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IDENTIFICATION OF BIOACTIVE COMPOUNDS IN GRAINS OF COLORED WHEAT VARIETIES DEVELOPED IN LITHUANIA AND IN SEEDS OF *FABACEAE* FAMILY PLANTS AND ENHANCEMENT OF THE ADDED VALUE OF THESE RAW MATERIALS FOR THEIR SUSTAINABLE APPLICATION IN THE FEED INDUSTRY

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LIETUVOJE SUKURTŲ SPALVOTŲJŲ KVIEČIŲ VEISLIŲ GRŪDŲ BEI *FABACEAE* ŠEIMOS AUGALŲ SĖKLŲ VEIKLIŲJŲ KOMPONENTŲ IDENTIFIKAVIMAS IR NAUJŲ ŽALIAVŲ PRIDĖTINĖS VERTĖS DIDINIMAS, SIEKIANT TVARAUS JŲ PANAUDOJIMO PAŠARŲ PRAMONĖJE

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LIST OF ABBREVIATIONS

AA – Amino acid
ABE – Acetone-butanol-ethanol fermentation
ANF – Antinutritional factors
BA – Biogenic amine
BCFA – Branched-chain fatty acid
BSTFA – *N,O* -Bis(trimethylsilyl)trifluoroacetamide
DON – Deoxynivalenol
DS – DS8558-1
FA – Fatty acid
FAA – Free amino acid
GABA – γ – aminobutyric acid
GAD – Glutamic acid decarboxylase
GC-MS – Gas chromatography - mass spectrometry
GRAS – Generally Recognized as Safe
IVD – *in vitro* digestion
IVF – *in vitro* fermentation
LAB – Lactic acid bacteria
LUHS122 – *Lactiplantibacillus plantarum*
LUHS210 – *Lacticaseibacillus casei*
LUHS245 – *Liquorilactobacillus uvarum*
LUHS29 – *Pediococcus acidilactici*
MUFA – Monounsaturated fatty acids
NOAEL – No observed adverse effect level
PLS-DA – Partial least square discriminant analysis
PUFA – Polyunsaturated fatty acids
QPS – Qualified Presumption of Safety
SCFA – Short-chain fatty acids
SFA – Saturated fatty acids
SMF – Submerged fermentation
SPME – Solid phase microextraction
SSF – Solid state fermentation
TBC – Total bacterial count
TTA – Total titratable acidity
VIP – Variable importance in projection
VOC – Volatile organic compound

INTRODUCTION

The importance of the sustainable and productive animal-based food production sector is exponentially growing as global demand is rapidly increasing due to globalization, urbanization and population growth [1, 2]. Such shortages can be overcome by implementing multiple strategies, involving selective breeding, integration of smart agriculture systems and genetic modifications of plants and animals [3-5]. One of the most significant aspects of the animal farming sector's advancement is to improve the nutritional value of the feed, since it is essential to ensure a proper development of the animals and their products [6, 7]. The improvement of the feed often involves the introduction of novel or nutritively valuable components in the feed formulation, thus providing vitamins, prebiotics, probiotics, phytobiotics, important fatty acids or enzymes [6-9]. Despite intense discussions about reducing or eliminating the use of animal products, plant-based products hold substantial relevance in the human diet, the animal-based products contain important essential nutrients that currently cannot be obtained through the plant-based diet alone [10, 11]. Some of such nutrients include iron, calcium, vitamin D, vitamin B12, thiamine, selenium, phosphorus [12, 13]. Additionally, many animal-based food sources contain significant amounts of omega-3 fatty acids, such as α-linolenic, eicosapentaenoic and docosahexaenoic acids [14, 15]. In case of protein intake, animal-based products have an abundance of all the essential amino acids, while plant-based products alone lack the amounts required for human consumption [16]. Deficiency of aforementioned essential nutrients can lead to serious complications, which include anemia, osteoporosis, megaloblastic anemia, rickets/osteomalacia, impaired immune system and other metabolic, neurologic and cardiovascular disorders [12, 17-20]. While deficiencies of essential nutrients can be managed by supplementation, trying to resolve an unbalanced diet in such a way might introduce additional issues, such as unintentional overdosing, possible drug-supplement interactions, side effects of the supplement components and more [21, 22].

In order to ensure a balance between the consumption of plant and animal products, solutions are being sought to use plants as efficiently as possible for animal nutrition, especially those that can also be consumed for human nutrition.

Wheat cereal continues to be a major cereal that is used for human as well as animal consumption [23]. While starch and other polysaccharides are the main components in their composition, wheat cereal also contains significant amounts of protein, fiber, B-group vitamins, phytochemicals (e.g.,

phenolic compounds, terpenoids) and minerals [24]. Leguminous crops, aka crops from the *Fabaceae* family, are gaining significant attention in the food and feed industries, due to their nutrient-rich composition, low oil and moisture content, and relatively low carbon footprint [25, 26]. Legumes generally contain over 18% of protein, over 14% of fiber, significant amounts of micro and macroelements (Ca, K, Mg, Fe, P), carotenoids (e.g., lutein, α-carotene, β-carotene), tocopherols, B-group vitamins, various flavonoids, and other phenolic compounds [27, 28]. While legumes are packed in essential nutrients, their bioavailability is severely limited due to antinutritional factors, which include phytates, saponins, lectins, tannins, and protease inhibitors [27, 29]. However, wheat cereal grains also contain some of these antinutritional factors, especially trypsin inhibitors, though most of those groups of compounds are significantly higher in leguminous crops [30]. Fortunately, there are abundance of methods that help to significantly reduce the antinutritional factors, which include milling, soaking, boiling, autoclaving, sprouting, extrusion, and fermentation [31]. Wheat and leguminous crops contain an abundance of phytochemicals, including carotenoids, anthocyanins, flavonoids, and other phenolic compounds that contribute to the color and organoleptic properties of the crops and can provide growth-promoting properties for the animals [32-34]. Some of those compounds also have antioxidant, anti-inflammatory, antibacterial, antifungal, antiprotozoal and even antiparasitic properties for the animals or even improve the quality of their products [34-36]. Colored wheat cereals are getting a lot of attention due to the anthocyanins contained inside aleurone and pericarp layers, that provide not just the color, but the additional health benefits, reducing risks of cardiovascular diseases, obesity, diabetes, provides antioxidant properties, and reduce methane production in ruminants, increase body weight gain, and improve the organoleptic properties of meat in poultry [37-40]. The majority of those compounds are derivatives of glucose, galactose, and rutinose with aglycones, such as cyanidin, malvidin, peonidin, delphinidin, and petunidin, ranging from 3 to over 100 mg/kg in the colored wheat [39, 41, 42]. Moreover, intensely pigmented legumes also contain anthocyanins, often in the form of glycosidic aglycones as well, that are stored mainly in the hulls of the seeds [32]. Similarly, the sugar moieties of the anthocyanins are mostly glucose and galactose, able to reach concentrations over 200 mg/kg [32, 43]. Though the *in vitro* digestion study showed a significant reduction in total anthocyanins, the significant accumulation of anthocyanins was found in liver, eye, and brain tissue *in vivo*, where its beneficial properties can be expressed [44, 45].

Fermentation has proven to be an efficient solution for the rising problems, helping to manage various shortcomings of certain feed components or

providing additional essential nutrients. It can reduce antinutritional factors (ANF), increase crude protein, hydrolyze protein and carbohydrates for improved digestibility, and improve nutrient availability [46, 47]. Fermentation also has the potential to reduce antigenic proteins, phytates, tannins, and enzyme inhibitors, increase vitamin B12, phosphorus, short-chain fatty acids, and other useful nutrients [48-50]. Additionally, the fermented feed provides prebiotics, probiotics, postbiotics, and parabiotics to the animal, while excreted short-chain fatty acids and bacteriocins provide inhibition of pathogenic bacteria [51-54].

It has been reported that fermented feed produced from various substrates, in either solid or liquid form, can increase the average daily gain and gain-to-feed ratio of weaned piglets, improve nutrient availability, average daily feed intake, apparent total tract digestibility, and increase the concentration of short-chain fatty acids (SCFAs) *in vivo* [55-57]. In addition, fermented feed can reduce the abundance of potential pathogens, such as *Salmonella* spp. and *Escherichia coli*, improve the integrity of the small intestinal barrier, and modulate the gut microbiota *in vivo* [46, 58]. The protective effect against pathogens is often attributed to the substantial reduction in pH resulting from the production of organic acids, which contribute to strengthening the intestinal barrier [46]. Moreover, fermented feed has been shown to significantly reduce the incidence of diarrhea in weaned piglets and decrease the emission of toxic compounds from fecal matter [59, 60]. Furthermore, the fermentation process enables the incorporation of various agro-industrial by-products into feed formulations at levels of up to 20% without compromising animal performance parameters [61].

The quality control of fermented products is of great importance as contamination with pathogenic bacteria and fungi can introduce unwanted toxic compounds, such as phytoestrogens, alkaloids, prussic acid, mycotoxins, and others [62, 63]. While other products, such as biogenic amines, are products of bacterial fermentation, which can cause nausea, heart palpitations, headaches, oral burning, and disruption of the gastrointestinal tract [63, 64]. Thus, careful monitoring of the fermentation process and its products is of the highest importance.

The two major types of fermentation that are utilized in the feed industry are submerged (SMF) and solid-state (SSF) fermentation [48]. During the SSF fermentation process, the bacterial and fungal cultures are growing in the absence of free water, while SMF utilizes free-flowing substrates or an abundance of liquid fermentation broth/media [48, 62, 65]. The SSF provides advantages, such as lower water and energy consumption, greater yields of the products, very low waste generation, fairly simple instrumentation, and the sterilization is optional [48, 62, 65]. Thus, making this process desirable for

industrial or even pilot batch applications. The SMF process is much easier to monitor and control, provides consistent productivity, and ease of product isolation, so it is often used in enzyme and secondary metabolite production [66, 67]. Additionally, such drastic differences in growing conditions require specific microorganisms that are able to grow in the absence (e.g., fungi, yeast and lactic acid bacteria (LAB) species) or abundance of free water, respectively [62].

Evidently, for centuries, LAB have been used extensively for the fermentation of various food and feed matrices, such as meat, fish, vegetables, milk, bread, and others [68]. The LAB strains can excrete proteolytic and carbohydrate-degrading enzymes, which improve digestibility, produce various metabolites that can reduce food spoilage and improve microbial safety, and organoleptic properties [68, 69]. Additionally, LAB strains have the status of Qualified Presumption of Safety (QPS) and/or Generally Recognized as Safe (GRAS) in dairy, bakery, meat, legume, and other industries [69].

Fermentation technology is characterized by its complexity. One of the key factors determining the quality of fermented materials is the detailed composition of the substrate. The scientific problem addressed in this study is related to the modeling of bioconversion schemes for cereal grains of newly developed Lithuanian wheat varieties and lentils by selecting technological parameters, including bacterial cultures and the water content of the substrate, with the aim of achieving the most sustainable bioconversion process possible. The available literature lacks data on the detailed chemical composition of the colored-cereal grain wheat varieties and *Fabaceae* family plants investigated in this study, as well as on the changes in their composition during fermentation. To ensure the effective application of these newly developed raw materials in the feed industry, it is necessary to identify appropriate technological solutions and evaluate the factors that determine the quality of the fermented substrate, including *in vitro* digestibility parameters.

New colored wheat lines developed by Lithuanian breeders are characterized by a higher content of biologically active compounds, especially anthocyanins, and may be a promising raw material for the production of value-added feed. However, their properties and technological potential have not been studied to date. Plants of the *Fabaceae* family were chosen for analysis in this dissertation due to their high content of protein, fiber, and other valuable components, but their use is limited by anti-nutritional factors. Although innovative technological solutions are required to increase their bioavailability in the aforementioned plant seeds. Comprehensive studies of these raw materials, including the assessment of detailed chemical composition, complement existing scientific knowledge and create the

prerequisites for creating more sustainable and higher value-added feed raw materials in a more sustainable way, i.e., by applying SSF schemes.

Aims of the study

The aim of the dissertation was to analyze the composition of newly bred Lithuanian colored wheat varieties and *Fabaceae* family crops, to develop technologies that increase their added value for sustainable application in the feed industry, and to apply an *in vitro* digestion model for evaluating wheat bioconversion and predicting potential *in vivo* effects.

Objectives of the study

1. To investigate the impact of selected LAB strains (LUHS29, LUHS245, LUHS122) on the bioconversion of different wheat varieties (*Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7, DS8535-2), with particular focus on modifying grain quality parameters, including free amino acids (FAAs), fatty acid (FA) composition, biogenic amines (BAs), and other metabolites, while evaluating the influence of wheat variety and LAB strain on these changes.
2. To evaluate the combined effect of LAB strains (LUHS122 and LUHS210) and substrate particle size reduction on the fermentation efficiency of red and green lentils.
3. To determine how processing of *Fabaceae* family lentil varieties (Danaja – both pure and intercropped systems) influences FAA, FA, volatile organic compound (VOC) profiles, and other physicochemical parameters under SSF and SMF fermentation conditions.
4. To adapt an *in vitro* digestion model for evaluating the bioconversion efficiency of colored wheat cereals and predicting potential *in vivo* changes by comparing digestion- and fermentation-related parameters, as well as metabolite profiles, in non-pretreated and fermented samples.

THE SCIENTIFIC NOVELTY

This dissertation provides a comprehensive evaluation of LAB-mediated bioconversion as an innovative approach for enhancing the functional and nutritional properties of cereal grains and legumes. The novelty lies in (i) the comparative assessment of multiple wheat varieties to distinguish the relative influence of genotype and LAB strains on biochemical modifications, (ii) the integration of substrate particle size reduction with both solid-state and submerged fermentation (SSF and SMF) to optimize lentil bioprocessing efficiency, and (iii) the application of an *in vitro* digestion model to systematically link bioconversion-induced changes with predicted *in vivo* outcomes. Furthermore, the study advances current knowledge by simultaneously analyzing a wide range of parameters, including FAAs, FAs, VOCs, and other metabolites, thereby providing a multidimensional understanding of fermentation-driven transformations in plant-based substrates. The results of this study offer practical solutions for improving the valorization of cereal and legume raw materials within the agricultural sectors. The demonstrated effectiveness of LAB-based bioconversion technologies supports their application as sustainable tools for enhancing protein quality (e.g., increasing essential FAAs and GABA) and overall feed value, particularly in wheat and lentil-based systems. The findings also highlight the importance of selecting appropriate raw materials and processing conditions to achieve optimal results. Additionally, the validated use of *in vitro* digestion models provides a cost-effective and efficient approach for predicting *in vivo* performance. Overall, this research contributes to the development of sustainable, circular-economy-based processing strategies that improve the utilization of plant-based resources, support the reduction of dependence on imported protein sources, and enhance the competitiveness and resilience of the agricultural and feed industries.

THE LAYOUT OF THE DISSERTATION

This dissertation is based on five published scientific articles (listed in the list of publications section). It is composed of four major parts: (1) investigation of the influence of colored wheat cereal bioconversion on the microbiological and physicochemical characteristics; (2) investigation of the influence of the particle size reduction and bioconversion of different lentil varieties on the microbiological and physicochemical characteristics; (3) investigation of the influence of the type of fermentation and growing conditions of lentils on the microbiological and physicochemical characteristics; (4) investigation of the influence of the bioconversion of traditional and colored wheat varieties on the *in vitro* digestion and fermentation parameters and their metabolites. The publications are published in peer-reviewed journals referred in *Web of Science*: *Fermentation*, MDPI; *Biology*, MDPI; *Frontiers in Nutrition*, FRONTIERS; *Foods*, MDPI; *Scientific Reports*, Springer Nature.

PHD CANDIDATE'S CONTRIBUTION

The contributions of author **Ernestas Mockus** to the publications (as listed in the List of Publications section) are presented bellow:

1. Contributed to the methodology of chromatographical methods used in the manuscript. Performed chromatographical analyses, data collection and visualization.
2. Contributed to the methodology of chromatographical methods, performed chromatographical analyses, data collection and visualization.
3. Contributed to the methodology of chromatographical methods. Performed chromatographical analyses, data collection and visualization. Contributed to original draft preparation.
4. Contributed to the methodology of chromatographical methods. Performed chromatographical analyses, data collection, statistical analysis and visualization. Contributed to original draft preparation.
5. Contributed to the conceptualization of the experiment. Contributed to the methodology of chromatographical methods. Performed chromatographical analyses, data collection, statistical analysis and visualization. Contributed to original draft preparation.

CO-AUTHORS' CONTRIBUTION

- **Elena Bartkienė** contributed to the conceptualization, supervision, preparation, review and editing of the original draft for all of the scientific articles.
- **Vytautas Ruzgas** contributed to the methodology, investigation, data curation, resources and reviewing-editing the original draft for the article No. 1-2; contributed to the resources and reviewing-editing the original draft for the article No. 5.
- **Žilvinas Liatukas** contributed to the methodology, investigation, data curation and reviewing-editing the original draft for the article No. 1-2; contributed to the methodology, resources and reviewing-editing the original draft for the article No. 5.
- **Vytautė Starkutė,** contributed to the formal analysis, investigation, data curation and original draft preparation for articles No. 1-4; contributed to the formal analysis, data curation and original draft preparation for the article No. 5.
- **Dovilė Klupšaitė** contributed to the formal analysis, investigation, data curation and original draft preparation for articles No 1-3; contributed to the formal analysis and investigation for the article No 4; contributed to the investigation, writing and editing original draft for the article No. 5.
- **Eglė Zokaitytė** contributed to the formal analysis, investigation, data curation, visualization, writing and reviewing-editing the original draft for the articles No. 1-3.
- **Lina Šarūnaitė** and **Aušra Arlauskienė** contributed to the formal analysis, investigation, resources and reviewing-editing the original draft for the article No. 4.
- **Romas Ruibys** contributed to the investigation and original draft preparation for the article No. 1; contributed to the formal analysis and original draft preparation for the article No. 3.
- **Vadims Bartkevics** contributed to the methodology, investigation, resources, data curation, writing and reviewing-editing the original draft for the articles No. 1-2; contributed to the methodology and reviewing-editing the original draft for the article No. 3; contributed to the resources, writing and reviewing-editing the original draft for the article No. 4.
- **Anastasija Borisova** contributed to the methodology, formal analysis, investigation and data curation for articles No. 1-2; contributed to the methodology, formal analysis and investigation in the article No. 4.

- **João Miguel Rocha** contributed to the data curation, reviewing-editing original draft and supervision of the project in the article No.1; contributed to the formal analysis and reviewing-editing original draft in the article No. 3; contributed to the reviewing-editing original draft in the article No. 4.
- **Romas Gružauskas** contributed to the reviewing-editing original draft in the article No. 2.
- **Modestas Ružauskas** contributed to the resources and reviewing-editing the original draft in the article No. 5.
- **Šarūnas Badaras** contributed to the resources and reviewing-editing the original draft in the article No. 5.

LIST OF PUBLICATIONS

This PhD thesis is based on the following publications:

1. **Mockus, E.**, Starkutė, V., Zokaitytė, E., Klupšaitė, D., Bartkevics, V., Borisova, A., Rocha, J. M., Ruibys, R., Liatukas, Ž., Ruzgas, V., & Bartkienė, E.. (2022). The Potential of Traditional 'Gaja' and New Breed Lines of Waxy, Blue and Purple Wheat in Wholemeal Flour Fermentation. Fermentation. Basel, Switzerland : MDPI, 2022, Vol. 8, No. 10., 1-23. https://doi.org/10.3390/fermentation8100563 [S1a] [M.kr.: A003, A001] [IF: 3.7, AIF: 5.5, quartile: Q2 (2022. InCites JCR SCIE)].
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4. **Mockus, E.**, Starkutė, V., Klupšaitė, D., Bartkevics, V., Borisova, A., Šarūnaitė, L., Arlauskienė, A., Rocha, J. M., & Bartkienė, E.. (2024). Changes in Chemical Composition of Lentils, Including GammaAminobutyric Acid and Volatile Compound Formation during Submerged and Solid-State Fermentation with Pediococcus acidilactici. Foods, 13(8), 1-24. https://doi.org/10.3390/foods13081249 [S1] [M.kr.: A003, A001] [IF: 5.1, AIF: 5.331, quartile: Q1 (2024. InCites JCR SCIE)].
5. **Mockus, E.**, Starkutė, V., Klupšaitė, D., Badaras, Š., Liatukas, Ž., Ruzgas, V., Ružauskas, M., & Bartkienė, E.. (2026). Comparative metabolomic assessment of bioconverted traditional and black wheat via the in vitro piglet digestion model. Scientific Reports (T. 00, Number 00). https://doi.org/10.1038/s41598-026-48313-9 [S1] [M.kr.: A003, A002] [IF: 3.9, AIF: 5.844, quartile: Q1 (2024. InCites JCR SCIE)]

INTERNATIONAL SCIENTIFIC CONFERENCES

1. Oral presentation. **Mockus, E.**, Starkutė, V., Zokaitytė, E., Klupšaitė, D., Bartkevics, V., Borisova, A., Liatukas, Žilvinas, Ruzgas, V., & Bartkienė, E.. (2023). Advantages of the wheat wholemeal fermentation. Tagungsband: 21. BOKU-Symposium Tierernährung Fütterungsstrategien in Zeiten Knapper Ressourcen : 20. April 2023, Wien Institut für Tierernährung, Tierische Lebensmittel Und Ernährungsphysiologie (TTE). Department für Agrarbiotechnologie, IFA-Tulln. Universität für Bodenkultur Wien ; Herausgebers: Pedro H S Ferzola, Christiane Schwarz, Martin Gierus. Wien : Institut für Tierernährung, Tierische Lebensmittel Und Ernährungsphysiologie (TTE), 2023.
2. Oral presentation. **Mockus, E.**, Starkutė, V., Klupšaitė, D., Bartkevics, V., Borisova, A., Šarunaitė, L., Arlauskienė, A., Rocha, J. M., & Bartkienė, E.. (2024). Technological potential to enhance the nutritional and functional value of lentils as a source of valuable animal feed. Veterinarija Ir Zootechnika : "Livestock Production – Recent Trends and Future Prospects" : 4th International Scientific Conference Lithuanian University of Health Sciences, Veterinary Academy, Faculty of Animal Sciences. 26-27 September 2024. Kaunas.

1. MATERIALS AND METHODS

1.1. Materials Used in the Experiments

Seeds of the *Fabaceae* family (lentil varieties: CDC Lemay, CDC Red Rider, Danaja (pure and intercropped)) and cereal grains of the *Poaceae* family (wheat varieties: standard – *Ada*, *Gaja*, *Herkus*; waxy wheat varieties: DS8888-3-6, *Sarta*; colored – DS8472-5, DS8526-2, DS8548-7, DS8535-2, DS8558-1) were used for the designed experiments. The LAB strains *Pediococcus acidilactici* (LUHS29), *Liquorilactobacillus uvarum* (LUHS245), *Lacticaseibacillus casei* (LUHS210) and *Lactiplantibacillus plantarum* (LUHS122) were used in the designed experiments. Bacterial strains were obtained from from the Lithuanian University of Health Sciences (Kaunas, Lithuania). The isolation of the strains, their biochemical properties, antifungal, and antibacterial activities were described by Bartkiene et al. (2019) [70].

1.2. Pre-treatments Used for Experimental Plant Material

Samples (lentils and wheat cereal grains) were milled with a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) to achieve a substrate particle size of 1–2 mm.

SMF and SSF protocols were used for bioconversion of the aforementioned substrates. SMF involved fermentation using a 1:5 ratio (w/w) of substrate with water, while in SSF, 1:1 and 10:6 ratios (w/w) were used. The fermentation was initiated via the addition of LAB suspension (3 % of the substrate by dry mass, 8.6–8.7 $\log_{10}$ CFU/mL). The fermentation durations tested in the experiments were 24 h and 48 h.

1.3. Principal Schemes of the Designed Experiments

The experimental scheme of wheat bioconversion is presented in Figure 1.3.1. The experiment involved the SSF of the milled wheat cereal (*Ada*, *Gaja*, DS8888-3-6, *Sarta*, DS8472-5, DS8526-2, DS8548-7, DS8535-2). For the bioconversion, *Pediococcus acidilactici*, *Liquorilactobacillus uvarum*, and *Lactiplantibacillus plantarum* were used. Cereal substrates were fermented at 30 ± 2 °C for 24 h. The SSF samples contained a substrate and water ratio of 10:6 (w/w). The fermentation was initiated via the addition of LAB suspension (3 % of the substrate by dry mass, 8.7 $\log_{10}$ CFU/mL). The samples consisted of bioconverted and control (without the addition of LAB suspension) samples. The microbiological (LAB count) and physicochemical

(pH, total titratable acidity (TTA), chromaticity characteristics, BAs, FAAs, micro- and macroelements, FA composition, VOCs) and mycotoxins concentration parameters were evaluated in the sample groups.

The experimental scheme of milled and non-milled lentil (varieties 'CDC Lemay' and 'CDC Red Rider') bioconversion is presented in Figure 1.3.2. The experiment involved SSF of milled and nonmilled lentil substrates, using *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* strains. The SSF samples contained a substrate and water ratio of 1:1 (w/w). The fermentation was initiated via the addition of LAB suspension (3 % of the substrate by dry mass, 8.6 $\log_{10}$ CFU/mL). Duration of the bioconversion was 24 h at 30 ± 2 °C. Samples consisted of SSF samples and corresponding control groups (without LAB suspension). The microbiological (LAB count) and physicochemical (pH, TTA, chromaticity characteristics, BAs, FAAs, FA composition, VOCs) parameters were evaluated in the sample groups.

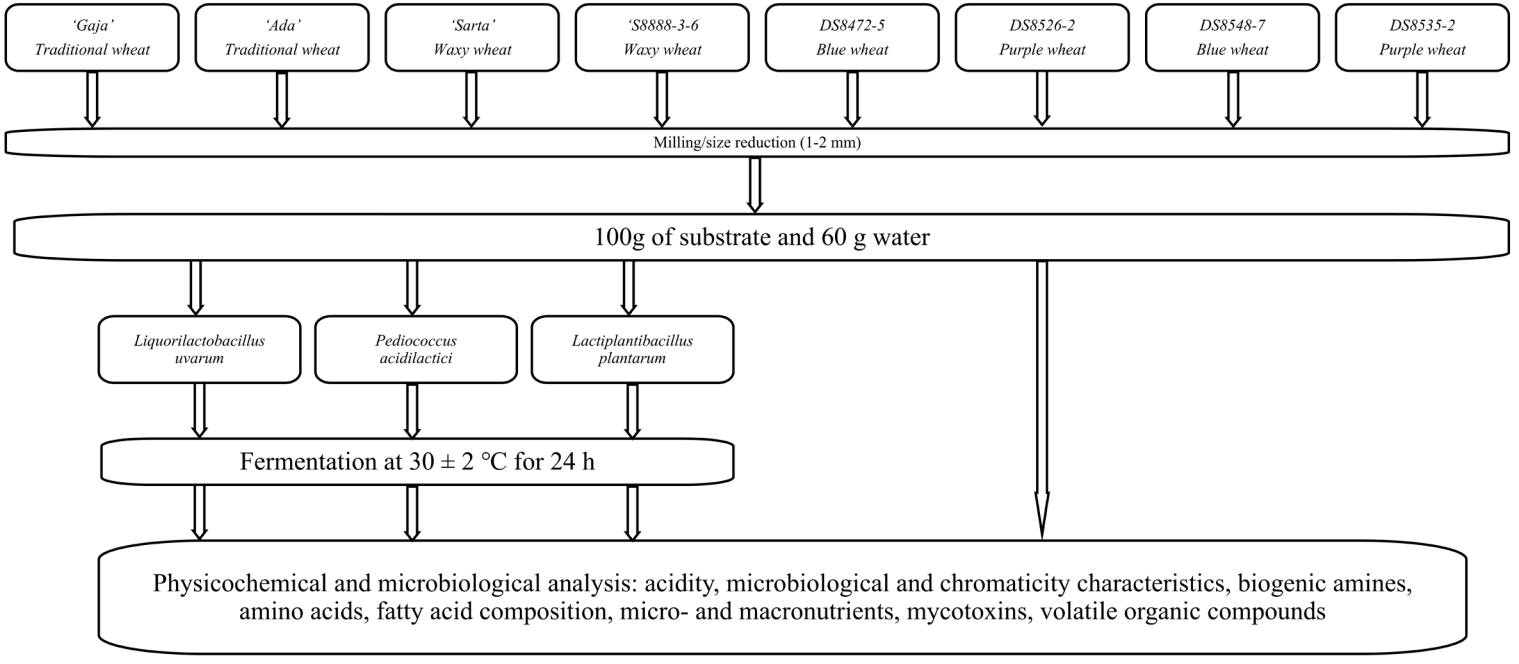

Fig. 1.3.1. *Experimental scheme of the wheat bioconversion*

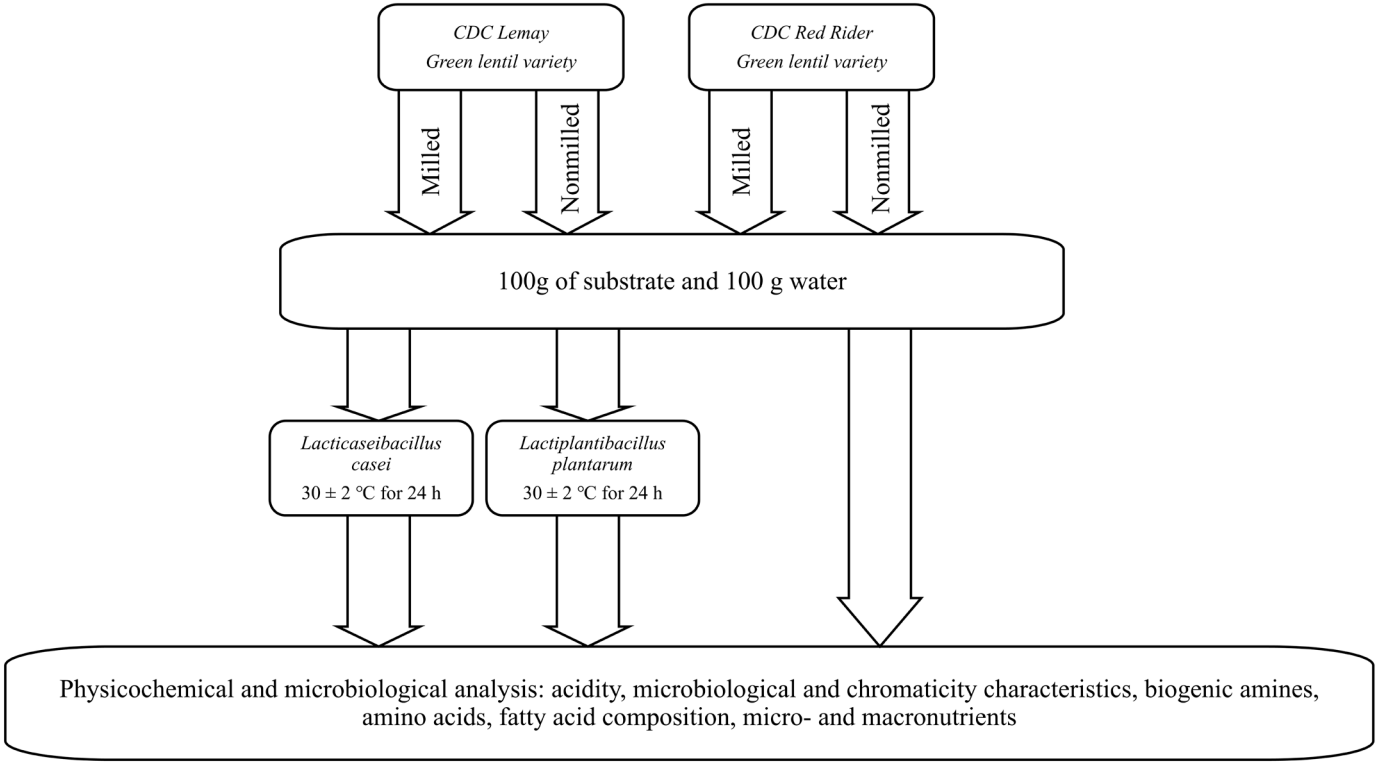

Fig. 1.3.2. *Experimental scheme of milled and non-milled lentils*

The experimental scheme of pure and intercropped lentil (variety Danaja) bioconversion, utilizing SMF and SSF protocols, is presented in Figure 1.3.3. The experiment consists of milled SMF and SSF samples, using substrate and water ratios of 1:5 and 1:1, respectively. Fermentation was initiated using LAB suspension (8.7 $\log_{10}$ CFU/mL). The duration of the bioconversion was 24 h and 48 h at 30 ± 2 °C. The corresponding control samples consisted of a substrate and water ratio without the LAB suspension. The microbiological (LAB count) and physicochemical (pH, TTA, chromaticity characteristics, BAs, FAAs, FA composition, VOCs, micro- and macroelements) parameters were evaluated in the sample groups.

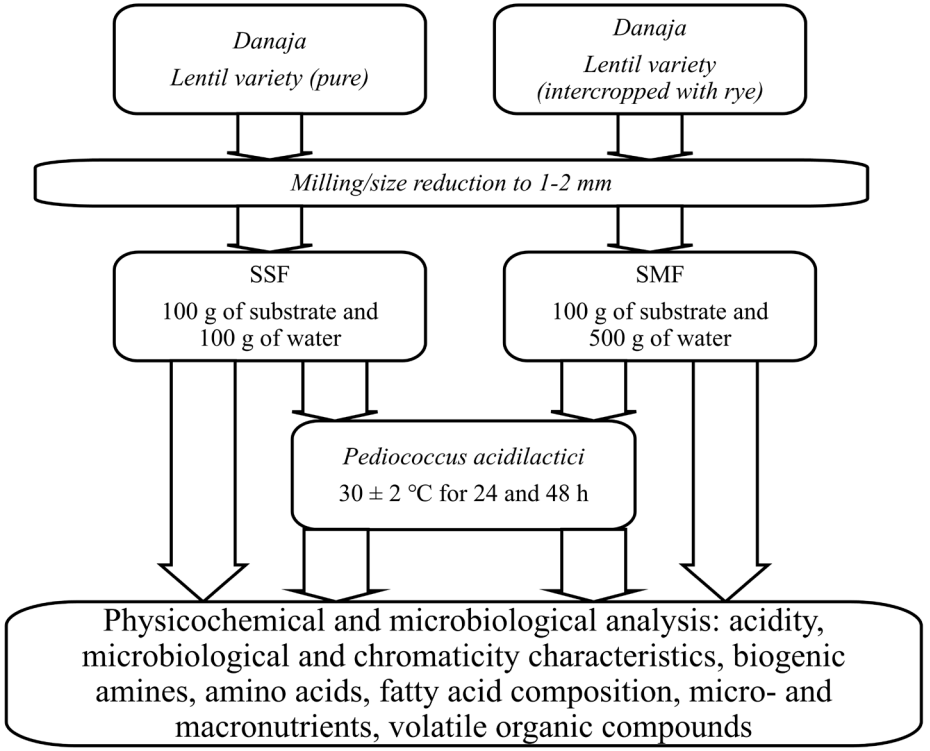

Fig. 1.3.3. *Experimental scheme of submerged and solid-state fermentation of lentil samples*

The experimental scheme of *in vitro* digestion and fermentation of bioconverted black (variety DS8558-1) and traditional (variety Herkus DS) wheat grains, using the *Sus scrofa domesticus* model, is presented in Figure 1.3.4. The experiment consisted of milling of the wheat grain samples and their bioconversion, using the SSF protocol (substrate/water ratio of 1:1 (w/w)) and *Lp. plantarum* strain suspension (3% of dry matter of the wholemeal flour, 8.7 $\log_{10}$ CFU/mL at (30 ± 2) °C for 24 h). The *in vitro* digestion step consisted of the modified INFOGEST model, described by Brodkorb et al. (2019) [71]. The *in vitro* fermentation step was adapted from the *in vitro* colonic batch fermentation protocol, described by Burillo et al. (2021) [72]. More detailed protocol of the experiment is described by Mockus et al. (2026) [73]. Bioconverted and control (without LAB suspension) samples were subjected to *in vitro* digestion and fermentation protocols. The total amino acids (AAs) were analysed in the SSF samples, *in vitro* digestion supernatant and residues. The FAAs were analysed in the *in vitro* digestion supernatant. The FAAs, BAs, VOCs, SCFAs, and other semi-volatile and non-volatile metabolites were analysed in the *in vitro* fermentation samples. Additionally, *in vitro* total digestibility, protein digestibility, and total fermentability were evaluated.

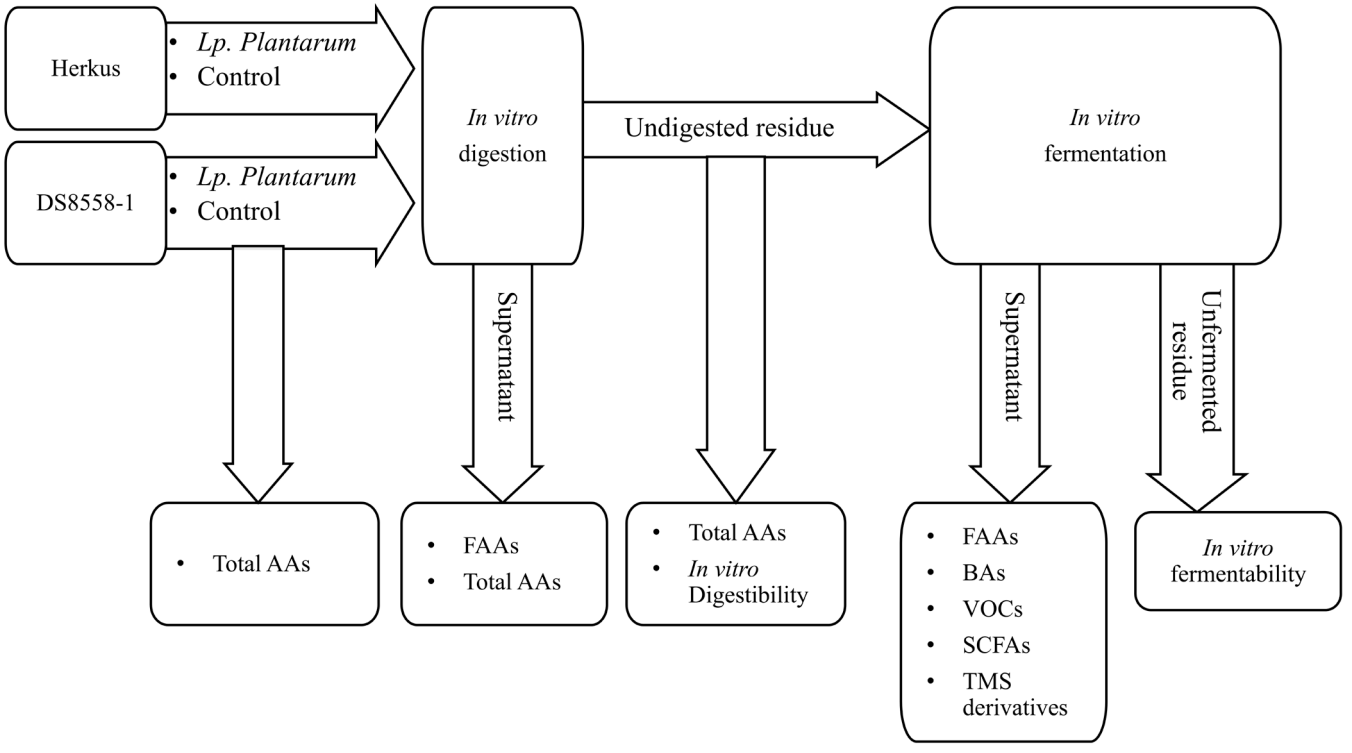

Fig. 1.3.4. *Schematic representation of the in vitro digestion/fermentation experiment*

AA – amino acid; FAA – free amino acid; BA – biogenic amine; VOC – volatile organic compound; SCFA – short-chain fatty acid; TMS – trimethylsilyl.

1.4. Methods Used for the Analysis of the Samples in the Designed Experiments

1.4.1. Evaluation of Acidity, Microbiological, and Chromaticity Characteristics

The pH of the samples in the designed experiments was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany). The TTA was evaluated for a 10 g analysed sample mixed with 90 mL of water; results were expressed as mL of 0.1 mol/L NaOH solution required to achieve a pH value of 8.2. The LAB count was determined according to the method described by Bartkiene et al. (2017) [74]. The TBC was determined on Plate count agar and was incubated under aerobic conditions at 32 °C for 24 to 48 h. The chromaticity characteristics of the samples were evaluated on the surface using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Japan). The results were expressed as the CIE color values L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness).

1.4.2. Determination of the Biogenic Amine Content

Analysis of the BAs was performed by extracting the samples with 0.4 M perchloric acid solution, containing 1,7-diaminoheptane as internal standard, and diluting to 30 mL of final volume. Afterwards, the pH of 1 mL of the derivatization solution was increased with 2 M NaOH and saturated $NaHCO_3$ solutions, prior to the addition of 2 mL of 10 mg/mL dansyl chloride solution in acetone. Derivatization was performed at 40 ℃ for 45 minutes. After the derivatization, excess reagent was quenched with 25 % ammonia solution and diluted with mobile phase. Derivatized analytes were analysed using a Varian Prostar HPLC-UV system (Varian Corp., Palo Alto, California, USA), using 254 nm for detection. A detailed description of the protocol is described by Bartkiene et al. (2022) [75].

1.4.3. Analysis of Free Amino Acids

For the analysis of FAAs, a homogenous portion of the sample (100–1000 mg) was extracted with a 0.1 M HCl solution (1–10 mL). The resulting supernatant (50–200 μL) was made alkaline, using 2 M NaOH and saturated $NaHCO_3$ solutions, and derivatized using a 10 mg/mL solution of dansyl chloride. Derivatization was performed at 60 ℃ for 30 minutes. The excess reagent was quenched using 25% ammonia solution. Derivatized analytes were analysed using an LC-MS system, consisting of Varian Prostar HPLC system (Varian Corp., Palo Alto, California, USA) with Thermo Scientific

LCQ FLEET mass spectrometer (Thermofisher Scientific, San Jose, California, USA). Detailed protocols and their modifications are described by Mockus et al. (2022, 2023, 2024) [76-78].

1.4.4. Analysis of Macro- and Microelements Using Inductively Coupled Plasma Mass Spectrometry

Macro- and microelement analysis was performed using Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan) with the corresponding software MassHunter Work Station for ICPMS, version B.01.01 (Agilent Technologies, Tokyo, Japan). The homogenous portion of the sample was treated with concentrated nitric acid and hydrogen peroxide in the microwave system until the reaction was finished. Afterwards, the mixture was heated at 150 ℃ for 30 minutes and another 30 minutes at 200 ℃. The cooled sample was filtered, diluted to 50 mL and analysed subsequently. Detailed description of the protocol can be found in Bartkiene et al. (2018) [79].

1.4.5. Analysis of Fatty Acid Composition (Hexane Extraction Method)

For analysis of FA composition, about 1 g of homogeneous sample was dispersed in 5 mL of 30% NaCl solution. Lipids were extracted by adding 5 mL of hexane and subsequent extraction via orbital laboratory shaker for 1 h. A portion of the upper layer (4 mL) was reacted with 300 µL of the methylation reagent (2 mol/L of KOH in methanol) by shaking for 1 h. The upper layer, containing derivatized FAs, was separated by centrifugation and used for the analysis. Gas chromatograph – mass spectrometer GCMSQP2010 (Shimadzu, Kyoto, Japan) was used to analyse the samples. A capillary Stabilwax-MS column (30 m × 0.25 mm ID × 0.25 µm film thickness) was used for the separation of the analytes. Concentrations were determined from calibration curves obtained from analytical standards at different concentrations, and results were expressed as a percentage of the total detected FAs. A detailed description is given by Bartkiene et al. (2022) [75].

1.4.6. Analysis of Fatty Acid Composition (Folch Extraction Method)

The Folch extraction method of the lipids involves the extraction of 1 g of the homogenous sample with 10 mL of the extraction solution, consisting of a chloroform and methanol mixture (2:1, v/v). Extraction is performed by shaking, using an orbital laboratory shaker for 1 h. The separated organic layer is washed with 0.9% NaCl solution. Separated organic layer is subsequently dried using nitrogen gas. The resulting residue is dissolved in 1 mL of hexane

and reacted with 70 µL of the methylation reagent by shaking for 30 minutes. The instrument, analytical column and data processing were identical as described in section 1.4.5. More details about the method can be found in Mockus et al. (2024) [78].

1.4.7. Analysis of Mycotoxins *via* High-Performance Liquid Chromatography Coupled to Triple Quadrupole Mass Spectrometry (LC-MS/MS)

For the analysis of mycotoxins, the QuEChERS extraction protocol was employed. In brief, about 2.5 g of the homogenous sample was extracted using 10 mL of water, 10 mL of acetonitrile, 20 µL of formic acid and QuEChERS buffer salts. After extraction, the upper layer of the supernatant was subjected to QuEChERS dispersive solid-phase extraction cleanup step and the upper layer was used for the analysis with UltiMate™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) HPLC coupled with a Thermo Scientific TSQ Quantiva mass spectrometer. The details of the analysis can be found by Bartkiene et al. (2022) [75] .

1.4.8. Analysis of Volatile Organic Compounds

Analysis of the VOCs in the samples involved dispersion of a homogenous portion of the sample (about 1–2 g), which was homogenized with buffering medium and transferred to 20 mL headspace vials, sealed with polytetrafluoroethylene septum. For the analysis, the autosampler transferred samples to the thermostat to heat up samples up to 60 °C and, subsequently, the solid phase microextraction (SPME) device fiber was exposed to the headspace of the vial to extract volatile compounds. Extracted VOCs were desorbed into the liner of the gas chromatograph for the analysis. Analysis was performed using a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph coupled with a mass spectrometer. The VOCs were identified using mass spectrum libraries (NIST11 and FFNSC2). Detailed protocols and modifications are described by Bartkiene et al. (2022) and Mockus et al. (2023, 2024) [75, 76, 78].

1.4.9. Analysis of Total Amino Acids

For analysis of AA hydrolysates, an appropriate amount of sample (50-500 mg depending on protein content) was hydrolyzed with 6 M HCl solution, containing 0.1% phenol, in a laboratory oven at 110 °C for 23 hours. Afterwards, the hydrolyzed mixture was cooled down and neutralized with 7 M NaOH solution. The hydrolysates were diluted to 50 mL with deionized

water and subsequently derivatized and analysed using the procedure, described in section 1.4.3.

1.4.10. Analysis of Free Amino Acids and Biogenic Amines in the *In Vitro* Fermented Samples

The protocol of the analysis is based on the derivatization with chloroformate and subsequent analysis using gas chromatography – mass spectrometry (GC-MS). In brief, the sample was diluted 10 times with 0.1 M HCl solution, and a 100 µL aliquot was subjected to the derivatization. After dilution to 200 µL with 0.1 M HCl, addition of internal standard (norleucine), alkalization with 2M NaOH solution, addition of methanol and pyridine mixture (4:1, v/v) and chloroform (500 µL), the mixture was derivatized with the addition of isobutylchloroformate (50 µL). The organic layer was separated and dried with anhydrous Na_2SO_4 afterwards. Analysis was performed on a GCMS-QP2010 (Shimadzu, Kyoto, Japan) GC-MS system, using a capillary Rxi®-5MS column (Restek, USA) (length 30 m, coating thickness 0.25 µm, inner diameter of 0.25 mm) for separation. Instrument operated in single ion monitoring mode for quantification. Results were calculated from the internal standard calibration curve. Detailed protocol description can be found in Mockus et al. (2026) [73].

1.4.11. Untargeted Analysis of Small Non-volatile and Semi-volatile Metabolites in the *In Vitro* Fermented Samples

Ten-fold dilution with methanol (containing internal standard ribitol) of the samples was performed with subsequent extraction in the sonication bath for 5 minutes. Aliquot of the separated supernatant (100 µL) was dried with nitrogen and subjected to derivatization with 50 µL of methoxyamine hydrochloride solution in pyridine (20 mg/mL) for 2 h at room temperature. The resulting mixture was subsequently derivatized with the addition of 50 µL of *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% of trimethylchlorosilane, for 1 h at 70 °C. Afterwards, the reaction mixture was diluted with 400 µL of hexane and used for the analysis with the GC-MS system. Analysis was performed on a GCMS-QP2010 (Shimadzu, Kyoto, Japan) GC-MS system, using a capillary Rxi®-5MS column (Restek, USA) (length 30 m, coating thickness 0.25 µm, inner diameter of 0.25 mm) for separation. Analytes were identified using mass spectrum libraries (NIST11 and FFNSC2). A detailed description of the protocol can be found in Mockus et al. (2026) [73].

1.4.12. Analysis of Short-chain Fatty Acids

The analysis of SCFAs was performed using a GC-MS system. In brief, the *in vitro* fermented samples were spiked with internal standard (3-methylvaleric acid) and diluted up to tenfold with 0.1 M HCl solution. The supernatant was directly analysed with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) GC-MS system, using a Stabilwax-Da column (Restek, USA) (length 30 m, coating thickness 0.25 μm-ϕ, inner diameter of 0.25 mm-ϕ) for separation. Instrument operated in single ion monitoring mode, and concentrations were obtained from internal standard calibration curves.

1.4.13. The *In Vitro* Digestion and Fermentation Protocol Adapted for Sus Scrofa Domesticus Model

Bioconverted and control substrate samples were subjected to the *in vitro* digestion and colonic fermentation protocol. The *in vitro* digestion protocol was adapted from the simulated static digestion INFOGEST protocol, described by Brodkorb et al. (2019) [71]. In brief, the substrate was subjected to simulated gastric digestion, involving simulated gastric fluid, low pH (pH = 3), porcine pepsin (enzymatic activity in the final mixture of 2000 U/mL) and was incubated at 39 ℃ for 2 hours. Simulated intestinal phase involved subsequent addition of simulated intestinal phase, bile salts, porcine pancreatin (enzymatic activity in final mixture of 100 U/mL), neutral pH and were incubated at 39 ℃ for 2 hours. Afterwards, samples were cooled down in an ice bath, and undigested matter was separated from the mixture and dried. The residue was used for the *in vitro* digestion evaluation and the *in vitro* fermentation. The supernatant was frozen and analysed according to the experimental scheme in Figure 1.3.4. The *in vitro* fermentation protocol was adapted from the protocol, described by Burillo et al. (2021) [72]. In brief, a portion of undigested residue (~400 mg) was subjected to the sterile fermentation medium (phosphate buffer pH = 7.00, reductive solution, resazurin, and peptone) and faecal inoculum. Samples were incubated at 39 °C for 48 h in a thermostat. Afterwards, the samples were cooled down in the ice bath and centrifuged at 500*g* to recover unfermented residue. The supernatant was divided into 1.5 mL aliquots and frozen at –20 °C until the analysis. The details of the protocol are described by Mockus et al. (2026) [73].

1.4.14. Statistical Analysis

Microbiological and physical chemical parameters data were expressed as the mean ± standard deviation. Statistical calculations were carried out

using R statistical programming software packages. In order to evaluate the effects of the different factors, the data were analysed using analysis of variance (ANOVA), and Tukey HSD (Tukey's honest significant difference) post hoc test was conducted to detect differences among sample groups. To determine the normality and homoskedasticity, the Shapiro–Wilk test and Levene test were performed, using "stats" (version 4.4.2) and "car" (version 3.1-3) packages. The linear Pearson's correlation coefficients were calculated and visualized using "psych" (version 2.5.6) and "corrplot" (version 0.95) packages. The results were recognized as statistically significant at $p < 0.05$. Heatmap visualization and analysis was carried out using the R statistical programming software package "ComplexHeatmap" (version 2.25.2). The partial least square discriminant analysis (PLS-DA) and variable importance in projection (VIP) were carried out using the "mixOmics" package (version 6.26.0). Volcano plot analysis and visualizations were carried out using the "EnhancedVolcano" package (version 1.24.0). Missing values for the volcano plot analysis were filled using the half minimum method.

2. RESULTS AND DISCUSSION

2.1. Influence of Wheat Cereal Grain Bioconversion on Their Microbiological and Physicochemical Parameters

Two experiments with different wheat varieties (*Ada*, *Sarta*, DS8472-5, DS8526-2; and *Gaja*, DS8888-3-6, DS8548-7, DS8535-2, respectively) were performed [75, 77]. Those studies involved bioconversion of traditional, waxy, and colored wheat varieties and evaluation of analyzed microbiological and physical chemical (pH, chromaticity characteristics, BAs, FAAs, VOCs, FA composition, mycotoxins, micro-and macroelements) parameters.

The pH, TTA, color characteristics and LAB count results are presented in Table 2.1.1. It was found that all LAB strains were able to propagate and reach over 8.00 $\log_{10}$ CFU/g. It shows that the substrate is suitable for fermentation with *P. acidilactici*, *Liq. uvarum*, and *Lp. plantarum* strains. Moreover, the pH of the bioconverted samples dropped from 4.67 to 3.55 for some samples. While very low pH can inhibit the growth of LAB strains, most of the strains are able to handle a pH as low as 3.2 [80, 81]. The low pH tolerance and effective propagation in the substrates show its potential probiotic properties [82]. The TTA and pH were negatively correlated ($r = -0.422$, $p = 0.003$), and the LAB strain was a significant factor for TTA ($p = 0.011$, $p \leq 0.001$). Though TTA is associated with the LAB activity via the production of organic acids, the substrate, temperature, or even the LAB strain itself can greatly affect the TTA [83, 84]. The wheat variety, LAB strain, and the combination of the factors were significant for color characteristics. Various compounds such as anthocyanins, carotenoids, and flavones, are responsible for the color of the cereals, while LAB strains have varied abilities to degrade those compounds and/or introduce their antioxidative effects during the fermentation [85, 86].

Table 2.1.1. *Microbiological and physicochemical parameters of the wheat samples*

Sample	pH	TTA, °N	L	a	b	LAB count, log_{10} CFU/g
Ada	6.68 ± 0.030 a	1.30 ± 0.020 m	52.8 ± 1.21 ab	5.79 ± 0.110 bc	16.6 ± 0.130 b	4.41 ± 0.110 f
Ada_LUHS122	3.99 ± 0.020 rs	5.10 ± 0.150 ij	51.9 ± 0.970 bcd	5.79 ± 0.130 bc	16.8 ± 0.190 b	8.72 ± 0.180 abcd
Ada_LUHS245	4.21 ± 0.010 klm	4.80 ± 0.090 k	50.6 ± 1.50 bcde	5.46 ± 0.160 d	15.7 ± 0.210 c	8.61 ± 0.140 abcde
Ada_LUHS29	4.29 ± 0.020 j	4.90 ± 0.080 jk	42.0 ± 1.10 kl	4.27 ± 0.100 ghi	8.88 ± 0.130 n	8.52 ± 0.150 abcde
Sarta	6.42 ± 0.030 b	1.00 ± 0.030 n	40.9 ± 1.19 lm	4.43 ± 0.090 g	8.10 ± 0.200 o	4.33 ± 0.090 f
Sarta_LUHS122	3.81 ± 0.020 t	5.70 ± 0.120 cde	48.9 ± 1.41 efg	3.94 ± 0.080 jk	14.3 ± 0.130 de	8.64 ± 0.160 abcde
Sarta_LUHS245	3.84 ± 0.010 t	5.50 ± 0.160 efgh	46.7 ± 1.17 ghi	3.72 ± 0.110 klm	9.89 ± 0.110 k	8.37 ± 0.180 bcde
Sarta_LUHS29	4.19 ± 0.020 lmn	4.50 ± 0.090 l	44.9 ± 0.940 ij	3.85 ± 0.070 jkl	9.59 ± 0.140 kl	8.11 ± 0.210 e
DS8472-5	6.09 ± 0.020 d	1.00 ± 0.020 n	47.6 ± 0.320 fgh	3.03 ± 0.070 p	11.3 ± 0.090 hi	4.30 ± 0.060 f
DS8472-5_LUHS122	3.94 ± 0.030 s	5.80 ± 0.04 cd	50.6 ± 0.340 bcde	4.76 ± 0.070 f	14.0 ± 0.320 ef	8.92 ± 0.210 ab
DS8472-5_LUHS245	4.15 ± 0.020 mno	5.10 ± 0.120 ij	50.3 ± 1.18 cde	6.85 ± 0.160 a	13.8 ± 0.210 f	8.53 ± 0.200 abcde
DS8472-5_LUHS29	4.07 ± 0.010 pq	5.40 ± 0.080 fgh	40.9 ± 0.270 lm	4.08 ± 0.060 ij	6.12 ± 0.140 qr	8.31 ± 0.120 cde
DS8526-2	6.18 ± 0.020 c	1.20 ± 0.030 mn	46.1 ± 1.32 hij	5.12 ± 0.110 e	9.52 ± 0.170 klm	4.53 ± 0.090 f
DS8526-2_LUHS122	4.67 ± 0.020 g	5.60 ± 0.140 def	39.8 ± 1.14 lmn	4.10 ± 0.090 hij	5.86 ± 0.140 rs	8.76 ± 0.090 abc
DS8526-2_LUHS245	4.86 ± 0.040 f	5.90 ± 0.120 bc	40.3 ± 0.890 lmn	3.58 ± 0.100 lmn	5.29 ± 0.120 t	8.45 ± 0.170 abcde
DS8526-2_LUHS29	4.24 ± 0.030 jkl	5.00 ± 0.110 ijk	38.6 ± 0.770 mn	4.25 ± 0.120 ghi	5.42 ± 0.080 st	8.31 ± 0.110 cde
Gaja	6.01 ± 0.010 e	1.23 ± 0.110 mn	41.9 ± 0.120 kl	4.39 ± 0.090 gh	9.07 ± 0.110 mn	4.54 ± 0.320 f
Gaja_LUHS122	4.44 ± 0.020 h	4.91 ± 0.090 jk	44.1 ± 0.140 jk	4.86 ± 0.070 ef	9.16 ± 0.090 lmn	8.48 ± 0.180 abcde
Gaja_LUHS245	4.37 ± 0.020 i	4.90 ± 0.060 jk	52.1 ± 0.230 bc	4.88 ± 0.060 ef	16.0 ± 0.130 c	8.59 ± 0.120 abcde
Gaja_LUHS29	4.01 ± 0.010 qr	5.00 ± 0.050 ijk	54.8 ± 0.150 a	5.89 ± 0.080 bc	18.9 ± 0.140 a	8.63 ± 0.140 abcde
DS8888-3-6	6.11 ± 0.030 d	1.20 ± 0.030 mn	52.3 ± 0.210 bc	3.06 ± 0.030 op	14.8 ± 0.220 d	4.19 ± 0.270 f
DS8888-3-6_LUHS122	4.09 ± 0.020 op	5.22 ± 0.050 hi	48.5 ± 0.350 efgh	3.36 ± 0.050 n	10.8 ± 0.180 j	8.08 ± 0.140 e
DS8888-3-6_LUHS245	4.20 ± 0.010 lm	5.43 ± 0.040 efgh	49.1 ± 0.210 efg	3.54 ± 0.060 mn	11.8 ± 0.210 h	8.48 ± 0.110 abcde
DS8888-3-6_LUHS29	3.99 ± 0.020 rs	5.61 ± 0.090 cdef	52.3 ± 0.190 bc	3.06 ± 0.090 op	14.8 ± 0.160 d	8.61 ± 0.160 abcde

Table 2.1.1 cont.

Sample	pH	TTA, °N	L	a	b	LAB count, log_{10} CFU/g
DS8535-2	5.98 ± 0.020 e	1.00 ± 0.050 n	45.1 ± 0.160 ij	5.93 ± 0.120 bc	11.2 ± 0.120 ij	4.41 ± 0.130 f
DS8535-2_LUHS122	4.40 ± 0.020 hi	5.52 ± 0.070 defg	38.7 ± 0.220 mn	6.08 ± 0.080 b	6.55 ± 0.070 q	8.74 ± 0.190 abc
DS8535-2_LUHS245	3.72 ± 0.030 u	6.21 ± 0.050 a	38.0 ± 0.130 n	5.67 ± 0.060 cd	5.08 ± 0.080 t	8.92 ± 0.210 ab
DS8535-2_LUHS29	3.61 ± 0.010 v	6.30 ± 0.110 a	42.0 ± 0.160 kl	5.95 ± 0.090 bc	8.10 ± 0.110 o	8.99 ± 0.170 a
DS8548-7	6.18 ± 0.020 c	1.02 ± 0.110 mn	49.5 ± 0.140 def	4.10 ± 0.050 hij	12.7 ± 0.150 g	4.69 ± 0.310 f
DS8548-7_LUHS122	4.13 ± 0.020 nop	5.23 ± 0.060 ghi	44.0 ± 0.230 jk	3.76 ± 0.110 klm	7.09 ± 0.090 p	8.16 ± 0.230 de
DS8548-7_LUHS245	4.27 ± 0.020 jk	5.42 ± 0.100 efgh	48.2 ± 0.170 efgh	2.93 ± 0.090 p	8.76 ± 0.080 n	8.49 ± 0.160 abcde
DS8548-7_LUHS29	3.55 ± 0.010 v	6.10 ± 0.140 ab	41.7 ± 0.250 kl	3.34 ± 0.040 no	7.65 ± 0.110 o	8.94 ± 0.220 ab

TTA – total titratable acidity; L* – lightness; a* – redness (-a* greenness); b* – yellowness (-b* blueness); LAB – lactic acid bacteria; CFU – colony forming units; LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–v Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Phenylethylamine was found in all wheat samples and was a major BA in the control samples (Table 2.1.2). Cadaverine formed in most of the samples fermented with *Liq. uvarum* and *Lp. plantarum* strains. Putrescine was found in all *Ada* and *Sarta* samples. Additionally, wheat variety, LAB strain, and the factors' interaction were significant for concentrations of BAs in the wheat wholemeal samples. BAs' production is mostly associated with bacterial activities during the fermentation through several mechanisms *via* the decarboxylation and transamination of amino acids and ketones/aldehydes, respectively [87, 88]. Though LAB has GRAS status, the production of the BAs should be highly monitored as their production highly depends on substrate, bacterial strain, temperature, and other factors [89].

Table 2.1.2. *Concentrations of biogenic amines (mg/kg) in the bioconverted wheat wholemeal samples*

Sample	Phenylethyl-amine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total BAs
Ada	72.8 ± 5.40 a	35.6 ± 2.90 d	ND h	ND f	ND c	23.4 ± 2.20 fg	ND b	131.8 ± 10.5 fgh
Ada_LUHS122	64.1 ± 4.70 abcde	98.1 ± 6.30 bc	205.3 ± 9.80 a	32.2 ± 2.60 d	ND c	ND h	ND b	399.7 ± 23.4 a
Ada_LUHS245	50.3 ± 3.40 fghijk	33.1 ± 2.50 de	102.3 ± 6.90 cde	ND f	ND c	32.6 ± 3.80 cd	ND b	218.3 ± 16.6 bc
Ada_LUHS29	56.9 ± 3.90 cdefgh	20.3 ± 1.80 e	ND h	ND f	ND c	36.1 ± 3.30 bc	ND b	113.3 ± 9.00 ghi
Sarta	68.1 ± 5.00 abcd	32.6 ± 2.60 de	ND h	ND f	ND c	ND h	ND b	57.2 ± 3.9 klm
Sarta_LUHS122	55.6 ± 3.60 defghi	26.6 ± 2.10 de	ND h	77.4 ± 5.40 a	ND c	ND h	ND b	188.6 ± 15.1 cde
Sarta_LUHS245	46.9 ± 3.10 ghijk	26.7 ± 1.9 de	ND h	ND f	ND c	31.9 ± 3.40 cd	ND b	98.7 ± 8.20 hijk
Sarta_LUHS29	68.6 ± 5.20 abc	28.9 ± 2.1 de	ND h	ND f	ND c	ND h	ND b	80.3 ± 6.70 ijklm
DS8472-5	57.2 ± 3.90 cdefg	ND f	ND h	ND f	ND c	ND h	ND b	79.4 ± 6.60 ijklm
DS8472-5_ LUHS122	68.7 ± 5.10 abc	ND f	88.0 ± 5.90 ef	ND f	ND c	31.9 ± 4.10 cd	ND b	254.1 ± 18.0 b
DS8472-5_ LUHS245	62.2 ± 4.40 abcdef	ND f	ND h	ND f	ND c	36.5 ± 3.80 bc	ND b	164.4 ± 14.0 def
DS8472-5_LUHS29	53.6 ± 3.50 efghij	ND f	ND h	ND f	ND c	26.7 ± 3.20 defg	ND b	101.1 ± 8.80 hij
DS8526-2	58.0 ± 4.30 bcdefg	ND f	ND h	ND f	ND c	21.4 ± 2.30 g	ND b	110.2 ± 6.90 ghij
DS8526-2_ LUHS122	51.9 ± 3.20 efghijk	ND f	99.9 ± 6.50 de	ND f	76.0 ± 5.40 a	26.3 ± 2.90 defg	ND b	46.1 ± 2.9 lm

Table 2.1.2 cont.

Sample	Phenylethyl-amine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total BAs
DS8526-2_ LUHS245	26.4 ± 2.40 m	26.8 ± 2.7 de	84.0 ± 5.80 f	ND f	ND c	27.2 ± 3.10 defg	ND b	147.0 ± 9.50 efg
DS8526-2_LUHS29	70.4 ± 5.30 ab	ND f	ND h	ND f	ND c	30.7 ± 3.5 cde	ND b	168.3 ± 12.9 def
Gaja	58.5 ± 3.70 bcdefg	19.9 ± 1.10 e	ND h	ND f	ND c	31.8 ± 2.10 cd	ND b	80.0 ± 6.20 ijklm
Gaja_LUHS122	46.1 ± 2.90 ghijk	ND f	ND h	ND f	ND c	ND h	ND b	42.9 ± 3.50 m
Gaja_LUHS245	52.6 ± 4.10 efghijk	ND f	94.4 ± 5.40 ef	ND f	ND c	ND h	ND b	213.3 ± 18.0 bc
Gaja_LUHS29	52.0 ± 4.80 efghijk	ND f	116.3 ± 8.10 bc	ND f	ND c	ND h	ND b	415.9 ± 33.0 a
DS8888-3-6	57.3 ± 4.50 cdefg	ND f	ND h	ND f	ND c	22.7 ± 1.70 fg	ND b	44.1 ± 3.20 lm
DS8888-3-6_ LUHS122	42.9 ± 3.50 ijkl	ND f	ND h	ND f	ND c	ND h	ND b	68.55 ± 5 jklm
DS8888-3-6_ LUHS245	45.9 ± 4.10 ghijk	103.8 ± 9.20 b	63.6 ± 4.70 g	ND f	ND c	ND h	ND b	203.3 ± 16.4 cd
DS8888-3-6_ LUHS29	41.8 ± 3.20 jkl	226.18 ± 18.5 a	127.9 ± 10.5 b	20.1 ± 0.800 e	ND c	ND h	ND b	407.3 ± 31.3 a
DS8535-2	44.1 ± 3.20 hijk	ND f	ND h	ND f	ND c	ND h	ND b	86.8 ± 6.40 ijkl
DS8535-2_ LUHS122	44.1 ± 3.50 hijk	ND f	ND h	ND f	ND c	24.5 ± 1.50 efg	ND b	70.0 ± 5.00 ijklm
DS8535-2_ LUHS245	50.9 ± 4.40 fghijk	ND f	122.6 ± 10.7 b	ND f	ND c	29.8 ± 1.30 cdef	ND b	135.0 ± 10.2 fgh
DS8535-2_LUHS29	30.9 ± 2.40 lm	87.3 ± 6.80 c	115.1 ± 9.80 bcd	66.3 ± 4.80 b	35.6 ± 2.40 b	51.8 ± 3.70 a	20.2 ± 1.40 a	226.6 ± 16.7 bc
DS8548-7	55.9 ± 4.70 cdefgh	ND f	ND h	ND f	ND c	30.9 ± 1.70 cde	ND b	131.8 ± 10.5 fgh

Table 2.1.2 cont.

Sample	Phenylethyl-amine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total BAs
DS8548-7_ LUHS122	40.5 ± 3.80 kl	ND f	ND h	ND f	ND c	29.5 ± 1.20 cdef	ND b	399.7 ± 23.4 a
DS8548-7_ LUHS245	42.0 ± 3.10 jkl	ND f	52.4 ± 4.20 g	ND f	ND c	40.6 ± 2.90 b	ND b	218.3 ± 16.6 bc
DS8548-7_LUHS29	39.3 ± 2.80 kl	27.2 ± 1.70 de	84.1 ± 7.10 f	49.9 ± 3.20 c	ND c	25.7 ± 1.90 defg	ND b	113.3 ± 9.00 ghi

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–l Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Micro- and macroelement concentrations in the wheat wholemeal samples are presented in Table 2.1.3, Table 2.1.4 and Table 2.1.5. Both experiments showed that wheat variety was a significant factor for the micro- and macroelement concentrations in the samples. While the LAB strain was not a significant factor in most cases.

***Table 2.1.3**. Macroelement concentrations in bioconverted wheat wholemeal samples*

Sample	Na, g/100g	Mg, g/kg	K, g/kg	Ca, g/kg
Ada	ND k	0.662 ± 0.066 abc	1.978 ± 0.194 abcdef	0.229 ± 0.023 b
Ada_LUHS122	0.070 ± 0.001 a	0.578 ± 0.058 abcdefg	1.785 ± 0.175 cdefg	0.197 ± 0.020 bcdef
Ada_LUHS245	0.070 ± 0.001 a	0.551 ± 0.055 abcdefgh	1.739 ± 0.170 defgh	0.197 ± 0.020 bcdef
Ada_LUHS29	0.010 ± 0.001 cde	0.601 ± 0.060 abcdef	1.905 ± 0.187 bcdefg	0.192 ± 0.019 bcdefg
Sarta	ND k	0.555 ± 0.056 abcdefgh	1.976 ± 0.194 abcdef	0.305 ± 0.031 a
Sarta_LUHS122	0.011 ± 0.001 bcd	0.373 ± 0.037 ij	1.15 ± 0.113 h	0.16 ± 0.016 defghi
Sarta_LUHS245	0.009 ± 0.001 def	0.479 ± 0.048 efghij	1.519 ± 0.152 fgh	0.225 ± 0.025 bc
Sarta_LUHS29	0.011 ± 0.001 bcd	0.607 ± 0.061 abcde	1.817 ± 0.178 bcdefg	0.293 ± 0.029 a
DS8472-5	ND k	0.401 ± 0.04 hij	1.45 ± 0.150 fgh	0.16 ± 0.016 defghi
DS8472-5_LUHS122	0.007 ± 0.002 efgh	0.456 ± 0.046 efghij	1.76 ± 0.180 defg	0.147 ± 0.015 efghi
DS8472-5_LUHS245	0.009 ± 0.001 def	0.431 ± 0.043 ghij	1.71 ± 0.170 efgh	0.126 ± 0.013 hi
DS8472-5_LUHS29	0.008 ± 0.001 defg	0.445 ± 0.045 ghij	1.96 ± 0.200 abcdefg	0.148 ± 0.015 efghi
DS8526-2	ND k	0.518 ± 0.025 bcdefghi	1.93 ± 0.190 bcdefg	0.146 ± 0.015 fghi
DS8526-2_LUHS122	0.005 ± 0.003 ghij	0.354 ± 0.084 j	1.377 ± 0.371 gh	0.132 ± 0.016 ghi
DS8526-2_LUHS245	0.003 ± 0.001 ijk	0.500 ± 0.05 defghij	2.00 ± 0.200 abcdef	0.129 ± 0.013 ghi
DS8526-2_LUHS29	0.005 ± 0.001 ghij	0.376 ± 0.038 ij	1.964 ± 0.160 abcdefg	0.124 ± 0.012 i
Gaja	ND k	0.682 ± 0.053 a	2.54 ± 0.220 a	0.21 ± 0.019 bcde
Gaja_LUHS122	0.010 ± 0.002 cde	0.638 ± 0.051 abcd	2.32 ± 0.190 abcd	0.199 ± 0.018 bcdef
Gaja_LUHS245	0.008 ± 0.001 defg	0.669 ± 0.042 ab	2.38 ± 0.130 ab	0.192 ± 0.019 bcdefg
Gaja_LUHS29	0.007 ± 0.002 efgh	0.641 ± 0.05 abcd	2.37 ± 0.210 abc	0.189 ± 0.02 bcdefgh
DS8888-3-6	ND k	0.448 ± 0.045 fghij	1.98 ± 0.170 abcdef	0.345 ± 0.031 a
DS8888-3-6_LUHS122	0.013 ± 0.001 bc	0.501 ± 0.041 defghij	2.38 ± 0.230 ab	0.310 ± 0.026 a
DS8888-3-6_LUHS245	0.011 ± 0.002 bcd	0.438 ± 0.040 ghij	2.20 ± 0.180 abcde	0.321 ± 0.027 a
DS8888-3-6_LUHS29	0.014 ± 0.001 b	0.509 ± 0.051 cdefghi	2.37 ± 0.230 abc	0.322 ± 0.030 a
DS8535-2	ND k	0.482 ± 0.041 efghij	1.75 ± 0.160 defg	0.173 ± 0.016 bcdefghi

Table 2.1.3 cont.

Sample	Na, g/100g	Mg, g/kg	K, g/kg	Ca, g/kg
DS8535-2_LUHS122	0.006 ± 0.001 fghi	0.458 ± 0.039 efghij	1.86 ± 0.200 bcdefg	0.162 ± 0.015 cdefghi
DS8535-2_LUHS245	0.006 ± 0.002 fghi	0.495 ± 0.036 defghij	1.77 ± 0.140 defg	0.163 ± 0.012 cdefghi
DS8535-2_LUHS29	0.006 ± 0.001 fghi	0.438 ± 0.034 ghij	1.84 ± 0.110 bcdefg	0.162 ± 0.013 cdefghi
DS8548-7	ND k	0.510 ± 0.048 cdefghi	1.93 ± 0.160 bcdefg	0.213 ± 0.018 bcd
DS8548-7_LUHS122	0.003 ± 0.001 ijk	0.511 ± 0.027 cdefghi	1.84 ± 0.110 bcdefg	0.205 ± 0.014 bcdef
DS8548-7_LUHS245	0.002 ± 0.002 jk	0.513 ± 0.022 cdefghi	1.88 ± 0.090 bcdefg	0.210 ± 0.016 bcde
DS8548-7_LUHS29	0.004 ± 0.001 hij	0.534 ± 0.043 abcdefgh	1.82 ± 0.100 bcdefg	0.214 ± 0.012 bcd

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–l Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

***Table 2.1.4**. Essential microelement concentrations in bioconverted wheat wholemeal samples*

Sample	Cr, mg/100g	Mn, mg/kg	Fe, mg/kg	Cu, mg/kg
Ada	ND c	8.21 ± 0.820 jklm	22.9 ± 2.30 bcde	1.85 ± 0.190 ab
Ada_LUHS122	0.014 ± 0.001 c	7.81 ± 0.780 klm	38.7 ± 3.90 a	1.61 ± 0.160 bc
Ada_LUHS245	ND c	5.83 ± 0.580 m	23.2 ± 2.30 bcd	1.59 ± 0.160 bc
Ada_LUHS29	ND c	9 ± 0.900 ijklm	28.3 ± 2.80 b	1.51 ± 0.150 bcd
Sarta	0.047 ± 0.005 b	12.8 ± 1.30 defgh	24.8 ± 2.10 bc	2.19 ± 0.220 a
Sarta_LUHS122	ND c	11.7 ± 1.20 fghij	22.4 ± 2.00 bcdef	2.01 ± 0.180 a
Sarta_LUHS245	ND c	11.7 ± 1.20 fghij	25.0 ± 2.30 bc	2.14 ± 0.160 a
Sarta_LUHS29	0.052 ± 0.005 b	11.5 ± 1.20 ghijk	24.8 ± 2.20 bc	2.23 ± 0.180 a
DS8472-5	0.015 ± 0.002 c	7.54 ± 0.710 lm	15.6 ± 1.40 hi	1.14 ± 0.110 defghi
DS8472-5_LUHS122	ND c	7.77 ± 0.800 lm	14.6 ± 1.30 hi	1.27 ± 0.130 cdef
DS8472-5_LUHS245	ND c	7.69 ± 0.920 lm	15.2 ± 1.70 hi	1.23 ± 0.120 cdefg
DS8472-5_LUHS29	ND c	7.71 ± 1.00 lm	15.9 ± 1.60 hi	1.22 ± 0.190 cdefgh
DS8526-2	ND c	10.4 ± 1.00 ghijkl	14.0 ± 1.40 hi	1.24 ± 0.120 cdefg
DS8526-2_LUHS122	ND c	8.25 ± 0.820 jklm	5.48 ± 0.550 j	0.83 ± 0.083 hij
DS8526-2_LUHS245	ND c	12.1 ± 1.20 efghi	22.0 ± 2.20 cdefg	1.27 ± 0.130 cdef
DS8526-2_LUHS29	ND c	13.4 ± 1.30 defgh	15.4 ± 1.50 hi	1.32 ± 0.130 cde
Gaja	ND c	10.7 ± 1.10 ghijkl	14.9 ± 1.50 hi	0.763 ± 0.076 ij
Gaja_LUHS122	ND c	14.0 ± 1.40 cdefg	15.6 ± 1.60 hi	0.850 ± 0.085 ghij
Gaja_LUHS245	ND c	10.9 ± 1.10 ghijkl	13.7 ± 1.40 hi	0.695 ± 0.070 j
Gaja_LUHS29	ND c	13.3 ± 1.30 defgh	16.6 ± 1.70 fgh	0.857 ± 0.086 ghij
DS8888-3-6	0.151 ± 0.014 a	15.3 ± 1.50 abcdef	16.5 ± 1.7 fgh	0.913 ± 0.091 fghij
DS8888-3-6_LUHS122	0.161 ± 0.007 a	10.2 ± 1.93 hijkl	17.1 ± 2.59 efgh	0.764 ± 0.037 ij
DS8888-3-6_LUHS245	0.145 ± 0.025 a	13.8 ± 1.40 cdefgh	15.9 ± 1.60 hi	0.848 ± 0.085 ghij
DS8888-3-6_LUHS29	0.158 ± 0.014 a	11.1 ± 1.10 ghijkl	10.3 ± 1.10 ij	0.692 ± 0.069 j
DS8535-2	ND c	17.8 ± 1.50 ab	16.6 ± 1.70 fgh	0.805 ± 0.081 ij

Table 2.1.4 cont.

Sample	Cr, mg/100g	Mn, mg/kg	Fe, mg/kg	Cu, mg/kg
DS8535-2_LUHS122	ND c	16.1 ± 1.30 abcd	17.3 ± 1.80 defgh	0.797 ± 0.080 ij
DS8535-2_LUHS245	ND c	17.9 ± 1.20 a	16.7 ± 1.50 fgh	0.775 ± 0.078 ij
DS8535-2_LUHS29	ND c	17.4 ± 1.20 abc	17.0 ± 1.30 efgh	0.764 ± 0.070 ij
DS8548-7	ND c	15.6 ± 0.30 abcde	16.2 ± 1.60 ghi	0.973 ± 0.097 efghij
DS8548-7_LUHS122	ND c	15.4 ± 1.20 abcdef	15.0 ± 1.10 hi	0.926 ± 0.073 efghij
DS8548-7_LUHS245	ND c	14.1 ± 1.10 bcdefg	16.7 ± 1.20 fgh	0.938 ± 0.074 efghij
DS8548-7_LUHS29	ND c	15.3 ± 1.00 abcdef	16.5 ± 1.20 fgh	0.921 ± 0.071 fghij

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–l Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 2.1.5. *Concentrations of non-essential microelements (mg/kg) in bioconveted wheat wholemeal samples*

Sample	As	Rb	Sr	Cd	Ba	Pb
Ada	ND d	ND b	1.000 ± 0.100 ij	0.038 ± 0.004 b	2.71 ± 0.270 abc	0.017 ± 0.002 e
Ada_LUHS122	ND d	ND b	0.909 ± 0.091 ij	0.033 ± 0.003 bc	2.30 ± 0.230 bcd	0.064 ± 0.006 a
Ada_LUHS245	0.009 ± 0.001 bc	ND b	0.854 ± 0.085 ij	0.038 ± 0.004 b	2.27 ± 0.230 bcd	0.057 ± 0.006 b
Ada_LUHS29	ND d	ND b	0.949 ± 0.095 ij	0.029 ± 0.003 bcd	2.36 ± 0.240 abcd	0.032 ± 0.003 c
Sarta	0.007 ± 0.001 c	1.44 ± 0.140 a	0.963 ± 0.091 ij	0.054 ± 0.005 a	2.35 ± 0.240 abcd	0.012 ± 0.001 e
Sarta_LUHS122	0.013 ± 0.001 a	1.36 ± 0.120 a	0.976 ± 0.083 ij	0.053 ± 0.006 a	2.21 ± 0.190 cd	0.014 ± 0.002 e
Sarta_LUHS245	ND d	1.41 ± 0.100 a	0.984 ± 0.074 ij	0.057 ± 0.004 a	2.33 ± 0.220 abcd	0.013 ± 0.001 e
Sarta_LUHS29	0.011 ± 0.001 ab	1.43 ± 0.110 a	0.962 ± 0.087 ij	0.058 ± 0.005 a	2.36 ± 0.230 abcd	0.012 ± 0.001 e
DS8472-5	ND d	ND b	1.50 ± 0.130 cdefg	0.029 ± 0.003 bcd	2.22 ± 0.210 cd	ND f
DS8472-5_LUHS122	ND d	ND b	1.46 ± 0.120 defg	0.033 ± 0.003 bc	2.45 ± 0.250 abcd	ND f
DS8472-5_LUHS245	ND d	ND b	1.42 ± 0.130 fgh	0.035 ± 0.004 bc	2.23 ± 0.220 cd	ND f
DS8472-5_LUHS29	ND d	ND b	1.52 ± 0.140 cdefg	0.034 ± 0.004 bc	2.35 ± 0.260 abcd	ND f
DS8526-2	ND d	ND b	1.22 ± 0.012 ghi	0.027 ± 0.003 cde	2.30 ± 0.230 bcd	0.031 ± 0.003 cd
DS8526-2_LUHS122	ND d	ND b	0.719 ± 0.072 j	0.012 ± 0.001 ghij	ND e	ND f
DS8526-2_LUHS245	ND d	ND b	1.02 ± 0.010 hij	0.028 ± 0.003 cde	2.11 ± 0.210 d	ND f
DS8526-2_LUHS29	ND d	ND b	1.20 ± 0.012 ghi	0.036 ± 0.004 bc	2.38 ± 0.240 abcd	ND f
Gaja	0.007 ± 0.001 c	ND b	1.46 ± 0.015 defg	0.009 ± 0.001 ijk	ND e	ND f
Gaja_LUHS122	0.009 ± 0.002 bc	ND b	1.67 ± 0.017 bcdef	0.012 ± 0.001 ghij	ND e	0.015 ± 0.002 e
Gaja_LUHS245	0.008 ± 0.001 c	ND b	1.44 ± 0.014 efg	0.009 ± 0.001 ijk	ND e	ND f
Gaja_LUHS29	0.008 ± 0.002 c	ND b	1.61 ± 0.016 bcdefg	0.01 ± 0.001 hij	ND e	ND f
DS8888-3-6	ND d	ND b	1.9 ± 0.019 bc	0.009 ± 0.001 ijk	ND e	0.025 ± 0.003 d
DS8888-3-6_LUHS122	ND d	ND b	1.492 ± 0.286 cdefg	ND k	ND e	ND f
DS8888-3-6_LUHS245	ND d	ND b	1.84 ± 0.018 bcde	0.009 ± 0.001 ijk	ND e	ND f
DS8888-3-6_LUHS29	ND d	ND b	1.45 ± 0.015 efg	0.005 ± 0.001 jk	ND e	ND f
DS8535-2	ND d	ND b	2.00 ± 0.200 b	0.019 ± 0.003 efgh	ND e	0.032 ± 0.003 c

Table 2.1.5 cont.

Sample	As	Rb	Sr	Cd	Ba	Pb
DS8535-2_LUHS122	ND d	ND b	1.97 ± 0.190 b	0.016 ± 0.002 fghi	ND e	0.029 ± 0.002 cd
DS8535-2_LUHS245	ND d	ND b	1.90 ± 0.140 bc	0.017 ± 0.003 fghi	ND e	0.031 ± 0.003 cd
DS8535-2_LUHS29	ND d	ND b	1.87 ± 0.170 bcd	0.016 ± 0.002 fghi	ND e	0.028 ± 0.003 cd
DS8548-7	ND d	ND b	2.58 ± 0.260 a	0.021 ± 0.002 defg	2.90 ± 0.290 a	ND f
DS8548-7_LUHS122	ND d	ND b	2.59 ± 0.200 a	0.02 ± 0.001 defg	2.78 ± 0.260 abc	ND f
DS8548-7_LUHS245	ND d	ND b	2.66 ± 0.190 a	0.022 ± 0.001 def	2.83 ± 0.200 ab	ND f
DS8548-7_LUHS29	ND d	ND b	2.63 ± 0.210 a	0.023 ± 0.002 def	2.69 ± 0.250 abcd	ND f

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–k Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The majority of the FA composition consisted of palmitic, octadecenoic, and linoleic acids in the wheat wholemeal samples. The saturated fatty acids (SFA) were between 20.8 and 39.8 %, monounsaturated fatty acids (MUFA) were between 14.1 and 28.8 %, and polyunsaturated fatty acids (PUFA) were between 36.9 and 64.5 % in the samples. The first experiment results showed that wheat variety and LAB strain interaction was significant for the majority of the FAs, except for palmitic, octadecenoic, α-linolenic, and linoleic acids. LAB strain was significant for octadecenoic acid concentrations. The second experiment showed that the LAB strain and its interaction with wheat variety were significant for SFA, MUFA, and omega-6 FAs in the wheat wholemeal samples. Additionally, the majority of detected FAs were significantly affected by both factors and their interaction, similarly lacking significance for palmitic and octadecenoic acids. As wheat cereals contain only up to 3.8 % of lipids, the majority stored in the germ, their composition can be easily affected by bacterial activity [90-92]. The literature reports similar values in terms of individual FAs and corresponding groups (SFA, MUFA, PUFA), denoting palmitic, oleic, and linoleic acids as major FAs and α-linolenic acid as a major omega-3 FA [91, 93].

Table 2.1.6. *Fatty acid compositions of the wheat wholemeal samples*

Sample	SFA	MUFA	PUFA	Omega 3	Omega 6	Omega 9
Ada	39.8 ± 0.14 a	23.1 ± 0.104 d	36.91 ± 0.210 r	2.11 ± 0.010 r	34.8 ± 0.200 r	23.065 ± 0.104 d
Ada_LUHS122	35.6 ± 0.400 d	17.7 ± 0.202 ij	46.9 ± 0.400 n	2.07 ± 0.100 r	44.8 ± 0.300 klm	17.7 ± 0.202 ij
Ada_LUHS245	31.4 ± 0.345 gh	21.2 ± 0.105 f	47.2 ± 0.300 mn	3.09 ± 0.100 l	44.1 ± 0.200 m	21.2 ± 0.105 f
Ada_LUHS29	29.7 ± 0.121 j	16.9 ± 0.100 klm	53.3 ± 0.220 hi	2.55 ± 0.020 pq	50.7 ± 0.200 g	16.9 ± 0.100 klm
Sarta	23.9 ± 0.128 n	15.4 ± 0.104 p	60.6 ± 0.245 c	6.23 ± 0.030 d	54.3 ± 0.215 de	15.4 ± 0.104 p
Sarta_LUHS122	27.9 ± 0.300 k	18.9 ± 0.103 h	53.1 ± 0.210 hi	2.94 ± 0.010 m	50.2 ± 0.200 g	18.9 ± 0.103 h
Sarta_LUHS245	31.0 ± 0.300 hi	21.1 ± 0.200 f	47.9 ± 0.320 klm	2.84 ± 0.020 mn	45.1 ± 0.300 jkl	21.1 ± 0.200 f
Sarta_LUHS29	21.4 ± 0.120 pqr	14.1 ± 0.110 q	64.5 ± 0.220 a	5.27 ± 0.020 e	59.2 ± 0.200 a	14.1 ± 0.110 q
DS8472-5	32.5 ± 0.320 f	19.9 ± 0.100 g	47.4 ± 0.310 mn	2.73 ± 0.010 no	44.7 ± 0.300 klm	19.9 ± 0.100 g
DS8472-5_LUHS122	25.0 ± 0.310 m	17.4 ± 0.100 jk	57.5 ± 0.220 e	3.38 ± 0.020 ij	54.1 ± 0.200 e	17.4 ± 0.100 jk
DS8472-5_LUHS245	31.9 ± 0.340 fg	19.1 ± 0.205 h	48.8 ± 0.320 kl	2.89 ± 0.020 m	45.9 ± 0.300 ij	19.1 ± 0.205 h
DS8472-5_LUHS29	25.5 ± 0.230 m	19.0 ± 0.102 h	55.4 ± 0.240 g	2.93 ± 0.040 m	52.5 ± 0.200 f	19.0 ± 0.102 h
DS8526-2	30.3 ± 0.280 ij	22.2 ± 0.206 e	47.4 ± 0.330 mn	2.69 ± 0.030 op	44.7 ± 0.300 klm	22.225 ± 0.206 e
DS8526-2_LUHS122	21.8 ± 0.220 opq	21.0 ± 0.203 f	57.1 ± 0.370 ef	3.28 ± 0.070 jk	53.8 ± 0.300 e	21.024 ± 0.203 f
DS8526-2_LUHS245	26.4 ± 0.300 l	24.4 ± 0.210 b	48.9 ± 0.323 k	2.80 ± 0.020 mno	46.1 ± 0.303 i	24.4 ± 0.210 b
DS8526-2_LUHS29	20.8 ± 0.130 r	28.8 ± 0.220 a	50.3 ± 0.250 j	7.45 ± 0.050 c	42.8 ± 0.200 n	28.8 ± 0.220 a
Gaja	29.9 ± 0.140 j	16.1 ± 0.201 o	53.9 ± 0.530 h	3.22 ± 0.030 kl	50.7 ± 0.500 g	16.1 ± 0.201 o
Gaja_LUHS122	26.4 ± 0.255 l	17.5 ± 0.106 ij	56.2 ± 0.452 fg	4.48 ± 0.050 g	51.7 ± 0.402 f	17.5 ± 0.106 ij
Gaja_LUHS245	26.3 ± 0.190 l	21.4 ± 0.206 f	52.3 ± 0.403 i	11.8 ± 0.100 a	40.5 ± 0.303 p	21.4 ± 0.206 f
Gaja_LUHS29	24.8 ± 0.180 m	24.9 ± 0.154 b	50.4 ± 0.290 j	6.20 ± 0.090 d	44.2 ± 0.200 lm	24.9 ± 0.154 b
DS8888-3-6	34.5 ± 0.240 e	16.5 ± 0.105 mno	48.9 ± 0.440 k	3.39 ± 0.040 ij	45.5 ± 0.400 ijk	16.5 ± 0.105 mno
DS8888-3-6_LUHS122	22.2 ± 0.160 o	15.1 ± 0.105 p	62.6 ± 0.441 b	4.78 ± 0.040 f	57.9 ± 0.401 b	15.1 ± 0.105 p
DS8888-3-6_LUHS245	21.0 ± 0.130 qr	15.2 ± 0.203 p	63.7 ± 0.350 ab	8.18 ± 0.050 b	55.5 ± 0.300 c	15.2 ± 0.203 p
DS8888-3-6_LUHS29	37.7 ± 0.360 c	23.9 ± 0.204 c	38.4 ± 0.224 q	2.17 ± 0.020 r	36.2 ± 0.204 q	23.9 ± 0.204 c
DS8535-2	32.1 ± 0.130 fg	21.3 ± 0.20 f	46.7 ± 0.230 n	4.71 ± 0.030 f	42.0 ± 0.200 no	21.3 ± 0.200 f

Table 2.1.6 cont.

Sample	SFA	MUFA	PUFA	Omega 3	Omega 6	Omega 9
DS8535-2_LUHS122	32.0 ± 0.140 fg	22.4 ± 0.200 e	45.6 ± 0.320 o	2.80 ± 0.020 mno	42.8 ± 0.300 n	22.4 ± 0.200 e
DS8535-2_LUHS245	21.8 ± 0.120 op	19.2 ± 0.200 h	59.0 ± 0.440 d	3.73 ± 0.040 h	55.3 ± 0.400 c	19.2 ± 0.200 h
DS8535-2_LUHS29	34.4 ± 0.254 e	18.0 ± 0.100 i	47.8 ± 0.240 lmn	3.66 ± 0.040 h	44.1 ± 0.200 m	18.0 ± 0.100 i
DS8548-7	38.1 ± 0.300 c	16.8 ± 0.100 lmn	45.1 ± 0.330 o	3.46 ± 0.030 i	41.6 ± 0.300 o	16.8 ± 0.100 lmn
DS8548-7_LUHS122	24.7 ± 0.143 m	16.3 ± 0.104 no	58.9 ± 0.420 d	3.80 ± 0.020 h	55.1 ± 0.400 cd	16.3 ± 0.104 no
DS8548-7_LUHS245	38.9 ± 0.230 b	17.2 ± 0.301 jkl	43.8 ± 0.320 p	2.53 ± 0.020 q	41.3 ± 0.300 op	17.2 ± 0.301 jkl
DS8548-7_LUHS29	33.9 ± 0.323 e	15.1 ± 0.100 p	51.0 ± 0.220 j	2.9 ± 0.020 m	48.1 ± 0.200 h	15.1 ± 0.100 p

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. The data are represented as means ± standard deviation. a–r Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The essential and non-essential FAA concentrations of the second experiment (involving *Gaja*, DS8888-3-6, DS8548-7, DS8535-2 varieties) are presented in Table 2.1.7 and Table 2.1.8, respectively. In almost all cases, the bioconverted sample groups contained significantly higher concentrations of essential amino acids, in comparison to respective control groups. LAB strain was significant for all essential amino acid concentrations. Additionally, wheat variety was significant for essential amino acid concentrations, except for phenylalanine and leucine/isoleucine, while the interaction of both factors was significant for methionine, valine, and histidine. Wheat variety was a significant factor for almost all of the non-essential amino acid concentrations, including γ – aminobutyric acid (GABA). Moreover, the LAB strain was significant for almost all non-essential and non-proteinogenic amino acid concentrations, except glutamic acid, while the interaction of both factors was not significant only for serine and aspartic acid. The significant increase in FAAs is generally associated with the LAB proteases' activity during the fermentation [94]. Furthermore, the amount of precipitation, temperature and wheat variety can greatly affect protein content and its composition in the cereals [95, 96]. GABA is a non-proteinogenic amino acid, which is associated with LAB activity via its production from glutamic acid, using glutamate decarboxylase [97]. GABA has GRAS status and has important properties as a neurotransmitter, providing beneficial effects, such as blood pressure reduction, improvement of discrimination learning, blood glucose reduction and is able to treat sleeplessness, depression, and other illnesses [97-99].

Table 2.1.7. *Concentrations of essential free amino acids (µmol/g) in the wheat wholemeal samples*

Sample	Threonine	Methionine	Valine	Phenyl-alanine	Leucine/ Isoleucine	Lysine	Histidine
Gaja	0.46 ± 0.026 gh	ND g	0.385 ± 0.020 h	ND f	0.185 ± 0.010 g	0.360 ± 0.021 gh	ND g
Gaja_LUHS122	0.823 ± 0.077 bc	0.303 ± 0.014 b	2.43 ± 0.16 b	1.177 ± 0.067 a	5.71 ± 0.217 a	1.51 ± 0.142 b	0.169 ± 0.007 b
Gaja_LUHS245	0.624 ± 0.034 ef	0.043 ± 0.003 f	1.95 ± 0.102 cd	1.251 ± 0.101 a	4.17 ± 0.249 bc	1.16 ± 0.046 c	ND g
Gaja_LUHS29	0.78 ± 0.074 bcd	0.146 ± 0.008 c	1.21 ± 0.041 g	0.767 ± 0.034 de	2.95 ± 0.17 ef	0.839 ± 0.044 de	0.083 ± 0.008 e
DS8888-3-6	0.423 ± 0.04 h	ND g	0.343 ± 0.034 h	ND f	0.188 ± 0.012 g	0.285 ± 0.02 h	ND g
DS8888-3-6_LUHS122	0.768 ± 0.029 bcde	0.117 ± 0.01 de	2.01 ± 0.174 c	0.813 ± 0.058 cde	4.67 ± 0.257 b	1.07 ± 0.061 cd	0.024 ± 0.001 f
DS8888-3-6_LUHS245	0.591 ± 0.035 fg	0.146 ± 0.008 c	1.65 ± 0.059 cdef	0.788 ± 0.065 de	3.53 ± 0.317 de	1.02 ± 0.043 cd	0.031 ± 0.003 f
DS8888-3-6_LUHS29	0.715 ± 0.054 cdef	ND g	1.99 ± 0.079 cd	0.695 ± 0.065 de	3.01 ± 0.255 ef	0.583 ± 0.045 fg	ND g
DS8535-2	0.440 ± 0.021 h	ND g	0.430 ± 0.035 h	ND f	0.103 ± 0.006 g	0.321 ± 0.028 h	ND g
DS8535-2_LUHS122	1.23 ± 0.094 a	0.477 ± 0.015 a	3.28 ± 0.312 a	0.978 ± 0.083 bc	5.46 ± 0.195 a	2.23 ± 0.202 a	0.297 ± 0.013 a
DS8535-2_LUHS245	0.791 ± 0.025 bcd	0.135 ± 0.007 cd	1.76 ± 0.08 cde	1.07 ± 0.103 ab	3.47 ± 0.303 de	1.16 ± 0.063 c	0.145 ± 0.005 c
DS8535-2_LUHS29	0.909 ± 0.038 b	0.1 ± 0.006 e	1.87 ± 0.109 cd	0.853 ± 0.03 cd	3.54 ± 0.345 cde	0.629 ± 0.049 ef	0.116 ± 0.008 d
DS8548-7	0.420 ± 0.019 h	ND g	0.422 ± 0.019 h	ND f	0.162 ± 0.013 g	0.224 ± 0.014 h	ND g
DS8548-7_LUHS122	0.656 ± 0.024 def	0.154 ± 0.009 c	1.63 ± 0.132 def	0.857 ± 0.078 cd	3.73 ± 0.140 cd	0.946 ± 0.056 cd	0.093 ± 0.004 e
DS8548-7_LUHS245	0.593 ± 0.044 fg	0.061 ± 0.005 f	1.368 ± 0.089 fg	0.743 ± 0.074 de	3.20 ± 0.110 de	0.702 ± 0.059 ef	0.027 ± 0.002 f

Table 2.1.7 cont.

Sample	Threonine	Methionine	Valine	Phenyl-alanine	Leucine/ Isoleucine	Lysine	Histidine
DS8548-7_LUHS29	0.685 ± 0.060 cdef	ND g	1.49 ± 0.126 efg	0.641 ± 0.028 e	2.47 ± 0.222 f	0.671 ± 0.065 ef	ND g

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data are represented as means ± standard deviation. a–h Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 2.1.8. *Concentrations of non-essential and non-proteinogenic free amino acids (µmol/g) in the wheat wholemeal samples*

Sample	Arginine	Gluta-mine	Serine	Apartic acid	Glutamic acid	Glycine	Alanine	Proline	Tyrosine	GABA
Gaja	0.521 ± 0.018 d	1.95 ± 0.138 fgh	0.971 ± 0.061 gh	0.793 ± 0.068 defg	0.581 ± 0.057 cde	0.895 ± 0.040 fg	1.74 ± 0.157 h	0.285 ± 0.015 g	ND h	2.43 ± 0.125 hij
Gaja_LUHS122	0.221 ± 0.013 gh	3.07 ± 0.150 bc	1.47 ± 0.054 bc	1.38 ± 0.090 b	0.780 ± 0.023 b	1.84 ± 0.124 bc	5.98 ± 0.319 b	1.55 ± 0.057 bcde	0.246 ± 0.01 bc	5.32 ± 0.297 cde
Gaja_LUHS245	1.09 ± 0.102 ab	1.12 ± 0.052 i	1.16 ± 0.079 defgh	0.686 ± 0.066 fgh	0.477 ± 0.016 efg	1.56 ± 0.125 cd	4.28 ± 0.162 cd	1.31 ± 0.070 de	0.279 ± 0.019 a	6.37 ± 0.409 b
Gaja_LUHS29	0.987 ± 0.071 b	0.452 ± 0.023 j	1.42 ± 0.133 bc	0.626 ± 0.020 gh	0.470 ± 0.038 efg	1.76 ± 0.107 bc	4.54 ± 0.396 c	0.972 ± 0.042 f	0.055 ± 0.003 g	6.51 ± 0.420 b
DS8888-3-6	0.578 ± 0.047 d	1.81 ± 0.095 gh	1.49 ± 0.113 b	0.906 ± 0.036 de	0.199 ± 0.019 i	0.527 ± 0.033 h	2.11 ± 0.097 gh	0.300 ± 0.025 g	ND h	2.51 ± 0.085 hi
DS8888-3-6_ LUHS122	0.236 ± 0.015 gh	2.66 ± 0.184 cd	1.35 ± 0.11 bcd	1.15 ± 0.09 c	0.585 ± 0.045 cde	1.39 ± 0.069 de	4.27 ± 0.176 cd	1.39 ± 0.066 cde	0.246 ± 0.007 bc	4.53 ± 0.392 ef
DS8888-3-6_ LUHS245	0.722 ± 0.054 c	1.75 ± 0.110 h	1.26 ± 0.089 bcdef	0.546 ± 0.051 hi	0.409 ± 0.03 fg	1.33 ± 0.07 de	4.07 ± 0.308 cd	1.35 ± 0.053 de	0.225 ± 0.007 cd	4.87 ± 0.171 def
DS8888-3-6_ LUHS29	0.227 ± 0.007 gh	1.97 ± 0.183 fgh	1.30 ± 0.109 bcde	0.425 ± 0.027 i	0.392 ± 0.033 fg	2.06 ± 0.172 b	4.51 ± 0.312 c	2.22 ± 0.174 a	0.191 ± 0.007 e	7.60 ± 0.243 a
DS8535-2	0.475 ± 0.041 de	2.01 ± 0.111 efgh	1.05 ± 0.064 efgh	0.739 ± 0.036 efg	0.245 ± 0.008 hi	0.663 ± 0.054 gh	1.72 ± 0.063 h	0.336 ± 0.031 g	ND h	1.56 ± 0.138 j
DS8535-2_ LUHS122	0.192 ± 0.007 h	4.31 ± 0.367 a	1.85 ± 0.109 a	1.72 ± 0.088 a	1.22 ± 0.114 a	2.41 ± 0.124 a	8.08 ± 0.309 a	2.16 ± 0.128 a	0.243 ± 0.023 bc	5.61 ± 0.557 bcd
DS8535-2_ LUHS245	1.21 ± 0.046 a	3.26 ± 0.299 b	1.22 ± 0.055 cdefg	0.894 ± 0.059 de	0.522 ± 0.021 def	1.72 ± 0.158 c	4.28 ± 0.155 cd	1.72 ± 0.152 b	0.268 ± 0.008 ab	3.97 ± 0.336 fg
DS8535-2_ LUHS29	0.105 ± 0.007 h	2.77 ± 0.173 bcd	1.10 ± 0.104 defgh	0.849 ± 0.062 def	0.783 ± 0.055 b	1.78 ± 0.107 bc	2.68 ± 0.208 fg	1.58 ± 0.150 bcd	0.227 ± 0.009 cd	2.82 ± 0.235 hi

Table 2.1.8 cont.

Sample	Arginine	Gluta-mine	Serine	Apartic acid	Glutamic acid	Glycine	Alanine	Proline	Tyrosine	GABA
DS8548-7	0.370 ± 0.033 ef	2.52 ± 0.234 de	1.03 ± 0.091 fgh	0.893 ± 0.057 de	0.368 ± 0.024 gh	0.494 ± 0.020 h	3.03 ± 0.253 ef	0.330 ± 0.028 g	ND h	2.27 ± 0.209 ij
DS8548-7_ LUHS122	0.337 ± 0.011 fg	2.32 ± 0.220 defg	0.919 ± 0.031 h	0.954 ± 0.043 d	0.677 ± 0.048 bc	1.25 ± 0.120 e	3.56 ± 0.207 de	1.26 ± 0.078 ef	0.198 ± 0.008 de	3.25 ± 0.290 gh
DS8548-7_ LUHS245	0.352 ± 0.035 efg	2.37 ± 0.078 def	0.960 ± 0.038 h	0.782 ± 0.070 defg	0.621 ± 0.026 cd	1.18 ± 0.102 ef	3.73 ± 0.373 de	1.33 ± 0.072 de	0.210 ± 0.010 de	3.35 ± 0.127 gh
DS8548-7_ LUHS29	0.846 ± 0.048 c	0.134 ± 0.004 j	1.13 ± 0.051 defgh	0.147 ± 0.013 j	0.154 ± 0.009 i	1.30 ± 0.047 de	4.15 ± 0.129 cd	1.65 ± 0.147 bc	0.108 ± 0.009 f	6.061 ± 0.466 bc

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain; GABA – γ – aminobutyric acid. The data are represented as means ± standard deviation. a–h Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The mycotoxin concentrations of the first experiment (involving *Ada*, *Sarta*, DS8472-5, DS8526-2 varieties) are presented in Table 2.1.9. Only deoxynivalenol (DON) was detected in the wheat wholemeal samples. The significant increases were observed in *Sarta*, DS8472-5, and DS8526-2 sample groups after fermentation in the majority of cases. Though LAB has the ability to neutralize mycotoxins via cell wall binding or its metabolism, the significant increase could be explained due to rehydration of fungal spores [100, 101].

Table 2.1.9. *Mycotoxin concentrations (µg/kg) in the wheat wholemeal samples*

Sample	DON	HT2	T2	FB1	FB2	ZEA	OTA
Ada	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Ada_LUHS122	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Ada_LUHS245	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Ada_LUHS29	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Sarta	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Sarta_LUHS122	<15 i	<1.5	<1.5	<15	<15	<3	<1.5
Sarta_LUHS245	17.4 ± 1.80 hi	<1.5	<1.5	<15	<15	<3	<1.5
Sarta_LUHS29	21.5 ± 2.10 h	<1.5	<1.5	<15	<15	<3	<1.5
DS8472-5	96.4 ± 4.20 g	<1.5	<1.5	<15	<15	<3	<1.5
DS8472-5_LUHS122	116.0 ± 9.20 fg	<1.5	<1.5	<15	<15	<3	<1.5
DS8472-5_LUHS245	134.0 ± 6.50 f	<1.5	<1.5	<15	<15	<3	<1.5
DS8472-5_LUHS29	163.0 ± 7.80 e	<1.5	<1.5	<15	<15	<3	<1.5
DS8526-2	274.4 ± 9.80 c	<1.5	<1.5	<15	<15	<3	<1.5
DS8526-2_LUHS122	226.0 ± 11.6 d	<1.5	<1.5	<15	<15	<3	<1.5
DS8526-2_LUHS245	323.9 ± 14.5 b	<1.5	<1.5	<15	<15	<3	<1.5
DS8526-2_LUHS29	392.3 ± 11.3 a	<1.5	<1.5	<15	<15	<3	<1.5

LUHS29 – fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245 – fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122 – fermented with *Lactiplantibacillus plantarum* LUHS122 strain; DON – deoxynivalenol; HT2 – HT2 toxin; T2 – T2 toxin; FB1 – fumonisin B1; FB2 – fumonisin B2; ZEA – zearalenone; OTA – ochratoxin A. The data are represented as means ± standard deviation. a–i Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The VOCs profile of the first experiment (involving *Ada*, *Sarta*, DS8472-5, DS8526-2 varieties) is depicted in Figure 2.1.1. Major VOCs detected in wheat wholemeal samples are various aliphatic acids, aldehydes, esters, and alcohols. Some of the major aliphatic acid VOCs include acetic, hexanoic, nonanoic, octanoic, heptanoic, and lactic acids. Short-chain aliphatic acids have pungent and acidic aroma, while longer-chain acids have more fatty,

green, and waxy notes [102, 103]. Short-chain aliphatic acids are products of carbohydrate fermentation, while longer-chain acids are products of fat metabolism [103, 104]. Alcohols, such as pentanol, hexanol, 1-octen-3-ol, are described as fruity, whiskey-like, sweet, floral, and balsamic [103]. Aldehydes in the matrix can be reduced by LAB activity into alcohols [105]. Aldehydes can be formed from α-keto acids, amino acids, and fatty acids by LAB activity [105, 106]. Esters are produced by LAB as well [102]. Bioconverted wheat wholemeal samples had more diverse VOC profiles. Additionally, wheat variety, LAB strain, and the interaction of both factors were significant for the VOC profile in the wheat wholemeal samples.

Fig. 2.1.1. *Heatmap of volatile organic compounds in the wheat wholemeal samples*

While fermentation scale-up does not require complex equipment to carry out, it introduces important aspects of industrial production. In addition to BA and mycotoxin contamination, a possible pathogenic contamination of fermentable substrate is one of the major issues of bioconverted matrices [107]. The pathogenic contamination is often a result of poor hygiene protocols, process control, or the use of contaminated materials, which can be controlled by applying tighter process control, the testing of substrate for harmful pathogens or their metabolites [107]. Moreover, the biosafety of the final product from the majority of the pathogens can be achieved when the pH of substrate reaches the pH of 4 [108]. Additionally, SSF, combined with an adequate inoculum of technological microorganisms (e.g., LAB), represents an effective strategy for preventing the overgrowth of pathogenic microorganisms. Furthermore, extrusion pretreatment prior to fermentation can be recommended as an effective strategy for reducing the biological contamination of the substrate before the fermentation process.

Bioconverted wheat samples showed great potential as a value-added, highly functional feed material due to the high survival rate of LAB under low-pH conditions. However, the bioconversion showed a significant increase in BAs for the most part, thus denoting their monitoring to be an important aspect of the fermented feed production process. The concentrations of micro- and macroelements were significantly affected only by the wheat variety for the most part, thus noting that bioconversion with selected LAB strains won't affect the final mineral feed material composition. Wheat bioconversion increased omega-3 concentrations in most fermented wheat samples, providing important nutrients to the animals. Additionally, fermented wheat samples exhibited significantly higher concentrations of essential and the majority of non-essential FAAs, indicating an enhanced biological value and nutritional quality of the resulting feed material. Moreover, LUHS29 strain was able to consistently produce the highest amounts of GABA, in most cases. However, the observed increase in DON concentrations in certain sample groups underscores the need for careful monitoring and control of mycotoxin levels throughout the bioconversion process. The majority of VOCs detected in the samples during fermentation were associated with lipid and carbohydrate metabolism. A greater diversity of VOCs in fermented cereals is generally considered indicative of improved sensory characteristics of the fermented substrate.

2.2. Influence of Size Reduction and LAB Strain on the Bioconverted Lentils' (*Lens culinaris* L.) Microbiological and Physicochemical Parameters

In this study stage, red and green lentil bioconversion with LAB strains (*Lactiplantibacillus plantarum* No.122 and *Lacticaseibacillus casei* No.210) and the size of the bioconverted matrix factors influence on the microbiological and physicochemical parameters (pH, chromaticity values, FAAs, BAs, FA composition and VOCs) were evaluated.

The chromaticity, pH, and LAB count values are presented in Table 2.2.1. The pH after 24 h of fermentation reached values under 4.5, and LAB count values were over 8 $\log_{10}$ CFU/g. Thus, the selected LAB strains were suitable for bioconversion as similar results of LAB count were found under SMF and SSF conditions elsewhere [109]. Only the milling factor was not significant for the pH values of all analyzed factors (lentil variety, LAB strain) and their interactions. Additionally, lentil variety, LAB strain, and lentil variety and milling factor interaction were significant for LAB count values. Strong negative correlation ($r = –0.973$, $p < 0.001$) between pH and LAB count was observed. The color coordinate values were significantly affected by LAB strain, milling, and lentil variety and LAB strain interaction. The main compounds, contributing to the color of the lentils, are tocopherols and carotenoids [110, 111]. Though significant reduction of those compounds via oxidation or even increasement can be observed in certain LAB strains, thus providing color changes [112, 113].

Table 2.2.1. *Chromaticity, pH and LAB count values in milled and non-milled lentil samples*

Sample	pH after 0 h	pH afer 24 h	Color coordinates, NBS			LAB count, log_{10}CFU/g
			L*	a*	b*	
Re	6.14 ± 0.020 b	-	55.9 ± 2.34 a	24.0 ± 0.98 a	26.4 ± 1.03 a	4.21 ± 0.330 b
Re_LUHS122_N	5.79 ± 0.010 ef	4.21 ± 0.020 d	53.2 ± 1.98 a	19.6 ± 1.32 bc	22.8 ± 0.870 b	8.04 ± 0.270 a
Re_LUHS210_N	5.98 ± 0.020 c	4.33 ± 0.010 c	53.2 ± 1.45 a	20.9 ± 1.96 b	23.5 ± 0.670 b	8.15 ± 0.190 a
Re_LUHS122_M	5.75 ± 0.030 fg	4.31 ± 0.030 c	44.2 ± 1.54 b	5.92 ± 0.470 e	17.5 ± 0.370 c	7.99 ± 0.340 a
Re_LUHS210_M	5.70 ± 0.020 g	4.43 ± 0.020 a	45.7 ± 2.01 b	6.10 ± 0.520 e	18.5 ± 0.380 c	8.16 ± 0.290 a
Gr	6.43 ± 0.020 a	-	42.9 ± 1.98 b	6.28 ± 0.380 e	14.3 ± 0.250 d	4.10 ± 0.250 b
Gr_LUHS122_N	5.81 ± 0.030 ef	4.37 ± 0.020 abc	58.6 ± 2.05 a	16.9 ± 1.30 cd	25.9 ± 0.580 a	8.34 ± 0.280 a
Gr_LUHS210_N	5.88 ± 0.010 d	4.40 ± 0.030 ab	58.0 ± 2.93 a	15.9 ± 1.22 d	25.8 ± 0.760 a	8.37 ± 0.300 a
Gr_LUHS122_M	5.99 ± 0.010 c	4.23 ± 0.010 d	52.6 ± 3.27 a	1.87 ± 0.110 f	18.9 ± 0.640 c	8.59 ± 0.360 a
Gr_LUHS210_M	5.82 ± 0.040 de	4.35 ± 0.020 bc	52.7 ± 2.62 a	1.40 ± 0.200 f	15.6 ± 0.310 d	8.60 ± 0.370 a

Re – non-treated red lentils; Gr – non-treated green lentils; LUHS122 – fermented with *Lactiplantibacillus plantarum* strain; LUSH210 – fermented with *Lacticaseibacillus casei* strain; M – milled lentils; N – non-milled lentils; L* – lightness; a* – redness (−a* greenness); b* – yellowness (−b* blueness); LAB – lactic acid bacteria; NBS – National Bureau of Standards units; CFU – colony forming units. Data are represented as means standard deviation. not analyzed; a–g Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Table 2.2.2. *Concentrations of essential, non-essential and non-proteinogenic amino acids (µmol/g) in the lentil samples*

Non-essential and non-proteinogenic amino acids										
Sample	**Serine**	**Aspartic acid**	**Glutamic acid**	**Glycine**	**Alanine**	**Proline**	**Tyrosine**	**Gluta-mine**	**Arginine**	**GABA**
Re	0.930 ± 0.080 fg	6.54 ± 0.830 a	9.80 ± 0.840 a	0.950 ± 0.140 b	2.44 ± 0.230 d	5.06 ± 0.440 ab	0.060 ± 0.010 e	5.27 ± 0.490 bc	8.52 ± 0.790 ab	ND d
Re_LUHS122_N	2.96 ± 0.230 a	1.48 ± 0.110 cd	3.44 ± 0.500 c	1.22 ± 0.100 b	6.60 ± 0.870 b	5.16 ± 0.520 a	0.210 ± 0.020 de	3.64 ± 0.520 cd	2.72 ± 0.390 f	4.53 ± 0.400 bc
Re_LUHS210_N	1.68 ± 0.170 de	0.750 ± 0.080 cd	2.86 ± 0.360 c	1.36 ± 0.200 b	7.26 ± 0.590 ab	3.97 ± 0.580 b	0.190 ± 0.010 de	2.91 ± 0.420 d	3.79 ± 0.380 ef	2.91 ± 0.290 c
Re_LUHS122_M	2.40 ± 0.220 bc	3.27 ± 0.260 b	7.94 ± 0.770 b	2.64 ± 0.210 a	8.95 ± 1.00 a	4.26 ± 0.340 ab	0.740 ± 0.090 b	4.13 ± 0.300 cd	5.16 ± 0.760 de	8.46 ± 1.14 a
Re_LUHS210_M	2.01 ± 0.270 cd	1.97 ± 0.280 bc	7.07 ± 0.920 b	2.54 ± 0.320 a	6.31 ± 0.910 bc	4.56 ± 0.660 ab	0.650 ± 0.090 b	3.13 ± 0.300 d	7.26 ± 0.860 bcd	8.13 ± 1.06 a
Gr	0.84 ± 0.120 g	7.47 ± 1.11 a	3.75 ± 0.400 c	1.21 ± 0.120 b	1.82 ± 0.230 d	2.24 ± 0.340 c	0.160 ± 0.020 de	6.09 ± 0.710 b	7.71 ± 0.890 abc	0.170 ± 0.020 d
Gr_LUHS122_N	1.13 ± 0.130 efg	0.450 ± 0.050 d	3.62 ± 0.320 c	0.820 ± 0.100 b	1.44 ± 0.120 d	2.38 ± 0.270 c	0.440 ± 0.040 c	5.24 ± 0.560 bc	5.86 ± 0.700 cde	9.35 ± 1.00 a
Gr_LUHS210_N	1.40 ± 0.130 ef	0.490 ± 0.040 d	2.85 ± 0.320 c	1.00 ± 0.120 b	1.66 ± 0.120 d	1.67 ± 0.200 c	0.350 ± 0.020 cd	9.38 ± 1.31 a	9.77 ± 1.07 a	8.48 ± 0.800 a
Gr_LUHS122_M	2.70 ± 0.230 ab	1.72 ± 0.240 cd	3.17 ± 0.260 c	2.20 ± 0.220 a	6.67 ± 0.570 b	2.12 ± 0.220 c	0.630 ± 0.090 b	3.22 ± 0.400 d	7.40 ± 0.570 bc	5.76 ± 0.470 b
Gr_LUHS210_M	1.61 ± 0.240 de	1.53 ± 0.110 cd	4.44 ± 0.460 c	2.16 ± 0.270 a	4.81 ± 0.600 c	1.56 ± 0.140 c	0.950 ± 0.130 a	3.55 ± 0.250 cd	5.16 ± 0.650 de	5.18 ± 0.400 b

Table 2.2.2 cont.

Essential amino acids							
Sample	**Histidine**	**Leucine/ Isoleucine**	**Lysine**	**Methionine**	**Threonine**	**Phenylalanine**	**Valine**
Re	1.05 ± 0.150 a	0.450 ± 0.040 c	0.410 ± 0.060 f	0.040 ± 0.010 cd	0.940 ± 0.110 cd	0.240 ± 0.030 e	1.11 ± 0.130 de
Re_LUHS122_N	0.770 ± 0.070 bcd	2.22 ± 0.170 b	1.08 ± 0.160 cd	0.270 ± 0.040 b	1.81 ± 0.210 b	0.730 ± 0.080 cd	2.14 ± 0.280 ab
Re_LUHS210_N	0.710 ± 0.100 cd	1.09 ± 0.160 c	0.780 ± 0.090 def	0.150 ± 0.020 c	1.12 ± 0.120 bcd	0.390 ± 0.050 e	1.08 ± 0.150 de
Re_LUHS122_M	1.01 ± 0.150 ab	4.23 ± 0.480 a	1.59 ± 0.230 ab	0.540 ± 0.080 a	3.12 ± 0.400 a	1.01 ± 0.070 ab	2.46 ± 0.180 a
Re_LUHS210_M	0.920 ± 0.070 abc	4.30 ± 0.370 a	1.60 ± 0.160 ab	0.580 ± 0.070 a	3.10 ± 0.270 a	1.08 ± 0.100 a	2.29 ± 0.280 ab
Gr	0.720 ± 0.050 cd	0.400 ± 0.050 c	0.590 ± 0.090 ef	0.030 ± 0.0001 d	0.440 ± 0.050 d	0.270 ± 0.030 e	0.720 ± 0.100 e
Gr_LUHS122_N	0.670 ± 0.060 cd	0.830 ± 0.070 c	1.34 ± 0.120 bc	0.110 ± 0.020 cd	1.33 ± 0.170 bc	0.350 ± 0.030 e	1.16 ± 0.170 de
Gr_LUHS210_N	0.620 ± 0.050 d	0.750 ± 0.100 c	0.920 ± 0.100 cde	0.140 ± 0.020 cd	1.1 ± 0.150 bcd	0.400 ± 0.060 e	1.08 ± 0.110 de
Gr_LUHS122_M	0.530 ± 0.060 d	2.28 ± 0.290 b	1.29 ± 0.150 bc	0.330 ± 0.050 b	3.13 ± 0.470 a	0.610 ± 0.090 d	1.90 ± 0.150 bc
Gr_LUHS210_M	0.730 ± 0.080 cd	4.42 ± 0.490 a	2.02 ± 0.230 a	0.360 ± 0.030 b	1.49 ± 0.210 bc	0.840 ± 0.008 bc	1.57 ± 0.130 cd

Re – non-treated red lentils; Gr – non-treated green lentils; LUHS122 – fermented with *Lactiplantibacillus plantarum* strain; LUSH210 – fermented with *Lacticaseibacillus casei* strain; M – milled lentils; N – non-milled lentils; GABA – γ – aminobutyric acid. Data are represented as means standard deviation. -not analyzed; a–g Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

The concentrations of the FAAs in the milled and non-milled lentil samples are presented in Table 2.2.2. The majority of essential FAAs, except histidine, phenylalanine, and valine, in the lentil samples were significantly higher in all fermented samples, in comparison to respective control samples. Additionally, phenylalanine and valine were significantly higher in all fermented milled sample groups, in comparison to respective controls. Most of the non-essential FAAs, except aspartic acid, glutamic acid, glycine, alanine, proline, glutamine, and arginine, were significantly higher in all fermented sample groups, in comparison to respective controls. GABA was significantly higher in all fermented sample groups. All analyzed factors were significant for all the FAAs, except for alanine, glutamine, and proline. Bacterial strains are able to produce FAAs via the breakdown of the protein and consume the FAAs if required [114, 115]. Thus, varied tendencies for certain FAAs can be observed during the bioconversion of the matrix.

Table 2.2.3. *Concentrations of biogenic amines (mg/kg) in the lentil samples*

Sample	PHE	PUT	TYR	TRY	CAD	HIST	SPRMD	SPRM
Re	7.43 ± 0.650 cd	83.9 ± 5.30 a	ND	ND	ND	ND	235.0 ± 15.3 a	69.1 ± 4.30 a
Re_LUHS122_N	5.52 ± 0.320 ef	59.8 ± 3.80 b	ND	ND	ND	ND	168.0 ± 9.32 cd	55.0 ± 4.80 b
Re_LUHS210_N	5.43 ± 0.410 ef	62.4 ± 4.50 b	ND	ND	ND	ND	172.0 ± 11.2 bc	55.8 ± 3.90 b
Re_LUHS122_M	9.27 ± 0.710 b	41.0 ± 2.90 de	ND	ND	ND	ND	142.0 ± 12.5 cde	36.1 ± 2.90 cd
Re_LUHS210_M	11.3 ± 0.110 a	42.0 ± 3.10 cde	ND	ND	ND	ND	134.0 ± 11.8 de	33.5 ± 3.20 cd
Gr	9.49 ± 0.760 b	82.8 ± 6.80 a	ND	ND	ND	ND	204.0 ± 16.3 ab	55.2 ± 4.60 b
Gr_LUHS122_N	6.57 ± 0.540 de	52.9 ± 3.90 bcd	ND	ND	ND	ND	138.0 ± 10.5 cde	42.7 ± 2.70 c
Gr_LUHS210_N	0.64 ± 0.030 g	53.8 ± 4.10 bc	ND	ND	ND	ND	139.0 ± 11.4 cde	43.6 ± 2.60 c
Gr_LUHS122_M	4.18 ± 0.290 f	39.3 ± 2.50 e	ND	ND	ND	ND	108.0 ± 9.10 e	29.2 ± 2.70 d
Gr_LUHS210_M	8.69 ± 0.620 bc	41.6 ± 2.90 de	ND	ND	ND	ND	108.0 ± 8.60 e	28.2 ± 2.10 d

Re – non-treated red lentils; Gr – non-treated green lentils; LUHS122 – fermented with *Lactiplantibacillus plantarum* strain; LUSH210 – fermented with *Lacticaseibacillus casei* strain; M – milled lentils; N – non-milled lentils; TRY – tryptamine; PHE – phenylethylamine; PUT – putrescine; CAD – cadaverine; HIST – histamine; TYR – tyramine; SPRMD – spermidine; SPRM – spermine. Data are represented as means standard deviation. -not analyzed; a–g Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Concentrations of BAs in the milled and non-milled lentil samples are presented in Table 2.2.3. Only phenylalanine, putrescine, spermidine, and spermine were detected in all lentil sample groups. Non-milled fermented samples contained significantly lower concentrations of the BAs, in comparison to control samples. All analyzed factors were significant for almost all detected BAs, except for putrescine. Its concentration was significantly affected only by LAB strain and milling factors. Significant negative correlations were observed between spermidine, spermine, putrescine and the LAB count ($r = -0.810$, $p < 0.001$; $r = -0.660$, $p < 0.001$; $r = -0.829$, $p < 0.001$, respectively). While fermentation is often a culprit of the production of BAs, certain LAB strains, such as *Lactiplantibacillus plantarum*, *Pediococcus acidilactici* and *Lactiplantibacillus pentosus*, can excrete amine-oxidase enzymes that degrade putrescine, tyramine, cadaverine, phenylethylamine and histamine [116, 117]. The activity of those enzymes can be affected by various factors, including pH, temperature, substrate and mediators [116, 117].

The FA composition results, expressed in percentage of total detected FAs, are presented in Table 2.2.4. A total of 5 individual FAs (palmitic, stearic, oleic, linoleic and α-linolenic acids) were detected in the lentil samples. All factors and their interactions were significant for almost all detected FAs, except for linoleic and oleic acids. Omega-3 FAs were affected by lentil variety, LAB strain, and interactions between aforementioned factors with milling. Major detected FAs in lentil samples are oleic and linoleic acids, as well as other notable FAs like palmitic, stearic, and α-linolenic acids, as reported in the literature [118]. Changes observed can be attributed to the endogenous lipoxygenases that are able to convert FAs into VOCs [119]. Additionally, FA composition changes can be assigned to LAB activity [104].

Table 2.2.4. *Fatty acid composition of the lentil samples*

Sample	SFA	MUFA	PUFA	Omega 3	Omega 6	Omega 9
Re	14.1 ± 1.34 cd	26.9 ± 0.290 d	59.1 ± 0.440 a	11.1 ± 0.090 a	48.0 ± 0.350 a	26.9 ± 0.290 d
Re_LUHS122_N	17.2 ± 0.320 a	30.0 ± 0.210 b	52.8 ± 0.690 e	8.13 ± 0.360 d	44.7 ± 0.330 c	30.0 ± 0.210 b
Re_LUHS210_N	14.9 ± 0.560 bc	28.4 ± 0.340 c	56.7 ± 0.290 c	10.3 ± 0.090 b	46.4 ± 0.200 b	28.4 ± 0.340 c
Re_LUHS122_M	13.4 ± 0.280 d	28.2 ± 0.270 c	58.4 ± 0.370 a	10.4 ± 0.050 b	48.1 ± 0.320 a	28.2 ± 0.270 c
Re_LUHS210_M	14.4 ± 0.160 bcd	26.9 ± 0.220 d	58.7 ± 0.610 a	10.8 ± 0.080 ab	47.8 ± 0.530 a	26.9 ± 0.220 d
Gr	13.8 ± 0.330 cd	28.0 ± 0.160 c	58.1 ± 0.380 ab	10.8 ± 0.070 ab	47.4 ± 0.310 a	28.0 ± 0.160 c
Gr_LUHS122_N	15.7 ± 0.220 ab	30.8 ± 0.150 a	53.5 ± 0.170 de	9.02 ± 0.060 c	44.5 ± 0.110 c	30.8 ± 0.150 a
Gr_LUHS210_N	15.3 ± 0.390 bc	27.8 ± 0.110 c	57.0± 0.300 bc	10.7 ± 0.080 ab	46.3 ± 0.220 b	27.8 ± 0.110 c
Gr_LUHS122_M	15.3 ± 0.150 bc	31.0 ± 0.180 a	53.7 ± 0.670 de	8.68 ± 0.320 cd	45.1 ± 0.350 c	31.0 ± 0.180 a
Gr_LUHS210_M	15.0 ± 0.130 bc	30.4 ± 0.140 ab	54.7 ± 0.640 d	8.68 ± 0.510 cd	46.0 ± 0.130 b	30.4 ± 0.140 ab

Re – non-treated red lentils; Gr – non-treated green lentils; LUHS122 – fermented with *Lactiplantibacillus plantarum* strain; LUSH210 – fermented with *Lacticaseibacillus casei* strain; M – milled lentils; N – non-milled lentils; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Data are represented as means standard deviation. -not analyzed; a–e Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

The VOCs of milled and non-milled lentil samples are expressed in area percentage of total identified analytes and are depicted as the heatmap in Figure 2.2.1. The majority of detected VOCs are aldehydes, organic acids, and monoterpenes. Hexanal, 1-hexanol, hexanoic acid, D-limonene, and (E)-2-nonen-1-ol were the compounds with the highest area percentage values in the lentil samples. The majority of detected VOCs were significantly correlated with LAB count, and the majority of aldehydes correlated with FAs as well. Though the most of the VOCs in legume seeds are similar, certain compounds, like D-limonene could be discriminatory for some lentil varieties [119, 120]. Additionally, most of the aldehydes are products of FA oxidation and terpenes are naturally occurring compounds in legume seeds [121].

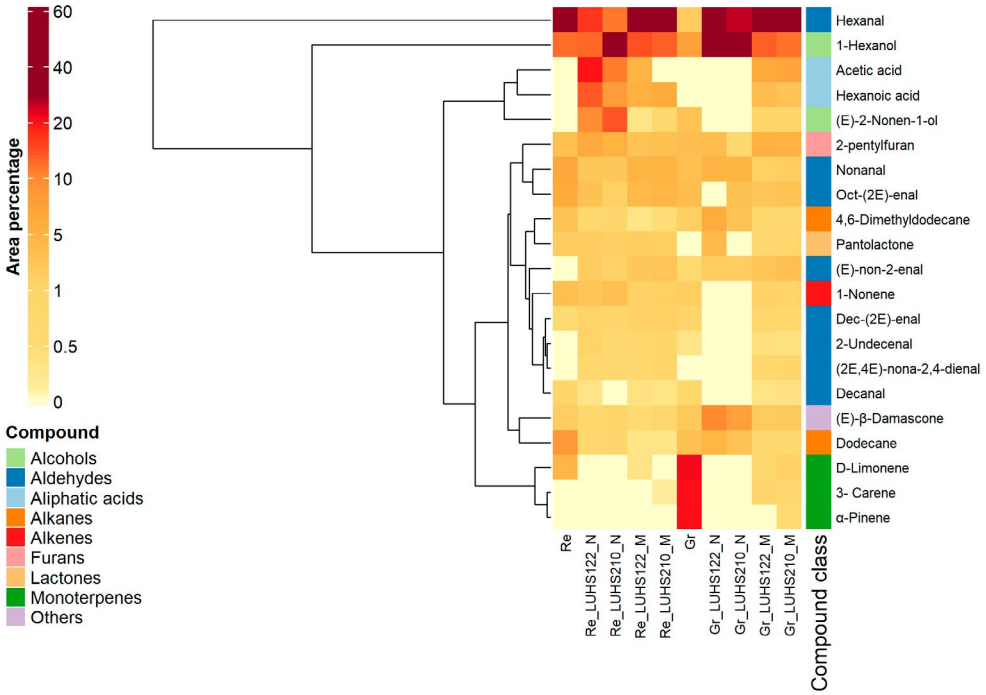

Fig. 2.2.1. *Heatmap of the volatile organic compounds in the milled and non-milled lentils*

The lentil substrates used in the experiment proved to be suitable for LAB fermentation with selected strains, in addition to being a potential probiotic due to high LAB survivability at low pH. All the analyzed factors, including the milling process, proved to have a significant effect on the majority of FAAs. In addition, the milling factor significantly increased the majority of the essential and non-essential FAAs. Therefore, the implementation of size reduction, in addition to other factors, can greatly improve the potential bioavailability

of important feed nutrients. The bioconversion of lentil substrates showed potential BA reduction. Fermented milled samples contained significantly lower concentrations of BAs for the most part. While the major FAs were not affected by the analyzed factors, omega-3 FAs were significantly affected by the lentil variety, thus noting potential variation within cultivated crops. The bioconversion of lentils produced new VOCs, potentially improving the sensory characteristics of the leguminous substrates.

2.3. Influence of the Type of the Fermentation and Growing Conditions on the Lentil Microbiological and Physicochemical Parameters

The aim of the experiment was to evaluate microbiological and physicochemical (pH, FAAs, FAs, VOCs, BAs, micro- and macroelements) of intercropped and normally grown lentils, bioconverted via the SMF and SSF methods, using *P. acidilactici* strain.

The pH of all bioconverted sample groups was significantly reduced, and LAB counts reaching at least 7.3 $\log_{10}$ CFU/g, on average (Table 2.3.1). Thus, it reached sufficient concentrations of desirable microorganisms so that bioconverted products of lentils can be considered as probiotics. The analyzed factors (type of fermentation, growing conditions, and fermentation duration) were significant for pH, while type of fermentation and fermentation duration were significant for LAB count. In a similar fashion, lentil flour, fermented in SMF via *P. acidilactici*, the pH reached down to 4.2, while viable cell count was over 12 log10 CFU/g, thus showing consistent ability to ferment leguminous seeds and tolerate low pH [122]. Similarly, SSF bioconverted legumes showed similar results [123]. Thus, low pH and high viable cell count (over 7.0 $\log_{10}$ CFU/g) showed that *P. acidilactici* strain is a suitable strain for lentil bioconversion.

All the analyzed factors and their interactions, except the growing conditions, type of fermentation, and fermentation duration interaction, were significant for all color coordinates in the lentil samples. Many of those compounds, responsible for the color of the legumes, such as anthocyanins and flavonoids, can be broken down by enzymes, produced by the LAB strains, thus producing color changes [124]. Anthocyanins and other flavonoids are typically broken down into aglycones by breaking glycosidic bonds and further broken down to small phenolic compounds by esterases, reductases and other enzymes, while carotenoids can be degraded under LAB activity, producing low molecular weight VOCs [125, 126].

Table 2.3.1. *The pH, microbiological and chromaticity characteristics of the lentil samples*

Sample	pH	Color coordinates, NBS			LAB count, log_{10} CFU/g
		L*	a*	b*	
LR_C_SM	6.12 ± 0.030 a	68.0 ± 0.310 b	1.85 ± 0.110 d	31.0 ± 0.290 d	4.80 ± 0.230 d
LR_C_SS	6.03 ± 0.010 b	67.9 ± 0.220 b	1.78 ± 0.120 d	31.3 ± 0.230 d	4.60 ± 0.190 de
LR_SMF_24	5.20 ± 0.030 e	63.2 ± 0.270 d	1.08 ± 0.070 e	23.5 ± 0.410 f	7.98 ± 0.210 ab
LR_SMF_48	4.36 ± 0.020 h	61.6 ± 0.430 e	2.96 ± 0.140 c	21.6 ± 0.360 g	8.27 ± 0.220 a
LR_SSF_24	5.22 ± 0.030 e	64.0 ± 0.250 d	1.19 ± 0.080 e	24.3 ± 0.250 f	7.63 ± 0.230 bc
LR_SSF_48	4.72 ± 0.020 f	50.9 ± 0.190 h	5.34 ± 0.210 b	15.6 ± 0.210 j	8.14 ± 0.190 ab
L_C_SM	6.02 ± 0.010 b	69.6 ± 0.250 a	1.01 ± 0.060 e	35.7 ± 0.370 a	4.02 ± 0.150 e
L_C_SS	5.94 ± 0.030 c	68.4 ± 0.390 b	1.08 ± 0.050 e	34.5 ± 0.410 b	4.87 ± 0.240 d
L_SMF_24	5.58 ± 0.020 d	67.7 ± 0.260 bc	1.98 ± 0.120 d	32.3 ± 0.320 c	7.61 ± 0.200 bc
L_SMF_48	4.45 ± 0.020 g	58.8 ± 0.320 f	5.12 ± 0.230 b	18.6 ± 0.090 h	8.41 ± 0.190 a
L_SSF_24	4.71 ± 0.030 f	66.8 ± 0.420 c	1.68 ± 0.140 d	29.7 ± 0.140 e	7.30 ± 0.220 c
L_SSF_48	4.46 ± 0.010 g	54.5 ± 0.290 g	7.03 ± 0.250 a	17.6 ± 0.110 i	8.10 ± 0.180 ab

C – control samples (non-fermented); L – lentils obtained from pure stands; LR – lentils obtained from relay incorporated with winter rye; SM – submerged conditions (lentils/water ratio 1:5, w/w); SS – submerged conditions (lentils/water ratio 1:1, w/w); SMF – fermented under submerged conditions; SSF – fermented under solid-state conditions; 24, 48 – duration of fermentation (in hours). L* – brightness or (-) darkness; a* – redness or (-) greenness; b* – yellowness or (-) blueness; NBS – National Bureau of Standards units; LAB – lactic acid bacteria; CFU – colony-forming units. Data are represented as means standard deviation. a–g Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Table 2.3.2. *Free essential, non-essential and non-proteinogenic amino acid concentrations (µmol/g) in lentil samples*

Non-essential and non-proteinogenic amino acids											
Sample	**Arginine**	**Glutamine**	**Asparagine**	**Glutamic acid**	**Serine**	**Aspartic acid**	**Glycine**	**Alanine**	**Proline**	**Tyrosine**	**GABA**
LR_C_SM	1.86 ± 0.028 d	0.943 ± 0.008 f	1.60 ± 0.019 fg	3.42 ± 0.085 f	0.819 ± 0.057 e	ND g	1.50 ± 0.091 g	3.62 ± 0.069 g	0.750 ± 0.027 f	0.476 ± 0.039 e	3.24 ± 0.090 gh
LR_C_SS	2.28 ± 0.044 c	1.96 ± 0.007 a	1.84 ± 0.085 ef	4.16 ± 0.134 e	1.03 ± 0.012 d	0.738 ± 0.027 e	1.91 ± 0.023 de	6.69 ± 0.176 c	0.990 ± 0.001 e	0.679 ± 0.009 c	3.61 ± 0.073 def
LR_SMF_24	ND f	0.172 ± 0.014 h	1.17 ± 0.026 h	4.85 ± 0.073 cd	0.128 ± 0.017 gh	0.879 ± 0.052 d	1.58 ± 0.027 fg	5.09 ± 0.078 f	0.760 ± 0.001 f	0.635 ± 0.011 cd	2.97 ± 0.001 i
LR_SMF_48	ND f	0.178 ± 0.01 h	1.33 ± 0.023 gh	5.00 ± 0.165 cd	ND h	1.08 ± 0.005 c	1.73 ± 0.026 ef	5.23 ± 0.077 f	0.960 ± 0.042 e	0.627 ± 0.013 cd	3.15 ± 0.011 hi
LR_SSF_24	0.750 ± 0.041 e	1.70 ± 0.059 b	1.97 ± 0.175 e	4.73 ± 0.120 d	1.44 ± 0.090 b	0.439 ± 0.012 f	2.26 ± 0.057 b	8.43 ± 0.035 b	1.37 ± 0.009 c	0.613 ± 0.011 d	5.88 ± 0.029 a
LR_SSF_48	ND f	1.11 ± 0.039 e	1.90 ± 0.138 ef	6.02 ± 0.074 a	1.67 ± 0.171 a	0.949 ± 0.036 d	2.67 ± 0.108 a	9.01 ± 0.022 a	1.56 ± 0.032 b	0.753 ± 0.003 b	4.73 ± 0.200 b
L_C_SM	2.99 ± 0.004 b	1.35 ± 0.027 c	3.63 ± 0.042 b	2.14 ± 0.051 h	0.893 ± 0.077 de	ND g	1.62 ± 0.001 fg	3.90 ± 0.090 g	0.752 ± 0.072 f	0.501 ± 0.042 e	3.65 ± 0.001 de
L_C_SS	4.35 ± 0.038 a	2.00 ± 0.112 a	4.68 ± 0.300 a	2.73 ± 0.029 g	1.25 ± 0.007 c	ND g	2.21 ± 0.080 bc	8.32 ± 0.090 b	1.11 ± 0.020 d	0.634 ± 0.014 cd	4.83 ± 0.145 b
L_SMF_24	ND f	0.694 ± 0.007 g	3.14 ± 0.023 d	4.95 ± 0.163 cd	0.269 ± 0.003 fg	1.39 ± 0.016 b	1.88 ± 0.036 de	5.90 ± 0.046 e	0.890 ± 0.002 e	0.595 ± 0.009 d	3.48 ± 0.026 efg
L_SMF_48	ND f	0.708 ± 0.005 g	3.23 ± 0.038 cd	5.67 ± 0.034 b	0.427 ± 0.021 f	1.57 ± 0.001 a	2.01 ± 0.117 d	6.29 ± 0.162 d	1.15 ± 0.052 d	0.632 ± 0.005 cd	3.38 ± 0.035 fgh
L_SSF_24	ND f	1.20 ± 0.052 de	3.41 ± 0.018 bcd	5.10 ± 0.022 c	1.34 ± 0.003 bc	0.928 ± 0.035 d	2.05 ± 0.014 cd	7.02 ± 0.246 c	1.44 ± 0.015 c	0.495 ± 0.022 e	3.82 ± 0.037 d
L_SSF_48	ND f	1.25 ± 0.016 cd	3.53 ± 0.049 bc	6.15 ± 0.032 a	1.74 ± 0.031 a	1.595 ± 0.044 a	2.513 ± 0.018 a	8.226 ± 0.056 b	1.70 ± 0.085 a	0.818 ± 0.019 a	4.13 ± 0.077 c

Table 2.3.2 cont.

Essential amino acids							
Sample	**Threonine**	**Valine**	**Methionine**	**Phenylalanine**	**Leucine/ Isoleucine**	**Lysine**	**Histidine**
LR_C_SM	1.15 ± 0.091 g	1.08 ± 0.086 h	0.115 ± 0.008 f	0.484 ± 0.040 f	1.27 ± 0.019 g	1.69 ± 0.118 f	0.620 ± 0.019 de
LR_C_SS	1.46 ± 0.028 ef	1.50 ± 0.039 g	0.195 ± 0.025 bc	0.632 ± 0.012 e	1.77 ± 0.036 f	2.04 ± 0.031 cd	0.860 ± 0.019 b
LR_SMF_24	1.39 ± 0.004 f	1.76 ± 0.016 ef	0.160 ± 0.002 de	0.697 ± 0.020 de	2.75 ± 0.017 e	1.8 ± 0.007 def	0.540 ± 0.012 e
LR_SMF_48	1.47 ± 0.011 ef	1.65 ± 0.008 fg	0.152 ± 0.008 de	0.685 ± 0.034 de	2.56 ± 0.108 e	1.98 ± 0.070 cde	0.580 ± 0.017 de
LR_SSF_24	2.10 ± 0.061 b	2.30 ± 0.001 c	0.112 ± 0.002 f	0.848 ± 0.006 bc	3.09 ± 0.021 d	2.77 ± 0.086 b	0.860 ± 0.004 b
LR_SSF_48	2.43 ± 0.046 a	2.98 ± 0.148 b	0.222 ± 0.003 ab	0.912 ± 0.024 b	4.39 ± 0.150 b	2.90 ± 0.007 b	0.730 ± 0.044 c
L_C_SM	1.21 ± 0.098 g	1.122 ± 0.103 h	0.168 ± 0.014 cde	0.428 ± 0.032 f	1.22 ± 0.101 g	1.72 ± 0.113 ef	0.580 ± 0.022 de
L_C_SS	1.59 ± 0.023 de	1.53 ± 0.030 g	0.219 ± 0.005 b	0.638 ± 0.018 e	1.61 ± 0.055 f	2.24 ± 0.041 c	1.01 ± 0.043 a
L_SMF_24	1.69 ± 0.016 d	1.94 ± 0.046 de	0.176 ± 0.012 cd	0.680 ± 0.005 de	2.69 ± 0.037 e	1.93 ± 0.014 def	0.550 ± 0.036 e
L_SMF_48	1.93 ± 0.047 c	2.05 ± 0.060 d	0.199 ± 0.002 bc	0.769 ± 0.025 cd	3.48 ± 0.026 c	2.73 ± 0.031 b	0.640 ± 0.009 d
L_SSF_24	2.14 ± 0.004 b	2.36 ± 0.122 c	0.141 ± 0.017 ef	0.834 ± 0.050 bc	3.38 ± 0.044 c	2.78 ± 0.189 b	0.730 ± 0.039 c
L_SSF_48	2.52 ± 0.023 a	3.33 ± 0.051 a	0.254 ± 0.001 a	1.09 ± 0.052 a	5.47 ± 0.164 a	3.38 ± 0.130 a	0.730 ± 0.022 c

C – control samples (non-fermented); L – lentils obtained from pure stands; LR – lentils obtained from relay incorporated with winter rye; SM – submerged conditions (lentils/water ratio 1:5, w/w); SS – submerged conditions (lentils/water ratio 1:1, w/w); SMF – fermented under submerged conditions; SSF – fermented under solid-state conditions; 24, 48 – duration of fermentation (in hours); GABA – γ – aminobutyric acid. Data are represented as means standard deviation. a–h Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Concentrations of FAAs in the SMF and SSF bioconverted lentil samples are presented in Table 2.3.2. In general, bioconverted lentil samples contained significantly lower concentrations of arginine, asparagine, glutamine, and aspartic acid concentrations, while glutamic acid, glycine, alanine, proline, threonine, valine, phenylalanine, and leucine/isoleucine concentrations were significantly higher than in respective control samples. Additionally, the growing conditions and the type of fermentation were significant for all FAAs, except for histidine, glycine, and alanine. Moreover, the fermentation duration was significant for all FAAs, except for histidine and asparagine. The interaction between type of fermentation and fermentation duration were significant for all FAAs, except for lysine, asparagine, alanine, and proline. The interaction of all analyzed factors was statistically significant for arginine, glutamine, GABA, and tyrosine.

The LAB strains are able to produce FAAs via the breakdown of proteins and by utilizing products of carbohydrate metabolism [127, 128]. Additionally, the LAB strain and the fermentation duration proved to be significant factors for the FAAs concentrations as our findings and literature suggest [129]. Significantly higher concentrations of products in SSF are often observed in literature, while the actual mechanisms are not fully understood, some explanations suggest that the process more closely mimics natural conditions [130-132]. Reduction of arginine during fermentation could be attributed to the production of BAs, while asparagine and glutamine reduction can be linked to bacterial asparaginase and glutaminase enzymes, which convert into aspartic and glutamic acids, respectively [133, 134]. GABA production is associated with glutamic acid conversion via glutamate decarboxylase, which is produced by LAB strains [135].

Only phenylethylamine, putrescine, spermidine, and spermine were detected in the lentil sample groups Table 2.3.3. All analyzed factors were significant for concentrations of phenylethylamine and putrescine in the lentil samples. The growing conditions and the type of fermentation factors' interaction were significant for all detected BAs' concentrations in the lentil samples. Additionally, all of the other factor interactions were significant for phenylethylamine and spermidine. Positive correlations between putrescine, spermine, and LAB count were established ($r = 0.761$, $p < 0.001$ and $r = 0.331$, $p = 0.049$, respectively), while putrescine was negatively correlated with the pH ($r = –0.719$, $p < 0.001$).

Table 2.3.3. *Concentrations of biogenic amines (mg/kg) in the lentil samples*

Sample	TRIP	PHE	PUT	CAD	HIS	TYR	SPRMD	SPRM
LR_C_SM	ND	ND b	ND f	ND	ND	ND	75.8 ± 5.06 e	13.6 ± 1.10 h
LR_C_SS	ND	ND b	14.6 ± 0.120 e	ND	ND	ND	110.1 ± 8.53 abc	20.7 ± 1.60 bcd
LR_SMF_24	ND	4.9 ± 0.41 a	36.2 ± 2.14 c	ND	ND	ND	87.8 ± 6.87 de	15.2 ± 1.20 fgh
LR_SMF_48	ND	ND b	41.8 ± 3.12 c	ND	ND	ND	84.7 ± 5.42 de	13.8 ± 1.10 gh
LR_SSF_24	ND	ND b	32.2 ± 2.81 cd	ND	ND	ND	129.0 ± 8.23 a	25.3 ± 1.60 a
LR_SSF_48	ND	ND b	37.2 ± 2.89 c	ND	ND	ND	116.7 ± 7.29 ab	22.4 ± 1.80 abc
L_C_SM	ND	ND b	ND f	ND	ND	ND	87.3 ± 5.32 de	ND i
L_C_SS	ND	ND b	22.1 ± 1.83 de	ND	ND	ND	117.1 ± 8.36 ab	23.9 ± 1.50 ab
L_SMF_24	ND	ND b	79.5 ± 5.45 b	ND	ND	ND	96.5 ± 7.21 cd	16.4 ± 1.30 efgh
L_SMF_48	ND	ND b	97.4 ± 6.84 a	ND	ND	ND	99.6 ± 6.81 bcd	17.9 ± 1.60 defg
L_SSF_24	ND	ND b	71.3 ± 5.42 b	ND	ND	ND	ND f	19.5 ± 1.70 cde
L_SSF_48	ND	ND b	77.2 ± 6.12 b	ND	ND	ND	101.5 ± 7.46 bcd	19.3 ± 1.80 cdef

C – control samples (non-fermented); L – lentils obtained from pure stands; LR – lentils obtained from relay incorporated with winter rye; SM – submerged conditions (lentils/water ratio 1:5, w/w); SS – submerged conditions (lentils/water ratio 1:1, w/w); SMF – fermented under submerged conditions; SSF – fermented under solid-state conditions; 24, 48 – duration of fermentation (in hours); TRIP – tryptamine; PHE – phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; SPRMD – spermidine; SPRM – spermine; BA – biogenic amines. ND – not detected. Data are represented as means standard deviation. a–i Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Putrescine production is associated with arginine metabolism in bacteria via multiple pathways [136]. This is further corroborated by the negative correlations established between putrescine and arginine in the lentil samples ($r = –0.686$, $p < 0.001$). Polyamines such as spermidine and spermine, however, are produced from putrescine via spermidine and spermine synthases, mainly of bacterial origin [137]. Aromatic and heterocyclic BAs, such as phenylethylamine, tryptamine, histamine, and tyramine, are associated with decarboxylation reactions of amino acids (phenylalanine, tryptophan, histidine, and tyrosine, respectively), which are often initiated by enzymes of bacterial origin [134]. The BAs have important biological roles in the host's organism, but overproduction of those compounds can introduce serious health issues [138, 139]. Some of those issues include headaches, intestinal mucosa damage, and digestive disorders [140, 141]. In addition, they can negatively affect the productivity parameters, such as body weight, feed conversion ratio, and reduced hatchings [142, 143]. The World Health Organization proposed maximum acceptable limit for only for histamine (50 ppm), while rat model suggested that no observed adverse effect level (NOAEL) of 2000 ppm for tyramine, putrescine, and cadaverine, 1000 ppm for cadaverine, and 200 ppm for spermine [144, 145]. Thus, the prepared samples in our experiment are safe for consumption from the BAs standpoint, though significant production of the BAs should be heavily monitored.

The predominant group of FAs in lentil samples was PUFAs (Table 2.3.4). The major component of PUFAs and omega-6 FAs was linoleic acid, ranging between 41.3 and 50.3 % in the sample groups. The majority of SFAs consisted of palmitic and stearic acids, ranging between 16.2–20.3 and 1.63 – 3.29 %, respectively. Only factor interactions were significant for concentrations of all detected SFAs. Additionally, interactions of all factors (type of fermentation, fermentation duration, and growing conditions) were significant for all analyzed FAs. Moreover, all factors and their interactions were significant for α-linolenic acid, a major component of omega-3 FAs in the lentil samples. The growing conditions and the type of fermentation were significant for concentrations of oleic acid in the lentil samples. Positive correlation between stearic acid and LAB count was established as well ($r = 0.363$, $p = 0.030$).

Table 2.3.4. *Fatty acid composition of the lentil samples*

Sample	SFA	MUFA	PUFA	Omega_3	Omega_6	Omega_9
LR_C_SM	19.4 ± 1.67 bcd	18.0 ± 1.38 bcdef	62.6 ± 2.88 ab	14.6 ± 0.582 abc	48.1 ± 4.49 ab	18.0 ± 1.45 abc
LR_C_SS	20.9 ± 2.04 b	16.9 ± 0.872 def	62.2 ± 5.12 ab	14.8 ± 1.128 ab	47.3 ± 3.15 ab	16.9 ± 0.783 bc
LR_SMF_24	17.2 ± 0.724 cd	15.8 ± 1.11 f	67.1 ± 2.21 a	15.8 ± 0.838 a	51.2 ± 3.11 a	15.8 ± 0.670 c
LR_SMF_48	19.5 ± 0.764 bcd	16.5 ± 0.711 ef	64.0 ± 3.08 ab	16 ± 0.502 a	48.0 ± 1.55 ab	16.5 ± 0.894 bc
LR_SSF_24	20.7 ± 1.26 bc	18.0 ± 1.64 bcdef	61.3 ± 5.73 ab	14.0 ± 1.202 abc	47.4 ± 3.66 ab	18.0 ± 0.884 abc
LR_SSF_48	20.9 ± 0.754 b	17.6 ± 1.26 cdef	61.4 ± 5.42 ab	14.0 ± 1.059 abc	47.4 ± 2.14 ab	17.6 ± 1.47 abc
L_C_SM	18.2 ± 1.29 bcd	19.2 ± 0.771 abcde	62.6 ± 4.68 ab	14.4 ± 1.054 abc	48.2 ± 1.84 ab	19.2 ± 1.76 abc
L_C_SS	17.8 ± 0.702 bcd	19.8 ± 0.836 abcd	62.4 ± 2.96 ab	14.0 ± 0.606 abc	48.4 ± 3.55 ab	19.8 ± 0.905 ab
L_SMF_24	26.2 ± 0.927 a	20.4 ± 0.779 abc	53.4 ± 5.23 b	12.1 ± 0.891 c	41.3 ± 3.75 b	20.4 ± 1.23 ab
L_SMF_48	15.9 ± 1.55 d	19.0 ± 1.15 abcde	65.1 ± 5.62 ab	14.8 ± 0.531 ab	50.3 ± 4.27 ab	19.0 ± 1.84 abc
L_SSF_24	19.9 ± 1.00 bc	21.0 ± 0.651 ab	59.2 ± 4.67 ab	12.5 ± 0.556 bc	46.6 ± 4.18 ab	21.0 ± 1.30 a
L_SSF_48	19.8 ± 1.49 bc	21.2 ± 1.29 a	59.0 ± 4.82 ab	12.6 ± 1.12 bc	46.4 ± 2.86 ab	21.2 ± 1.99 a

C – control samples (non-fermented); L – lentils obtained from pure stands; LR – lentils obtained from relay incorporated with winter rye; SM – submerged conditions (lentils/water ratio 1:5, w/w); SS – submerged conditions (lentils/water ratio 1:1, w/w); SMF – fermented under submerged conditions; SSF – fermented under solid-state conditions; 24, 48 – duration of fermentation (in hours); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Data are represented as means standard deviation. a–i Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

The main detected FAs in the lentil samples were in accordance with the findings elsewhere [119, 120]. The differences in the FA composition between intercropped and normally grown lentils were already observed in the literature; furthermore, noting that geographical location and cultivar can be significant factors as well [110, 146]. It was also established that fermentation can significantly affect concentrations of FAs in the leguminous crops [147, 148].

Intercropped lentils contained significantly lower concentrations of the majority of macroelements (Na, K, and Ca) (Table 2.3.5). Regarding the essential microelement concentrations, intercropped lentils contained significantly lower concentrations of P, Mn, and Se, while Fe and Ni concentrations were significantly higher, in comparison to pure stand grown lentil samples. The majority of non-essential microelements were not detected in the lentil samples.

Table 2.3.5. *Concentrations of micro- and macroelements in the lentil samples*

	LR	**L**
Macroelements, mg/kg (D.M.)		
Na	7.12 ± 0.450 b	21.0 ± 1.85 a
Mg	676.0 ± 32.1 a	726.0 ± 56.3 a
K	4118 ± 115 b	4925 ± 136 a
Ca	510.0 ± 49.1 b	747.0 ± 57.3 a
Essential microelements, mg/kg (D.M.)		
P	3406 ± 123 b	3899 ± 142 a
Cr	0.405 ± 0.390 a	0.405 ± 0.028 a
Mn	8.96 ± 0.520 b	13.9 ± 0.930 a
Fe	116.0 ± 9.35 a	96.3 ± 7.41 b
Co	0.059 ± 0.040 a	0.064 ± 0.007 a
Ni	1.43 ± 0.110 a	0.893 ± 0.090 b
Cu	7.29 ± 0.620 a	7.12 ± 0.410 a
Zn	22.0 ± 1.93 a	24.6 ± 1.84 a
Se	ND b	0.014 ± 0.002 a
Non-essential microelements, mg/kg (D.M.)		
Li	ND	ND
Be	ND	ND
B	2.48 ± 0.120 b	6.62 ± 0.580 a
Al	88.3 ± 1.69 a	25.3 ± 1.32 b
Ti	3.19 ± 0.250 a	0.767 ± 0.052 b
V	ND	ND
Ga	ND	ND
As	0.019 ± 0.002 a	0.017 ± 0.002 a
Rb	7.22 ± 0.520 a	6.33 ± 0.290 a

Table 2.3.5 cont.

	LR	L
Sr	2.65 ± 0.220 a	3.02 ± 0.300 a
Mo	2.09 ± 0.19 a	1.84 ± 0.170 a
Pd	ND	ND
Ag	ND	ND
Cd	ND	ND
Sn	ND	ND
Sb	ND	ND
Cs	ND	ND
Ba	0.865 ± 0.023 b	1.49 ± 0.110 a
Hg	ND	ND
Pb	0.177 ± 0.012 a	0.036 ± 0.003 b
In	ND	ND
Pt	ND	ND
Tl	ND	ND
Bi	ND	ND
U	ND	ND

L – lentils obtained from pure stands; LR – lentils obtained from relay incorporated with winter rye; D. M. – dry matter; ND – not detected. Data are represented as means standard deviation. a–b Means with different letters in the column are significantly different all sample groups. Results are statistically significant when $p < 0.05$.

Lentils are already well known for containing significant amounts of various micro- and macroelements, such as potassium, iron, zinc, copper, selenium, magnesium, and others, though their concentrations can vary due to many factors, such as rainfall, soil, and temperature [149, 150]. Thus, differences of micro- and macroelements between lentil samples of different growing conditions can be attributed to intercropping, as significant differences were already described in the literature [151, 152].

The VOCs of the lentil samples, expressed as area percentage of total identified VOCs, are presented in Figure 2.3.1. Most of the detected VOCs in the lentil samples belonged to alcohols (1-hexanol, 11-hexadecyn-1-ol, phenethyl alcohol), aldehydes (hexadecanal, pentadecanal, nonanal, dodecanal), organic acids (hexanoic, octanoic, nonanoic acids), ketones (β-damascenone), and allylbenzenes (eugenol and anethole). The higher number of VOCs was detected in fermented samples in most of the cases, in comparison to respective control samples. Additionally, a higher number of VOCs were detected in SSF lentil samples, in comparison to SMF lentil samples. All factors and their interactions were significant for most of the VOCs in the lentil samples.

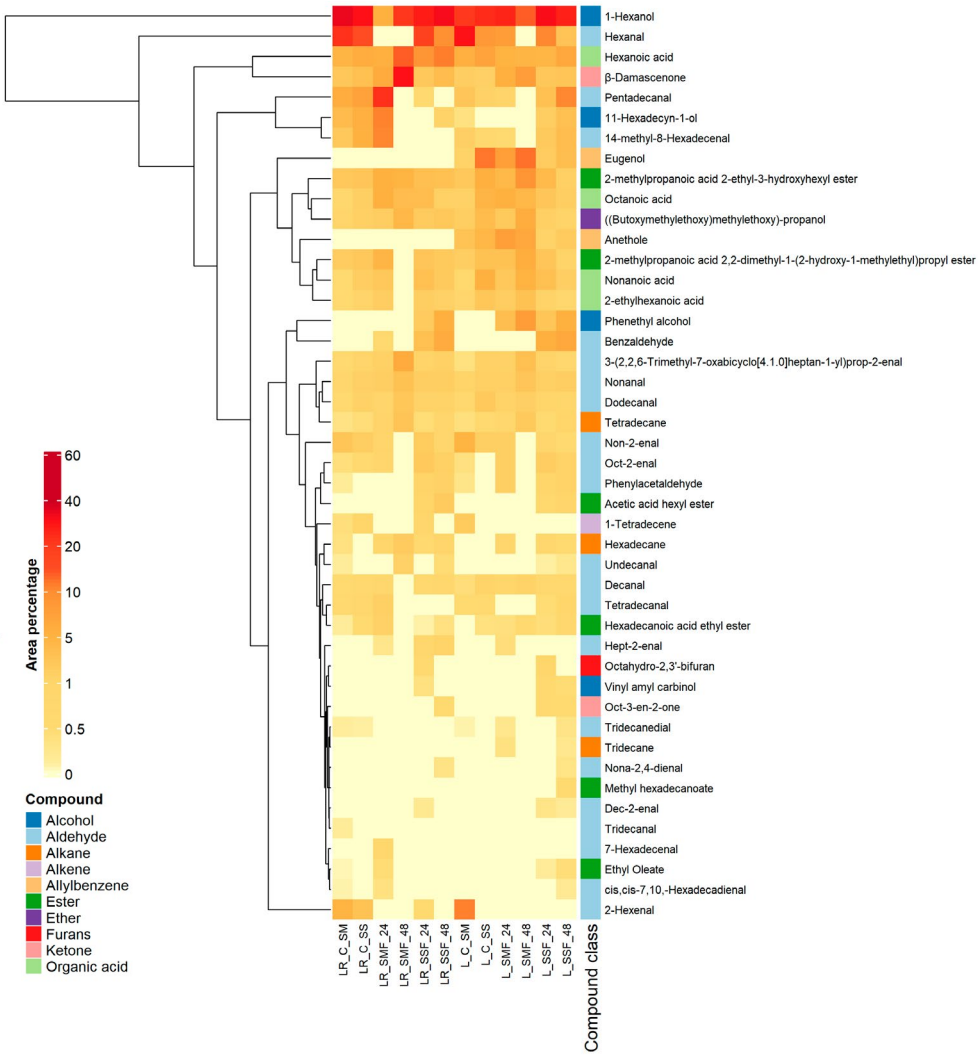

Fig. 2.3.1. *Heatmap of volatile organic compounds in the lentil samples*

Detected VOCs contribute to flavor and aroma of the final product, like alcohols, such as 1-hexanol providing light apple, sweet, fruity flavor and herbal, oily aroma; aldehydes, such as hexanal and others, providing fatty, grassy, and fresh aroma; organic acids, such as hexanoic and octanoic acids, contributing sour, fatty, and cheesy flavor [153-155]. Aldehyde production is associated with fatty acid oxidation, while aromatic aldehydes and alcohols can be linked to aromatic amino acids metabolism [156, 157]. Additionally, statistically significant correlations between benzaldehyde, phenylacetaldehyde, phenylethyl alcohol, and phenylalanine were established

(r = 0.824, $p < 0.001$, r = 0.569, $p < 0.001$, r = 0.660, $p < 0.001$, respectively). Alkanes and alkenes are generally attributed to FA metabolism by bacteria [158]. Higher VOC profile complexity of the SSF lentil samples can be attributed to the overall higher production rates of the enzymes, which results in higher yields of the products [159].

The selected LAB strain was able to successfully propagate in all of the bioconverted lentil samples, reaching substantial LAB count values (>6.0 $\log_{10}$ CFU/g) at low pH. Additionally, the type of fermentation and fermentation duration proved to be significant for LAB count, noting that the selection of an appropriate fermentation method with the optimized fermentation duration can significantly improve the probiotic performance of the feed in more sustainable manner (SSF conditions). The bioconversion in general increased almost all of the essential FAA concentrations. The growing conditions, fermentation duration, and type of fermentation significantly affected the majority of the FAA concentrations, thus implying the importance of potential crop selection and fermentation conditions for the bioavailability of AAs in the final feed material. In general, increased fermentation duration increased concentrations of the essential and non-essential FAAs. Moreover, SSF samples contained significantly higher amounts of FAAs, for the most part. However, the bioconversion increased the concentrations of BAs and must be carefully monitored. All analyzed factors significantly affected omega-3 FA composition of the lentils, so a careful selection and optimization of substrate selection and fermentation conditions can significantly improve the nutritive and functional value of the feed material. Additionally, lentils growing conditions showed significant differences in the micro- and macroelemental composition. The SSF of the lentil substrates provided a more diverse VOC profile, in comparison to SMF. In addition to potential improvement of the sensory properties in SSF samples, the overall higher concentrations of FAAs were observed, thus denoting the higher rate of production over the SMF.

2.4. Influence of the Bioconversion and Wheat Variety on the Digestion Parameters *In Vitro*

The aim of the experiment was to investigate differences in metabolism between different winter wheat cereals of different varieties, Herkus and DS8558-1 (DS), and different treatments, non-pretreated and bioconverted using *Lp. plantarum* strain. The investigation of the metabolism was conducted by applying the piglet *in vitro* digestion and colonic fermentation model. The colonic fermentation stage was conducted using the faecal inoculum of weaning piglets, approximately 42 days of age. During the experiment, free and total AAs concentrations in the digesta; total AAs in the undigested

residue and bioconverted/control wheat wholemeal substrates; SCFAs, VOCs, FAAs, BAs and other small non-volatile metabolites in the *in vitro* fermentation (IVF) samples were evaluated. Factor effects (bioconversion and substrate) and their interaction were evaluated for the analyzed parameters.

The bioconverted samples featured low pH (below 4) and high LAB count values (over 8.4 $\log_{10}$ CFU/g) (Table 2.4.1), thus emphasizing the *Lp. plantarum* good survivability at low pH and great ability to ferment starchy substrates, as denoted in the literature [109, 160].

Table 2.4.1. *Lactic acid bacteria count, total titratable acidity and the pH of the wheat wholemeal samples*

Sample	pH	TTA°	LAB Count, $\log_{10}$ CFU/g
DS_F	3.94 ± 0.090 c	9.07 ± 0.153 b	8.50 ± 0.020 a
DS_NF	5.64 ± 0.040 b	2.38 ± 0.076 c	4.40 ± 0.040 b
Herkus_F	3.95 ± 0.030 c	9.90 ± 0.100 a	8.48 ± 0.070 a
Herkus_NF	5.94 ± 0.045 a	1.92 ± 0.076 d	4.42 ± 0.040 b

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample; TTA° – total titratable acidity; LAB – lactic acid bacteria. a–d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Most of the AAs in the wheat wholemeal samples were not significantly different, in comparison to the respective control samples (Table 2.4.2). The interaction for both factors was significant for concentrations of all the AAs. The concentrations of FAAs in the *in vitro* digestion (IVD) samples are presented in Table 2.4.3. The bioconversion and substrate were significant for concentrations of GABA, proline and glutamine, while the concentrations of glutamic acid and glycine were significantly affected by bioconversion alone. While certainly concentrations of total AAs in the IVD samples are significantly higher than in blank samples, the majority of the AAs did not differ significantly between sample groups (Table 2.4.4). Thus bioconversion factor was only significant for the concentration of glycine, while substrate was significant factor for the concentrations of alanine, glutamic acid, threonine, proline, valine, methionine, phenylalanine, leucine/isoleucine, tyrosine, and total AAs in the IVD samples. The total AAs in the undigested residue of the IVD samples are presented in Table 2.4.5. The fermented sample groups contained significantly higher concentrations of threonine, in comparison to the respective control group samples. Additionally, the bioconverted Herkus samples contained significantly higher concentrations of glutamic acid, glycine, alanine, and proline, and total AAs, in comparison to Herkus control

group samples. The bioconversion factor was significant for the concentrations of the majority of the AAs in the hydrolysates, except for serine, valine, phenylalanine, and leucine/isoleucine. However, only glutamic and aspartic acids, threonine, proline, histidine, and total AAs were significantly affected by the substrate factor. The interaction of the aforementioned factors was significant for the concentrations of arginine, proline, and histidine in the IVD residue samples.

Table 2.4.2. *Total amino acid concentrations (g/100g) in the wheat wholemeal samples*

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	0.406 ± 0.021 a	0.408 ± 0.001 a	0.348 ± 0.014 b	0.351 ± 0.0003 b
Glutamic acid	2.539 ± 0.024 a	2.616 ± 0.080 a	1.820 ± 0.110 b	1.892 ± 0.067 b
Serine	0.354 ± 0.029 a	0.363 ± 0.004 a	0.291 ± 0.043 a	0.288 ± 0.009 a
Aspartic acid	0.313 ± 0.028 a	0.309 ± 0.004 a	0.259 ± 0.035 a	0.258 ± 0.010 a
Threonine	0.225 ± 0.007 a	0.222 ± 0.001 a	0.167 ± 0.001 b	0.158 ± 0.008 b
Glycine	0.287 ± 0.017 a	0.300 ± 0.004 a	0.221 ± 0.017 b	0.233 ± 0.009 b
Alanine	0.281 ± 0.019 a	0.268 ± 0.018 ab	0.227 ± 0.004 ab	0.217 ± 0.010 b
Proline	1.66 ± 0.032 a	1.75 ± 0.133 a	1.17 ± 0.028 b	1.12 ± 0.035 b
Valine	0.285 ± 0.018 a	0.298 ± 0.022 a	0.231 ± 0.009 a	0.224 ± 0.024 a
Methionine	0.068 ± 0.01 a	0.074 ± 0.011 a	0.043 ± 0.005 a	0.054 ± 0.008 a
Phenylalanine	0.411 ± 0.009 a	0.415 ± 0.001 a	0.312 ± 0.039 b	0.256 ± 0.013 b
Leucine/Isoleucine	0.961 ± 0.010 a	0.951 ± 0.039 a	0.741 ± 0.010 b	0.610 ± 0.045 c
Cystine	0.100 ± 0.002 a	0.115 ± 0.009 a	0.072 ± 0.008 b	0.092 ± 0.001 ab
Lysine	0.219 ± 0.005 a	0.207 ± 0.007 a	0.197 ± 0.013 ab	0.164 ± 0.007 b
Histidine	0.158 ± 0.0004 a	0.154 ± 0.008 a	0.128 ± 0.011 ab	0.110 ± 0.015 b
Tyrosine	0.305 ± 0.005 ab	0.325 ± 0.027 a	0.268 ± 0.003 ab	0.265 ± 0.002 b
Total amino acids	8.57 ± 0.162 a	8.77 ± 0.204 a	6.49 ± 0.161 b	6.29 ± 0.149 b

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 2.4.3. *Free amino acid concentrations (mg/L) in the in vitro digestion samples*

Sample	Blank	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	256.1 ± 45.5 b	516.4 ± 21.9 a	555.0 ± 52.0 a	498.5 ± 38.1 a	511.1 ± 50.5 a
Glutamine	125.4 ± 15.0 d	620.6 ± 38.2 bc	743.76 ± 33.13 a	550.2 ± 44.8 c	643.0 ± 12.1 b
Asparagine	91.7 ± 10.7 a	108.0 ± 17.9 a	119.3 ± 15.0 a	107.3 ± 14.4 a	116.3 ± 4.34 a
Glutamic acid	148.3 ± 20.2 b	179.2 ± 31.5 ab	154.3 ± 14.3 ab	200.3 ± 6.42 a	176.2 ± 6.96 ab
Serine	86.7 ± 19.0 b	106.0 ± 16.7 ab	115.6 ± 17.1 ab	121.8 ± 7.11 ab	131.8 ± 12.3 a
Aspartic acid	80.8 ± 15.2 a	100.4 ± 13.7 a	96.3 ± 4.46 a	97.8 ± 25.3 a	92.3 ± 27.5 a
Threonine	75.5 ± 15.4 b	113.4 ± 1.05 a	126.4 ± 3.07 a	111.2 ± 12.7 a	119.7 ± 14.8 a
Glycine	119.5 ± 8.71 b	143.0 ± 9.39 a	131.2 ± 5.11 ab	144.6 ± 6.30 a	131.2 ± 5.55 ab
Alanine	108.0 ± 10.6 b	182.3 ± 17.8 a	170.9 ± 13.8 a	188.1 ± 6.62 a	169.9 ± 11.8 a
GABA	2.35 ± 0.380 d	20.0 ± 2.61 b	9.84 ± 0.970 c	30.3 ± 2.13 a	16.7 ± 0.750 b
Proline	5.23 ± 0.530 d	43.2 ± 2.28 a	19.8 ± 0.740 c	37.7 ± 1.21 b	16.3 ± 1.16 c
Tryptophan	109.8 ± 19.4 b	139.0 ± 22.6 ab	139.8 ± 20.8 ab	177.0 ± 11.5 a	180.3 ± 10.4 a
Valine	111.7 ± 21.1 b	194.5 ± 10.6 a	216.4 ± 19.3 a	205.5 ± 27.2 a	231.1 ± 8.7 a
Methionine	29.6 ± 7.21 b	90.6 ± 9.74 a	88.9 ± 19.7 a	88.6 ± 13.9 a	91.6 ± 1.87 a
Phenylalanine	146.4 ± 11.0 b	361.0 ± 68.1 a	371.1 ± 75.9 a	341.4 ± 16.3 a	341.8 ± 21.9 a
Leucine/Isoleucine	368.9 ± 36.4 b	811.1 ± 143.1 a	770.4 ± 68.9 a	778.3 ± 80.9 a	850.0 ± 8.59 a
Cystine	26.9 ± 6.47 a	22.5 ± 1.24 a	24.8 ± 2.67 a	27.5 ± 3.04 a	29.2 ± 0.990 a
Lysine	275.7 ± 27.8 b	389.8 ± 55.1 a	372.0 ± 58.1 ab	390.2 ± 16.7 a	385.6 ± 15.8 a
Histidine	64.4 ± 8.30 b	108.4 ± 18.0 a	116.7 ± 15.0 a	109.4 ± 3.97 a	120.4 ± 7.66 a
Tyrosine	226.0 ± 26.9 a	344.4 ± 62.8 a	323.5 ± 65.9 a	253.5 ± 34.53 a	302.8 ± 56.0 a
Total AA	2458.9 ± 304.9 b	4593.5 ± 492.6 a	4665.8 ± 360.1 a	4459.3 ± 176.1 a	4657.2 ± 93.6 a

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with Lactiplantibacillus plantarum; NF – control sample; GABA – γ-aminobutyric acid. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 2.4.4. *Total amino acids (mg/L) in the in vitro digestion samples*

Sample	Blank	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	409.1 ± 7.97 b	885.5 ± 14.4 a	916.4 ± 50.8 a	847.6 ± 116.5 a	718.0 ± 84.8 a
Glutamic acid	739.9 ± 54.8 b	3538.6 ± 93.7 a	3544.2 ± 257.1 a	2901.4 ± 289.8 a	2882.2 ± 363.5 a
Serine	405.7 ± 39.4 b	828.8 ± 80.9 a	821.8 ± 125.8 a	731.2 ± 89.9 a	820.3 ± 131.6 a
Aspartic acid	770.5 ± 1.61 b	1130.7 ± 92.7 a	1074.8 ± 75.4 a	1066.7 ± 90.3 a	1073 ± 127.9 a
Threonine	294.7 ± 32.1 b	594.4 ± 28.0 a	555.9 ± 33.6 a	521.0 ± 50.7 a	517.5 ± 9.7 a
Glycine	531.04 ± 9.81 c	904.6 ± 46.0 a	850.8 ± 45.4 ab	863.3 ± 63.9 ab	780.7 ± 11.9 b
Alanine	284.9 ± 9.84 b	580.3 ± 34.6 a	582.4 ± 21.1 a	565.4 ± 54.9 a	524.4 ± 32.4 a
Proline	557.2 ± 84.2 c	2633.8 ± 71.6 ab	2786.5 ± 147.3 a	1953.2 ± 158.1 ab	2009 ± 44.7 ab
Valine	323.4 ± 41.6 b	801.1 ± 77.6 a	776.2 ± 29.8 a	657.8 ± 58.6 a	697.4 ± 69.8 a
Methionine	46.2 ± 15.0 b	182.8 ± 2.90 a	178.4 ± 11.2 a	131.7 ± 35.5 a	133.0 ± 10.0 a
Phenylalanine	238.4 ± 30.8 d	699.8 ± 18.0 ab	757.8 ± 79.5 a	554.3 ± 64.9 c	596.9 ± 29.5 bc
Leucine/Isoleucine	678.0 ± 17.5 d	1813.9 ± 73.1 ab	1910.7 ± 205.9 a	1458.0 ± 131.1 c	1479.8 ± 40.4 bc
Cystine	129.0 ± 17.9 b	238.2 ± 7.50 a	237.4 ± 10.2 a	240.2 ± 19.8 a	225.5 ± 22.3 a
Lysine	383.1 ± 41.34 b	642.3 ± 40.4 a	693.5 ± 55.4 a	666.1 ± 25.3 a	664.7 ± 51.1 a
Histidine	126.6 ± 5.06 b	345.1 ± 16.9 a	355.6 ± 35.8 a	306.9 ± 31.8 a	326.0 ± 31.4 a
Tyrosine	359.5 ± 28.31 c	771.7 ± 28.9 a	789.9 ± 22.8 a	506.0 ± 109.0 bc	641.2 ± 81.6 ab
Total AA	6304.3 ± 436.9 c	16667.3 ± 244.8 a	16893.7 ± 586.7 a	14010.4 ± 1183.7 b	14156.7 ± 886.7 b

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 2.4.5. *Total amino acids (g/100g) in the in vitro undigested residue samples*

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	0.524 ± 0.036 a	0.527 ± 0.029 a	0.549 ± 0.039 a	0.420 ± 0.025 b
Glutamic acid	1.45 ± 0.128 a	1.33 ± 0.065 a	1.33 ± 0.086 a	0.971 ± 0.144 b
Serine	0.495 ± 0.114 a	0.415 ± 0.035 a	0.430 ± 0.049 a	0.337 ± 0.051 a
Aspartic acid	0.931 ± 0.136 a	0.677 ± 0.038 b	0.782 ± 0.089 ab	0.560 ± 0.076 b
Threonine	0.333 ± 0.036 a	0.216 ± 0.020 bc	0.241 ± 0.007 b	0.154 ± 0.029 c
Glycine	0.493 ± 0.039 ab	0.435 ± 0.046 b	0.526 ± 0.018 a	0.409 ± 0.028 b
Alanine	0.432 ± 0.019 a	0.345 ± 0.023 ab	0.434 ± 0.054 a	0.310 ± 0.042 b
Proline	0.960 ± 0.045 a	0.880 ± 0.008 a	0.934 ± 0.052 a	0.652 ± 0.058 b
Valine	0.429 ± 0.036 a	0.390 ± 0.033 a	0.406 ± 0.051 a	0.390 ± 0.046 a
Methionine	ND	ND	ND	ND
Phenylalanine	0.361 ± 0.026 a	0.322 ± 0.024 a	0.336 ± 0.059 a	0.312 ± 0.029 a
Leucine/Isoleucine	0.860 ± 0.078 a	0.746 ± 0.065 a	0.850 ± 0.19 a	0.684 ± 0.032 a
Cystine	ND	ND	ND	ND
Lysine	0.502 ± 0.084 a	0.392 ± 0.031 ab	0.437 ± 0.031 ab	0.331 ± 0.051 b
Histidine	0.126 ± 0.026 a	0.092 ± 0.011 a	0.131 ± 0.018 a	ND b
Tyrosine	0.572 ± 0.017 a	0.507 ± 0.058 a	0.597 ± 0.047 a	0.503 ± 0.044 a
Total AA	8.48 ± 0.146 a	7.27 ± 0.182 ab	7.98 ± 0.728 a	6.03 ± 0.635 b

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample; ND – not detected. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The *in vitro* digestibility was significantly higher in the bioconverted sample groups, in comparison to the control sample groups, while the opposite trend was observed for the *in vitro* fermentability of the IVD residue samples (Table 2.4.6). The lowest total AA digestibility was observed in the Herkus_NF samples. Both factors were significant for the aforementioned parameters, except digestibility. Only bioconversion and interaction of both factors were significant for the *in vitro* digestibility. While the majority of the individual AA digestibilities were not significantly different between sample groups, the bioconversion factor was a significant factor for the digestibility of all AAs, except glutamic acid, serine, aspartic acid, threonine, glycine, and alanine. The substrate factor was significant for almost all of the AA digestibilities, except of arginine, glutamic acid, serine, aspartic acid, threonine, and lysine.

Similarly, in cases of spontaneous fermentation, no significant increase in the total protein can be observed, while as the fermentation duration increases up to 48 h, significantly higher concentrations of protein can be observed [161, 162]. The differences in FAAs in the IVD samples could be attributed

to the activity of the LAB strain, as such strains as *L. plantarum* are known to excrete enzymes that degrade proteinaceous substrates, which produces glumatate that is converted to GABA via glutamic acid decarboxylase (GAD) [163, 164]. The IVD residue analysis showed significant alteration of the digestibility of some non-essential (arginine, proline, tyrosine) and essential (valine, phenylalanine, leucine/isoleucine, lysine, histidine) amino acids of the bioconverted samples. Significant differences between bioconverted and control samples could be attributed to bacterial activity, as it is able to excrete proteolytic and amylolytic enzymes that help to degrade food matrix [165, 166]. Substrate factor effects on AA digestibility could be attributed to differences in cultivars, as certain differences were observed in the literature [167, 168]. Lysine is often reported as having the lowest bioavailability as it is contained mostly in the aleurone layer [167, 169]. Our research results showed that bioconversion can improve its digestibility, as well as other essential AAs, such as phenylalanine, histidine, leucine/isoleucine, and valine.

Table 2.4.6. *The in vitro digestibility and fermentability parameters*

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
In vitro digestibility, % DM	83.9 ± 0.490 a	78.8 ± 0.650 b	84.8 ± 0.530 a	77.6 ± 0.490 b
Total AA digested, %	91.9 ± 0.180 a	91.1 ± 0.490 ab	90.6 ± 0.610 ab	89.0 ± 1.41 b
In vitro fermentability, % DM	42.7 ± 0.240 c	48.1 ± 1.27 b	49.5 ± 1.60 b	57.5 ± 0.830 a
Total *in vitro* digestibility, % DM	90.8 ± 0.310 b	89.0 ± 0.220 c	92.3 ± 0.250 a	90.5 ± 0.210 b

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample; AA – amino acids; DM – dry matter. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The FAAs and BAs in the IVF samples are presented in Table 2.4.7. The Herkus samples contained significantly higher concentrations of the majority of FAAs, such as glycine, valine, leucine, serine, proline, aspartic acid, glutamic acid, methionine, phenylalanine, histidine, tryptophan, and tyrosine, in comparison to DS sample groups. Factor analysis showed that bioconversion was a significant factor for all FAAs and BAs, except for alanine, valine, leucine, threonine, aspartic acid, phenylalanine, tyramine, histidine, and tryptophan. Although the substrate factor significantly affected almost all concentrations of the analytes, except for lysine. The BAs play important roles in the animal, such as acidity regulation, immunoregulation, though a putrefactive fermentation can produce the abundance of the BAs

that can cause serious problems to the host [138, 139, 170]. Elevated levels of BAs are associated with pathogens, as most of the BAs produced in piglets' colon are of bacterial origin [170, 171]. Additionally, some BAs (putrescine, cadaverine, histamine) negatively correlated with TBC count, providing a link between bacterial activity and its products. We failed to establish any significant correlations between BAs and LAB count, suggesting modulation of certain bacterial communities other than LAB.

Table 2.4.7. *Concentrations of free amino acids and biogenic amines (mg/L) in the in vitro fermentation samples*

Sample	B0	Bl	DS_F	DS_NF	Herkus_F	Herkus_NF
Alanine	111.9 ± 4.66 bc	1.67 ± 0.830 d	109.1 ± 16.0 c	153.7 ± 18.5 abc	175.1 ± 21.6 ab	186.7 ± 39.1 a
Glycine	38.5 ± 2.58 b	5.33 ± 0.710 c	8.21 ± 3.10 c	56.1 ± 9.18 ab	71.8 ± 2.72 a	72.4 ± 15.4 a
Valine	102.5 ± 7.40 a	9.19 ± 1.76 cd	2.38 ± 0.150 d	1.48 ± 0.090 d	27.5 ± 3.73 bc	40.2 ± 19.8 b
Leucine	231.4 ± 9.75 a	9.92 ± 1.61 c	2.30 ± 0.350 c	2.02 ± 0.330 c	30.9 ± 2.23 b	37.4 ± 15.9 b
Isoleucine	74.5 ± 1.76 a	8.09 ± 1.16 c	5.18 ± 0.050 c	4.21 ± 0.670 c	5.96 ± 0.630 c	41.7 ± 23.4 b
GABA	ND c	ND c	44.0 ± 4.89 c	303.6 ± 105.2 b	385.3 ± 50.9 b	628.0 ± 77.0a
Serine	65.0 ± 19.0 a	ND c	ND c	ND c	27.7 ± 0.61 b	33.9 ± 3.34 b
Threonine	47.0 ± 14.4 a	ND c	14.6 ± 1.06 bc	11.6 ± 0.440 bc	25.6 ± 1.47 ab	39.7 ± 16.4 a
Proline	24.7 ± 0.050 bc	25.3 ± 5.04 bc	2.66 ± 0.810 c	55.0 ± 13.0 b	331.8 ± 13.9 a	345.4 ± 17.4 a
Asparagine	ND	ND	ND	ND	ND	ND
Aspartic acid	26.4 ± 3.31 a	5.20 ± 0.200 b	8.85 ± 0.120 b	7.86 ± 4.50 b	35.1 ± 8.24 a	26.0 ± 8.73 a
Phenylethylamine	1.82 ± 0.130 a	ND b	ND b	ND b	ND b	ND b
Glutamic acid	55.4 ± 19.2 c	23.7 ± 10.0 c	13.8 ± 0.480 c	9.90 ± 2.31 c	358.3 ± 50.2 a	185.4 ± 13.9 b
Methionine	42.1 ± 11.4 ab	9.25 ± 0.240 c	11.2 ± 0.260 c	16.2 ± 5.24 c	31.9 ± 3.88 b	48.1 ± 7.06 a
Phenylalanine	81.0 ± 30.3 bc	9.47 ± 3.27 c	6.40 ± 0.780 c	105.0 ± 32.5 b	290.4 ± 19.6 a	254.7 ± 53.6 a
Putrescine	ND c	ND c	116.5 ± 6.63 b	107.9 ± 12.7 b	160.3 ± 16.5 a	107.2 ± 11.5 b
Cadaverine	5.38 ± 0.210 c	ND c	63.2 ± 6.41 b	60.0 ± 5.48 b	89.7 ± 4.15 a	60.0 ± 8.06 b
Histamine	ND c	ND c	23.3 ± 1.97 b	24.0 ± 1.15 b	38.0 ± 4.78 a	26.3 ± 5.58 b
Tryptamine	ND c	ND c	5.69 ± 0.060 b	5.76 ± 0.750 b	18.1 ± 3.29 a	6.39 ± 0.630 b
Lysine	57.6 ± 11.1 a	ND d	39.6 ± 0.060 bc	27.1 ± 0.290 c	42.9 ± 3.88 ab	33.1 ± 8.83 bc
Tyramine	ND b	ND b	4.25 ± 0.04 b	7.42 ± 3.10 b	39.39 ± 19.1 a	10.89 ± 4.31 b
Histidine	375.0 ± 34.9 a	ND c	ND c	ND c	336.5 ± 6.93 ab	291.1 ± 52.2 b
Tryptophan	31.9 ± 2.72 b	ND d	ND d	11.5 ± 3.50 c	43.0 ± 2.92 a	33.2 ± 4.17 b
Tyrosine	74.1 ± 25.2 a	5.96 ± 0.340 b	28.5 ± 0.170 b	11.9 ± 5.00 b	87.4 ± 18.6 a	66.2 ± 4.56 a

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample; Bl – fermentation blank at 48 h; B0 – fermentation blank at 0 h. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

The highest concentrations of acetic acid were observed in the DS samples (Table 2.4.8). The Herkus_NF samples contained the highest concentrations of butanoic and pentanoic acids. The bioconversion significantly affected branched-chain SCFAs (2-methylpropanoic and 3-methylbutanoic acids), butanoic and pentanoic acids, while substrate was a significant factor for almost all SCFAs, except for propanoic and hexanoic acids. The interaction of both factors showed a significant effect on the concentrations of propanoic, butanoic, pentanoic, and hexanoic acids in the IVF samples.

The concentrations of alkanes, such as tridecane, octadecane, hexadecane, dodecane, tetradecane, aromatic and heterocyclic compounds, such as benzothiazole, 2-phenoxyethanol, naphthalene, skatole, 4-ethylphenol, 2,4-ditertbutylphenol, some organic acids and alcohols, such as nonanoic acid, 4-methylpentanoic acid, 3-hexadecanol, were significantly lower in almost all IVF DS and Herkus samples, in comparison to blank after 24 h IVF samples (Figure 2.4.1). Statistical analysis showed that the bioconversion factor was significant for the majority of VOCs (over 58% of analytes). Additionally, substrate factor significantly affected over 67% of the VOCs detected in the IVF samples. However, the interaction of these factors was significant only for six organic acids (propanoic, 2-methylpropanoic, butanoic, 3-methylbutanoic, pentanoic, and hexanoic acid), indole, hydrocinnamic acid, *p*-cresol, pentyl levulinate, tetradecanal, and octadecane.

Table 2.4.8. *Concentrations of short-chain fatty acids (mmol/L) in the in vitro fermentation samples*

Sample	B0	Bl	DS_F	DS_NF	Herkus_F	Herkus_NF
Acetic acid	6.16 ± 0.142 c	34.4 ± 10.5 b	86.5 ± 7.91 a	74.8 ± 8.17 a	47.4 ± 12.0 b	50.4 ± 0.989 b
Propanoic acid	2.06 ± 0.094 c	8.08 ± 0.167 c	39.3 ± 2.66 b	51.6 ± 3.23 a	51.3 ± 7.01 a	46.9 ± 1.62 ab
2-methylpropanoic acid	0.232 ± 0.032 d	2.61 ± 0.063 b	3.26 ± 0.135 a	2.78 ± 0.180 ab	2.62 ± 0.195 b	1.95 ± 0.295 c
Butanoic acid	1.31 ± 0.041 d	11.2 ± 0.266 c	23.7 ± 0.585 b	23.5 ± 2.13 b	23.8 ± 4.02 b	38.4 ± 3.55 a
3-methylbutanoic acid	0.253 ± 0.025 c	3.82 ± 0.078 ab	4.50 ± 0.102 a	4.15 ± 0.168 a	3.98 ± 0.24 ab	3.25 ± 0.661 b
Pentanoic acid	0.376 ± 0.030 e	6.46 ± 0.187 d	12.3 ± 0.726 c	16.4 ± 0.194 b	16.6 ± 1.74 b	26.0 ± 1.88 a
4-methylpentanoic acid	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hexanoic acid	<0.1 c	0.085 ± 0.02 c	0.752 ± 0.064 a	0.525 ± 0.155 ab	0.342 ± 0.116 bc	0.711 ± 0.166 a
Heptanoic acid	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Herkus – wheat variety Herkus DS; DS – wheat variety DS8558-1; F – sample fermented with *Lactiplantibacillus plantarum*; NF – control sample; Bl – fermentation blank at 48 h; B0 – fermentation blank at 0 h. a-e Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

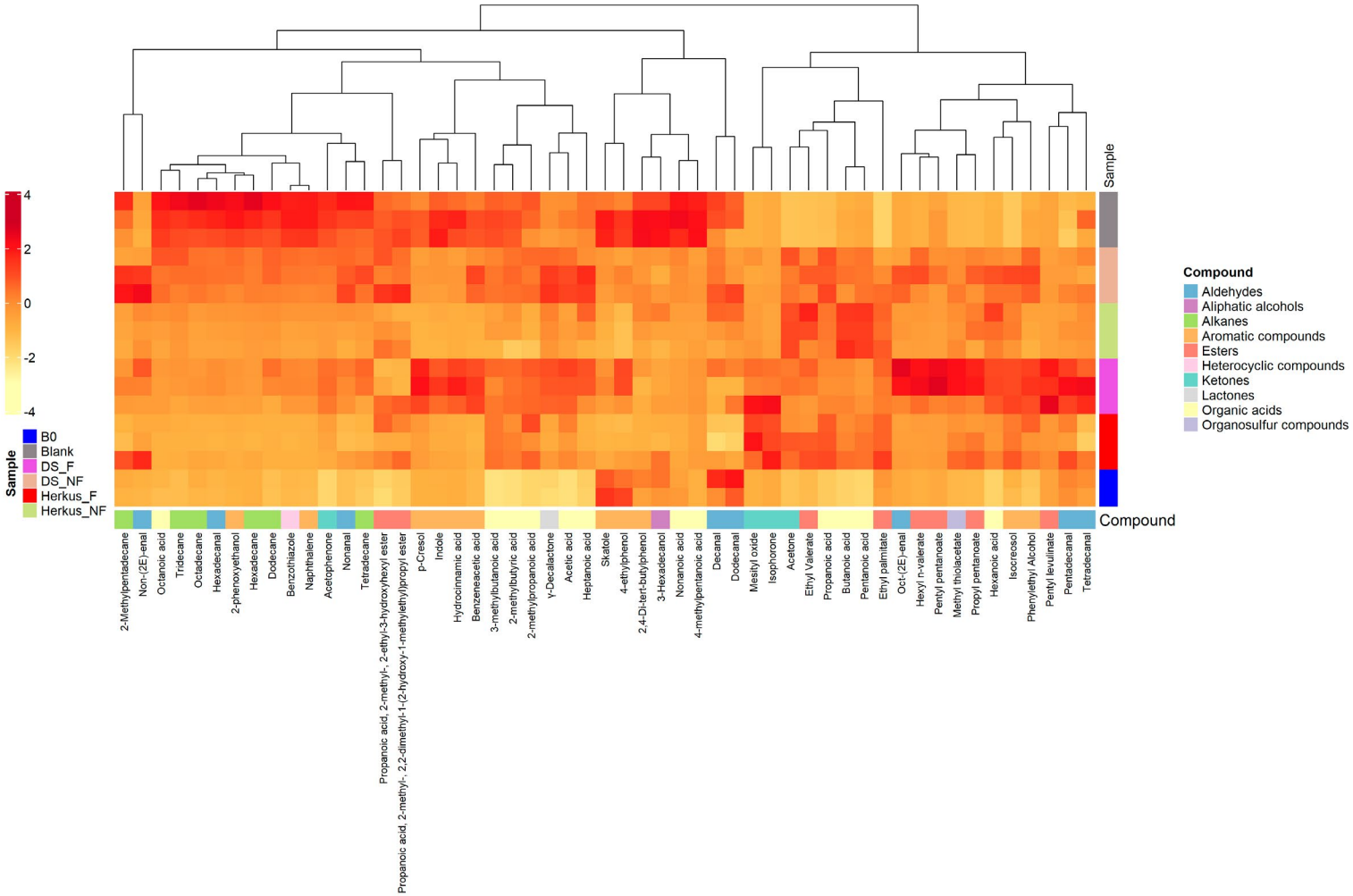

Fig. 2.4.1. *Heatmap of volatile organic compounds in the in vitro fermentation samples*

The PLS-DA plots showed clear separation between groups, in the case of bioconversion (Figure 2.4.2-a) or substrate (Figure 2.4.2-c) is used as a classifier. The VIP analysis showed that VOCs isophorone, mesityl oxide, acetone, pentyl levulinate, methyl thioacetate, aliphatic organic acids (pentanoic, butanoic, nonanoic, octanoic, 2-methylpropanoic, 3-methylbutanoic acids), alkanes (hexadecane, tridecane, octadecane, dodecane), aldehydes (pentadecanal, hexadecanal, dodecanal, decanal), aromatic compounds (p-cresol, isocreosol, 2-phenoxyethanol, 2,4-ditertbutylphenol) were contributing the most for the PLS-DA model, where classifier was bioconversion (Figure 2.4.2-b). However, aromatic VOCs (acetophenone, benzeneacetic acid, hydrocinnamic acid, naphthalene, 4-ethylphenol, phenylethyl alcohol, 2-phenoxyethanol, *p*-cresol), indoles (skatole, indole), aliphatic organic acids (acetic, 4-methylpentanoic, nonanoic, pentanoic, octanoic, 2-methylbutyric, heptanoic acids), alkanes and aliphatic aldehydes (nonanal, hexadecanal, octadecane, tetradecane, dodecane, oct-(2E)-enal), γ – decalactone, benzothiazole, hexyl n-valerate, and ethyl palmitate were the most significant contributors to the differences between different substrates (Figure 2.4.2-d).

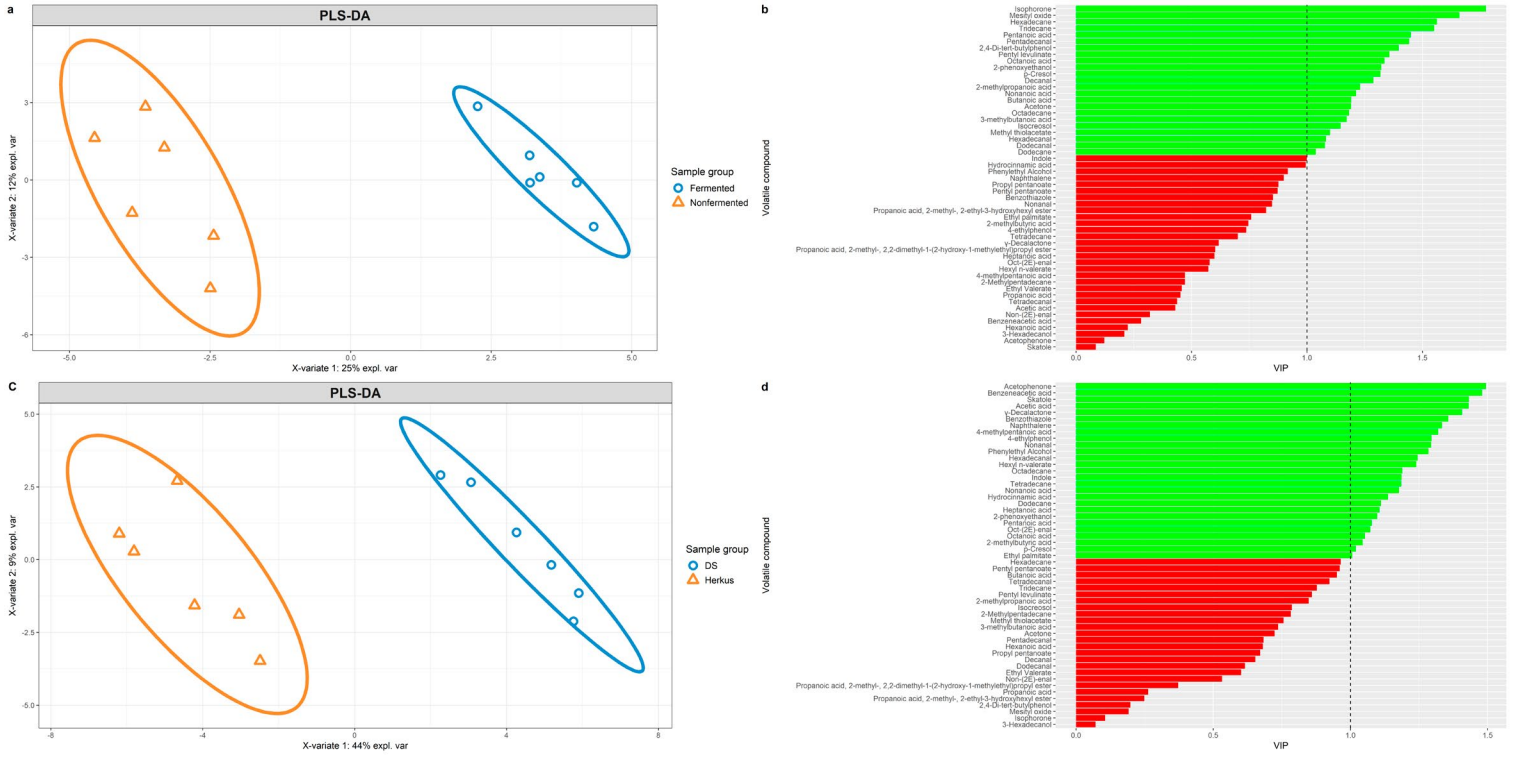

Fig. 2.4.2. *PLS-DA and VIP analysis of the volatile organic compounds in the in vitro fermentation samples*

a, c – PLS-DA analysis plots, where categorical values are bioconversion and substrate; b, d – VIP plots of the respective PLS-DA analyses.

Volcano plot analysis depicted that *p*-cresol, mesityl oxide, isophorone, and pentyl levulinate were significantly upregulated in the fermented IVF samples (Figure 2.4.3-a). However, benzenacetic acid, hexyl n-valerate, hydrocinnamic acid, indole, *p*-cresol, oct-(2E)-enal, and pentyl pentanoate were significantly upregulated in the DS samples, in comparison to the Herkus IVF samples.

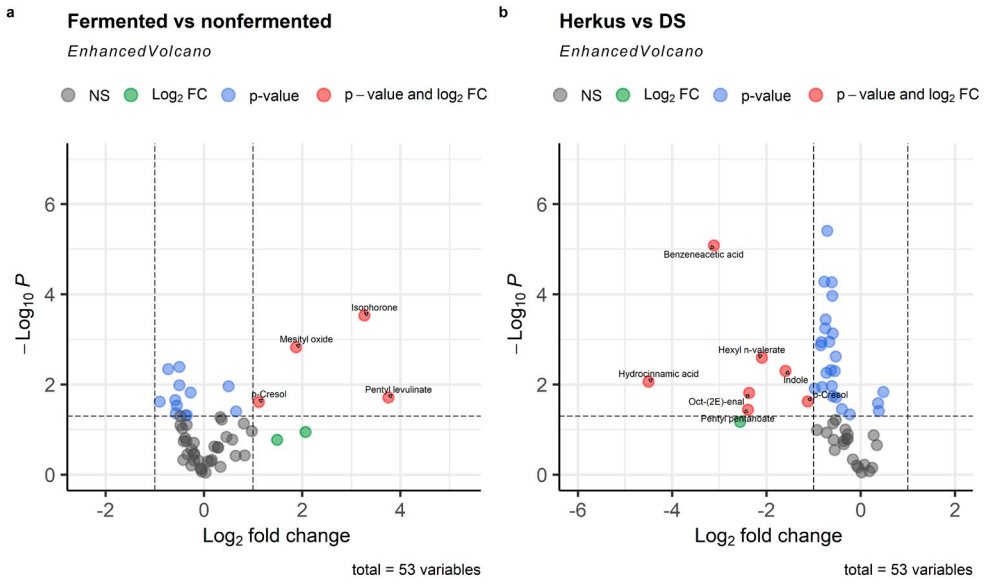

Fig. 2.4.3. *Volcano plots of the volatile organic compounds in the in vitro fermentation samples*

Propanoic, pentanoic, nonanoic, and 2-methylpropanoic acids were significantly different between bioconverted and control samples, and their VIP values were greater than 1, denoting their significant contribution to the PLS-DA models. Additionally, we found that acetic, 4-methylpentanoic, nonanoic, pentanoic, octanoic, 2-methylbutyric, and heptanoic acids were significantly different between substrates as well. The SCFAs are generally products of carbohydrate fermentation, which provide many benefits to the host, ranging a from food source to epithelium to the provision of a favorable environment to the beneficial bacteria [138, 172]. Branched-chain fatty acids (BCFA) are associated with the fermentation of AAs in the colon [138, 172]. In addition, we found significant negative correlations between some AAs (valine, leucine, isoleucine, serine, threonine, and histidine) and BCFAs (2-methylpropanoic, 2-methylbutyric, and 3-methylbutanoic acid) in the IVF samples, thus aligning with the literature. Additionally, some SCFAs (butanoic, propanoic, pentanoic, and hexanoic acids) negatively

correlated with total bacterial count (TBC) and BCFAs (2-methylpropanoic and 3-methylbutanoic acids) negatively correlated with LAB counts. Such correlations might indicate certain microbiota modulation mechanisms, as similar observations were made in the literature as well [173, 174].

The majority of detected analytes of the untargeted analysis of BTSFA derivatives were significantly higher in DS and Herkus sample groups, in comparison to the blank samples (Figure 2.4.4). The bioconversion factor showed to be significant for the concentrations of aliphatic acids (succinic, glyceric, 2-deoxytetronic, α-hydroxyglutaric acids), aromatic acids (dihydrocinnamic, 4-hydroxyphenylacetic, 3-hydroxyphenylpropionic acids), and heterocyclic acids (pyroglutamic, indole-3-acetic acids) in the IVF samples. Moreover, substrate was significant for concentrations of certain aliphatic acids (lactic, glycolic, 4-hydroxybutyric, succinic, glyceric, 2-deoxytetronic, α-hydroxyglutaric, 5-aminopentanoic acids), aromatic acids (β-phenyllactic, 3-hydroxyphenylpropionic, 4-hydroxyhydrocinnamic, dihydroferulic, benzeneacetic, dihydrocinnamic acids), and the same heterocyclic acids in the IVF samples. PLS-DA analysis of the BSTFA derivatives showed clear separation between different substrates (Herkus and DS), while bioconverted and control samples were partially overlapped (Figure 2.4.5-a,c). VIP analysis results showed that certain aromatic acids (4-hydroxyphenylacetic, 3-hydroxyphenylpropionic, dihydrocinnamic acids), aliphatic acids (2-deoxytetronic, succinic, α-hydroxyglutaric acids), ethanolamine, pyroglutamic acid, and propane-1,3-diol had the highest VIP values for the fermented – nonfermented PLS-DA model. Additionally, compounds that contributed most to the Herkus – DS PLS-DA model were aliphatic acids (5-aminopentanoic, 4- hydroxybutyric, glyceric, lactic, α-hydroxyglutaric, glycolic, and succinic acids), aromatic acids (benzeneacetic, β-phenyllactic, 4-hydroxyhydrocinnamic, dihydroferulic, dihydrocinnamic acids), and indole-3-acetic acid.

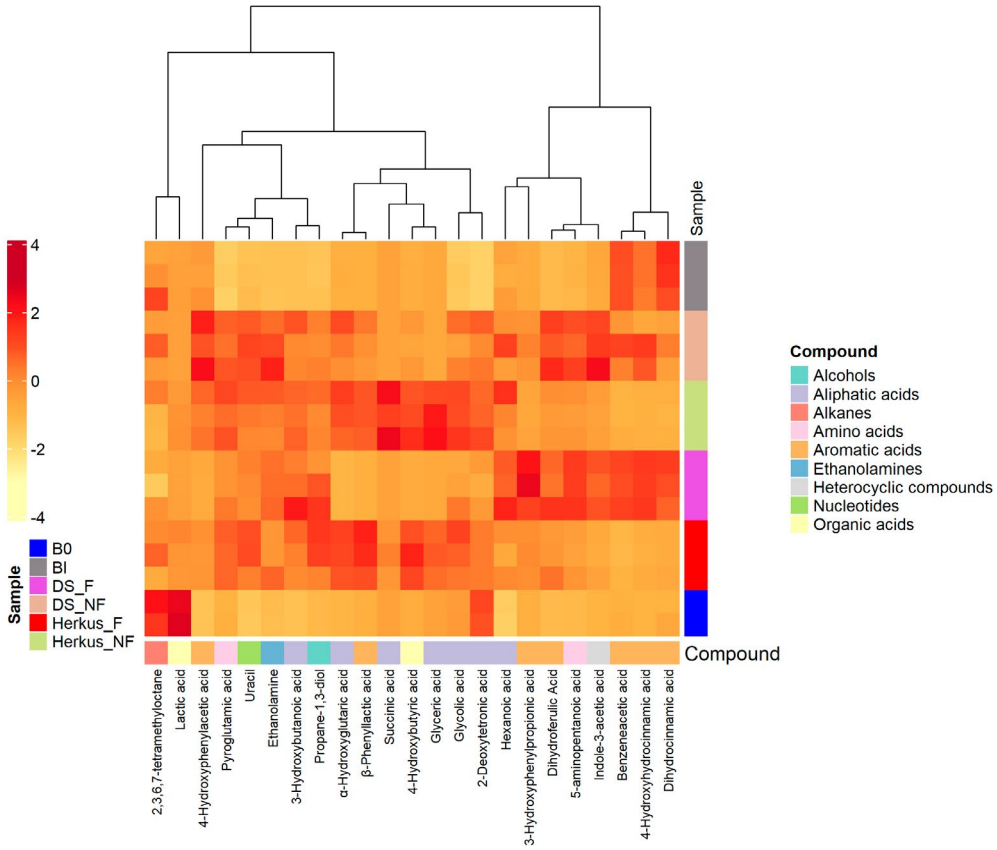

Fig. 2.4.4. *Heatmap of untargeted analysis results in the in vitro fermentation samples*

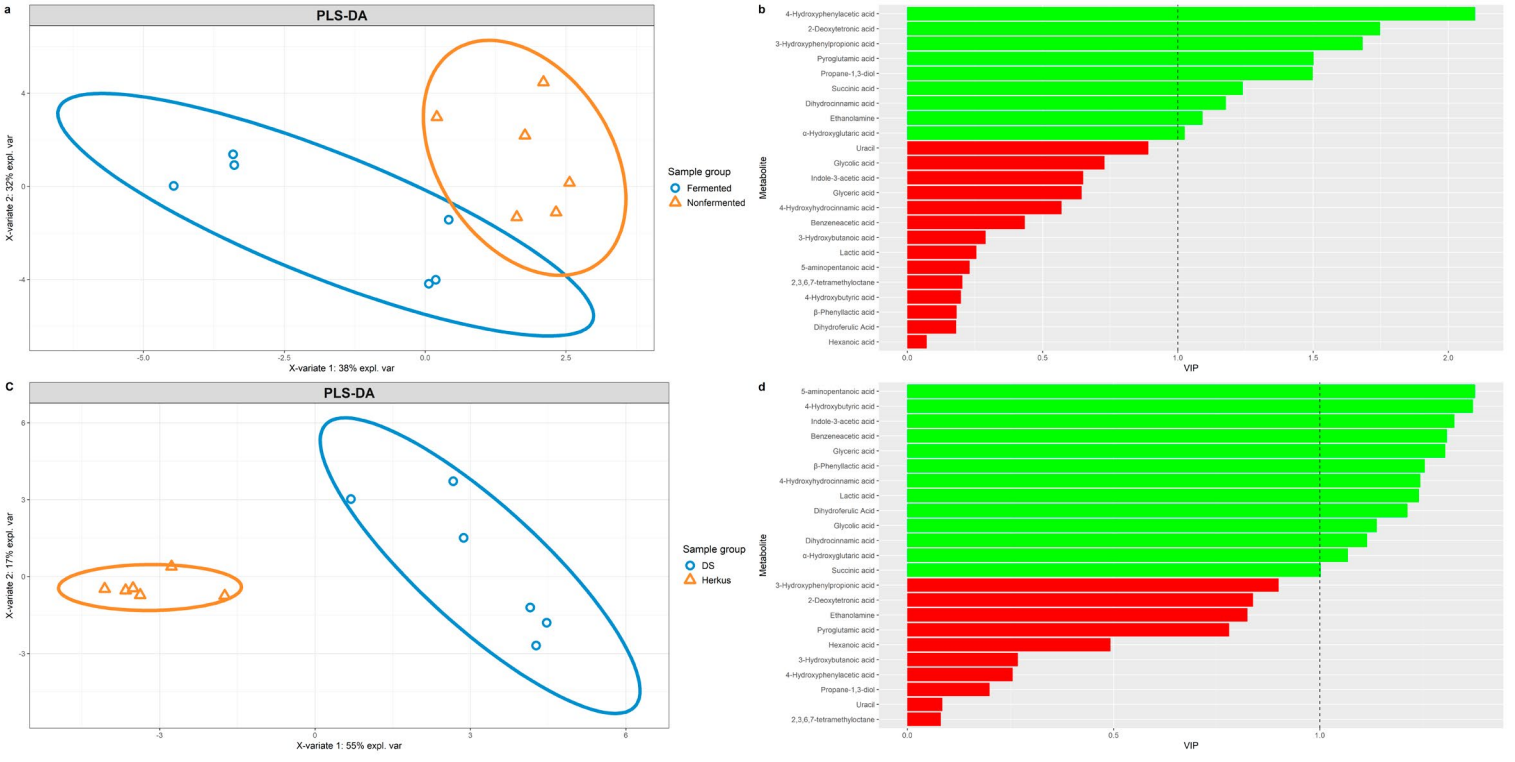

Fig. 2.4.5. *PLS-DA and VIP plots of the untargeted metabolites of the in vitro fermentation samples*

a, c – PLS-DA analysis plots, where categorical values are bioconversion and substrate; b, d – VIP plots of the respective PLS-DA analyses.

Volcano plot depicts significantly lower concentrations of 4-hydroxyphenylacetic acid and higher concentrations of 3-hydroxyphenylpropionic acid in fermented IVF samples (Figure 2.4.6-a). Moreover, aliphatic acids (glyceric, succinic, lactic, α-hydroxyglutaric acids) and β- phenyllactic acid were upregulated in the Herkus samples, while aromatic acids (benzenacetic, 4-hydroxycinnamic, dihydroferulic, dihydrocinnamic, 3-hydroxyphenylpropionic acids), 5-aminopentanoic, and indole-3-acetic acid were downregulated, in comparison to the DS samples (Figure 2.4.6-b).

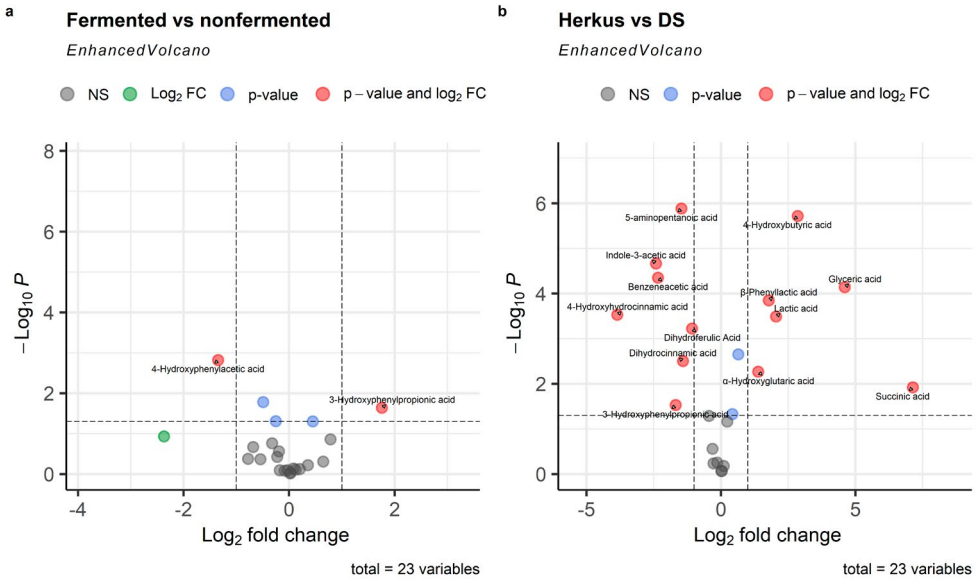

Fig. 2.4.6. *Volcano plots of the untargeted metabolites of the in vitro fermentation samples*

We found that some aliphatic aldehydes and alkanes were contributing to the differences between bioconversion and substrate factors in the PLS-DA models. This could suggest certain differences in lipid metabolism between sample groups, as those compounds are generally attributed to the degradation of lipids [175-177]. Another important phenolic amino acid degradation products by gut bacteria, such as *Escherichia coli, Clostridium sporogenes, Clostridium difficile*, include *p*-cresol and 4-ethylphenol [178, 179]. While 4-ethylphenol is a naturally occurring compound, also having a fairly unpleasant odor in high concentrations, its ability to cross the blood-brain barrier on high consumption could lead to serious health complications [180, 181]. *P*-cresol was downregulated, and 4-hydroxyphenylacetic acid, precursor to *p*-cresol, was upregulated in the non-bioconverted samples, thus possibly hinting certain at inhibition of its conversion to *p*-cresol in non-

bioconverted samples. Moreover, we found significant negative correlations between *p*-cresol and LAB count, hinting at the *p*-cresol ability to modulate gut microbiome, especially LAB strains, as similar observations were made in the literature [182]. Additionally, various phenolic metabolites, such as 3-hydroxyphenylpropionic, benzenacetic, 4-hydroxyhydrocinnamic, and β-phenyllactic acids are products of bacterial degradation of phenolic amino acids, such as phenylalanine and tyrosine [183-186]. While some of the aforementioned metabolites, in addition to hydrocinnamic and dihydroferulic acids, can also be obtained through bacterial degradation of ferulic and caffeic acids, which are abundant in wheat cereals [187-189]. We found benzenacetic, dihydrocinnamic, and 4-hydroxyhydrocinnamic acids upregulated in DS samples and significant negative correlations with phenolic amino acids, thus possibly hinting its origin to the metabolism of amino acids. The higher concentrations of β-phenyllactic acid were observed in Herkus samples, and significant negative correlations with TBC were observed, thus hinting at its bacterial community modulation properties as they were established earlier [190].

Acetone production in the IVF samples could be attributed to the acetone-butanol-ethanol fermentation (ABE) of solventogenic clostridia [191, 192]. Acetone was significant for the PLS-DA bioconverted – non-bionconverted model, thus the bioconversion of substrate could affect further metabolism of feed in the gut. Glyceric acid is a product of glycerol and carbohydrate fermentation, which can cause serious health issues in high doses [193]. We found its concentrations upregulated in the Herkus samples, in addition to all analyzed factors and their interactions being significant to its concentrations in the IVF samples. Thus, the bioconversion and substrate management can help to manage its production in the gut.

We found lactic acid upregulated production in the Herkus samples. It is a classical product of bacterial fermentation in the gut, though it can originate from carbohydrate or amino acid fermentation [194, 195]. However, significant positive correlations between lactic acid and branched-chain AAs suggest its origin from the carbohydrate fermentation, especially when wheat substrates have an abundance of it.

The upregulation of 5-aminopentanoic acid and downregulation of succinic and α-hydroxyglutaric acids in the DS samples could suggest possible inhibition of 5-aminopentanoic acid conversion to succinic acid [196]. Substrate fermentation produced significant amounts of GABA, and both factors were significant for its concentrations in the IVF samples.

The bioconversion of selected wheat substrates successfully reached appropriate LAB count values at low pH, providing evidence for the LAB strain's ability to ferment selected substrates and provide functional properties

to the feed material. The interaction of both analyzed factors significantly affected the total AA composition, thus pointing out the importance of the selection of substrate and the optimization of fermentation conditions to improve the nutritive quality of the feed material. In addition, the bioconversion factor was significant for the majority of the essential AA *in vitro* digestibility, including lysine, so its bioavailability can be significantly improved by utilizing the bioconversion methods. Moreover, the bioconversion significantly improved the total *in vitro* digestibility, while reducing the *in vitro* fermentability. Both factors and their interaction significantly affected the majority of the analyzed SCFAs. Also, we found significant differences in concentrations of aliphatic aldehydes and alkanes, suggesting altered lipid metabolism. Additionally, we observed significant upregulation of phenolic metabolites in DS samples, which could be linked to protein degradation. These findings indicate substrate selection and bioconversion significance on overall nutrient bioavailability and microbiota activity.

CONCLUSIONS

1. Bioconversion of cereal grains from wheat varieties *Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7, and DS8535-2 using selected LAB strains (LUHS29, LUHS245, LUHS122) is a suitable technology for modifying wheat grain parameters in a desirable way, except for micro- and macroelement concentrations. Wheat variety was a significant factor influencing these concentrations in the samples, whereas the LAB strain was not significant in most cases:
   1.1. Effective fermentation was confirmed by achieving high viable counts of LAB (>8.00 $\log_{10}$ CFU/g and by a decrease in pH from 4.67 to 3.55 in some samples.
   1.2. The main BAs detected in the wheat samples were phenylethylamine, putrescine, spermidine, and cadaverine. The concentrations of putrescine, spermidine, cadaverine, and the total BAs were significantly higher in the bioconverted wheat wholemeal samples and the LAB strain, wheat variety, and their interaction was significant ($p < 0.05$) for all of the BAs concentrations in the wheat wholemeal samples.
   1.3. The SFAs, MUFAs, and PUFAs in the wheat wholemeal samples were 20.8-39.8%, 14.1–28.8%, and 36.9–64.5%, respectively, and, the LAB strain and its interaction with wheat variety were significant ($p < 0.05$) for SFA, MUFA, and omega-6 FAs in the wheat wholemeal samples.
   1.4. The significant increase in essential FAAs were observed in bioconverted wheat wholemeal samples, and the LAB strain and wheat variety were significant ($p < 0.05$) for concentrations of the most of the essential FAAs. Both of the factors were significant ($p < 0.05$) for the most of the non-essential FAAs.
   1.5. Fermentation led to higher GABA content in bioconverted wheat wholemeal samples and both of the analyzed factors were significant ($p < 0.05$) for its concentration in the wheat wholemeal samples.
   1.6. The increasement of DON was observed in bioconverted wheat wholemeal samples.
   1.7. Fermentation led to more diverse profile of the VOCs, and the majority of the VOCs were aliphatic organic acids, esters, alcohols, and aldehydes.

2. The combination of red and green lentil bioconversion using selected LAB strains (LUHS122 and LUHS210) with substrate particle size reduction is a suitable technology for increasing fermentation efficiency:
   2.1. The pH of fermented lentil samples dropped from 4.43 to 4.21, and the LAB count values reached about 8.0 $\log_{10}$CFU/g, on average, thus confirming the ability of the selected LAB strains to ferment proteinaceous substrates. The lentil variety and milling factor interaction were significant ($p < 0.05$) for the LAB count.
   2.2. The LAB strain, particle size, and lentil variety and LAB strain interaction significantly ($p < 0.05$) affected the chromaticity characteristics of the lentil samples.
   2.3. The most of the essential FAAs and GABA were significantly ($p < 0.05$) higher in the fermented samples and the lentil variety, particle size, and LAB strain factors were significant ($p < 0.05$) for almost all the concentrations of FAAs in the lentil samples.
   2.4. The four BAs, phenylalanine, putrescine, spermidine, and spermine, were detected in the lentil samples. The non-milled fermented samples contained significantly lower concentrations of the BAs, in comparison to the control samples and all of the analyzed factors were significant ($p < 0.05$) for all the detected BAs in the lentil samples, except for putrescine. Negative Pearson's correlations between the BAs (spermidine, spermine, putrescine) and the LAB count ($r = -0.810$, $p < 0.001$; $r = -0.660$, $p < 0.001$; $r = -0.829$, $p < 0.001$, respectively) were observed.
   2.5. All factors and their interactions were significant ($p < 0.05$) for palmitic, stearic, and α-linolenic acids in the lentil samples. The LAB strain, lentil variety, and their interactions with particle size factor were significant ($p < 0.05$) for the omega-3 FA concentrations in the lentils.
   2.6. The profile of the lentils VOC mainly consisted of aliphatic aldehydes, organic acids, and monoterpenes, the significant correlations between most of the VOCs and LAB count, and aldehydes with the FAs.

3. The use of SSF and SMF for seeds of the *Fabaceae* family lentils (variety Danaja – pure and intercropped) processing allows modulation of FAA, FA, and VOC profiles, as well as other physicochemical parameters in most cases:
   3.1. The pH of lentil samples reached down to 4.3, and the LAB count values were over 7.3 $\log_{10}$ CFU/g, on average. The type of fermentation, fermentation duration, and growing conditions

factors were significant ($p < 0.05$) for the pH values, while LAB count values were significantly ($p < 0.05$) affected by the type of fermentation (SSF or SMF) and fermentation duration.

3.2. The essential FAAs were significantly ($p < 0.05$) higher in the SMF and SSF samples in most cases, in comparison with non-fermented ones. Also, SSF samples contained significantly higher concentrations of FAAs and GABA in most of the cases, in comparison with SMF samples. All of the analyzed factors were significant ($p < 0.05$) for the concentrations of FAAs and GABA in the lentil samples. The type of fermentation and fermentation duration interaction, as well as interaction of all of the analyzed factors were significant ($p < 0.05$) for the majority of the analyzed FAAs in the lentil samples.

3.3. Phenylethylamine, putrescine, spermidine, and spermine of all analyzed BAs were detected in lentil samples. The fermented samples contained significantly ($p < 0.05$) higher amounts of the BA in most of the cases. The factor interaction of the growing conditions and the type of fermentation significantly ($p < 0.05$) affected the concentrations of all detected BAs in the lentil samples.

3.4. The major FAs in the lentil samples were linoleic, palmitic, and stearic acids, ranging between 41.3–50.3, 16.2–20.3, and 1.63–3.29%, respectively. The interactions of analyzed factors significantly ($p < 0.05$) affected all of the analyzed FAs in lentil samples. All of the factors and their interactions were significant ($p < 0.05$) for the concentrations of α-linolenic acid in the lentil samples.

3.5. The growing conditions showed significant ($p < 0.05$) differences in micro- and macroelement concentrations in the lentil samples.

3.6. Most of the detected VOCs in the lentil samples were aliphatic acids, ketones, aldehydes, and alcohols, and the SSF samples showed higher diversity of detected VOCs, in comparison to the SMF samples. All of the analyzed factors and their interactions significantly ($p < 0.05$) affected VOC concentrations in the lentils, in most of the cases.

4. The application of an *in vitro* digestion model for the evaluation of wheat sample bioconversion is a suitable approach for predicting potential *in vivo* changes in both non-bioconverted and fermented substrates, as significant differences were observed in digestion- and fermentation-related parameters, as well as in other analyzed metabolites:

4.1. The bioconversion did not significantly alter the concentrations of AAs in the wheat wholemeal samples, however, the wheat variety and bioconversion factors' interaction significantly affected ($p < 0.05$) most of the AAs in the substrates.
4.2. The concentrations of FAAs in the IVD digesta samples, such as GABA, proline, and glutamine were significantly affected by the bioconversion and substrate factors. Only bioconversion factor was significant ($p < 0.05$) for the concentrations of glutamic acid and glycine. The most of the total AA concentrations in the IVD undigested residue samples were significantly ($p < 0.05$) affected by bioconversion, while the substrate was significant only for threonine, proline, histidine, glutamic and aspartic acids, and total AAs. The digestibility of the essential AAs, such as lysine, phenylalanine, and valine, were significantly ($p < 0.05$) increased.
4.3. The concentrations of the most of the FAAs and BAs in the IVF samples were significantly ($p < 0.05$) affected by the bioconversion and substrate factors. The GABA concentration in the IVF samples were significantly ($p < 0.05$) affected by the both factors. The significant ($p < 0.05$) negative correlations between some of the BAs (putrescine, cadaverine, histamine) and the TBC were found.
4.4. The concentrations of branched-chain SCFAs, butanoic, and pentanoic acids in the IVF samples were significantly ($p < 0.05$) affected by the bioconversion factor. The substrate factor was significant ($p < 0.05$) for all almost all SCFAs, except for propanoic and hexanoic acids.
4.5. The bioconversion significantly ($p < 0.05$) increased the *in vitro* digestibility of the wheat wholemeal samples, while the same factor significantly ($p < 0.05$) reduced their *in vitro* fermentability.
4.6. The PLS-DA and VIP analysis results of the IVF samples pointed out differences in aliphatic alkanes and aldehydes, suggesting differences in lipid metabolism between the different sample groups. The significant ($p < 0.05$) differences of the phenolic metabolites, such as benzenacetic, dihydrocinnamic, and 4-hydroxy-hydrocinnamic acids, suggesting differences in phenolic AA metabolism.

RECOMMENDATIONS

1. Bioconversion of cereal grains from wheat varieties *Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7, and DS8535-2 using selected LAB strains (LUHS29, LUHS245, LUHS122) is recommended as an effective approach for modifying grain characteristics in a desirable manner, particularly for increasing essential FAAs and GABA concentrations.
2. To improve the characteristics of red and green lentils, especially by enhancing essential FAAs and GABA concentrations, a combination of bioconversion using selected LAB strains (LUHS122 and LUHS210) and substrate particle size reduction is recommended. However, the formation of BAs during the process should be controlled.
3. The SSF process is recommended for the bioconversion of wheat cereal grains and lentil seeds because it promotes a higher production of functional molecules than the SMF while providing a more sustainable processing approach due to its lower water requirements.
4. The application of an *in vitro* digestion model for evaluating wheat cereal bioconversion is recommended as a primary step for predicting potential *in vivo* changes in both non-bioconverted and fermented substrates.

SUMMARY IN LITHUANIAN

SANTRUMPOS

AA – Aminorūgštis
ABE – Acetono-butanolio-etanolio fermentacija
ANF – Antimitybiniai veiksniai
BA – Biogeniniai aminai
BCFA – Šakotos grandinės riebalų rūgštys
BSTFA – *N,O*-Bis(trimetilsilil)trifluoroacetamidas
DON – Deoksinivalenolis
DS – Kviečių veislė Nr. DS8558-1
FA – Riebalų rūgštis
FAA – Laisvoji aminorūgštis
GABA – γ – Aminobutyrinė rūgštis
GAD – Glutamo rūgšties dekarboksilazė
GC-MS – Dujų chromatografija – masių spektrometrija
GRAS – Visuotinai pripažintas saugiu
IVD – *In vitro* virškinimas
IVF – *In vitro* fermentacija
LAB – Pieno rūgšties bakterija
LUHS122 – *Lactiplantibacillus plantarum*
LUHS210 – *Lacticaseibacillus casei*
LUHS245 – *Liquorilactobacillus uvarum*
LUHS29 – *Pediococcus acidilactici*
MUFA – Mononesočiosios riebalų rūgštys
NOAEL – Nepastebėto neigiamo poveikio lygis
PLS-DA – Dalinė mažiausių kvadratų diskriminantinė analizė
PUFA – Polinesočiosios riebalų rūgštys
QPS – Kvalifikuota saugos prezumpcija
SCFA – Trumpiosios grandinės riebalų rūgštis
SFA – Sočiosios riebalų rūgštys
SMF – Tradicinė fermentacija skystoje fazėje
SPME – Kietafazė mikroekstrakcija
SSF – Kietafazė fermentacija
TBC – Bendras bakterijų skaičius
TTA – Bendras titruojamasis rūgštingumas
VIP – Kintamųjų svarba projekcijoje
VOC – Lakusis organinis junginys

ĮVADAS

Didėjant žmonių populiacijai, efektyvus visuomenės aprūpinimas gyvūninės kilmės maistu tampa vis didesniu iššūkiu [1, 2]. Ši problema sprendžiama, taikant selektyvų veisimą, bei integruojant išmaniąsias agrotechnologijas [3-5]. Siekiant užtikrinti gyvulininkystės plėtrą, pašarų efektyvus panaudojimas tampa vienu pagrindinių prioritetų [6, 7]. Gyvūnų mitybos raciono praturtinimui naudojami vitaminai, prebiotikai, probiotikai, fitobiotikai, įvairios riebalų rūgštys, fermentai ir kt. [6-9]. Nors vyksta diskusijos dėl gyvūninės kilmės produktų vartojimo mažinimo žmonių mityboje, tačiau gyvūninės kilmės maisto produktai išlieka svarbūs, dėl savo unikalios sudėties ir įsisavinamumo [10-16]. Su gyvūniniais produktais gaunamų veikliųjų komponentų trūkumas žmonių mityboje gali sukelti įvairias sveikatos problemas [12, 17-20]. Nors šių medžiagų poreikį mityboje galima patenkinti vartojant papildus, tačiau pastarieji gali kelti rizikų, susijusių su netyčiniu perdozavimu, nepageidaujama vaistų ir papildų veikliųjų komponentų sąveika, nenumatytais šalutiniais poveikiais ir kt. [21, 22]. Dėl šių priežasčių, siekiant užtikrinti pilnavertę žmonių mitybą, svarbu kurti tvarias gyvūnų auginimo technologijas, apimančias efektyvų pašarinių žaliavų panaudojimą gyvūnų sveikatingumui bei produktyvumui užtikrinti.

Kviečiai yra pagrindinė grūdinė kultūra žmonių bei gyvūnų mityboje [23]. Be krakmolo ir kitų polisacharidų, kviečiuose yra baltymų, B grupės vitaminų, skaidulinių medžiagų, funkcionaliosiomis savybėmis pasižyminčių molekulių (pvz., fitoestrogenų, flavonoidų ir kt.), mineralinių medžiagų ir kt. [24]. Kita svarbi augalų grupė, tai *Fabaceae* šeimos augalai. Juose yra didelis baltymų, mažas riebalų ir drėgmės kiekis [25, 26], vidutiniškai, 14 proc. skaidulinių medžiagų, reikšmingi kiekiai mikro- ir makroelementų, karotenoidų, B grupės vitaminų, flavonoidų ir kitų fenolinių junginių [27, 28]. Tačiau *Fabaceae* šeimos augalų maistinių medžiagų biologinį prieinamumą mažina juose esantys antimitybiniai veiksniai: fitatai, saponinai, lektinai, taninai, proteazės inhibitoriai ir kt. [27, 29]. Kviečių grūduose taip pat yra antimitybinių veiksnių, pvz., tripsino inhibitorių [30]. Antimitybinių veiksnių koncentracijos mažinimui taikomi įvairūs technologiniai sprendimai: malimas, mirkymas, virimas, autoklavavimas, daiginimas, ekstruzija, fermentacija ir kt. [31].

Be pagrindinių energinę vertę suteikiančių komponentų, kviečiuose ir ankštiniuose augaluose yra įvairių biologiškai aktyvių molekulių: karotenoidų, antocianinų, flavonoidų ir kt. [32-34]. Dalis šių junginių pasižymi antioksidacinėmis, priešuždegiminėmis, antibakterinėmis, priešgrybelinėmis, antiprotozoinėmis, antiparazitinėmis ir kitomis savybėmis [34-36]. Spalvotieji kviečiai sulaukia vis daugiau dėmesio dėl aleurono ir perikarpio sluoksniuose esančių antocianinų [37-40]. Pastarieji junginiai pasižymi pageidautinu fizi-

ologiniu poveikiu, pvz., mažina metano gamybą atrajojantiems gyvuliams, didina prieaugį, gerina produkcijos juslines savybes [37-40]. Dauguma jų yra gliukozės, galaktozės ar rutinozės bei aglikonų (cianidino, malvidino, peonidino, delfinidino ir petunidino) dariniai, o jų koncentracijos kviečiuose siekia nuo 3 iki 100 ar daugiau mg/kg [39, 41, 42]. Spalvotose ankštinių augalų sėklose yra antocianinų (dažniausiai glikozidiniai aglikonų dariniai) [32]. Atitinkamai, gliukozė ir galaktozė yra pagrindiniai sacharidų fragmentai antocianinų molekulėse, jų koncentracijos ankštinių augalų sėklose gali viršyti 200 mg/kg [32, 43]. Nors *in vitro* virškinimo metu antocianinų koncentracija reikšmingai sumažėja, pranešama apie *in vivo* šių junginių kumuliaciją akių, kepenų ir smegenų audiniuose [44, 45].

Efektyvesniam pašarų maistinių medžiagų įsisavinamumo gerinimui taikomi įvairūs technologiniai sprendimai, iš kurių fermentacija apibūdinama, kaip yra efektyvus procesas antimitybinių veiksnių (ANF) mažinimui, žalių baltymų kiekio didinimui bei baltymų ir angliavandenių virškinamumo gerinimui [46, 47]. Taikant fermentaciją galima sumažinti antigeninių baltymų, fitatų, taninų ir fermentų inhibitorių kiekius bei padidinti vitaminų, fosforo, trumposios grandinės riebalų rūgščių koncentracijas [48-50]. Fermentuotas pašaras gali pasižymėti prebiotinėmis, probiotinėmis, postbiotinėmis bei parabiotinėmis savybėmis, o bakterijų susintetintos trumposios grandinės riebalų rūgštys ir bakteriocinai gali inhibuoti patogeninius mikroorganizmus [51-54].

Nepaisant pageidaujamos pašarų cheminių junginių konversijos bei sintezės fermentacijos metu, fermentuojamo substrato užterštumas patogeniniais mikroorganizmais ir jų simbiotinė veikla su technologiniais mikroorganizmais gali lemti ir toksinių junginių susidarymą [62, 63]. Fermentuotame substrate labai svarbi ir biogeninių aminų (BA) kontrolė [63, 64]. Pastarųjų junginių sintezę gali lemti tiek technologiniai mikroorganizmai (pvz., LAB), tiek patogeniniai oportunistiniai.

Pašarų gamybai gali būti taikoma kietafazė fermentacija (SSF) bei tradicinė (SMF) skystoje fazėje [48]. Vykdant SSF, mikroorganizmai auga ant substrato esant labai mažam laisvo vandens kiekiui, o SMF substratas, dažnu atveju, yra skystas [48, 62, 65]. SSF procesui reikalingas mažesnis vandens bei energijos kiekis, gaunamos didesnės metabolitų koncentracijos, naudojama nesudėtinga įranga, nebūtinas substrato sterilizavimas, lyginant su SMF [48, 62, 65]. Dėl šių priežasčių SSF procesas yra patrauklus pramoninei gamybai. Tačiau SMF procesą lengviau kontroliuoti, užtikrinti pastovų našumą ir tikslinio produkto išskyrimą iš substrato, todėl šis procesas dažnai naudojamas fermentų ir antrinių metabolitų gamybai [66, 67]. Technologiniai skirtumai tarp SMF ir SSF procesų apima ir mikroorganizmų kultūrų parinkimą [62]. Pieno rūgšties bakterijos (LAB) plačiai naudojamos įvairių substratų

fermentacijai [68]. LAB išskiria fermentus, kurie pagerina substrato angliavandenių ir baltymų virškinamumą [68, 69] bei sintetina įvairius metabolitus, kurie gerina fermentuojamo substrato biosaugos rodiklius ir juslines savybes [68, 69]. LAB padermės „visuotinai pripažintos saugiomis“ (GRAS) ir turi „kvalifikuotos saugos prezumpcijos“ (QPS) statusą [69].

Fermentacijos technologija pasižymi kompleksiškumu. Vienas iš veiksnių, lemiančių fermentuotų žaliavų kokybę yra substrato sudėtis. Šiame darbe sprendžiama mokslinė problema siejama su Lietuvoje sukurtų naujų veislių kviečių grūdų bei lęšių biokonversijos schemų modeliavimu, parenkant technologinius rodiklius: bakterijų kultūras bei vandens kiekį substrate, siekiant kuo tvaresnio biokonversijos būdo. Literatūroje nėra duomenų apie šiame darbe analizuojamų spalvotagrūdžių kviečių ir *Fabaceae šeimos* augalų detalią cheminę sudėtį bei jos pokyčius fermentacijos metu. Siekiant efektyvaus naujai sukurtų žaliavų pritaikomumo pašarų pramonėje, iškyla būtinybė ieškoti technologinių sprendimų bei įvertinti veiksnius, lemiančius fermentuoto substrato kokybę, įskaitant *in vitro* virškinimo rodiklius.

Lietuvos selekcininkų sukurtos spalvotųjų kviečių linijos pasižymi itin dideliu biologiškai aktyvių junginių kiekiu, ypač antocianinų, kurie gali būti perspektyvūs didesnės pridėtinės vertės pašarinių žaliavų kūrimui. Ankštinių augalų sėklos pasižymi dideliu baltymų, skaidulinių medžiagų ir kitų funkcionaliųjų komponentų kiekiu. Tačiau jų bioprieinamumą riboja antimitybiniai veiksniai. Šiai problemai išspręsti kuriami inovatyvūs technologiniai sprendimai. Gauti išsamūs duomenys apie šių žaliavų detalią cheminę sudėtį papildys mokslinių žinių bazę bei sudarys prielaidas jų efektyvesniam panaudojimui, kuriant didesnės pridėtinės vertės pašarines žaliavas.

Disertacijos tikslas

Disertacijos tikslas – išanalizuoti naujai sukurtų lietuviškų spalvotųjų kviečių veislių ir *Fabaceae* šeimos augalų sudėtį, sukurti technologijas, didinančias jų pridėtinę vertę tvaresnių pašarinių žaliavų gamybai bei pritaikyti *in vitro* virškinimo modelį, siekiant prognozuoti šių pašarinių žaliavų *in vivo* efektyvumą.

Darbo uždaviniai

1. Ištirti pasirinktų LAB padermių (LUHS29, LUHS245, LUHS122) poveikį skirtingų kviečių veislių (*Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7, DS8535-2) grūdų biokonversijai, ypatingą dėmesį skiriant laisvųjų aminorūgščių (FAA), riebalų rūgščių (FA), biogeninių aminų (BA) ir kt. metabolitų analizei, vertinant kviečių veislės ir

LAB padermės įtakos reikšmingumą substrato fizikiniams cheminiams pokyčiams.
2. Išanalizuoti LAB padermių (LUHS122 ir LUHS210) ir substrato dalelių dydžio poveikį raudonųjų ir žaliųjų lęšių sėklų (CDC Lemay ir CDC Red Rider) fermentacijos efektyvumui.
3. Įvertinti veiksnių: skirtingų *Fabaceae* šeimos lęšių veislių (Danaja), tradicinio ir tarpinio pasėlio auginimo sistemų, SSF ir SMF sąlygų įtaką, tiriant lęšių sėklų substrato FAA, FA, lakiųjų organinių junginių (VOC) profilius, bei kt. fizikinius cheminius rodiklius.
4. Pritaikyti *in vitro* virškinimo modelį spalvotųjų kviečių grūdų biokonversijos vertinimui ir galimiems *in vivo* pokyčiams prognozuoti, lyginant virškinimo ir fermentacijos rodiklius bei metabolitų profilius, susidarančius iš neapdorotų ir fermentuotų mėginių.

Mokslinis naujumas ir praktinis naudingumas

Disertaciniame darbe pateikiamas išsamus naujai sukurtų kviečių grūdų ir ankštinių augalų sėklų biokonversijos atrinktomis LAB padermėmis įvertinimas, kaip novatoriškas sprendimas, siekiant pagerinti pašarinių žaliavų funkcionalumą ir maistinę vertę. Disertacijos mokslinis naujumas glūdi (I) naujai sukurtų kviečių grūdų veislių ir jų biokonversijos atrinktomis LAB padermėmis įtakos substratų biocheminėms modifikacijoms vertinime, (II) lęšių sėklų substrato daugiafaktoriniame apdorojime, taikant SSF ir SMF bei substrato dalelių dydžio modifikacijas, (III) neapdorotiems ir biokonvertuotiems spalvotiesiems kviečių grūdams *in vitro* virškinimo modelio taikyme, siekiant prognozuoti pašarinių žaliavų *in vivo* efektyvumą. Disertacijoje analizuojamas taikytų kompleksinių technologinių sprendimų poveikis plačiam spektrui jautrių substrato rodiklių: FAA, FA, VOC ir kt. Sukurtos žinios suteikia daugiamatį supratimą apie augalinių žaliavų pokyčius biokonversijos metu ir lemia praktinių rekomendacijų, skirtų efektyvinti kviečių grūdų ir lęšių sėklų panaudojimą gyvulininkystėje. Disertaciniame darbe skelbiamais rezultatais įrodytas LAB pagrindu vykdomų biokonversijos schemų efektyvumas patvirtina jų galimą pritaikomumą augalinių žaliavų baltymų kokybės gerinimui (didinant FAA ir GABA koncentracijas). Disertacijoje akcentuojama žaliavų sudėties ir apdorojimo sąlygų parinkimo svarba, siekiant optimalių rodiklių. Atkreiptinas dėmesys, kad validuotų *in vitro* virškinimo modelių taikymas suteikia ekonomiškai ir praktiškai efektyvias galimybes prognozuoti poveikį *in vivo*. Disertacijoje skelbiami rezultatai reikšmingai prisideda prie tvarių, žiedine ekonomika pagrįstų, perdirbimo strategijų kūrimo, siejamų su augalinės kilmės išteklių efektyviu naudojimu bei gali prisidėti prie priklausomy-

bės nuo importuojamų baltymų šaltinių mažinimo ir žemės ūkio bei pašarų pramonės konkurencingumo bei atsparumo išoriniams veiksniams didinimo.

1. MEDŽIAGOS IR METODAI

1.1. Eksperimente naudotos medžiagos

Eksperimentuose naudotos kviečių grūdų veislės: tradicinės: *Ada*, *Gaja*, *Herkus*; vaškiniai kviečiai: DS8888-3-6, *Sarta*; spalvotieji: DS8472-5, DS8526-2, DS8548-7, DS8535-2, DS8558-1) ir *Fabaceae* šeimos augalų (lęšių) sėklos: veislės – CDC Lemay, CDC Red Rider, Danaja (tradicinio ir tarpinio sėjimo).

Substratų biokonversijai naudotos LAB padermės: *Pediococcus acidilactici* (LUHS29), *Liquorilactobacillus* uvarum (LUHS245), *Lacticaseibacillus casei* (LUHS210) ir *Lactiplantibacillus pl*antarum (LUHS122) [70].

1.2. Augalinių žaliavų paruošimas eksperimetui

Grūdai ir sėklos susmulkinti laboratoriniu malūnu iki 1–2 mm skersmens dalelių dydžio. Biokonversijai: SMF – substrato ir vandens santykis 1:5 (m:m), SSF – 1:1 (m:m) ir 10:6 (m:m). Fermentacijai inicijuoti, į substratus pridėta 3 proc. (nuo sausosios masės) 8,6–8,7 $\log_{10}$ KSV/ml) atitinkamos LAB padermės grynos kultūros. Substratų biokonversija vykdyta 24 ir/arba 48 valandas.

1.3. Principinės eksperimentų schemos

Principinė kviečių grūdų biokonversijos schema pavaizduota 1.3.1 pav. Šiame etape buvo tirta SSF LUHS29, LUHS245 ir LUHS122 LAB padermėmis įtaka skirtingų rūšių kviečių grūdų (*Ada*, *Gaja*, DS8888-3-6, *Sarta*, DS8472-5, DS8526-2, DS8548-7, DS8535-2) mikrobiologiniams ir fizikiniams cheminiams rodikliams: LAB KSV/g skaičiui, pH, bendram titruojamajam rūgštingumui (TTA), spalvų koordinatėms, BA, FAA, mikro- ir makroelementų, FA, VOC ir mikotoksinų koncentracijai.

Lęšių veislių ‘CDC Lemay’ ir ‘CDC Red Rider’ sėklų tyrimų principinė eksperimento schema pateikta 1.3.2 pav. Šiame etape analizuota substrato dalelių dydžio ir SSF LUHS122 ir LUHS210 LAB padermėmis įtaka mikrobiologiniams ir fizikiniams cheminiams sėklų rodikliams: LAB KSV/g skaičiui, pH, TTA, spalvų koordinatėms, BA, FAA, FA ir VOC sudėčiai.

Tradicinio ir tarpinio sėjimo lęšių sėklų (Danaja) tyrimų principinė eksperimento schema pateikta 1.3.3 pav. Šiame etape atlikta lęšių sėklų SMF ir SSF LUHS29 LAB paderme. Substrato rodikliai analizuoti po 24 ir 48 valandų,

tiriant LAB KSV/g skaičių, pH, TTA, spalvų koordinates, BA, FAA, mikro- ir makroelementų koncentraciją, FA ir VOC profilių pokyčius.

Kviečių (spalvotųjų DS8558-1 ir tradicinių žieminių Herkus DS) *in vitro* virškinimo principinė eksperimento schema pateikta 1.3.4 pav. Tyrimas atliktas pagal Brodkorb et al. (2019) [71] metodiką, fermentacijos protokolas vykdytas pagal Burillo et al. (2021) [72] metodą. Metodikos modifikuotos, kad tiksliau atitiktų *Sus Scrofa Domesticus* modelį [72]. *In vitro* virškinimo mėginiuose analizuotos laisvosios ir suminės aminorūgščių (AA) koncentracijos; *in vitro* fermentacijos mėginiuose analizuota FAA, BA, VOC, trumposios grandinės riebalų rūgštys (SCFA) ir kiti nelakūs ir pusiau lakūs metabolitai.

1.4. Analizės metodai

1.4.1. Mikrobiologinių ir fizikinių cheminių tyrimų metodai

Mėginių pH vertės nustatytos pH elektrodu (PP-15, Sartorius, Getingenas, Vokietija). TTA analizei taikytas titrimetrinis metodas.

Mėginių spalvų koordinačių analizei naudotas kolorimetras „CromaMeter CR-400“ (Konica Minolta, Japonija). TBC ir LAB skaičius buvo nustatytas auginant sėjimus ant Petri lėkštelių anaerobinėse sąlygose, naudojant atitinkamai MRS ir nespecifinius agarus.

BA kokybinei ir kiekybinei analizei naudota Varian Prostar efektyviosios skysčių chromatografijos sistema su UV detektoriumi (Varian Corp., Palo Altas, Kalifornija, JAV).

Laisvųjų aminorūgščių nustatymui mėginiai buvo ekstrahuojami 0,1 M HCl tirpalu, ištirpintos analitės derivatizuotos dansilchlorido tirpalu (20 mg/ml acetonitrile) ir analizuojami Varian Prostar skysčių chromatografu (Varian Corp., Palo Altas, Kalifornija, JAV) ir Thermo Scientific LCQ FLEET (Thermofisher Scientific, San Chosė, Kalifornija, JAV) masių spektrometru.

Mikro- ir makro elementų koncentracijos nustatytos Agilent 7700x ICP-MS (Agilent Technologies, Tokijas, Japonija) induktyviai susietos plazmos masių spektrometru. Mėginiai buvo veikiami azoto rūgštimi ir vandenilio peroksidu, o po to kaitinami 30 minučių 150 ℃ temperatūroje ir dar 30 minučių 200 ℃ temperatūroje. Detali metodika aprašyta Bartkiene et al. (2018) [79].

Riebalų rūgščių sudėties analizė heksano ekstrakcijos metodu vykdyta FA ekstrahuojant heksanu, o gautą tirpalą derivatizuojant 2 M KOH tirpalu kambario temperatūroje. Gautas tirpalas analizuotas GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru [75].

Riebalų rūgščių sudėties analizė Folch ekstrakcijos metodu vykdyta FA ekstrahuojant chloroformo: metanolio mišiniu (2:1 tūrio santykiu). Gautas

mišinys išvalytas 0.9 proc. NaCl tirpalu. Dalis organinio sluoksnio išgarinta azoto srove, o sausasis likutis derivatizuotas 2 M KOH tirpalu kambario temperatūroje. Gautas tirpalas analizuotas GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru [78].

Mikotoksinų analizei mėginio ekstrakcija atlikta pagal QuEChERS protokolą. Mėginiai analizuoti efektyviosios skysčių chromatografijos sistema su Thermo Scientific TSQ Quantiva (ThermoFisher Scientific, Voltamas, Masačusetsas, JAV) trigubo kvadrupolio masių spektrometru [75].

VOC analizei, apie 1–2 g mėginio disperguojama 10-yje ml fosfatinio buferio (pH = 3,00). Analitės iš viršerdvės ekstrahuotos kietafazės mikroekstrakcijos prietaisu. Adsorbuotos analitės analizuotos GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru [68,69,71].

Suminių AA analizė atlikta hidrolizuojant mėginius 6 M HCl tirpalu, turinčiu 0,1 proc. fenolio, 110 °C temperatūroje 23 valandas. Gautas mišinys neutralizuotas ir analizuotas efektyviosios skysčių chromatografijos – masių spektrometrijos metodu, aprašytu anksčiau.

Laisvųjų aminorūgščių ir biogeninių aminų analizei *in vitro* fermentacijos mėginiuose, 0,1 M HCl tirpalu ekstrahuotos analitės derivatizuojamos izobutilchloroformatu, gauti derivatai analizuoti GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru [73].

Netikslinė pusiau lakių ir nelakių metabolitų analizė *in vitro* fermentacijos mėginiuose atlikta metanolinius mėginių ekstraktus derivatizuojant *N,O*-Bis(trimetilsilil)trifluoracetamidu (BSTFA). Gauti derivatai analizuoti GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru [73].

SCFA analizei mėginiai buvo skiedžiami 0.1 M HCl tirpalu dešimt kartų ir analizuojami GCMS-QP2010 (Shimadzu, Kiotas, Japonija) dujų chromatografu – masių spektrometru.

In vitro virškinimo protokolas buvo adaptuotas pagal Brodkorb et al. (2019) [71]. Esmė – mėginiai veikiami pepsino ir pankreatino fermentais, atitinkamai simuliuojant skrandžio ir žarnyno virškinimo etapus. Nesuvirškintas likutis apdorojamas, pagal Burillo et al. (2021) metodiką [72]. Nesuvirškintas likutis buvo fermentuojamas paršelių fekalijų inokuliatu 39 °C temperatūroje 48 valandas termostate.

1.4.2. Statistiniai metodai

Mikrobiologinių ir fizikinių cheminių rodiklių duomenys pateikti kaip vidurkis ± standartinis nuokrypis. Statistiniai skaičiavimai atlikti naudojant R statistinio programavimo programinės įrangos paketus. Veiksnių poveikiui analizuotiems rodikliams įvertinti naudota dispersinė analizė (ANOVA), o

skirtumams tarp imčių grupių nustatyti – Tukey HSD (Tukey sąžiningo reikšmingo skirtumo) post hoc testas. Normalumui ir homoskedastiškumui nustatyti atlikti Shapiro-Wilko ir Levene testai, naudojant „stats“ (4.4.2 versija) ir „car“ (3.1-3 versijos) paketus. Linijiniai Pearsono koreliacijos koeficientai apskaičiuoti ir vizualizuoti, naudojant „psych“ (2.5.6 versija) ir „corrplot“ (0.95 versija) paketus. Rezultatai interpretuoti kaip statistiškai reikšmingi, kai $p < 0,05$. „Heatmap“ vizualizavimas ir analizė atlikti naudojant R statistinio programavimo programinės įrangos paketą „ComplexHeatmap“ (2.25.2 versija). Dalinė mažiausių kvadratų diskriminantinė analizė (PLS-DA) ir kintamųjų svarbos projekcijoje (VIP) buvo atliktos naudojant „mixOmics“ paketą (6.26.0 versija). „Volcano plot“ analizė ir vizualizacijos atliktos naudojant „EnhancedVolcano“ paketą (1.24.0 versija). Trūkstamos „Volcano plot“ analizei reikalingos reikšmės buvo užpildytos, naudojant pusės minimumo metodą.

2. REZULTATAI

2.1. Biokonversijos įtaka kviečių grūdų mikrobiologiniams ir fizikiniams cheminiams rodikliams

Įvertinus pagrindinius fermentacijos rodiklius (LAB skaičių ir rūgštingumo rodiklius) Nustatyta, kad visuose fermentuotuose mėginiuose LAB skaičius viršijo 8,00 $\log_{10}$ KSV/g, o jų pH vertės varijavo nuo 3,55 iki 4,67. Nustatyta reikšminga neigiama koreliacija tarp mėginių TTA ir pH ($r = -0,422$, $p = 0,003$), o LAB padermė buvo reikšmingas veiksnys TTA ($p = 0,011$, $p < 0,001$). Kviečių veislė, LAB padermė bei šių veiksnių sąveika turėjo reikšmingos įtakos substrato spalvų koordinačių pokyčiams.

Iš visų tirtų BA, tik feniletilaminas nustatytas visuose kviečių grūdų mėginiuose (2.1.2 lentelė). Kadaverinas susidarė mėginiuose, apdorotuose *Liq. uvarum* ir *Lp. Plantarum.* Putrescino aptikta visuose *Ada* ir *Sarta* kviečių grūdų mėginiuose. Kviečių veislė, LAB padermė ir analizuotų veiksnių sąveika buvo reikšminga visų BA koncentracijai kviečių mėginiuose.

Mikro- ir makroelementų koncentracijos kviečių grūdų mėginiuose pateiktos 2.1.3, 2.1.4 ir 2.1.5 lentelėse. Nustatyta, kad kviečių veislė yra reikšmingas veiksnys visų tirtų mikro- ir makroelementų koncentracijai mėginiuose, priešingai, veiksnys – LAB padermė daugumai nustatytų mikro- ir makroelementų koncentracijai reikšmingos įtakos neturėjo.

Pagrindinės FA kviečių grūdų mėginiuose buvo palmitino, oktadecenoinė ir linolo rūgštys. Kviečių veislės ir LAB padermės veiksnių sąveika buvo reikšminga daugeliui identifikuotų kviečių grūduose FA, išskyrus paltimino, oktadecenoinę, α –linoleno ir linolo. LAB padermė buvo reikšmingas veiks-

nys oktadecenoinės rūgšties koncentracijai. Antrojo eksperimento rezultatai patvirtino, kad LAB padermė ir jos sąveika su kviečių veisle yra reikšmingi veiksniai sočiosioms (SFA), mononesočiosioms (MUFA) ir omega-6 FA kviečių mėginiuose.

Antrojo eksperimento FAA rezultatai pateikti 2.1.7 ir 2.1.8 lentelėje. Analizuojant FAA, nustatyta, kad LAB padermė yra reikšmingas veiksnys visoms nepakeičiamoms ir pakeičiamoms FAA, išskyrus, glutamo rūgštį.

Iš visų analizuotų mikotoksinų, kviečių grūduose aptiktas tik deoksinivalenolis (DON). Daugeliu atvejų fermentuotuose mėginiuose DON koncentracija nustatyta didesnė, lyginant su kontroliniais mėginiais.

Fermentacija turėjo įtakos kviečių grūdų VOC profiliui, o fermentuotų grūdų VOC profilis buvo įvairesnis (2.1.1 pav.). Pagrindinės VOC grupės identifikuotos kviečių grūduose: alifatinės organinės rūgštys, aldehidai, esteriai ir alkoholiai. Visi analizuoti veiksniai ir jų sąveikos buvo reikšmingi identifikuotiems VOC analizuotuose mėginiuose.

2.2. Fermentacijos tipo, LAB padermės ir substrato dalelių dydžio įtaka lęšių sėklų mikrobiologiniams ir fizikiniams cheminiams rodikliams

Šiame etape buvo analizuota veiksnių: fermentacijos tipo, LAB padermės ir substrato dalelių dydžio bei jų sąveikos įtaka žaliųjų ir raudonųjų lęšių mikrobiologiniams ir fizikiniams cheminiams rodikliams (2.2.1–2.2.4 lentelės ir 2.2.1 pav.).

Įvertinus pagrindinius fermentacijos efektyvumo rodiklius, nustatyta, kad LUHS122 ir LUHS210 padermės yra tinkamos lęšių apdorojimui (po 24 valandų fermentacijos lęšių mėginių pH vertės <4,5, LAB skaičiaus >8 $\log_{10}$ KSV/g. Substrato dalelių dydis neturėjo reikšmingos įtakos lęšių mėginių pH vertėms. Lęšių veislė, LAB padermė bei lęšių veislės ir substrato dalelių dydžio veiksnių sąveika turėjo reikšmingos įtakos LAB skaičiui mėginiuose. Nustatyta neigiama labai stipri koreliacija tarp pH ir LAB skaičiaus ($r = –0,973$, $p < 0,001$). LAB padermė, substrato dalelių dydis bei lęšių veislės ir LAB padermės veiksnių sąveika buvo reikšminga lęšių spalvų koordinačių pokyčiams. Fermentuotuose mėginiuose, daugeliu atvejų, nustatytos didesnės nepakeičiamų FAA ir GABA koncentracijos (2.2.2 lentelė). Visi analizuoti turėjo reikšmingos įtakos daugeliui FAA, išskyrus alaniną, proliną ir glutaminą. Iš tirtų BA, feniletilaminas, putrescinas, spermidinas ir sperminas nustatyti lęšių mėginiuose (2.2.3 lentelė). Visi analizuoti veiksniai buvo reikšmingi daugeliui BA, išskyrus putresciną, o LAB padermė ir substrato dalelių dydis buvo reikšmingi veiksniai putrescino koncentracijai. Nustatytos neigiamos koreliacijos tarp LAB skaičiaus mėginiuose ir spermidino, spermino bei putrescino ($r = −0,810$, $p < 0,001$; $r = −0,660$, $p < 0,001$;

r = −0,829, $p < 0,001$). Lęšių mėginių FA profilyje identifikuotos penkios pagrindinės FA: palmitino, stearino, oleino, linolo ir α-linoleno rūgštys. Analizuoti veiksniai ir jų sąveika buvo reikšmingi daugeliui identifikuotų FA, išskyrus linolo ir oleino. Lęšių veislė, LAB padermė, substrato dalelių dydis bei lęšių veislės ir LAB padermės sąveika buvo reikšminga omega-3 FA koncentracijai lęšių mėginiuose. Įvertinus lęšių mėginių VOC, nustatyta, kad dauguma identifikuotų lęšių VOC profilio aldehidų koreliavo su FA. Taip pat, nustatytos koreliacijos tarp daugelio VOC ir LAB skaičiaus mėginiuose.

2.3. Agrarinių veiksnių ir fermentacijos tipo įtaka lęšių sėklų mikrobiologiniams ir fizikiniams cheminiams rodikliams

Lęšių, užaugintų, taikant tradicinio ir tarpinio sėjimo metodus bei apdorotų SMF ir SSF mikrobiologiniai ir fizikiniai cheminiai rodikliai pateikti 2.3.1–2.3.5 lentelėse ir 2.3.1 paveiksle.

Fermentuotuose mėginiuose visais atvejais nustatytas >7,3 $\log_{10}$ KSV/g LAB skaičius ir <5,22 pH vertės (2.3.1 lentelė). Analizuotų veiksnių (fermentacijos tipo, fermentacijos trukmės ir auginimo sąlygų) įtaka buvo reikšminga mėginių pH vertėms. Tačiau augimo sąlygos nebuvo reikšmingas veiksnys LAB skaičiui mėginiuose. Visi analizuoti veiksniai ir jų sąveikos (išskyrus visų veiksnių sąveiką), buvo reikšmingi lęšių spalvų koordinačių reikšmėms.

Daugeliu atvejų fermentuotuose lęšių mėginiuose nustatytos didesnės nepakeičiamųjų FAA koncentracijos (2.3.2 lentelė). Augimo sąlygos ir fermentacijos tipas turėjo reikšmingą įtaką daugeliui FAA, išskyrus histidiną, gliciną ir alaniną. Fermentacijos trukmė buvo reikšmingas veiksnys daugelio FAA koncentracijai, išskyrus histidino ir asparagino. Fermentacijos trukmės ir fermentacijos tipo veiksnių bei visų analizuotų veiksnių sąveikos buvo reikšmingos daugeliui identifikuotų FAA koncentracijų lęšių mėginiuose. Iš visų analizuotų BA, feniletilaminas, spermidinas, sperminas ir putrescinas buvo nustatyti lęšių mėginiuose (2.3.3 lentelė). Visi analizuoti veiksniai turėjo reikšmingos įtakos putrescino ir feniletilamino koncentracijoms lęšių mėginiuose. Augimo sąlygų ir fermentacijos tipo veiksnių sąveika turėjo reikšmingos įtakos visų aptiktų BA koncentracijoms lęšiuose. Nustatytos teigiamos koreliacijos tarp LAB skaičiaus ir BA (putrescino ir spermino) ir (atitinkamai, r = 0,761, $p < 0,001$ ir r = 0,331, $p = 0,049$). Linolo rūgštis, priklausanti PUFA, sudarė didžiausią koncentraciją analizuotų lęšių junginių FA sudėtyje (2.3.4 lentelė). Stearino ir palmitino FA sudarė didžiąją dalį lęšių sėklų SFA profilio. Analizuotų veiksnių sąveikos buvo reikšmingos visų aptiktų SFA koncentracijoms lęšių mėginiuose. Visų veiksnių sąveika buvo reikšminga visoms aptiktoms FA lęšių mėginiuose. Visi veiksniai ir jų sąveikos buvo reikšmingi α-linoleno FA koncentracijai. Analizuojant mikro ir makroelementų

koncentracijas lęšių sėklų mėginiuose, nustatyta, kad tarpinio pasėlio lęšių mėginiuose yra reikšmingai mažesnės Na, K ir Ca koncentracijos (2.3.5 lentelė) bei nepakeičiamųjų mikroelementų P, Mn ir Se. Tačiau Fe ir Ni kiekiai tarpinio sėjimo lęšiuose nustatyti reikšmingai didesni, lyginant su tradicinio sėjimo būdu užaugintų pasėlių sėklomis. Pagrindiniai VOC, aptikti lęšių mėginiuose, priklausė alifatinių alkoholių, aldehidų ir organinių rūgščių klasėms (2.3.1 pav.). Fermentuotuose mėginiuose nustatyta didesnė VOC įvairovė, o lyginant SMF ir SSF mėginius, didesnė VOC įvairovė nustatyta SSF mėginių VOC profilyje. Analizuoti veiksniai ir jų sąveikos turėjo reikšmingos įtakos daugeliui identifikuotų VOC lęšių mėginių VOC profilyje. Tarp fenilalanino ir fenolinių VOC (benzaldehido, fenilacetaldehido, feniletilalkoholio) nustatyti koreliaciniai ryšiai ($r = 0{,}824$, $p < 0{,}001$, $r = 0{,}569$, $p < 0{,}001$, $r = 0{,}660$, $p < 0{,}001$).

2.4. Biokonversijos ir kviečių veislės įtaka virškinamumo rodikliams *in vitro*

Fermentuotų kviečių grūdų mikrobiologiniai ir fizikiniai cheminiai rodikliai pateikti 2.4.1–2.4.8 lentelėse ir 2.4.1–2.4.6 paveiksluose. LAB skaičius fermentuotuose mėginiuose visais atvejais buvo >8,4 $\log_{10}$ KSV/g) (2.4.1 lentelė). Nors suminis AA kiekis daugeliu atvejų tarp mėginių reikšmingai nesiskyrė, tačiau biokonversijos ir veislės veiksnių sąveika buvo reikšminga visoms suminių AA koncentracijoms kviečių mėginiuose (2.4.2 lentelė). Nustatyta reikšminga biokonversijos ir substrato įtaka GABA, prolino ir glutamino koncentracijoms *in vitro* virškinimo (IVD) mėginiuose (2.4.3 lentelė). Biokonversija turėjo reikšmingos įtakos glutamo rūgšties ir glicino FAA koncentracijoms IVD mėginiuose. Dauguma suminių AA koncentracijų, lyginant skirtingų grupių IVD mėginius, reikšmingai nesiskyrė (2.4.4 lentelė). Biokonversija turėjo reikšmingos įtakos suminei glicino koncentracijai, o kviečių veislė buvo reikšmingas veiksnys alanino, glutamo rūgšties, treonino, prolino, valino, metionino, fenilalanino, leucino/izoleucino, tirozino ir bendrai suminei AA koncentracijai IVD mėginiuose. Biokonvertuotuose nesuvirškintų IVD likučių mėginiuose nustatyta reikšmingai didesnė treonino koncentracija, lyginant su kontrolinėmis grupėmis (2.4.5 lentelė). Nustatyta reikšminga biokonversijos įtaka daugelio suminių AA koncentracijoms nesuvirškintų IVD likučių mėginiuose, išskyrus seriną, valiną, fenilalaniną ir leuciną/izoleuciną. Veislė buvo reikšmingas veiksnys glutamo ir asparto rūgščių, treonino, prolino, histidino koncentracijai ir suminiam AA kiekiui nesuvirškintuose IVD likučių mėginiuose.

Biokonvertuotų kviečių mėginių *in vitro* virškinamumas buvo didesnis, tačiau šie mėginiai pasižymėjo mažesniu *in vitro* fermentuojamumu (2.4.6

lentelė). Mažiausias bendras AA virškinamumas nustatytas kontrolinių Herkus veislės mėginių. Abu analizuoti veiksniai turėjo reikšmingos įtakos *in vitro* fermentuojamumui ir AA virškinamumui. Biokonversija ir abiejų veiksnių sąveika buvo reikšminga *in vitro* virškinamumui. Biokonversija buvo reikšmingas veiksnys daugelio AA (išskyrus glutamo ir asparto rūgšties, serino, treonino, glicino ir alanino) *in vitro* virškinamumui. Daugelio AA (išskyrus glutamo, asparto, arginino, serino, treonino ir lizino) virškinamumui kviečių veislė buvo reikšmingas veiksnys. Tyrimo rezultatai parodė, kad biokonversija gali reikšmingai pagerinti lizino ir kitų nepakeičiamųjų AA virškinamumą.

FFA ir BA koncentracijos *in vitro* fermentacijos (IVF) mėginiuose pateiktos 2.4.7 lentelėje. Herkus veislės kviečių mėginiuose, daugeliu atvejų, nustatytos reikšmingai didesnės FAA koncentracijos, lyginant su DS8558-1 (DS) kviečių veislės grūdų mėginių grupėmis. Biokonversija turėjo reikšmingos įtakos daugumai FAA ir BA, išskyrus alaniną, valiną, leuciną, treoniną, asparto rūgštį, feniletilalaniną, tiraminą, histidiną ir triptofaną. Kviečių veislė buvo reikšmingas veiksnys daugumai FAA ir BA IVF mėginiuose, išskyrus liziną. DS kviečių veislės mėginių grupės pasižymėjo didžiausiu acto rūgšties kiekiu (2.4.8 lentelė). Kontroliniuose Herkus veislės kviečių mėginiuose nustatyti didžiausi butano ir pentano rūgšties kiekiai. Biokonversija buvo reikšmingas veiksnys šakotos grandinės SCFA bei butano ir pentano rūgšties koncentracijoms, o kviečių veislė buvo reikšmingas veiksnys daugeliui SCFA, išskyrus propano ir heksano rūgštims. Veiksnių sąveika buvo reikšminga propano, butano, pentano ir heksano rūgščių koncentracijoms IVF kviečių mėginiuose.

VOC analizės rezultatai pateikti 2.4.1 pav. Analizuoti veiksniai (biokonversija ir kviečių veislė) buvo reikšmingi daugumai identifikuotų VOC IVF mėginiuose. Šių veiksnių sąveika buvo reikšminga šešių alifatinių organinių rūgščių (propano, 2-metilpropano, butano, 3-metilbutano, pentano ir heksano rūgšties), indolo, hidrocinamono rūgšties, *p*-krezolio, pentillevulinato, tetradekanalio ir oktadekano koncentracijoms mėginių VOC profilyje. Dalinės mažiausių kvadratų diskriminantinės analizės (PLS-DA) rezultatai parodė skirtumus tarp grupių, pagal analizuotus veiksnius: biokonversijos (2.4.2-a pav.) ir kviečių veislės (2.4.2-c pav.). Kintamųjų svarbos projekcijoje (VIP) rezultatai parodė kai kurių VOC (izoforono, mezitilo oksido, acetono, pentillevulinato, metiltioacetato, dalies alifatinių organinių rūgščių, alkanų, aldehidų ir kt.) skirtumus PLS-DA modelyje tarp biokonvertuotų ir kontrolinių mėginių (2.4.2-b pav.). Kai kurie VOC (acetofenonas, benzenacto rūgštis, hidrocinamono rūgštis, naftalenas, 4-etilfenolis, feniletilalkoholis, 2-fenoksietanolis, *p*-krezolis), indolai, keletas alifatinių organinių rūgščių, aldehidų, esterių ir alkanų bei kt., buvo reikšmingi PLS-DA modelui tarp Herkus ir DS mėginių grupių (2.4.2-d pav.). „Volcano plot“ analizės rezultatai parodė,

kad reikšmingai didesnės *p*-krezolio, mezitilo oksido, izoforono ir pentillevulinato koncentracijos yra biokonvertuotuose IVF mėginiuose (2.4.3-a pav.). Reikšmingai didesnės benzenacto, hidrocinamono rūgščių, heksilvalerato, indolo, *p*-krezolio, okten-(2E)-alio ir pentilpentanoato koncentracijos nustatytos DS mėginių grupėje, lyginant su Herkus grupe (2.4.3-b pav.). SCFA analizė PLS-DA modeliu parodė skirtumus tarp grupių (paminėtini skirtumai tarp acto, 4-metilpentano, propano, pentano, oktano, nonano ir 2-metilpropano rūgščių). Nustatytos neigiamos koreliacijos tarp kai kurių AA ir BCFA. Tarp LAB skaičiaus ir propano, butano, pentano ir heksano rūgščių nustatytos neigiamos koreliacijos.

Dauguma identifikuotų BTSFA derivatų koncentracijų buvo reikšmingai didesnės DS ir Herkus mėginių grupėse, lyginant su tuščiais IVF mėginiais (2.4.4 pav.). Biokonversijos veiksnys buvo reikšmingas alifatinių (gintaro, glicerino, 2-deoksitetrono, α-hidroksiglutaro), aromatinių (dihidrocinamono, 4-hidroksifenilacto, 3-hidroksifenilpropiono) rūgščių bei heterociklinių junginių (piroglutamo ir indol-3-acto rūgšties) koncentracijoms. Kviečių veislė buvo reikšmingas veiksnys identifikuotoms alifatinių (pieno, glikolio, 4-hidroksisviesto, gintaro, glicerino, 2-deoksitetrono, α-hidroksiglutaro, 5-aminopentano) ir aromatinių (β-fenilpieno, 3-hidroksifenilpropiono, 4-hidroksihidrocinamono, dihidroferulo, benzenacto, dihidrocinamono) rūgščių ir heterociklinių junginių koncentracijoms IVF mėginiuose. PLS-DA analizės rezultatai parodė aiškų grupių atsiskyrimą tarp DS ir Herkus mėginių (2.4.5-a,c pav.). Aromatinės (4-hidroksifenilacto, 3-hidroksifenilpropiono, dihidrocinamo) ir alifatinės (2-deoksitetrono, gintaro, α-hidroksiglutaro) rūgštys, etanolaminas, piroglutamo rūgštis ir propan-1,3-diolis skyrėsi biokonvertuotų ir kontrolinių mėginių grupių PLS-DA modelyje (2.4.5-b pav.). PLS-DA analizė, lyginant Herkus ir DS mėginius parodė skirtumus tarp alifatinių ir aromatinių (5-aminopentano, 4-hidroksisviesto, glicerino, pieno, α-hidroksiglutaro, glikolio, gintaro, benzenacto, β-fenilpieno, 4-hidroksihidrocinamono, dihidroferulo, dihidrocinamono) rūgščių bei indol-3-acto rūgšties (2.4.5-d pav.). Biokonvertuotuose IVF mėginiuose nustatyta mažesnė 4-hidroksifenilacto rūgšties ir didesnė 3-hidroksifenilpropiono rūgšties koncentracija (2.4.6-a pav.). Alifatinių rūgščių (glicerino, gintaro, pieno, α-hidroksiglutaro rūgščių) ir β-fenilpieno rūgšties koncentracijos Herkus grupės mėginiuose nustatytos didesnės, o aromatinių rūgščių (benzenacto, 4-hidroksicinamono, dihidroferulo, dihidrocinamono, 3-hidroksifenilpropiono rūgščių), 5-aminopentano ir indol-3-acto rūgščių - mažesnės, lyginant su DS mėginiais (2.4.6-b pav.). Nustatyta neigiama koreliacija tarp *p*-krezolio ir LAB skaičiaus. Benzenacto, dihidrocinamono ir 4-hidroksihidrocinamono rūgštys neigiamai koreliavo su aromatinėmis AA. Biokonversija ir kviečių veislė turėjo reikšmingos įtakos GABA koncentracijai IVF mėginiuose.

3. REZULTATŲ APTARIMAS

Disertacinio darbo rezultatai pagrindžia analizuotų augalinių žaliavų biokonversijos potencialą ir atkreipia dėmesį į fermentacijos proceso kompleksiškumą bei technologinių veiksnių įtaką biokonvertuotų žaliavų rodikliams. Apibendrinant visų eksperimentų duomenis, galima išskirti kelias pagrindines tendencijas, susijusias su fermentacijos efektyvumu, fizikinių cheminių rodiklių pokyčiais bei *in vitro* virškinamumo pokyčiais.

Visuose fermentuotuose mėginiuose nustatytas >6 $\log_{10}$ KSV/g LAB kiekis (>7–8 $\log_{10}$ KSV/g) ir reikšmingai sumažėjusios pH vertės patvirtina šiame darbe analizuotų fermentacijos procesų efektyvumą. Tai rodo, kad eksperimentams naudotos LAB padermės yra tinkamos disertaciniame darbe nagrinėtų kviečių ir lęšių veislių fermentacijai [80-82, 109, 122, 123, 160]. Nustatytos neigiamos koreliacijos tarp pH ir LAB skaičiaus (ypač stiprios lęšių atveju, $r = -0{,}973$) patvirtina klasikinį fermentacijos mechanizmą, kai didėjant LAB kiekiui – intensyvėja organinių rūgščių gamyba ir mažėja terpės pH. Toks pH pokytis yra svarbus ne tik substrato saugos rodikliams, bet ir jo stabilumui bei galimam fiziologiniam poveikiui [109, 160].

BA analizė atskleidė, kad jų susidarymas priklauso nuo kelių veiksnių – žaliavos, LAB padermės ir jų sąveikos. Nors kai kurie BA (pvz., feniletilaminas) buvo aptikti visuose mėginiuose, kitų (pvz., kadaverino ar putrescino) susidarymas buvo labiau selektyvus. Tai rodo, kad fermentacijos procesas gali turėti dvejopą poveikį: viena vertus, gerinti maistinę ir funkcionaliąją vertę, kita vertus – skatinti nepageidaujamų junginių susidarymą [87, 88]. Kai kuriais atvejais nustatytos neigiamos koreliacijos tarp LAB ir BA, ypač lęšių mėginiuose, leidžia daryti prielaidą, kad tinkamai parinktos padermės gali slopinti šių junginių kaupimąsi [116, 117].

Riebalų rūgščių (FA) ir laisvųjų aminorūgščių (FAA) pokyčiai rodo reikšmingą fermentacijos įtaką substrato biocheminiams rodikliams. Kviečių grūdų mėginiuose dominavo palmitino, oleino ir linolo rūgštys, o lęšių sėklose nustatyta ir α-linoleno rūgštis. Pastarosios FA lęšiuose rodo šių ankštinių augalų sėklų, kaip omega-3 šaltinio potencialą. Biokonversija parinktomis LAB padermėmis, turėjo reikšmingos įtakos tiek sočiųjų, tiek nesočiųjų riebalų rūgščių kiekiams. Taip pat nustatytas FAA padidėjimas fermentuotuose mėginiuose, kuris gali būti siejamas su baltymų hidrolize fermentacijos metu [94]. FAA gerina produkto biologinę vertę, įskaitant virškinamumą.

Mikro- ir makroelementų analizė parodė, kad didžiausią įtaką jų koncentracijoms turėjo veislė ir auginimo sąlygos, fermentacija nebuvo reikšmingas veiksnys. Tai leidžia daryti išvadą, kad mineralinė sudėtis yra labiau genetiškai ir agronomiškai nulemta, nors fermentacija gali turėti įtakos jų biologi-

niam prieinamumui, tačiau nėra reikšmingas veiksnys šių elementų koncentracijoms [149-152].

Didesnė VOC įvairovė nustatyta fermentuotuose mėginiuose, tiek kviečiuose, tiek lęšiuose. Identifikuotos pagrindinės junginių grupės – aldehidai, alkoholiai, esteriai ir organinės rūgštys yra svarbios formuojant produkto aromatą ir juslines savybes [102, 103, 153-155]. Taip pat, jos gali būti interpretuojamos ir kaip jautrūs biocheminiai žymenys substrato kitimams vertinti [103, 104, 121, 156-158]. Nustatytos koreliacijos tarp VOC ir FA ar FAA rodo, kad fermentacijos metu vyksta kompleksinės biocheminės reakcijos, lemiančios naujų junginių susidarymą.

In vitro virškinamumo analizė parodė, kad biokonversija gali pagerinti AA, ypač nepakeičiamųjų (pvz., lizino), virškinamumą. Nors virškinamumas didėja, fermentuojamumas gali mažėti, kas rodo galimus pokyčius substrato prieinamume žarnyno mikrobiotai. Taip pat nustatyta, kad kviečių veislė yra svarbus veiksnys, lemiantis tiek aminorūgščių profilio sudėtį, tiek jų įsisavinimą. SCFA ir kitų metabolitų analizė *in vitro* fermentacijos modelyje parodė, kad biokonversija ir žaliavos tipas turi reikšmingos įtakos metabolitų profiliui. Nustatytos koreliacijos tarp LAB ir atskirų metabolitų rodo, kad mikroorganizmai gali moduliuoti metabolinį atsaką fermentacijos metu [173, 174].

Apibendrinant galima teigti, kad biokonversijos technologinių veiksnių modeliavimas: nuo substrato cheminės sudėties, dalelių dydžio, vandens kiekio ir kt., turi reikšmingą potencialą augalinių žaliavų kokybei gerinti. Šiame disertaciniame darbe gauti rezultatai pagrindžia LAB padermės pasirinkimo, substrato tipo, jo dalelių dydžio bei agrarinių veiksnių kompleksinę įtaką galutiniams žaliavų rodikliams. Gauti rezultatai svarbūs efektyvesniam žemės ūkio resursų panaudojimui.

IŠVADOS

1. Kviečių veislių *Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7 ir DS8535-2 grūdų biokonversija, naudojant pasirinktas LAB padermes (LUHS29, LUHS245, LUHS122) yra tinkama technologija modifikuoti grūdų sudėtį palankia linkme, išskyrus mikro- ir makroelementų koncentracijas. Kviečių veislė, daugeliu atvejų, yra reikšmingas veiksnys analizuotiems kviečių grūdų rodikliams, priešingai nei LAB padermė, pastarasis veiksnys, daugeliu atvejų, nebuvo reikšmingas:
   1.1. Gyvybingų LAB skaičius nustatytas substratuose >8,00 $\log_{10}$ KSV/g, pH vertės sumažėjo nuo 4,67 iki 3,55.
   1.2. Pagrindiniai kviečių grūdų mėginiuose identifikuoti BA feniletilaminas, putrescinas, spermidinas ir kadaverinas. Reikšmingai didesnės putrescino, spermidino, kadaverino ir suminė BA kiekio

koncentracija nustatyta biokonvertuotų kviečių grūdų mėginiuose, o LAB padermė, kviečių grūdų veislė ir šių veiksnių sąveika buvo reikšmingi ($p < 0,05$) visiems identifikuotiems BA.

1.3. Kviečių grūdų SFA, MUFA ir PUFA sudarė atitinkamai 20,8–39,8 proc., 14,1–28,8 proc. ir 36,9–64,5 proc.; veiksnys LAB padermė ir veiksnio LAB padermė sąveika su veiksniu „kviečių veislė“ buvo reikšminga ($p < 0,05$) SFA, MUFA ir omega-6 FA koncentracijoms kviečių grūdų mėginiuose.

1.4. Biokonvertuotų kviečių grūdų mėginiuose nustatytas reikšmingas nepakeičiamųjų FAA koncentracijos padidėjimas; LAB padermė ir kviečių veislė buvo reikšmingi veiksniai ($p < 0.05$) daugumos nepakeičiamųjų ir pakeičiamųjų FAA koncentracijų pokyčiams substrate.

1.5. Fermentacija padidino GABA koncentraciją mėginiuose; analizuoti veiksniai (LAB padermė ir kviečių grūdų veislė) buvo reikšmingi ($p < 0.05$) šiam rodikliui.

1.6. Fermentuotuose mėginiuose nustatytas didesnis DON kiekis, lyginant su nefermentuotais mėginiais.

1.7. Fermentacija lėmė įvairesnį VOC profilį, kuriame dominavo alifatinės organinės rūgštys, esteriai, alkoholiai ir aldehidai.

2. Raudonųjų ir žaliųjų lęšių biokonversija parinktomis LAB padermėmis (LUHS122 ir LUHS210) ir substrato dalelių dydžio mažinimas yra efektyvi technologija žaliavos sudėties modeliavimui:

2.1. Gyvybingų LAB skaičius substratuose nustatytas >8,00 $\log_{10}$ KSV/g, pH sumažėjimas iki 4,21 – 4,43; Lęšių veislės ir substrato dalelių dydžio veiksnių sąveika buvo statistiškai reikšminga ($p < 0,05$) LAB skaičiui mėginiuose.

2.2. LAB padermė, substrato dalelių dydžio ir lęšių veislės bei LAB padermės veiksnių sąveika buvo reikšmingi ($p < 0,05$) lęšių mėginių spalvų koordinačių vertėms.

2.3. Daugelis nepakeičiamųjų FAA ir GABA kiekis biokonvertuotuose mėginiuose nustatytas didesnis ($p < 0,05$), lyginant su kontroliniais mėginiais; lęšių veislės, dalelių dydžio ir LAB padermės įtaka daugeliui FAA lęšių mėginiuose buvo reikšminga ($p < 0,05$).

2.4. Dominuojantys BA lęšių mėginiuose: fenilalaninas, putrescinas, spermidinas ir sperminas. Nesmulkinto fermentuoto substrato mėginiuose BA koncentracijos nustatytos mažesnės, lyginant su smulkintais fermentuotais mėginiais; visi analizuoti veiksniai turėjo reikšmingos įtakos BA koncentracijai ($p < 0,05$) lęšių mėginiuose, išskyrus putrescino kiekiui. Nustatyta neigiama koreliacija

tarp LAB skaičiaus ir atskirų BA (spermidino, spermino, putrescino, atitinkamai, r = −0,810, $p < 0,001$; r = −0,660, $p < 0,001$; r = −0,829, $p < 0,001$).

2.5. Visi analizuoti veiksniai ir jų sąveika buvo reikšmingi ($p < 0,05$) palmitino, stearino ir α-linoleno rūgščių kiekiui lęšių mėginiuose. LAB padermė, lęšių veislė ir šių veiksnių sąveika su dalelių dydžio veiksniu buvo reikšmingi ($p < 0,05$) omega-3 FA koncentracijoms lęšių mėginiuose.

2.6. Lęšių VOC profilyje dominavo alifatiniai aldehidai, organinės rūgštys ir monoterpenai. Nustatytos koreliacijos ($p < 0,05$) tarp daugumos VOC ir LAB skaičiaus mėginiuose bei tarp aldehidų ir FA.

3. SSF ir SMF taikymas *Fabaceae* šeimos skirtingų lęšių veislių sėkloms apdoroti yra tinkama priemonė FAA, FA ir VOC profilių bei kitų fizikinių cheminių rodiklių modeliavimui palankia linkme:

3.1. Gyvybingų LAB skaičius substratuose nustatytas >7,3 $\log_{10}$ KSV/g, pH sumažėjo iki 4,3; Fermentacijos tipas, fermentacijos trukmė ir lęšių auginimo technologija turėjo reikšmingos ($p < 0,05$) įtakos substratų pH vertėms, o fermentacijos tipas ir fermentacijos trukmė turėjo reikšmingos įtakos LAB skaičiui mėginiuose ($p < 0,05$).

3.2. Daugeliu atvejų nepakeičiamųjų FAA kiekis SSF ir SMF mėginiuose nustatytas reikšmingai ($p < 0,05$) didesnis, lyginant su nefermentuotais mėginiais. SSF mėginiuose daugeliu atvejų FAA ir GABA koncentracijos nustatytos didesnės, lyginant su SMF mėginiais. Visi analizuoti veiksniai buvo reikšmingi ($p < 0,05$) FAA ir GABA koncentracijoms lęšių mėginiuose. Fermentacijos tipo ir fermentacijos trukmės sąveika bei visų analizuotų veiksnių sąveika buvo reikšminga ($p < 0,05$) daugumai analizuotų FAA koncentracijoms lęšių mėginiuose.

3.3. Lęšių mėginiuose nustatyti feniletilaminas, putrescinas, spermidinas ir sperminas; daugeliu atvejų didesnė BA koncentracija nustatyta fermentuotuose mėginiuose. Auginimo sąlygų ir fermentacijos tipo sąveika buvo reikšminga ($p < 0,05$) BA koncentracijai lęšių mėginiuose.

3.4. Pagrindinės FA lęšių mėginiuose nustatytos linolo, palmitino ir stearino; jų kiekis, atitinkamai, varijavo nuo 41,3–50,3 proc., 16,2–20,3 proc. ir 1,63–3,29 proc. Analizuotų veiksnių sąveika buvo reikšminga ($p < 0,05$) visų FA kiekiui lęšių mėginių FA pro-

filyje. Visi analizuoti veiksniai ir jų sąveikos buvo reikšmingos ($p < 0,05$) α-linoleno rūgšties koncentracijai lęšių mėginiuose.

3.5. Lęšių auginimo technologija turėjo reikšmingos įtakos ($p < 0,05$) mikro- ir makroelementų koncentracijai lęšių sėklose.

3.6. Daugumoje lęšių mėginių identifikuoti VOC, tai alifatinės rūgštys, ketonai, aldehidai ir alkoholiai; SSF mėginiuose nustatyta didesnė VOC įvairovė, lyginant su SMF mėginiais. Visi analizuoti veiksniai ir jų sąveikos daugeliu atvejų turėjo reikšmingos įtakos ($p < 0,05$) VOC koncentracijoms.

4. *In vitro* virškinimo modelio taikymas kviečių mėginių biokonversijai įvertinti yra tinkamas metodas prognozuoti galimus neapdorotų ir fermentuotų kviečių grūdų *in vivo* pokyčius; nustatyti reikšmingi skirtumai tarp *in vitro* virškinamumo ir fermentacijos rodiklių bei kitų analizuotų metabolitų:

4.1. Biokonversija neturėjo reikšmingos įtakos aminorūgščių koncentracijai kviečių grūdų mėginiuose. Kviečių veislės ir biokonversijos veiksnių sąveika buvo reikšminga daugumai aminorūgščių analizuotuose substratuose.

4.2. GABA, prolino ir glutamino koncentracijoms *in vitro* virškinimo mėginiuose reikšmingos įtakos turėjo biokonversija ir kviečių veislė. Biokonversija buvo reikšminga ($p < 0,05$) glutamo rūgšties ir glicino koncentracijoms. Daugeliui suminių aminorūgščių koncentracijų *in vitro* virškinimo nesuvirškintų likučių mėginiuose reikšmingos įtakos turėjo biokonversija ($p < 0,05$); substratas buvo reikšmingas veiksnys treonino, prolino, histidino, glutamo ir asparto rūgščių koncentracijoms bei suminiams aminorūgščių kiekiams. Biokonvertų grūdų mėginių nepakeičiamųjų aminorūgščių: lizino, fenilalanino ir valino, virškinamumas nustatytas reikšmingai didesnis ($p < 0,05$).

4.3. Daugeliui FAA, BA ir GABA koncentracijoms IVF mėginiuose reikšmingos ($p < 0,05$) įtakos turėjo biokonversijos ir kviečių veislės veiksniai. Nustatytos ($p < 0,05$) neigiamos koreliacijos tarp TBC ir putrescino, kadaverino bei histamino).

4.4. BCFA, butano ir pentano rūgščių koncentracijoms IVF mėginiuose reikšmingos ($p < 0,05$) įtakos turėjo biokonversijos veiksnys. Kviečių veislė buvo reikšmingas veiksnys ($p < 0,05$) daugeliui SCFA, išskyrus propano ir heksano rūgštis.

4.5. Biokonversija reikšmingai padidino ($p < 0,05$) kviečių grūdų *in vitro* virškinamumą, tačiau biokonversijos veiksnys reikšmingai sumažino ($p < 0,05$) kviečių grūdų *in vitro* fermentuojamumą.

4.6. IVF mėginių PLS-DA ir VIP analizės rezultatai parodė alifatinių alkanų ir aldehidų skirtumus, rodančius lipidų metabolizmo skirtumus tarp skirtingų mėginių grupių. Reikšmingi ($p < 0,05$) fenolinių metabolitų (benzenacto, dihidrocinamono ir 4-hidroksihidrocinamono rūgščių) skirtumai rodo fenolinių aminorūgščių metabolizmo skirtumus.

REKOMENDACIJOS

1. Rekomenduojamas kviečių veislių *Ada*, *Sarta*, DS8472-5, DS8526-2, *Gaja*, DS8888-3-6, DS8548-7 ir DS8535-2 grūdų biokonversijos metodas, naudojant LAB padermes LUHS29, LUHS245, LUHS122, nes tai veiksminga technologija modifikuoti kviečių grūdų substratus, suteikiant pridėtinę vertę, t.y., padidinant nepakeičiamųjų FAA ir GABA koncentracijas.
2. Norint pagerinti raudonųjų ir žaliųjų lęšių sėklų maistines savybes, ypač padidinti nepakeičiamų amino rūgščių ir GABA koncentracijas, rekomenduojama taikyti mažesnių dalelių dydžio substrato konversiją LAB padermėmis LUHS122 ir LUHS210. Proceso metu rekomenduojama vykdyti BA stebėseną.
3. SSF rekomenduojamas šiame darbe analizuotų kviečių grūdų ir lęšių sėklų biokonversijai, nes, lyginant su SMF, užtikrina didesnę funkcionaliųjų molekulių sintezę substrate ir yra tvaresnis dėl mažesnio vandens poreikio, reikalingo biokonversijai užtikrinti.
4. Rekomenduojama taikyti *in vitro* virškinimo modelį kviečių grūdų mėginių biokonversijos efektyvumui įvertinti, kaip tinkamą įrankį prognozuojant galimus pašarinių žaliavų *in vivo* pokyčius.

REFERENCES

1. Georganas A, Giamouri E, Pappas AC, Papadomichelakis G, Galliou F, Manios T, Tsiplakou E, Fegeros K, Zervas G. Bioactive compounds in food waste: A review on the transformation of food waste to animal feed. Foods 2020;9(3):291.
2. der Poel AFBv, Abdollahi MR, Cheng H, Colovic R, den Hartog LA, Miladinovic D, Page G, Sijssens K, Smillie JF, Thomas M, Wang W, Yu P, Hendriks WH. Future directions of animal feed technology research to meet the challenges of a changing world. Anim Feed Sci Technol 2020;270:114692.
3. Forabosco F, Löhmus M, Rydhmer L, Sundström LF. Genetically modified farm animals and fish in agriculture: A review. Livestock Science 2013;153(1):1–9.
4. Hume DA, Whitelaw C, Archibald AL. The future of animal production: improving productivity and sustainability. The Journal of Agricultural Science 2011;149(S1):9–16.
5. Dayoub M, Shnaigat S, Tarawneh RA, Al-Yacoub AN, Al-Barakeh F, Al-Najjar K. Enhancing animal production through smart agriculture: possibilities, hurdles, resolutions, and advantages. Ruminants 2024;4(1):22–46.
6. Hernandez-Patlan D, Tellez-Isaias G, Hernandez-Velasco X, Solis-Cruz B. Technological strategies to improve animal health and production. Frontiers in veterinary science 2023;10:1206170.
7. Balehegn M, Duncan A, Tolera A, Ayantunde AA, Issa S, Karimou M, Zampaligré N, André K, Gnanda I, Varijakshapanicker P, Kebreab E, Dubeux J, Boote K, Minta M, Feyissa F, Adesogan AT. Improving adoption of technologies and interventions for increasing supply of quality livestock feed in low- and middle-income countries. Global Food Security 2020;26:100372.
8. Prates JA. Enhancing meat quality and nutritional value in monogastric livestock using sustainable novel feed ingredients. Foods 2025;14(2):146.
9. Mohammadi Gheisar M, Kim IH. Phytobiotics in poultry and swine nutrition – a review. Italian Journal of Animal Science 2018;17(1):92–99.
10. Alao BO, Falowo AB, Chulayo A, Muchenje V. The potential of animal by-products in food systems: Production, prospects and challenges. Sustainability 2017;9(7):1089.
11. Chuang W, Lin L, Shih H, Shy Y, Chang S, Lee T. The potential utilization of high-fiber agricultural by-products as monogastric animal feed and feed additives: A review. Animals 2021;11(7):2098.
12. Fouillet H, Dussiot A, Perraud E, Wang J, Huneau J, Kesse-Guyot E, Mariotti F. Plant to animal protein ratio in the diet: nutrient adequacy, long-term health and environmental pressure. Frontiers in nutrition 2023;10:1178121.
13. Sheffield S, Fiorotto ML, Davis TA. Nutritional importance of animal-sourced foods in a healthy diet. Frontiers in nutrition 2024;11:1424912.
14. Lane KE, Wilson M, Hellon TG, Davies IG. Bioavailability and conversion of plant based sources of omega-3 fatty acids – a scoping review to update supplementation options for vegetarians and vegans. Crit Rev Food Sci Nutr 2022;62(18):4982–4997.
15. Sardi L, Martelli G, Lambertini L, Parisini P, Mordenti A. Effects of a dietary supplement of DHA-rich marine algae on Italian heavy pig production parameters. Livestock Science 2006;103(1):95–103.
16. Ewy MW, Patel A, Abdelmagid MG, Mohamed Elfadil O, Bonnes SL, Salonen BR, Hurt RT, Mundi MS. Plant-based diet: is it as good as an animal-based diet when it comes to protein? Curr Nutr Rep 2022;11(2):337–346.
17. Obeid R, Heil SG, Verhoeven MM, Van den Heuvel EG, De Groot LC, Eussen SJ. Vitamin B12 intake from animal foods, biomarkers, and health aspects. Frontiers in nutrition 2019;6:93.

18. Cashman KD. Vitamin D: dietary requirements and food fortification as a means of helping achieve adequate vitamin D status. J Steroid Biochem Mol Biol 2015;148:19–26.
19. Pecoraro BM, Leal DF, Frias-De-Diego A, Browning M, Odle J, Crisci E. The health benefits of selenium in food animals: A review. J Animal Sci Biotechnol 2022;13(1):58.
20. Smith TJ, Johnson CR, Koshy R, Hess SY, Qureshi UA, Mynak ML, Fischer PR. Thiamine deficiency disorders: a clinical perspective. Ann N Y Acad Sci 2021;1498(1):9–28.
21. Lentjes MA. The balance between food and dietary supplements in the general population. Proc Nutr Soc 2019;78(1):97–109.
22. Ronis MJ, Pedersen KB, Watt J. Adverse effects of nutraceuticals and dietary supplements. Annu Rev Pharmacol Toxicol 2018;58(1):583–601.
23. Stas EB, DeRouchey JM, Goodband RD, Tokach MD, Woodworth JC, Gebhardt JT. Nutritional guide to feeding wheat and wheat co-products to swine: a review. Translational Animal Science 2024;8:txae106.
24. Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food and energy security 2015;4(3):178–202.
25. David LS, Nalle CL, Abdollahi MR, Ravindran V. Feeding value of lupins, field peas, faba beans and chickpeas for poultry: an overview. Animals 2024;14(4):619.
26. Stagnari F, Maggio A, Galieni A, Pisante M. Multiple benefits of legumes for agriculture sustainability: an overview. Chemical and Biological Technologies in Agriculture 2017;4(1):2.
27. Carbas B, Machado N, Pathania S, Brites C, Rosa EA, Barros AI. Potential of legumes: Nutritional value, bioactive properties, innovative food products, and application of eco-friendly tools for their assessment. Food Rev Int 2023;39(1):160–188.
28. Vaz Patto MC, Amarowicz R, Aryee ANA, Boye JI, Chung H, Martín-Cabrejas MA, Domoney C. Achievements and Challenges in Improving the Nutritional Quality of Food Legumes. Crit Rev Plant Sci 2015;34(1-3):105–143.
29. Arbab Sakandar H, Chen Y, Peng C, Chen X, Imran M, Zhang H. Impact of Fermentation on Antinutritional Factors and Protein Degradation of Legume Seeds: A Review. Food Rev Int 2023;39(3):1227–1249.
30. Ram S, Narwal S, Gupta OP, Pandey V, Singh GP. 4 - Anti-nutritional factors and bioavailability: approaches, challenges, and opportunities. In: Gupta OP, Pandey V, Narwal S, Sharma P, Ram S, Singh GP, editors. Wheat and Barley Grain Biofortification: Woodhead Publishing; 2020. p. 101–128.
31. Duraiswamy A, Sneha A NM, Jebakani K S, Selvaraj S, Pramitha J L, Selvaraj R, Petchiammal K I, Kather Sheriff S, Thinakaran J, Rathinamoorthy S, Kumar P. R. Genetic manipulation of anti-nutritional factors in major crops for a sustainable diet in future. Frontiers in plant science 2023;13:1070398.
32. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition and antioxidant potential of grain legume seeds: A review. Food Res Int 2017;101:1–16.
33. Saini P, Kumar N, Kumar S, Mwaurah PW, Panghal A, Attkan AK, Singh VK, Garg MK, Singh V. Bioactive compounds, nutritional benefits and food applications of colored wheat: a comprehensive review. Crit Rev Food Sci Nutr 2021;61(19):3197–3210.
34. Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Domínguez-Díaz D, González-Ríos H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol 2017;59(1):8.
35. Villalba JJ, Costes-Thiré M, Ginane C. Phytochemicals in animal health: diet selection and trade-offs between costs and benefits. Proc Nutr Soc 2017;76(2):113–121.
36. Achilonu M, Shale K, Arthur G, Naidoo K, Mbatha M. Phytochemical benefits of agroresidues as alternative nutritive dietary resource for pig and poultry farming. Journal of Chemistry 2018;2018(1):1035071.

37. Reis JF, Monteiro VVS, de Souza Gomes R, do Carmo MM, da Costa GV, Ribera PC, Monteiro MC. Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. J Transl Med 2016;14(1):315.
38. Tian X, Lu Q. Anthocyanins in dairy cow nutrition: a review. Agriculture 2022;12(11):1806.
39. Garg M, Kaur S, Sharma A, Kumari A, Tiwari V, Sharma S, Kapoor P, Sheoran B, Goyal A, Krishania M. Rising demand for healthy foods-anthocyanin biofortified colored wheat is a new research trend. Frontiers in Nutrition 2022;9:878221.
40. Changxing L, Chenling M, Alagawany M, Jianhua L, Dongfang D, Gaichao W, Wenyin Z, Syed SF, Arain MA, Saeed M, HASSAN FU, CHAO S. Health benefits and potential applications of anthocyanins in poultry feed industry. Worlds Poult Sci J 2018;74(2):251–264.
41. Padhy AK, Kaur P, Singh S, Kashyap L, Sharma A. Colored wheat and derived products: key to global nutritional security. Crit Rev Food Sci Nutr 2024;64(7):1894–1910.
42. Garg M, Chawla M, Chunduri V, Kumar R, Sharma S, Sharma NK, Kaur N, Kumar A, Mundey JK, Saini MK, Singh SP. Transfer of grain colors to elite wheat cultivars and their characterization. J Cereal Sci 2016;71:138–144.
43. Kan L, Nie S, Hu J, Wang S, Bai Z, Wang J, Zhou Y, Jiang J, Zeng Q, Song K. Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. Food Chem 2018;260:317–326.
44. Kalt W, Blumberg JB, McDonald JE, Vinqvist-Tymchuk MR, Fillmore SA, Graf BA, O'Leary JM, Milbury PE. Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. J Agric Food Chem 2008;56(3):705–712.
45. Liu Y, Zhang D, Wu Y, Wang D, Wei Y, Wu J, Ji B. Stability and absorption of anthocyanins from blueberries subjected to a simulated digestion process. Int J Food Sci Nutr 2014;65(4):440–448.
46. Missotten JA, Michiels J, Degroote J, De Smet S. Fermented liquid feed for pigs: an ancient technique for the future. J Animal Sci Biotechnol 2015;6(1):4.
47. Olukomaiya O, Fernando C, Mereddy R, Li X, Sultanbawa Y. Solid-state fermented plant protein sources in the diets of broiler chickens: A review. Animal Nutrition 2019;5(4):319–330.
48. Zhang Y, Ishikawa M, Jiang N, Zhang X. Fermentation-Based Strategies for the Feed Industry: Nutritional Augmentation, Environmental Sustainability. Fermentation 2026;12(2):103.
49. Shi C, Zhang Y, Lu Z, Wang Y. Solid-state fermentation of corn-soybean meal mixed feed with Bacillus subtilis and Enterococcus faecium for degrading antinutritional factors and enhancing nutritional value. Journal of Animal science and Biotechnology 2017;8(1):50.
50. Rollán GC, Gerez CL, LeBlanc JG. Lactic fermentation as a strategy to improve the nutritional and functional values of pseudocereals. Frontiers in Nutrition 2019;6:98.
51. Wang C, Shi C, Zhang Y, Song D, Lu Z, Wang Y. Microbiota in fermented feed and swine gut. Appl Microbiol Biotechnol 2018;102(7):2941–2948.
52. Dawood MA, Koshio S. Application of fermentation strategy in aquafeed for sustainable aquaculture. Reviews in Aquaculture 2020;12(2):987–1002.
53. Moradi M, Kousheh SA, Almasi H, Alizadeh A, Guimarães JT, Yılmaz N, Lotfi A. Postbiotics produced by lactic acid bacteria: The next frontier in food safety. Comprehensive reviews in food science and food safety 2020;19(6):3390–3415.
54. Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: The new horizons in microbial biotherapy and functional foods. Microb Cell Fact 2020;19(1):168.

55. Xu B, Li Z, Wang C, Fu J, Zhang Y, Wang Y, Lu Z. Effects of fermented feed supplementation on pig growth performance: A meta-analysis. Anim Feed Sci Technol 2020;259:114315.
56. Yuan L, Chang J, Yin Q, Lu M, Di Y, Wang P, Wang Z, Wang E, Lu F. Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets. Animal Nutrition 2017;3(1):19–24.
57. Xin H, Wang M, Xia Z, Yu B, He J, Yu J, Mao X, Huang Z, Luo Y, Luo J, Yan H, Wang H, Wang Q, Zheng P, Chen D. Fermented diet liquid feeding improves growth performance and intestinal function of pigs. Animals 2021;11(5):1452.
58. Liu S, Xiao H, Xiong Y, Chen J, Wu Q, Wen X, Jiang Z, Wang L. Effects of fermented feed on the growth performance, intestinal function, and microbiota of piglets weaned at different age. Frontiers in veterinary science 2022;9:841762.
59. Huang L, Ren P, Ouyang Z, Wei T, Kong X, Li T, Yin Y, He S, Yang C, He Q. Effect of fermented feed on growth performance, holistic metabolism and fecal microbiota in weanling piglets. Anim Feed Sci Technol 2020;266:114505.
60. Zhang D, Ji H, Wang S, Liu Y, Chen M, Liu H. Lactobacillus-driven feed fermentation regulates microbiota metabolism and reduces odor emission from the feces of pigs. Msystems 2023;8(6):988.
61. Sossidou EN, Banias GF, Batsioula M, Termatzidou S, Simitzis P, Patsios SI, Broom DM. Modern pig production: Aspects of animal welfare, sustainability and circular bioeconomy. Sustainability 2025;17(11):5184.
62. Dai Z, Cui L, Li J, Wang B, Guo L, Wu Z, et al. Chapter 24 - Fermentation techniques in feed production. In: Bazer FW, Lamb GC, Wu G, editors. Animal Agriculture: Academic Press; 2020. p. 407–429.
63. Chin XH, Elhalis H, Chow Y, Liu SQ. Enhancing food safety in soybean fermentation through strategic implementation of starter cultures. Heliyon 2024;10(2):e25007.
64. Licandro H, Ho PH, Nguyen TKC, Petchkongkaew A, Nguyen HV, Chu-Ky S, Nguyen TVA, Lorn D, Waché Y. How fermentation by lactic acid bacteria can address safety issues in legumes food products? Food Control 2020;110:106957.
65. Kalaiselvan P, Devi NC, Deepti M, Devi AA, Akamad K, Dheeran P, Debbarma S, Vadivel D, Rajesh D. Solid-state fermentation – a sustainable future technology in aquafeeds? Frontiers in Marine Science 2025;12:1669719.
66. Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol 2010;46(7):541–549.
67. Kapoor M, Panwar D, Kaira GS. Chapter 3 - Bioprocesses for Enzyme Production Using Agro-Industrial Wastes: Technical Challenges and Commercialization Potential. In: Dhillon GS, Kaur S, editors. Agro-Industrial Wastes as Feedstock for Enzyme Production San Diego: Academic Press; 2016. p. 61–93.
68. Behera SS, Ray RC, Zdolec N. Lactobacillus plantarum with functional properties: an approach to increase safety and shelf-life of fermented foods. BioMed research international 2018;2018(1):9361614.
69. Abedin MM, Chourasia R, Phukon LC, Sarkar P, Ray RC, Singh SP, Rai AK. Lactic acid bacteria in the functional food industry: biotechnological properties and potential applications. Crit Rev Food Sci Nutr 2024;64(29):10730–10748.
70. Bartkiene E, Lele V, Ruzauskas M, Domig KJ, Starkute V, Zavistanaviciute P, Bartkevics V, Pugajeva I, Klupsaite D, Juodeikiene G, Mickiene R, Rocha JM. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. Microorganisms 2019;8(1):64.

71. Brodkorb A, Egger L, Alminger M, Alvito P, Assuncao R, Ballance S, Bohn T, Bourlieu-Lacanal C, Boutrou R, Carriere F, Clemente A, Corredig M, Dupont D, Dufour C, Edwards C, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie AR, Martins C, Marze S, McClements DJ, Menard O, Minekus M, Portmann R, Santos CN, Souchon I, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Recio I. INFOGEST static in vitro simulation of gastrointestinal food digestion. 2019.
72. Pérez-Burillo S, Molino S, Navajas-Porras B, Valverde-Moya ÁJ, Hinojosa-Nogueira D, López-Maldonado A, Pastoriza S, Rufián-Henares JÁ. An in vitro batch fermentation protocol for studying the contribution of food to gut microbiota composition and functionality. Nat Protoc 2021;16(7):3186–3209.
73. Mockus E, Starkute V, Klupsaite D, Badaras S, Liatukas Z, Ruzgas V, Ruzauskas M, Bartkiene E. Comparative metabolomic assessment of bioconverted traditional and black wheat via the in vitro piglet digestion model. Sci Rep 2026 April 15.
74. Bartkiene E, Bartkevics V, Pugajeva I, Krungleviciute V, Mayrhofer S, Domig K. The contribution of P. acidilactici, L. plantarum, and L. curvatus starters and L-(+)-lactic acid to the acrylamide content and quality parameters of mixed rye - Wheat bread. Food science & technology 2017 Jul 1;80:43–50.
75. Bartkiene E, Starkute V, Zokaityte E, Klupsaite D, Mockus E, Bartkevics V, Borisova A, Gruzauskas R, Liatukas Ž, Ruzgas V. Comparison Study of Nontreated and Fermented Wheat Varieties 'Ada', 'Sarta', and New Breed Blue and Purple Wheat Lines Wholemeal Flour. Biology (Basel, Switzerland) 2022 Jun 27;11(7):966.
76. Mockus E, Zokaityte E, Starkute V, Klupsaite D, Ruibys R, Rocha JM, Bartkevics V, Bartkiene E. Influence of different lactic acid bacteria strains and milling process on the solid-state fermented green and red lentils (Lens culinaris L.) properties including gamma-aminobutyric acid formation. Frontiers in nutrition (Lausanne) 2023 Apr 13;10:1118710.
77. Mockus E, Starkute V, Zokaityte E, Klupsaite D, Bartkevics V, Borisova A, Rocha JM, Ruibys R, Liatukas Z, Ruzgas V, Bartkiene E. The Potential of Traditional 'Gaja' and New Breed Lines of Waxy, Blue and Purple Wheat in Wholemeal Flour Fermentation. Fermentation (Basel) 2022 Oct 1;8(10):563.
78. Mockus E, Starkute V, Klupsaite D, Bartkevics V, Borisova A, Sarunaite L, Arlauskiene A, Rocha JM, Bartkiene E. Changes in chemical composition of lentils, including gamma-aminobutyric acid and volatile compound formation during submerged and solid-state fermentation with Pediococcus acidilactici. Foods 2024;13(8):1249.
79. Elena Bartkiene, Vadims Bartkevics, Laura Elīna Ikkere, Iveta Pugajeva, Paulina Zavistanaviciute, Vita Lele, Modestas Ruzauskas, Jurga Bernatoniene, Valdas Jakstas, Dovile Klupsaite, Daiva Zadeike, Pranas Viskelis, Grazina Juodeikiene. The effects of ultrasonication, fermentation with Lactobacillus sp., and dehydration on the chemical composition and microbial contamination of bovine colostrum. Journal of Dairy Science 2018 Aug;101(8):6787–6798.
80. Lin W, Hwang C, Chen L, Tsen H. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. Food Microbiol 2006;23(1):74–81.
81. Stanzer D, Ivanuša I, Kazazić S, Hanousek Čiča K, Mrvčić J. Diversity of lactic acid bacteria on organic flours and application of isolates in sourdough fermentation. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam 2017;12(1-2):44–51.
82. Jukonyte R, Zadeike D, Bartkiene E, Lele V, Cernauskas D, Suproniene S, Juodeikiene G. A potential of brown rice polish as a substrate for the lactic acid and bioactive compounds production by the lactic acid bacteria newly isolated from cereal-based fermented products. LWT 2018;97:323–331.

83. De Vuyst L, Van Kerrebroeck S, Harth H, Huys G, Daniel H-, Weckx S. Microbial ecology of sourdough fermentations: Diverse or uniform? Food Microbiol 2014;37:11–29.
84. Chavan RS, Chavan SR. Sourdough Technology-A Traditional Way for Wholesome Foods: A Review. Comprehensive Reviews in Food Science and Food Safety 2011 May;10(3):169–182.
85. Capuani A, Behr J, Vogel RF. Influence of lactic acid bacteria on the oxidation–reduction potential of buckwheat (Fagopyrum esculentum Moench) sourdoughs. Eur Food Res Technol 2012 Dec 1;235(6):1063–1069.
86. Kidoń M, Grabowska J. Bioactive compounds, antioxidant activity, and sensory qualities of red-fleshed apples dried by different methods. LWT 2021;136:110302.
87. K. B. Biji, C. N. Ravishankar, R. Venkateswarlu, C. O. Mohan, T. K. Srinivasa Gopal. Biogenic amines in seafood: a review. Journal of Food Science and Technology 2016 May;53(5):2210–2218.
88. Apetrei IM, Apetrei C. Amperometric biosensor based on diamine oxidase/platinum nanoparticles/graphene/chitosan modified screen-printed carbon electrode for histamine detection. Sensors 2016;16(4):422.
89. Özogul F, Hamed I. The importance of lactic acid bacteria for the prevention of bacterial growth and their biogenic amines formation: A review. Critical reviews in food science and nutrition 2018 Jul 3;58(10):1660–1670.
90. Engels W, Siu J, van Schalkwijk S, Wesselink W, Jacobs S, Bachmann H. Metabolic Conversions by Lactic Acid Bacteria during Plant Protein Fermentations. Foods 2022 Mar 29;11(7):1005.
91. Narducci V, Finotti E, Galli V, Carcea M. Lipids and fatty acids in Italian durum wheat (Triticum durum Desf.) cultivars. Foods 2019;8(6):223.
92. Sissons M. Durum wheat chemistry and technology. : Academic Press; 2016.
93. Mandrioli M, Poggi GM, Cai G, Faleri C, Maccaferri M, Tuberosa R, Aloisi I, Toschi TG, Corneti S. Lipids and Fatty Acid Composition Reveal Differences between Durum Wheat Landraces and Modern Cultivars. Plants (Basel) 2024 Jul 1;13(13):1817.
94. Aung T, Park S, Kim M. Influence of Lactobacillus (LAB) fermentation on the enhancement of branched chain amino acids and antioxidant properties in bran among wheat by-products. Fermentation 2022;8(12):732.
95. Szuba-Trznadel A, Gałka B, Kamińska J, Jama-Rodzeńska A, Król Z, Jarki D, Fuchs B. Diversity of chemical composition and nutritional value in grain from selected winter wheat cultivars grown in south-western Poland. Sci Rep 2024;14(1):2630.
96. Zhang M, Ma D, Wang C, Zhao H, Zhu Y, Guo T. Responses of amino acid composition to nitrogen application in high-and low-protein wheat cultivars at two planting environments. Crop Sci 2016;56(3):1277–1287.
97. Ohmori T, Tahara M, Ohshima T. Mechanism of gamma-aminobutyric acid (GABA) production by a lactic acid bacterium in yogurt-sake. Process Biochemistry 2018;74:21–27.
98. Kim M, Kim K. Isolation and identification of γ-aminobutyric acid (GABA)-producing lactic acid bacteria from Kimchi. Journal of the Korean Society for Applied Biological Chemistry 2012;55(6):777–785.
99. Pannerchelvan S, Rios-Solis L, Wong FWF, Zaidan UH, Wasoh H, Mohamed MS, Tan JS, Mohamad R, Halim M. Strategies for improvement of gamma-aminobutyric acid (GABA) biosynthesis via lactic acid bacteria (LAB) fermentation. Food & Function 2023;14(9):3929–3948.
100. Tang EN, Ndindeng SA, Onaga G, Ortega-Beltran A, Falade TD, Djouaka R, Frei M. Mycotoxin concentrations in rice are affected by chalkiness, grain shape, processing type, and grain origin. Mycotoxin Res 2025;41(1):163–177.

101. Dalié DKD, Deschamps AM, Richard-Forget F. Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. Food Control 2010;21(4):370–380.
102. Lancetti R, Sciarini L, Pérez GT, Salvucci E. Technological performance and selection of lactic acid bacteria isolated from argentinian grains as starters for wheat sourdough. Curr Microbiol 2021;78(1):255–264.
103. Foss K, Starowicz M, Kłębukowska L, Sawicki T. Effect of lactic acid fermentation of red beetroot juice on volatile compounds profile and content. Eur Food Res Technol 2023 Sep;249(9):2401–2418.
104. Wang Y, Wang H, Wu Y, Xiang H, Zhao Y, Chen S, Qi B, Li L. Insights into lipid oxidation and free fatty acid profiles to the development of volatile organic compounds in traditional fermented golden pomfret based on multivariate analysis. Lwt 2022;171:114112.
105. Kaseleht K, Paalme T, Mihhalevski A, Sarand I. Analysis of volatile compounds produced by different species of lactobacilli in rye sourdough using multiple headspace extraction. International Journal of Food Science and Technology 2011;46(9):1940–1946.
106. Helinck S, Le Bars D, Moreau D, Yvon M. Ability of thermophilic lactic acid bacteria to produce aroma compounds from amino acids. Appl Environ Microbiol 2004;70(7):3855–3861.
107. Niyigaba T, Küçükgöz K, Kołożyn-Krajewska D, Królikowski T, Trząskowska M. Advances in Fermentation Technology: A Focus on Health and Safety. Applied sciences 2025 Mar 1;15(6):3001.
108. Dujardin M, Elain A, Lendormi T, Le Fellic M, Le Treut Y, Sire O. Keeping under control a liquid feed fermentation process for pigs: A reality scale pilot based study. Anim Feed Sci Technol 2014;194:81–88.
109. Chen K, Gao C, Han X, Li D, Wang H, Lu F. Co-fermentation of lentils using lactic acid bacteria and Bacillus subtilis natto increases functional and antioxidant components. J Food Sci 2021;86(2):475–483.
110. Zhang B, Deng Z, Tang Y, Chen P, Liu R, Ramdath DD, Liu Q, Hernandez M, Tsao R. Fatty acid, carotenoid and tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities. Food Chem 2014;161:296–304.
111. Zhang B, Peng H, Deng Z, Tsao R. Phytochemicals of lentil (Lens culinaris) and their antioxidant and anti-inflammatory effects. Journal of Food Bioactives 2018;1:93–103.
112. Liu QH, Shao T, Bai YF. The effect of fibrolytic enzyme, Lactobacillus plantarum and two food antioxidants on the fermentation quality, alpha-tocopherol and beta-carotene of high moisture napier grass silage ensiled at different temperatures. Anim Feed Sci Technol 2016;221:1–11.
113. Antognoni F, Mandrioli R, Potente G, Taneyo Saa DL, Gianotti A. Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. Food Chem 2019;292:211–216.
114. Osman MA. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. Journal of the Saudi Society of Agricultural Sciences 2011;10(1):1–6.
115. Pranoto Y, Anggrahini S, Efendi Z. Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. Food Bioscience 2013;2:46–52.
116. Li B, Lu S. The Importance of Amine-degrading Enzymes on the Biogenic Amine Degradation in Fermented Foods: A review. Process Biochemistry 2020;99:331–339.
117. Ma X, Wang Y, Liu Y, Li X, Wang F, Huang Y, Shi P, Brennan CS, Wang M. Mechanisms and factors influencing the ability of lactic acid bacteria on reducing biogenic amines in fermented food: A mini review. LWT 2024;197:115890.

118. Lastras C, Revilla I, González-Martín MI, Vivar-Quintana AM. Prediction of fatty acid and mineral composition of lentils using near infrared spectroscopy. Journal of Food Composition and Analysis 2021;102:104023.
119. Khrisanapant P, Kebede B, Leong SY, Oey I. A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. Foods 2019;8(12):651.
120. Paucean A, Moldovan OP, Mureșan V, Socaci SA, Dulf FV, Alexa E, Man SM, Mureșan AE, Muste S. Folic acid, minerals, amino-acids, fatty acids and volatile compounds of green and red lentils. Folic acid content optimization in wheat-lentils composite flours. Chemistry Central Journal 2018;12(1):88.
121. Azarnia S, Boye JI. Flavour compounds in legumes: Chemical and sensory aspects. Progress in Food Science and Technology 2011;1:1–34.
122. Byanju B, Hojilla-Evangelista MP, Lamsal BP. Fermentation performance and nutritional assessment of physically processed lentil and green pea flour. J Sci Food Agric 2021;101(14):5792–5806.
123. De Pasquale I, Pontonio E, Gobbetti M, Rizzello CG. Nutritional and functional effects of the lactic acid bacteria fermentation on gelatinized legume flours. Int J Food Microbiol 2020;316:108426.
124. Leonard W, Zhang P, Ying D, Adhikari B, Fang Z. Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. Biotechnol Adv 2021;49:107763.
125. Mapelli-Brahm P, Barba FJ, Remize F, Garcia C, Fessard A, Mousavi Khaneghah A, Sant'Ana AS, Lorenzo JM, Montesano D, Meléndez-Martínez AJ. The impact of fermentation processes on the production, retention and bioavailability of carotenoids: An overview. Trends Food Sci Technol 2020;99:389–401.
126. Yang F, Chen C, Ni D, Yang Y, Tian J, Li Y, Chen S, Ye X, Wang L. Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. Foods 2023;12(17):3315.
127. Shimizu K, Matsuoka Y. Feedback regulation and coordination of the main metabolism for bacterial growth and metabolic engineering for amino acid fermentation. Biotechnol Adv 2022;55:107887.
128. Emkani M, Oliete B, Saurel R. Effect of lactic acid fermentation on legume protein properties, a review. Fermentation 2022;8(6):244.
129. Toe CJ, Foo HL, Loh TC, Mohamad R, Abdul Rahim R, Idrus Z. Extracellular proteolytic activity and amino acid production by lactic acid bacteria isolated from Malaysian foods. International journal of molecular sciences 2019;20(7):1777.
130. Chilakamarry CR, Mimi Sakinah AM, Zularisam AW, Sirohi R, Khilji IA, Ahmad N, Pandey A. Advances in solid-state fermentation for bioconversion of agricultural wastes to value-added products: Opportunities and challenges. Bioresour Technol 2022;343:126065.
131. Pandey A. Solid-state fermentation. Biochem Eng J 2003;13(2):81–84.
132. Kumar V, Ahluwalia V, Saran S, Kumar J, Patel AK, Singhania RR. Recent developments on solid-state fermentation for production of microbial secondary metabolites: Challenges and solutions. Bioresour Technol 2021;323:124566.
133. Fernández M, Zúñiga M. Amino acid catabolic pathways of lactic acid bacteria. Crit Rev Microbiol 2006;32(3):155–183.
134. Barbieri F, Montanari C, Gardini F, Tabanelli G. Biogenic amine production by lactic acid bacteria: A review. Foods 2019;8(1):17.
135. Yogeswara IBA, Maneerat S, Haltrich D. Glutamate decarboxylase from lactic acid bacteria – A key enzyme in GABA synthesis. Microorganisms 2020;8(12):1923.
136. Benkerroum N. Biogenic amines in dairy products: origin, incidence, and control means. Comprehensive Reviews in Food Science and Food Safety 2016;15(4):801–826.

137. Kalač P, Krausová P. A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. Food Chem 2005;90(1):219–230.
138. Vasquez R, Oh JK, Song JH, Kang D. Gut microbiome-produced metabolites in pigs: a review on their biological functions and the influence of probiotics. Journal of Animal Science and Technology 2022;64(4):671.
139. Sudo N. Biogenic amines: signals between commensal microbiota and gut physiology. Frontiers in endocrinology 2019;10:504.
140. Carnino BB, Muro BB, Carnevale RF, Coelho FA, da Silva CA, Andretta I, Millet S, Garbossa CA. Feeding weanling piglets for optimal health and performance: what can we learn from research on complex diets? Nutrition Research Reviews 2025;38(2):1–70.
141. Ma Y, Han X, Fang J, Jiang H. Role of dietary amino acids and microbial metabolites in the regulation of pig intestinal health. Animal Nutrition 2022;9:1–6.
142. Barnes DM, Kirby YK, Oliver KG. Effects of Biogenic Amines on Growth and The Incidence of Proventricular Lesions in Broiler Chickens1. Poult Sci 2001;80(7):906–911.
143. Wójcik W, Łukasiewicz-Mierzejewska M, Damaziak K, Bień D. Biogenic amines in poultry meat and poultry products: formation, appearance, and methods of reduction. Animals 2022;12(12):1577.
144. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of biogenic amines in food – existing and emerging approaches. J Food Sci 2010;75(7):R139–R150.
145. Costa MP, Rodrigues BL, Frasao BS, Conte-Junior CA. Chapter 2 - Biogenic Amines as Food Quality Index and Chemical Risk for Human Consumption. In: Holban AM, Grumezescu AM, editors. Food Quality: Balancing Health and Disease: Academic Press; 2018. p. 75–108.
146. Zaeem M, Nadeem M, Pham TH, Ashiq W, Ali W, Gillani SSM, Moise E, Elavarthi S, Kavanagh V, Cheema M, Galagedara L, Thomas R. Corn-soybean intercropping improved the nutritional quality of forage cultivated on Podzols in boreal climate. Plants 2021;10(5):1015.
147. Adebo JA, Njobeh PB, Gbashi S, Oyedeji AB, Ogundele OM, Oyeyinka SA, Adebo OA. Fermentation of cereals and legumes: Impact on nutritional constituents and nutrient bioavailability. Fermentation 2022;8(2):63.
148. Ayllón-Parra N, Castellari M, Gou P, Ribas-Agustí A. Effects of solid-state fermentation with Pleurotus ostreatus on the nutritional and techno-functional properties of alternative protein ingredients. Food Chem 2025;490:145090.
149. Türk Z. Effects of different harvesting times on protein content and mineral nutrient values of some lentil (Lens culinaris) cultivars seeds. J Plant Nutr 2020;43(4):563–577.
150. Benayad A, Aboussaleh Y. Mineral composition of lentils: Physiological functions, antinutritional effects, and bioavailability enhancement. J Food Qual 2021;2021(1):5515654.
151. Musa EM, Elsheikh EA, Mohamed Ahmed IA, Babiker EE. Intercropping sorghum (Sorghum bicolor L.) and cowpea (Vigna unguiculata L.): Effect of Bradyrhizobium inoculation and fertilization on minerals composition of sorghum seeds. International Scholarly Research Notices 2012;2012(1):356183.
152. Gunes A, Inal A, Adak MS, Alpaslan M, Bagci EG, Erol T, Pilbeam DJ. Mineral nutrition of wheat, chickpea and lentil as affected by mixed cropping and soil moisture. Nutr Cycling Agroecosyst 2007;78(1):83–96.
153. Rajendran S, Silcock P, Bremer P. Flavour volatiles of fermented vegetable and fruit substrates: A review. Molecules 2023;28(7):3236.

154. Starowicz M, Rostek D, Lenkiewicz M, Yaneva TG, Wronkowska M. Profile of volatile organic compounds (VOCs) produced by selected Lactobacillus strains in buckwheat substrates. J Cereal Sci 2023;109:103588.
155. Pétel C, Onno B, Prost C. Sourdough volatile compounds and their contribution to bread: A review. Trends Food Sci Technol 2017;59:105–123.
156. Ardö Y. Flavour formation by amino acid catabolism. Biotechnol Adv 2006;24(2):238–242.
157. Chen Q, Kong B, Han Q, Xia X, Xu L. The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. LWT 2017;77:389–396.
158. Collins YF, McSweeney PLH, Wilkinson MG. Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. Int Dairy J 2003;13(11):841–866.
159. Latha Ravi J, Ghosh P, Ahmad F, Haque S, Barciela P, Chamorro F, Jorge AOS, Prieto MA, Rana SS. Microbial conversion of vegetable waste for flavor additives via solid-state fermentation: a comprehensive review. Frontiers in Nutrition 2025;12:1445189.
160. Sionek B, Szydłowska A, Trząskowska M, Kołożyn-Krajewska D. The impact of physicochemical conditions on lactic acid bacteria survival in food products. Fermentation 2024;10(6):298.
161. Feng X, Ng K, Ajlouni S, Zhang P, Fang Z. Effect of Solid-State Fermentation on Plant-Sourced Proteins: A Review. Food Rev Int 2024;40(9):2580–2617.
162. Islam S, Miah MAS, Islam MF, Bhuiyan MNI, Tisa KJ, Naim MR. Exploring the effects of spontaneous and solid-state fermentation on the physicochemical, functional and structural properties of whole wheat flour (Triticum aestivum L.). Innovative Food Science & Emerging Technologies 2024;97:103798.
163. Cui Y, Miao K, Niyaphorn S, Qu X. Production of gamma-aminobutyric acid from lactic acid bacteria: A systematic review. International Journal of Molecular Sciences 2020;21(3):995.
164. Reffai YM, Fechtali T. A critical review on the role of lactic acid bacteria in sourdough nutritional quality: Mechanisms, potential, and challenges. Appl Microbiol 2025;5(3):74.
165. Yin Y, Wang J, Yang S, Feng J, Jia F, Zhang C. Protein degradation in wheat sourdough fermentation with Lactobacillus plantarum M616. Interdiscip Sci Comput Life Sci 2015;7(2):205–210.
166. Ayyash M, Johnson SK, Liu S, Mesmari N, Dahmani S, Al Dhaheri AS, Kizhakkayil J. In vitro investigation of bioactivities of solid-state fermented lupin, quinoa and wheat using Lactobacillus spp. Food Chem 2019;275:50–58.
167. Zhao J, Tang S, Zhou X, Dong W, Zhang S, Huang C. Determination of chemical composition, energy content, and amino acid digestibility in different wheat cultivars fed to growing pigs. J Anim Sci 2019;97(2):714–726.
168. García-Pérez P, Giuberti G, Sestili F, Lafiandra D, Botticella E, Lucini L. The functional implications of high-amylose wholegrain wheat flours: An in vitro digestion and fermentation approach combined with metabolomics. Food Chem 2023;418:135959.
169. Rowan AM, Moughan PJ, Wilson MN, Maher K, Tasman-Jones C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. Br J Nutr 1994;71(1):29–42.
170. Kiarie E, Slominski BA, Nyachoti CM. Tissue fatty acid profiles, plasma biochemical characteristics and cecal biogenic amines in piglets fed diets containing flaxseed and carbohydrase enzymes. Livestock Science 2009;121(1):1–6.
171. Tan B, Xiao D, Wang J, Tan B. The Roles of Polyamines in Intestinal Development and Function in Piglets. Animals (Basel) 2024 Apr 1;14(8):1228.

172. Martin-Gallausiaux C, Marinelli L, Blottière HM, Larraufie P, Lapaque N. SCFA: mechanisms and functional importance in the gut. Proc Nutr Soc 2021;80(1):37–49.
173. Gojda J, Cahova M. Gut microbiota as the link between elevated BCAA serum levels and insulin resistance. Biomolecules 2021;11(10):1414.
174. Bourdeau-Julien I, Castonguay-Paradis S, Rochefort G, Perron J, Lamarche B, Flamand N, Di Marzo V, Veilleux A, Raymond F. The diet rapidly and differentially affects the gut microbiota and host lipid mediators in a healthy population. Microbiome 2023;11(1):26.
175. Fu W, Chi Z, Ma Z, Zhou H, Liu G, Lee C, Chi Z. Hydrocarbons, the advanced biofuels produced by different organisms, the evidence that alkanes in petroleum can be renewable. Appl Microbiol Biotechnol 2015;99(18):7481–7494.
176. Choi YJ, Lee SY. Microbial production of short-chain alkanes. Nature 2013;502(7472):571–574.
177. Chang X, Xiang H, Wu S, Fan L, Fang Y, Zhong R. Gut Microbiota and Lipid Metabolism: Impacts on Lamb Flavor Precursors. Comprehensive Reviews in Food Science and Food Safety 2025;24(6):e70307.
178. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, Paul H. Patterson, Sarkis K. Mazmanian. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155(7):1451–1463.
179. Zhang Y, Xu X, Fan X, Wu Y, Zhang X, Wu Y, Li X, Zou Q. Unraveling p-Cresol: from biosynthesis to biological and biochemical activities. Frontiers in Pharmacology 2025;16:1665421.
180. Kridelbaugh D, Hughes S, Allen T, Doerner KC. Production of 4-ethylphenol from 4-hydroxycinnamic acid by Lactobacillus sp. isolated from a swine waste lagoon. J Appl Microbiol 2010;109(1):190–198.
181. Day F, O'Sullivan J, Pook C. 4-Ethylphenol – fluxes, metabolism and excretion of a gut microbiome derived neuromodulator implicated in autism. Frontiers in Molecular Biosciences 2023;10:1267754.
182. Nowak A, Libudzisz Z. Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. Anaerobe 2006;12(2):80–84.
183. Xiong X, Liu D, Wang Y, Zeng T, Peng Y. Urinary 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-hydroxyphenylacetic acid, and 3-Hydroxyhippuric acid are elevated in children with autism spectrum disorders. BioMed research international 2016;2016(1):9485412.
184. Lambert MA, Moss CW. Production of p-hydroxyhydrocinnamic acid from tyrosine by Peptostreptococcus anaerobius. J Clin Microbiol 1980;12(2):291–293.
185. Chen L, Yang P, Hu L, Yang L, Chu H, Hou X. Modulating phenylalanine metabolism by L. acidophilus alleviates alcohol-related liver disease through enhancing intestinal barrier function. Cell Biosci 2023;13(1):24.
186. Zhu Y, Dwidar M, Nemet I, Buffa JA, Sangwan N, Li XS, Anderson JT, Romano KA, Fu X, Funabashi M, Zeneng Wang, Pooja Keranahalli, Shawna Battle, Aaron N. Tittle, Adeline M. Hajjar, Valentin Gogonea, Michael A. Fischbach, Joseph A. DiDonato, Stanley L. Hazen. Two distinct gut microbial pathways contribute to meta-organismal production of phenylacetylglutamine with links to cardiovascular disease. Cell host & microbe 2023;31(1):18–32. e9.
187. Vollmer M, Schröter D, Esders S, Neugart S, Farquharson FM, Duncan SH, Schreiner M, Louis P, Maul R, Rohn S. Chlorogenic acid versus amaranth's caffeoylisocitric acid – Gut microbial degradation of caffeic acid derivatives. Food Res Int 2017;100:375–384.

188. Leonard W, Zhang P, Ying D, Fang Z. Hydroxycinnamic acids on gut microbiota and health. Comprehensive reviews in food science and food safety 2021;20(1):710–737.
189. Koistinen VM, Hedberg M, Shi L, Johansson A, Savolainen O, Lehtonen M, Aura A, Hanhineva K, Landberg R. Metabolite Pattern Derived from Lactiplantibacillus plantarum – Fermented Rye Foods and In Vitro Gut Fermentation Synergistically Inhibits Bacterial Growth. Molecular Nutrition & Food Research 2022;66(21):2101096.
190. Kim D, Xu H, Li O, Xue M, Bao Z, Yang F. Phenyllactic acid modulates the gut microbiota, enhances intestinal health, and alleviates physical frailty in aging mice. Eur J Pharmacol 2024;985:177105.
191. Ezeji T, Milne C, Price ND, Blaschek HP. Achievements and perspectives to overcome the poor solvent resistance in acetone and butanol-producing microorganisms. Appl Microbiol Biotechnol 2010;85(6):1697–1712.
192. Datta R, Zeikus J. Modulation of acetone-butanol-ethanol fermentation by carbon monoxide and organic acids. Appl Environ Microbiol 1985;49(3):522–529.
193. Du J, Zhang Z, Tan L, Yang J, Yang R, Chen Y, Tan G, Li J, Li W, Yang L, Cai J, Shen D, Zhu H, Fan Z, Yuan M, Zhang W. Gut microbiota dysbiosis and metabolic perturbations of bile/glyceric acids in major depressive disorder with IBS comorbidity. Mbio 2025;16(11):2447.
194. Abedi E, Hashemi SMB. Lactic acid production–producing microorganisms and substrates sources-state of art. Heliyon 2020;6(10):e04974.
195. Starke S, Harris DM, Zimmermann J, Schuchardt S, Oumari M, Frank D, Bang C, Rosenstiel P, Schreiber S, Frey N, Franke A, Aden K, Waschina S. Amino acid auxotrophies in human gut bacteria are linked to higher microbiome diversity and long-term stability. ISME J 2023;17(12):2370–2380.
196. Knorr S, Sinn M, Galetskiy D, Williams RM, Wang C, Müller N, Mayans O, Schleheck D, Hartig JS. Widespread bacterial lysine degradation proceeding via glutarate and L-2-hydroxyglutarate. Nat Commun 2018;9(1):5071.

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The Potential of Traditional 'Gaja' and New Breed Lines of Waxy, Blue and Purple Wheat in Wholemeal Flour Fermentation

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Abstract: The aim of this study was to analyse and compare the acidity, microbiological and colour characteristics, fatty (FA) and amino (AA) acid profiles, biogenic amine (BA) and gamma-aminobutyric acid (GABA) concentrations, and macro- and microelement contents in non-treated (non-fermented) and fermented wholemeal cereal flours of 'Gaja' (traditional wheat) and new breed lines DS8888-3-6 (waxy wheat), DS8548-7 (blue wheat) and DS8535-2 (purple wheat). Independent fermentations were undertaken with selected strains of *Pediococcus acidilactici*, *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum*. The results revealed that all the wholemeal cereal flours of the analysed wheat varieties are suitable for fermentation with the selected strains because all the fermented samples showed lactic acid bacteria (LAB) viable counts higher than 8.00 $\log_{10}$ CFU/g and desirable low pH values. In most of the cases, fermentation increased the concentration of essential amino acids in the wholemeal cereal samples, and the LAB strain used for fermentation proved to be a significant factor in all the essential amino acid content of wholemeal wheat ($p \leq 0.0001$). When comparing the non-fermented samples, the highest GABA content was found in 'Gaja' and waxy wheat samples (2.47 µmol/g, on average), and, in all the cases, fermentation significantly increased GABA concentration in the wholemeal cereals. On the other hand, total levels of biogenic amines in wholemeal samples ranged from 22.7 to 416 mg/kg. The wheat variety was a significant factor in all the analysed macro- and microelement contents ($p \leq 0.0001$) in the wholemeal cereals. Furthermore, fermentation showed to be a significant factor in most of the FA content of the wholemeal cereal samples. Finally, fermentation can also contribute to improving the biological and functional value of wholemeal wheat flours (by increasing essential amino acids and GABA concentrations); however, safety parameters (e.g., biogenic amines) also should be taken into consideration when optimizing the most appropriate technological parameters.

Keywords: waxy wheat; purple wheat; blue wheat; fermentation; lactic acid bacteria; chemical composition

1. Introduction

Nowadays, consumers, as well as food and livestock producers, are looking for higher-value foodstuffs to meet the requirements of a balanced diet for both human and animal nutrition. Cereal grains are the most important food and feed material around the world due to their possible long-term storage, relatively easy transportation and concentrated nutritional and energy value. In addition to the most traditional cereals, progress in the development of new cereal varieties can be seen often, and, as a result, new and higher-value cereal grains are suggested for consumption. However, despite new varieties or hybrid lines that may show good chemical composition and a higher content of bioactive and functional compounds, data concerning its changes during the fermentation process are scarce, which is the main technology used in the cereal industry for both food and feed processing.

Additionally, to the chemical composition evaluation of new breed lines of wheat cereals, we hypothesized that different wheat varieties (traditional, waxy, blue, purple) may constitute a significant factor in the chemical composition of fermented cereal flours, including fatty and amino acid profiles, biogenic amines (BA) and gamma-aminobutyric acid (GABA) formation. Taking into consideration that lactic acid bacteria (LAB), as technological microorganisms used for cereal flour fermentation, are sensitive to environment conditions (including the nutrient composition of fermentable substrates), fermentation of different cereal grain varieties is very likely to lead to different final compositions of the fermented substrate/matrix. Moreover, in addition to the desirable microbial metabolites (e.g., organic acids and free amino acids), toxic compounds can be formed, such as biogenic amines. Finally, experimental data on the chemical composition of new wheat grain varieties (non-fermented and fermented flours with different LAB strains) can be important for their better application in the food and feed industries.

Waxy wheat was developed in 1995 [1]. Its unique properties, including the quantity of amylose, alter the texture parameters, stability and viscosity of the resulting food products [2–5]. Waxy starch has a higher swelling capacity in comparison with common starch [6]. Moreover, waxy wheat starch can be used as a thickener in the food and feed industry [7]. It was reported that the supplementation of waxy wheat flour with traditional ones can improve the quality and sensory properties of quick-frozen products [8]. The low amylose concentration of waxy wheat grain leads to better quality bread, including a longer shelf-life [9]. Additionally, the supplementation of waxy wheat flour to the main bread formula leads to a better flavour of the products [10]. Taking into consideration specific properties of the waxy wheat starch, we hypothesized that these characteristics could have a greater influence on LAB activity and viability, as well as on the metabolites released by them to the fermentable substrate.

Along with waxy wheat, coloured wheat grain has recently gained important attention from researchers and technologists [11–14]. The purple colour of wheat grain is explained by the presence of anthocyanin in the pericarp, and the blue colour in the aleurone [15]. The grain of coloured wheat has shown anti-inflammatory, antimicrobial and other desirable biological activities for human and animal nutrition [11,13,14,16–19]. However, presently, the data about the chemical composition of coloured wheat are scarce [20]. Furthermore, changes in the chemical composition of coloured wheat grains during the fermentation process was not reported so far. Taking into consideration specific compounds of the coloured wheat, which can induce inhibition of microorganisms, we hypothesized that differences can be attained in coloured wheat fermentation in comparison with traditional ('Gaja') and waxy wheat.

However, the chemical composition of the main substrate (i.e., wholemeal wheat grain) and the microorganisms used for fermentation are key factors. In this study, three LAB strains were selected for wholemeal wheat fermentation because of their proven good technological properties [21,22], desirable antimicrobial and antifungal characteristics [23], as well as a positive influence in vivo on piglets microbiota and other health parameters [24].

The aim of this study was to analyse and compare the acidity, microbiological and colour characteristics, fatty (FA) and amino (AA acid) profiles, BA and GABA concentration, macro- and microelement contents in non-treated (non-fermented or raw material) and fermented wholemeal cereal flours of 'Gaja' (traditional wheat) and new breed lines of DS8888-3-6 (waxy wheat), DS8548-7 (blue wheat) and DS8535-2 (purple wheat), and using three LAB strains, *viz. Pediococcus acidilactici*, *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum* strains. This study will also contribute to the creation of a database on the chemical composition of the new breed lines of wheat grains.

2. Materials and Methods

2.1. Wheat Varieties Used in the Experiments

The schematic representation of the experimental design is given in Figure 1. The grains of wheat varieties 'Gaja' (traditional wheat) and new breed lines DS8888-3-6 (waxy wheat), DS8548-7 (blue wheat) and DS8535-2 (purple wheat) were provided by the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (LAMMC, Akademija, Kėdainiai distr., Lithuania). Field trials were conducted during 2020–2021 at the experimental base of the LAMMC in Akademija, Kėdainiai distr., Lithuania (55°39′ N 23°57′ E). Field experiment description in detail is given in Supplementary File S1.

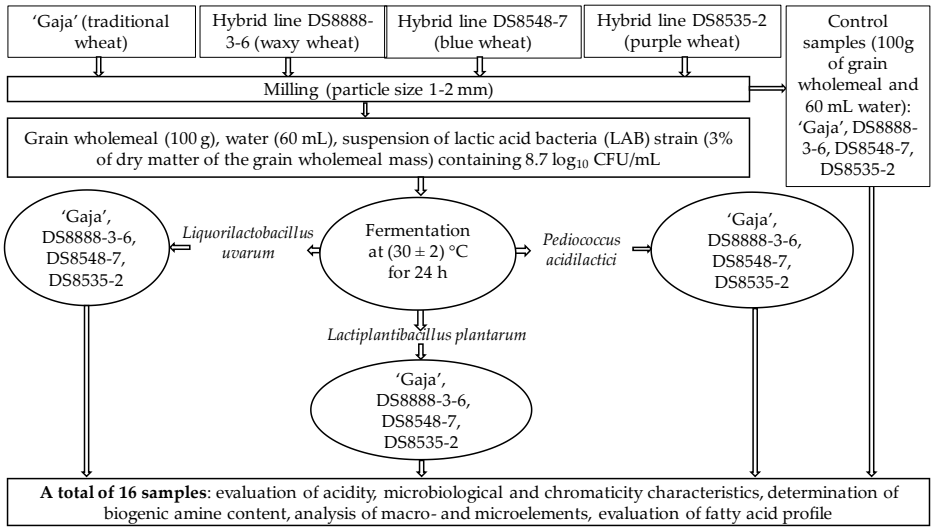

Figure 1. Experimental design of this study.

Wheat grains (moisture content of the wheat grain was 14%) were milled with Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particle size was 1–2 mm. The moisture content of the grain was determined by using Infratec™ 1241 Grain Analyser (FOSS, Foss Allé DK-3400, Hilleroed, Denmark).

2.2. Lactic Acid Bacteria Strains (LAB) Used in the Experiments and Wholemeal Wheat Flour Fermentation

The LAB strains *Pediococcus acidilactici*, *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum* were used for wholemeal wheat flour fermentation. The LAB strains, possessing antibacterial and antifungal properties [23] as well as good technological characteristics for wheat bread preparation [25,26], were obtained from the Lithuanian University of

Health Sciences (Kaunas, Lithuania). Before the experiments, the LAB strains stored at −80 °C (Microbank system, Pro-Lab Diagnostics, Birkenhead, UK) were activated and multiplied in de Man–Rogosa–Sharpe (MRS) broth (Oxoid Ltd., Hampshire, UK) at (30 ± 2) °C for 24 h, under aerobiosis, before use in the wholemeal cereal flour fermentation. The wholemeal wheat flours, water and a suspension of LAB strain (3% of dry matter of the wholemeal flour) containing 8.7 $\log_{10}$ CFU/mL were fermented at (30 ± 2) °C for 24 h. For 100 g of wholemeal wheat flour, 60 mL of water was added and mixed. Non-fermented wholemeal samples were analysed as a control. The analysis of total titratable acidity, pH, microbiological and colour characteristics was performed directly after fermentation, while the rest of samples were vacuum packed and frozen at –18 °C until further analysis of other compounds.

2.3. Evaluation of Total Titratable Acidity (TTA), pH, Microbiological and Colour Characteristics of Wholemeal Wheat Flour Samples

The total titratable acidity (TTA) was evaluated for a 10 g wholemeal wheat flour mixed with 90 mL of water. Results were expressed as the volume in mL of 0.1 mol/L NaOH solution required to achieve a pH value of 8.2. The pH of wholemeal samples was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany). The LAB viable count was determined according to the method described by Bartkiene et al. [22]. The colour characteristics of wholemeal samples were evaluated on the sample surface using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Tokyo, Japan). The results were expressed as the CIE colour values, i.e., L* (brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness).

2.4. Analysis of the Amino Acids (AA) and Gamma-Aminobutyric Acid (GABA) of Wholemeal Wheat Flour Samples

Sample preparation and dansylation were performed according to the method of Hua-Lin Cai et al. [27], with some modifications, which are given in Supplementary File S2.

2.5. Analysis of the Biogenic Amine (BA) Content of Wholemeal Wheat Flour Samples

The extraction and determination of BA in non-fermented and fermented wholemeal wheat flour followed the procedures developed by Ben-Gigirey et al. [28]. Detailed description of the method is given in Supplementary File S3.

2.6. Analysis of Macro- and Microelements Content of Wholemeal Wheat Flour Samples

For macro- and microelement analysis in wholemeal wheat flour samples, an Agilent 7700x ICP-MS (Agilent Technologies, Hachioji, Japan) and software Mass Hunter Work Station for ICP-MS, version B.01.01 (Agilent Technologies, Hachioji, Japan) were used. Description of the method in detail is given in Supplementary File S4.

2.7. Fatty Acid (FA) Composition Analysis of Wholemeal Wheat Flour Samples

The fatty acid (FA) composition of the wholemeal wheat grain samples was determined using GC-MS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph with a mass spectrometer. The fatty acid methyl esters (FAME) concentration was determined using a prepared calibration curve, and results were expressed as the percentage of total FAME concentration in the sample. Description of the method in detail is given in Supplementary File S5.

2.8. Statistical Analysis

Microbiological results were expressed as the mean (n = 5) ± standard error (SE), and physicochemical results were expressed as the mean (n = 3) ± standard error (SE). The experiment was performed by preparing three parallel samples (/replicates) for fermentation. In order to evaluate the effects of the different wheat varieties and different LAB strains used for wheat grain wholemeal fermentation, the data were analysed by multivariate analysis of variance (ANOVA). Shapiro–Wilk Test was used to determine the normality, whereas for homoscedasticity evaluation, the homoscedasticity test was performed by

using SPSS Statistical Package. The linear Pearson's correlation coefficients were calculated using the statistical package SPSS for Windows [version 28.0.1.0 (142), SPSS, Chicago, IL, USA]. Correlation level interpretation was performed in accordance with the procedure by Evans [29]. The results were recognized as statistically significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Acidity, Microbiological and Chromaticity Characteristics of Wholemeal Wheat Flour Samples

The pH, total titratable acidity (TTA), colour and microbiological characteristics of the wheat grain wholemeal are shown in Table 1. Significant differences between the control group (non-fermented) and fermented dough samples were found when comparing the acidity, and the lowest pH was achieved with the wholemeal 'Gaja' and DS8535—2 (purple wheat) samples (on average, 6.00). However, higher TTA values were found in 'Gaja' and DS8888-3-6 (waxy wheat) samples (on average, 1.22° N), in comparison with DS8548—7 (blue wheat) and DS8535—2 (purple wheat) (on average, 1.01° N). When comparing the pH of non-treated and fermented samples, in all the fermented groups, the lowest pH was reached in samples fermented with *Pediococcus acidilactici* strain. In addition, most of the latter samples (fermented ones) showed the highest TTA, as expected. The test between the subjects showed that the LAB strain used for fermentation was a significant factor in TTA ($p \leq 0.0001$). Moreover, a moderate positive correlation was established (r = 0.552, $p \leq 0.001$) between the TTA and type of LAB. The concentration of organic acids in fermented wholemeal flours depend on numerous factors, including LAB strain used, processing parameters and flour composition. [30]. However, controlling excessive acidity is one of the major challenges in fermented wheat, especially when they are used as a sourdough for breadmaking. Previous studies reported the influence of the interaction of processing factors on fermented cereal acidity parameters, microorganism growth, volatile compounds and amino acids formation [30]. However, the changes in waxy and coloured wheat grain during the fermentation process were not described. Thus, this study demonstrated that the cereal variety is not a significant factor in the acidity parameters (pH and TTA) of the fermented wholemeal wheat flours when using *Pediococcus acidilactici*, *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum*. It was also possible to conclude that all these LAB strains are suitable for acidic fermentation of the new varieties of wholemeal wheat grains under scrutiny in this research study.

When analysing the colour characteristics of non-fermented and fermented wholemeal wheat samples, with all the tested LAB strains, the fermented 'Gaja' samples showed higher L* (lightness) coordinates in comparison with non-fermented ones. However, opposite tendencies of the waxy, blue and purple wholemeal wheat cereals were found, as in the latter samples, L* decreased after fermentation (except DS8888-3-6 fermented with *Lactiplantibacillus plantarum*). Cereal variety proved to be a significant factor in the L* coordinates of the samples (p = 0.023). Different trends were established when comparing a* (redness) coordinates. After fermentation, a* coordinates showed tendencies to increase in all fermented 'Gaja' samples, in wholemeal waxy wheat fermented with *Pediococcus acidilactici*, wholemeal waxy wheat cereal fermented with *Liquorilactobacillus uvarum* and in wholemeal purple wheat fermented with *Pediococcus acidilactici*. However, fermentation decreased a* coordinates in all the fermented wholemeal blue wheat cereal samples. Comparing the b* (yellowness) coordinates, they were lower in both fermented wholemeal coloured wheat in comparison with non-fermented ones. However, in traditional wheat 'Gaja', fermentation with *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum* increased b* coordinates by 76.5 and 108.7%, respectively. Opposite trends were found in wholemeal waxy wheat samples, as fermentation with *Pediococcus acidilactici* and *Liquorilactobacillus uvarum* decreased b* values by 27.1 and 20.2%, respectively, in comparison with non-fermented ones. Variation in colour components in the cereal grain depends on genetic factors, growing conditions, as well as on technological processes [12]. Yellow C-glycosides of flavones are mainly present in the outer layer of cereal grains. However, carotenoids are located in the endosperm [12]. In addition, there is a great diversity of anthocyanin composition

among various cereals [31], and the stability of these compounds can be influenced by substrate pH, temperature, the presence of ascorbic acid, oxygen and enzymes, among other factors [32]. This study showed that the LAB strain used for fermentation, cereal variety and the interaction between these factors had a significant impact on a* coordinates of wholemeal cereal samples ($p = 0.021$, $p \leq 0.023$ $p \leq 0.001$, respectively), and in most of the cases, fermented samples showed lower redness and yellowness.

Table 1. pH, total titratable acidity (TTA), colour and microbiological characteristics of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	pH	TTA, °N	Colour Coordinates, NBS			LAB Viable Counts, $\log_{10}$ CFU/g
			L*	a*	b*	
Traditional Wheat						
'Gaja'	$6.01 \pm 0.01^{d,A}$	$1.23 \pm 0.11^{a,B}$	$41.94 \pm 0.12^{a,A}$	$4.39 \pm 0.09^{a,C}$	$9.07 \pm 0.11^{a,A}$	$4.54 \pm 0.32^{a,A}$
'Gaja' Pa	$4.44 \pm 0.02^{c,B}$	$4.91 \pm 0.09^{b,A}$	$44.11 \pm 0.14^{b,B}$	$4.86 \pm 0.07^{b,C}$	$9.16 \pm 0.09^{a,C}$	$8.48 \pm 0.18^{b,A,B}$
'Gaja' Li.u	$4.37 \pm 0.02^{b,D}$	$4.90 \pm 0.06^{b,A}$	$52.11 \pm 0.23^{c,D}$	$4.88 \pm 0.06^{b,C}$	$16.01 \pm 0.13^{b,D}$	$8.52 \pm 0.21^{b,A,B}$
'Gaja' La.p	$4.01 \pm 0.01^{a,C}$	$5.00 \pm 0.05^{b,A}$	$54.78 \pm 0.15^{d,C}$	$5.89 \pm 0.08^{c,C}$	$18.93 \pm 0.14^{c,D}$	$8.63 \pm 0.14^{b,A}$
Waxy Wheat						
DS8888-3-6	$6.11 \pm 0.03^{d,B}$	$1.20 \pm 0.03^{a,B}$	$52.29 \pm 0.21^{c,D}$	$3.06 \pm 0.03^{a,A}$	$14.78 \pm 0.22^{c,D}$	$4.19 \pm 0.27^{a,A}$
DS8888-3-6 Pa	$4.09 \pm 0.02^{b,A}$	$5.22 \pm 0.05^{b,B}$	$48.48 \pm 0.35^{a,C}$	$3.36 \pm 0.05^{b,A}$	$10.77 \pm 0.18^{a,D}$	$8.08 \pm 0.14^{b,A}$
DS8888-3-6 Li.u	$4.20 \pm 0.01^{c,B}$	$5.43 \pm 0.04^{c,B}$	$49.06 \pm 0.21^{b,C}$	$3.54 \pm 0.06^{c,B}$	$11.79 \pm 0.21^{b,C}$	$8.48 \pm 0.11^{c,A}$
DS8888-3-6 La.p	$3.99 \pm 0.02^{a,C}$	$5.61 \pm 0.09^{d,B}$	$52.29 \pm 0.19^{c,B}$	$3.06 \pm 0.09^{a,A}$	$14.78 \pm 0.16^{c,C}$	$8.61 \pm 0.16^{c,A}$
Blue Wheat						
DS8548-7	$6.18 \pm 0.02^{d,C}$	$1.02 \pm 0.11^{a,A}$	$49.51 \pm 0.14^{d,C}$	$4.10 \pm 0.05^{d,B}$	$12.68 \pm 0.15^{d,C}$	$4.69 \pm 0.31^{a,A}$
DS8548-7 Pa	$4.13 \pm 0.02^{b,A}$	$5.23 \pm 0.06^{b,B}$	$44.04 \pm 0.23^{b,B}$	$3.76 \pm 0.11^{c,B}$	$7.09 \pm 0.09^{a,B}$	$8.16 \pm 0.23^{b,A}$
DS8548-7 Li.u	$4.27 \pm 0.02^{c,C}$	$5.42 \pm 0.10^{c,B}$	$48.21 \pm 0.17^{c,B}$	$2.93 \pm 0.09^{a,A}$	$8.76 \pm 0.08^{c,B}$	$8.49 \pm 0.16^{b,A}$
DS8548-7 La.p	$3.55 \pm 0.01^{a,A}$	$6.10 \pm 0.14^{d,C}$	$41.69 \pm 0.25^{a,A}$	$3.34 \pm 0.04^{b,B}$	$7.65 \pm 0.11^{b,A}$	$8.94 \pm 0.22^{c,A}$
Purple Wheat						
DS8535-2	$5.98 \pm 0.02^{d,A}$	$1.00 \pm 0.05^{a,A}$	$45.14 \pm 0.16^{d,B}$	$5.93 \pm 0.12^{b,D}$	$11.16 \pm 0.12^{d,B}$	$4.41 \pm 0.13^{a,A}$
DS8535-2 Pa	$4.40 \pm 0.02^{c,B}$	$5.52 \pm 0.07^{b,C}$	$38.68 \pm 0.22^{b,A}$	$6.08 \pm 0.08^{b,D}$	$6.55 \pm 0.07^{b,A}$	$8.74 \pm 0.19^{b,B}$
DS8535-2 Li.u	$3.72 \pm 0.03^{b,A}$	$6.21 \pm 0.05^{c,C}$	$38.00 \pm 0.13^{a,A}$	$5.67 \pm 0.06^{a,D}$	$5.08 \pm 0.08^{a,A}$	$8.92 \pm 0.21^{b,B}$
DS8535-2 La.p	$3.61 \pm 0.01^{a,B}$	$6.30 \pm 0.11^{c,C}$	$41.98 \pm 0.16^{c,A}$	$5.95 \pm 0.09^{b,C}$	$8.10 \pm 0.11^{c,B}$	$8.99 \pm 0.17^{b,A}$

TTA—total titratable acidity; L*—lightness; a*—redness (−a* greenness); b*—yellowness (−b* blueness); LAB—lactic acid bacteria; CFU—colony forming units; Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data of pH, TTA and colour coordinates are represented as means ($n = 3$) ± SE. The data of LAB viable counts are represented as means ($n = 5$) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

Comparing LAB viable counts in fermented wholemeal wheat flours, the purple wheat samples fermented with *Pediococcus acidilactici* and *Liquorilactobacillus uvarum* showed the highest numbers of viable LAB (on average, 8.83 $\log_{10}$ CFU/g). As a matter of fact, technological microorganisms play a major role in developing the characteristics of fermented cereal flours [33]. A moderate positive correlation was established between the LAB viable counts and TTA (r = 0.552, $p \leq 0.001$). In addition, the type of LAB strain used for fermentation was a significant factor on samples TTA ($p \leq 0.0001$). Fermented cereal dough is a stressful ecosystem that is characterized by a low pH, high carbohydrate concentrations, oxygen limitation and viable cell counts of LAB $\geq 10^8$ CFU/g [34]. In this study, all the fermented wholemeal wheat flour samples showed LAB viable counts higher than 8.00 $\log_{10}$ CFU/g, and the type of LAB strain used for fermentation was a significant factor in LAB viable counts of wholemeal fermented flours ($p \leq 0.0001$). Finally, despite the colour intensity of the colored wheats being reduced during fermentation, all the tested wholemeal wheat flour samples showed desirable low pH values and a high number of viable LAB after fermentation.

3.2. Amino Acids (AA), γ-Aminobutyric Acids (GABA) and Biogenic Amines (BA) of Wholemeal Wheat Flour Samples

Essential amino acid concentrations of the wholemeal wheat grain samples are shown in Table 2. When comparing non-fermented samples, no significant differences were found in threonine, methionine, phenylalanine and histidine concentrations. However, in comparison with traditional and waxy wheat samples, higher valine concentration in both colored wholemeal wheat cereals was established (on average, by 14.6% higher). In contrast to these findings, in comparison with both colored wheat, traditional ('Gaja') and waxy wheat samples showed higher concentrations of leucine/isoleucine. Simultaneously, the highest concentration of lysine was found in 'Gaja' samples (0.360 µmol/g). In most of the cases, fermentation increased essential amino acid concentration in wholemeal wheat flour samples, but methionine in DS8888-3-6 La.p and in DS8548-7 La.p, and histidine in 'Gaja'Li.u, DS8888-3-6 La.p. and DS8548-7 La.p. The LAB strain used for fermentation proved to constitute a significant factor on all the essential amino acid concentrations in wholemeal wheat grains ($p \leq 0.0001$). On the other hand, wheat variety was a significant factor on threonine, methionine, valine, lysine and histidine concentrations ($p = 0.002$, $p \leq 0.0001$, $p = 0.002$, $p = 0.034$, $p \leq 0.0001$, respectively), and interaction of these factors was showed to be significant on methionine, valine and histidine contents ($p \leq 0.0001$, $p \leq 0.0001$, and $p \leq 0.01$, respectively).

Table 2. Essential amino acid concentration of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Concentration Essential of Amino Acids in Sample, µmol/g						
	Threonine	Methionine	Valine	Phenylalanine	Leucine/ Isoleucine	Lysine	Histidine
				Traditional Wheat			
'Gaja'	0.460 ± 0.026 ^{a,A}	<0.020	0.385 ± 0.020 ^{a,A}	<0.020	0.185 ± 0.010 ^{a,B,C}	0.360 ± 0.021 ^{a,C}	<0.020
'Gaja' Pa	0.823 ± 0.077 ^{d,C}	0.303 ± 0.014 ^{c,C}	2.43 ± 0.16 ^{d,C}	1.18 ± 0.07 ^{b,C}	5.71 ± 0.22 ^{d,C}	1.51 ± 0.14 ^{d,B}	0.169 ± 0.007 ^{b,C}
'Gaja' Li.u	0.624 ± 0.034 ^{b,A}	0.043 ± 0.003 ^{a,A}	1.95 ± 0.10 ^{c,C}	1.25 ± 0.10 ^{b,C}	4.17 ± 0.25 ^{c,B}	1.16 ± 0.05 ^{c,B}	<0.020
'Gaja' La.p	0.780 ± 0.074 ^{c,A}	0.146 ± 0.008 ^{b,B}	1.21 ± 0.04 ^{b,A}	0.767 ± 0.034 ^{a,B}	2.95 ± 0.17 ^{b,B}	0.839 ± 0.044 ^{b,B}	0.083 ± 0.008 ^{a,A}
				Waxy Wheat			
DS8888-3-6	0.423 ± 0.040 ^{a,A}	<0.020	0.343 ± 0.034 ^{a,A}	<0.020	0.188 ± 0.012 ^{a,C}	0.285 ± 0.020 ^{a,B}	<0.020
DS8888-3-6 Pa	0.768 ± 0.029 ^{c,B}	0.117 ± 0.010 ^{a,A}	2.01 ± 0.17 ^{d,B}	0.813 ± 0.058 ^{b,A}	4.67 ± 0.26 ^{c,B}	1.07 ± 0.06 ^{c,A}	0.024 ± 0.001 ^{a,A}
DS8888-3-6 Li.u	0.591 ± 0.035 ^{b,A}	0.146 ± 0.008 ^{b,C}	1.65 ± 0.06 ^{b,B}	0.788 ± 0.065 ^{a,b,A}	3.53 ±0.32 ^{b,A}	1.02 ± 0.04 ^{c,B}	0.031 ± 0.003 ^{b,A}
DS8888-3-6 La.p	0.715 ± 0.054 ^{c,A}	<0.020	1.99 ± 0.08 ^{c,C}	0.695 ± 0.065 ^{a,A}	3.01 ± 0.26 ^{b,B,C}	0.583 ± 0.045 ^{b,A}	<0.020
				Blue Wheat			
DS8548-7	0.420 ± 0.019 ^{a,A}	<0.020	0.422 ± 0.019 ^{a,B}	<0.020	0.162 ± 0.013 ^{a,B}	0.224 ± 0.014 ^{a,A}	<0.020
DS8548-7 Pa	0.656 ± 0.024 ^{c,A}	0.154 ± 0.009 ^{b,B}	1.63 ± 0.13 ^{c,A}	0.857 ± 0.078 ^{b,A,B}	3.73 ± 0.14 ^{d,A}	0.946 ± 0.056 ^{d,A}	0.093 ± 0.004 ^{b,B}
DS8548-7 Li.u	0.593 ± 0.044 ^{b,A}	0.061 ± 0.005 ^{a,B}	1.37 ± 0.09 ^{b,A}	0.743 ± 0.074 ^{a,b,A}	3.20 ± 0.11 ^{c,A}	0.702 ± 0.059 ^{c,A}	0.027 ± 0.002 ^{a,A}
DS8548-7 La.p	0.685 ± 0.060 ^{c,A}	<0.020	1.49 ± 0.13 ^{c,B}	0.641 ± 0.028 ^{a,A}	2.47 ± 0.22 ^{b,A}	0.671 ± 0.065 ^{b,A}	<0.020
				Purple Wheat			
DS8535-2	0.440 ± 0.021 ^{a,A}	<0.020	0.430± 0.035 ^{a,B}	<0.020	0.103 ± 0.006 ^{a,A}	0.321 ± 0.028 ^{a,B,C}	<0.020
DS8535-2 Pa	1.23 ± 0.09 ^{d,D}	0.477 ± 0.015 ^{c,D}	3.28 ± 0.31 ^{c,D}	0.978 ± 0.083 ^{b,B}	5.46 ± 0.20 ^{c,C}	2.23 ± 0.20 ^{d,C}	0.297 ± 0.013 ^{c,D}
DS8535-2 Li.u	0.791 ± 0.025 ^{b,B}	0.135 ± 0.007 ^{b,C}	1.76 ± 0.08 ^{b,B}	1.07 ± 0.10 ^{b,B}	3.47 ± 0.30 ^{b,A}	1.16 ± 0.06 ^{c,B}	0.145 ± 0.005 ^{b,B}
DS8535-2 La.p	0.909 ± 0.038 ^{c,B}	0.100 ± 0.006 ^{a,A}	1.87 ± 0.11 ^{b,C}	0.853 ± 0.030 ^{a,C}	3.54 ± 0.35 ^{b,C}	0.629 ± 0.049 ^{b,A}	0.116 ± 0.008 ^{a,B}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data are represented as means ($n = 3$) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

Nonessential amino acid and GABA concentrations of the wholemeal wheat grain samples are shown in Table 3. Regarding the nonessential amino acid concentrations in non-fermented samples, 'Gaja' and waxy wholemeal wheat flours showed higher arginine content, in comparison with both wholemeal coloured wheat, and wheat variety has shown to be a significant factor on arginine content ($p = 0.21$).

Table 3. Nonessential amino acid and γ-aminobutyric acid concentrations of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Concentration Nonessential of Amino Acids in Sample, μmol/g									GABA, μmol/g
	Arginine	Glutamine	Serine	Aspartic Acid	Glutamic Acid	Glycine	Alanine	Proline	Tyrosine	
Traditional Wheat										
'Gaja'	0.521 ± 0.018 ^{b,C}	1.95 ± 0.14 ^{c,A}	0.971 ± 0.061 ^{a,A}	0.793 ± 0.068 ^{b,A}	0.581 ± 0.057 ^{b,D}	0.895 ± 0.040 ^{a,C}	1.74 ± 0.16 ^{a,A}	0.285 ± 0.015 ^{a,A}	<0.020	2.43 ± 0.13 ^{a,C}
'Gaja' Pa	0.221 ± 0.013 ^{a,B}	3.07 ± 0.15 ^{d,C}	1.47 ± 0.05 ^{b,B}	1.38 ± 0.09 ^{c,C}	0.780 ± 0.023 ^{c,C}	1.84 ± 0.12 ^{c,B}	5.98 ± 0.32 ^{c,C}	1.55 ± 0.06 ^{d,B}	0.246 ± 0.010 ^{b,B}	5.32±0.30 ^{b,C}
'Gaja' Li.u	1.09 ± 0.10 ^{c,C}	1.12±0.05 ^{b,A}	1.16 ± 0.08 ^{a,B}	0.686 ± 0.066 ^{a,B}	0.477 ± 0.016 ^{a,B}	1.56 ± 0.13 ^{b,B}	4.28 ± 0.16 ^{b,A}	1.31 ± 0.07 ^{c,A}	0.279 ± 0.019 ^{b,B}	6.37 ± 0.41 ^{c,D}
'Gaja' La.p	0.987 ± 0.071 ^{c,D}	0.452 ± 0.023 ^{a,B}	1.42 ± 0.13 ^{a,b,B}	0.626 ± 0.020 ^{a,C}	0.470 ± 0.038 ^{a,C}	1.76 ± 0.11b, ^{c,B}	4.54 ± 0.40 ^{b,C}	0.972 ± 0.042 ^{b,A}	0.055 ± 0.003 ^{a,A}	6.51 ± 0.42 ^{c,B}
Waxy Wheat										
DS8888-3-6	0.578 ± 0.047 ^{b,C}	1.81 ± 0.10 ^{a,A}	1.49 ± 0.11 ^{a,b,B}	0.906 ± 0.036 ^{c,B}	0.199 ± 0.019 ^{a,A}	0.527a ± 0.033 ^{A}	2.11 ± 0.10 ^{a,B}	0.300 ± 0.025 ^{a,A,B}	<0.020	2.51 ± 0.09 ^{a,C}
DS8888-3-6 Pa	0.236 ± 0.015 ^{a,B}	2.66 ± 0.18 ^{b,B}	1.35 ± 0.11 ^{a,b,B}	1.15 ± 0.09 ^{c,B}	0.585 ± 0.045 ^{c,A}	1.39b ± 0.07 ^{A}	4.27 ± 0.18 ^{b,B}	1.39 ± 0.07 ^{b,A}	0.246 ± 0.007 ^{c,B}	4.53 ± 0.39 ^{b,B}
DS8888-3-6 Li.u	0.722 ± 0.054 ^{c,B}	1.75 ± 0.11 ^{a,B}	1.26 ± 0.09 ^{a,B}	0.546 ± 0.051 ^{b,A}	0.409 ± 0.030 ^{b,A}	1.33b ± 0.07 ^{A}	4.07 ± 0.31 ^{b,A}	1.35 ± 0.05 ^{b,A}	0.225 ± 0.007 ^{b,A}	4.87 ± 0.17 ^{b,C}
DS8888-3-6 La.p	0.227 ± 0.007 ^{a,B}	1.97 ± 0.18 ^{a,C}	1.30 ± 0.11 ^{a,b,B}	0.425 ± 0.027 ^{a,B}	0.392 ± 0.033 ^{b,B}	2.06 ± 0.17 ^{c,C}	4.51 ± 0.31 ^{b,C}	2.22 ± 0.17 ^{c,C}	0.191 ± 0.007 ^{a,C}	7.60 ± 0.24 ^{c,C}
Blue Wheat										
DS8548-7	0.370 ± 0.033 ^{b,A}	2.52 ± 0.23 ^{b,C}	1.03 ± 0.09 ^{b,A}	0.893 ± 0.057 ^{c,B}	0.368 ± 0.024 ^{b,C}	0.494 ± 0.020 ^{a,A}	3.03 ± 0.25 ^{a,C}	0.330 ± 0.028 ^{a,B}	<0.020	2.27 ± 0.21 ^{a,B}
DS8548-7 Pa	0.337 ± 0.011 ^{a,C}	2.32 ± 0.22 ^{b,A}	0.919 ± 0.031 ^{a,A}	0.954 ± 0.043 ^{d,A}	0.677 ± 0.048 ^{c,B}	1.25 ± 0.12 ^{b,A}	3.56 ± 0.21 ^{a,b,A}	1.26 ± 0.08 ^{b,A}	0.198 ± 0.008 ^{b,A}	3.25 ± 0.29 ^{b,A}
DS8548-7 Li.u	0.352 ± 0.035 ^{a,b,A}	2.37 ± 0.08 ^{b,C}	0.960 ± 0.038 ^{a,b,A}	0.782 ± 0.07 ^{b,C}	0.621 ± 0.026 ^{c,D}	1.18 ± 0.10 ^{b,A}	3.73 ± 0.37 ^{b,A}	1.33 ± 0.07 ^{b,A}	0.210 ± 0.010 ^{b,A}	3.35 ± 0.13 ^{b,A}
DS8548-7 La.p	0.846 ± 0.048 ^{c,C}	0.134 ± 0.004 ^{a,A}	1.13 ± 0.05 ^{b,A}	0.147 ± 0.013 ^{a,A}	0.154 ± 0.009 ^{a,A}	1.30 ± 0.047 ^{b,A}	4.15 ± 0.13 ^{c,B}	1.65 ± 0.15 ^{c,B}	0.108 ± 0.009 ^{a,B}	6.06 ± 0.47 ^{c,B}
Purple Wheat										
DS8535-2	0.475 ± 0.041 ^{c,B}	2.01 ± 0.11 ^{a,B}	1.05 ± 0.06 ^{a,A}	0.739 ± 0.036 ^{a,A}	0.245 ± 0.008 ^{a,B}	0.663 ± 0.054 ^{a,B}	1.72 ± 0.06 ^{a,A}	0.336 ± 0.031 ^{a,B}	<0.020	1.56 ± 0.14 ^{a,A}
DS8535-2 Pa	0.192 ± 0.007 ^{b,A}	4.31 ± 0.37 ^{d,D}	1.85 ± 0.11 ^{c,C}	1.72 ± 0.09 ^{c,D}	1.22 ± 0.114 ^{d,D}	2.41 ± 0.12 ^{c,C}	8.08 ± 0.31 ^{d,D}	2.16 ± 0.13 ^{c,C}	0.243 ± 0.023 ^{a,B}	5.61 ± 0.56 ^{d,C}
DS8535-2 Li.u	1.21 ± 0.05 ^{d,C}	3.26 ± 0.30 ^{c,D}	1.22 ± 0.06 ^{b,B}	0.894 ± 0.059 ^{b,D}	0.522 ± 0.021 ^{b,C}	1.72 ± 0.16 ^{b,B}	4.28 ± 0.16 ^{c,A}	1.72 ± 0.15 ^{b,B}	0.268 ± 0.008 ^{b,B}	3.97 ± 0.34 ^{c,B}
DS8535-2 La.p	0.105 ± 0.007 ^{a,A}	2.77 ± 0.17 ^{b,D}	1.10 ± 0.10 ^{a,b,A}	0.849 ± 0.062 ^{b,D}	0.783 ± 0.055 ^{c,D}	1.78 ± 0.11 ^{b,B}	2.68 ± 0.21 ^{b,A}	1.58 ± 0.15 ^{b,B}	0.227 ± 0.009 ^{a,D}	2.82 ± 0.24 ^{b,A}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. GABA—γ-aminobutyric acid. The data are represented as means ($n = 3$) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

The highest glutamine concentration was found in wholemeal blue wheat (2.52 μmol/g), and the wheat variety was a significant factor in glutamine content ($p \leq 0.0001$). The highest glutamic acid and glycine concentration was obtained in the 'Gaja' samples. The highest serine content was found in wholemeal waxy wheat, and the highest alanine content was established in wholemeal blue wheat. Wheat variety was a significant factor in the content of most of these amino acids, but alanine ($p = 0.015$, $p = 0.028$ and $p = 0.009$, respectively). The aspartic acid concentration in 'Gaja' and purple wheat samples was, on average, 15.5% lower in comparison with wholemeal waxy and blue wheat grain samples. The lowest proline concentration was found in the 'Gaja' samples (0.285 μmol/g). In all the cases, tyrosine content in non-fermented samples was lower than 0.020 μmol/g. When comparing fermented samples, different tendencies were found, and the type of LAB strain used for fermentation represented a significant factor in arginine, glutamine, aspartic acid, glycine, alanine, proline and tyrosine content ($p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, $p = 0.004$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, respectively). The interaction between LAB strain used for fermentation and wheat variety was significant with regards to the arginine, glutamine, glutamic acid, alanine, proline and tyrosine concentrations ($p \leq 0.0001$, $p \leq 0.0001$, $p = 0.027$, $p = 0.005$, $p = 0.003$, $p = 0.039$, respectively).

Despite wheat grains being a good source of protein, the amounts of essential amino acids (especially lysine) are low [35]. Finally, the quality of the protein must be taken into consideration because the cereal-based diet tends to be deficient in some essential amino acids. Having in mind that the amino acid profile (especially essential amino acids) shows the quality of protein, the use of fermentation in this study showed prospective results, as most of the essential amino acid concentrations in wholemeal wheat cereal samples increased. Cereal grain proteins supply 47% of total global protein intake [36], and cereal-based proteins are a predominant protein source in developing countries [37]. For this reason, finding technological solutions to increase the biological value of wheat cereal varieties becomes very important. Protein content in wheat grains can vary due to genetic differences, agronomic factors, cultivation conditions, etc., but essential amino acids are the most important parameter from nutritional and physiological points of view [36,38,39]. Better knowledge of the amino acid profile of different wheat varieties is beneficial not only to wheat farmers but also to food and feed manufacturers [40]. The recommended daily intake of total essential amino acids is 83.5 mg/kg of body weight [41]. In addition to lysine, threonine, which is present at low concentrations in cereals, is very important from a nutritional standpoint [42]. Recommended daily intake for threonine is about 19 mg/kg [43]. Recommended daily intake for histidine is 8 and 12 mg/kg of body weight/day in adults [44–46]. Phenylalanine is essential for the growth and development of children [47]. Leucine stimulates muscle protein synthesis [44,48]. Recommended daily intake for leucine is 39 mg/kg body weight per day [41].

It was reported that in wheat cereal grains, the predominant nonessential amino acid is glutamic, which concentration can range from 24.79 to 37.05 g/100 g protein [49]. Glutamic acid plays a major role in amino acid metabolism in the body and shows neurotransmitter function [47]. The mean daily intake of glutamic acid is 15 g/day [44]. Moreover, usually, wheat grains show low content of cysteine, methionine and histidine [50].

The results of this study showed that the quality of wholemeal wheat grain proteins can be significantly improved trough the modification of the amino acid profile by using fermentation biotechnology with selected LAB strains. However, it should be pointed out that for a feed industry, these results can be of great interest because one can obtain products with higher biological added value for feed purposes. However, for the food industry, fermented wholemeal wheat grain should be further tested (e.g., in bread production), and the quality, as well as the food safety parameters of the end-products, must be taken into consideration because, during the thermal treatment, various (desirable and not) changes may occur.

When comparing GABA concentration in non-fermented samples, the highest content was found in wholemeal 'Gaja' and waxy wheat samples (on average, 2.47 μmol/g).

Conversely, fermentation increased GABA concentration in all wholemeal cereal doughs. In addition, the LAB strain used for fermentation, the wheat variety and the interaction of these factors were shown to be significant on GABA concentration in wholemeal wheat grain samples ($p \leq 0.0001$, $p = 0.002$ and $p = 0.004$, respectively).

GABA is a non-protein amino acid compound produced during the decarboxylation of L-glutamic acid [51]. GABA shows many desirable effects in the body, including neurotransmission and regulation of brain metabolism [52].

Due to the activity of LAB (decarboxylation, deamination, transamination, etc.), the production of several functional compounds in cereal grains may occur [53–57], including GABA [58,59]. It was reported that during the fermentation with LAB, GABA could be formed [60–62], and, as a matter of fact, fermented food is a good source of dietary GABA [53]. The selection of cereal varieties, based on their nutritional and functional potential, may be very useful in improving the features of food and feed products. Finally, our study showed that fermentation with selected LAB increases GABA concentration in all the tested wholemeal wheat cereal samples and can be a very prospective technology to increase the functional value of cereals.

Biogenic amine concentration in wholemeal wheat cereal samples is shown in Table 4. Phenylethylamine was found in all the wholemeal cereal samples (fermented and non-fermented), and a higher concentration of this compound was disclosed in non-fermented samples of the wholemeal 'Gaja', waxy and blue wheat flour (on average, by 14.2, 24.1 and 27.2%, respectively, higher in comparison with fermented ones). However, opposite trends were found in wholemeal purple wheat cereal samples, and the lowest phenylethylamine concentration was found in samples fermented with *Lactiplantibacillus plantarum* (30.9 mg/kg). Putrescine was observed in non-fermented 'Gaja' samples, in waxy wheat cereal samples fermented with *Liquorilactobacillus uvarum*, and in wholemeal waxy, blue and purple wheat cereals fermented with *Lactiplantibacillus plantarum*. Waxy wheat samples fermented with *Lactiplantibacillus plantarum* showed 8.3 and 2.3 times higher concentrations of putrescine, in comparison with wholemeal blue and purple wheat, respectively, fermented in the same conditions. Cadaverine was formed in all samples fermented with *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum* strains. When comparing cadaverine concentration between different cereal groups fermented with the same LAB strain, the highest concentration was found in purple wheat samples fermented with *Liquorilactobacillus uvarum* (122.6 mg/kg). Furthermore, the cereal group consisting of wholemeal 'Gaja', waxy and purple wheat samples fermented with *Lactiplantibacillus plantarum* showed, on average, a concentration as high as 120.0 mg/kg cadaverine. Histamine was just formed in wholemeal waxy, blue and purple wheat cereal samples fermented with *Lactiplantibacillus plantarum*, and the highest concentration of histamine was reached in wholemeal purple wheat samples (66.3 mg/kg).

Tyramine (35.6 mg/kg) was formed in wholemeal purple wheat cereal samples fermented with *Lactiplantibacillus plantarum*, and it also displayed the highest spermidine concentration (51.8 mg/kg). When comparing spermidine in all wholemeal cereal groups, non-fermented wholemeal 'Gaja', waxy and blue flours contained spermidine concentrations above 20.0 mg/kg. Nevertheless, spermidine was not found in non-fermented whole purple wheat samples. In opposite to these trends, spermidine was found in all the fermented whole purple wheat samples, and its content ranged from 24.5 to 51.8 mg/kg (in fermented samples with *Pediococcus acidilactici* and *Lactiplantibacillus plantarum*, respectively). Spermine was just found in wholemeal purple wheat flour fermented with *Lactiplantibacillus plantarum* (20.2 mg/kg). Multivariate analyses of between-subjects effects indicated that the LAB strain used for fermentation, the wheat variety and their interaction were significant factors in the concentration of all biogenic amines ($p \leq 0.0001$).

Table 4. Biogenic amine concentration of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Biogenic Amines, mg/kg							
	Tryptamine	Phenyl-ethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
Traditional Wheat								
'Gaja'	<0.1	58.5 ± 3.7 ^{b,B}	19.9 ± 1.1	<0.1	<0.1	<0.1	31.8 ± 2.9 ^{B}	<0.1
'Gaja' Pa	<0.1	46.1 ± 2.9 ^{a,A}	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
'Gaja' Li.u	<0.1	52.6 ± 4.1 ^{a,b,B}	<0.1	94.4 ± 5.4 ^{a,C}	<0.1	<0.1	<0.1	<0.1
'Gaja' La.p	<0.1	52.0 ± 4.8 ^{a,b,C}	<0.1	116.3 ± 8.1 ^{b,B}	<0.1	<0.1	<0.1	<0.1
Waxy Wheat								
DS8888-3-6	<0.1	57.3 ± 4.5 ^{b,B}	<0.1	<0.1	<0.1	<0.1	22.7 ± 1.6 ^{A}	<0.1
DS8888-3-6 Pa	<0.1	42.9 ± 3.8 ^{a,A}	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
DS8888-3-6 Li.u	<0.1	45.9 ± 4.1 ^{a,A,B}	103.8 ± 7.2a	63.6 ± 4.9 ^{a,B}	<0.1	<0.1	<0.1	<0.1
DS8888-3-6 La.p	<0.1	41.8 ± 3.5 ^{a,B}	226.2 ± 14.1 ^{b,C}	127.9 ± 8.2 ^{b,B}	20.1 ± 1.7 ^{A}	<0.1	<0.1	<0.1
Blue Wheat								
DS8548-7	<0.1	55.9 ± 4.8 ^{b,B}	<0.1	<0.1	<0.1	<0.1	30.9 ± 2.5 ^{a,B}	<0.1
DS8548-7 Pa	<0.1	40.5 ± 3.1 ^{a,A}	<0.1	<0.1	<0.1	<0.1	29.5 ± 2.2 ^{a,B}	<0.1
DS8548-7 Li.u	<0.1	42.0 ± 2.9 ^{a,A}	<0.1	52.4 ± 4.7 ^{a,A}	<0.1	<0.1	40.6 ± 3.1 ^{b,B}	<0.1
DS8548-7 La.p	<0.1	39.7 ± 3.0 ^{a,B}	27.2 ± 1.7 ^{A}	84.1 ± 5.3 ^{b,A}	49.9 ± 3.8 ^{B}	<0.1	25.7 ± 1.3 ^{a,A}	<0.1
Purple Wheat								
DS8535-2	<0.1	44.1 ± 3.5 ^{b,A}	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
DS8535-2 Pa	<0.1	44.1 ± 4.2 ^{b,A}	<0.1	<0.1	<0.1	<0.1	24.5 ± 2.2 ^{a,A}	<0.1
DS8535-2 Li.u	<0.1	50.9 ± 4.7 ^{b,B}	<0.1	122.6 ± 8.4 ^{a,D}	<0.1	<0.1	29.8 ± 2.8 ^{b,A}	<0.1
DS8535-2 La.p	<0.1	30.9 ± 2.9 ^{a,A}	87.3 ± 6.3 ^{B}	115.1 ± 9.1 ^{a,B}	66.3 ± 5.1 ^{C}	35.6 ± 3.2	51.8 ± 4.9 ^{c,B}	20.2 ± 1.3

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data are represented as means (n = 3) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

Increased concentrations of cadaverine, putrescine and spermidine in products are associated with microbial activity [63]. There are a few mechanisms of action for the accumulation of biogenic amines in food, which are explained by their possible synthesis via the decarboxylation of amino acids, as well as by transamination of aldehydes or ketones [64,65]. There are published works highlighting the negative effects of tyramine and phenylethylamine on human [66] and animal health [67]. In addition to the negative effect on health, putrescine and cadaverine are characterised as specifically smelly molecules [68], whose presence in food or feed may reduce their acceptability. The total amount of biogenic amines in food is very important because they can increase toxicity [66,69]. Arginine can be metabolised via bacterial action to putrescine; lysine can be converted to cadaverine, histidine to histamine, tyrosine to tryptophan and phenylalanine to tyramine, tryptamine and β-phenylethylamine, respectively [70]. Histamine is the most toxic biogenic amine [71–73]. Tyramine is related to migraines, and cadaverine and putrescine can increase the toxic effect of other biogenic amines [74]. The Food and Drug Administration (FDA) suggested that the maximum permitted histamine in food should be limited to 500mg/kg, and a level of >1000 mg/kg should be considered, for safety for consumption purposes, as unfit for human consumption [75,76]. The European Union (EU) regulation suggests values between 100 and 200 mg of histamine per kilogram of food for the *Scomberesocidae* and *Scombridae* fish families [77], as well as the highest concentration of 300 mg/kg for the total biogenic amine content in fish and derivatives.

According to the results obtained in this study, the formation of biogenic amines in whole wheat cereal products should be taken into consideration, as the total levels of biogenic amine ranged from 22.7 (in non-fermented DS8888-3-6 samples) to 416 mg/kg (in samples DS8548-7 fermented with *Lactiplantibacillus plantarum*) (Figure 2). Moderate positive correlations were found between cadaverine concentration, TTA and viable LAB viable counts (r = 0.308, p = 0.033 and r = 0.581, p = 0.0001, respectively). However, a moderate positive correlation between histamine concentration and LAB viable counts was revealed (r = 0.306, p = 0.034).

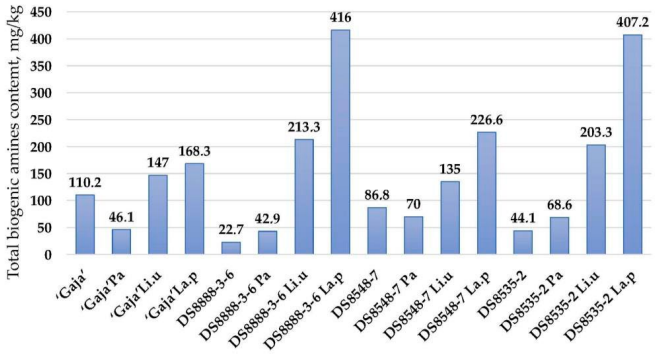

Figure 2. Total biogenic amine content (mg/kg) of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs). Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*.

3.3. Macro- and Microelements of Wholemeal Wheat Flour Samples

Macroelement concentrations in wholemeal wheat cereal samples are shown in Table 5. Significant differences were found in the Na content when comparing the macroelement concentrations in non-fermented and fermented groups, which, in all the cases, was lower in non-fermented samples. These differences can be explained by the addition of the LAB suspension, which contained a physiological (NaCl) solution.

When comparing the varieties of wholemeal wheat cereals under scrutiny, the highest Mg and K concentrations were obtained in wholemeal 'Gaja' wheat. However, the highest Ca content was found in wholemeal waxy wheat samples. Multivariate test of between-subject effects showed that the LAB strain used for fermentation and the interaction with the wheat variety had a significant impact on the Na concentration in wholemeal cereals ($p \leq 0.0001$) and showed as well that the wheat variety was a significant factor on the concentration of all the analysed macroelements ($p \leq 0.0001$).

Essential microelement concentrations of the wholemeal wheat cereal samples (except Co, Ni and Se, whose concentrations were lower than 0.010, 0.5 and 0.20 mg/kg, respectively) are given in Table 6. A concentration higher than 0.010 mg/100 g Cr was only detected in wholemeal waxy wheat samples (on average, 0.154 mg/100 g), and fermentation was not a significant factor towards the Cr content. Wholemeal blue and purple wheat samples showed the highest Mn concentration (on average, 16.2 mg/kg), and, comparatively, in wholemeal 'Gaja' and waxy wheats, the Mn concentration, on average, was 21.0 and 53.5% lower, respectively. The highest Fe content was found in wholemeal 'Gaja' (on average, 24.8 mg/kg), and s waxy, blue and purple wheats showed, on average, 35.1% lower Fe concentrations. Moreover, wholemeal 'Gaja' showed the highest Cu content (on average, 2.19 mg/kg), and wholemeal waxy, blue and purple wheat samples showed, on average, 47.9, 55.6 and 63.2% lower Cu concentrations, respectively. The highest concentration of Zn was found in wholemeal purple wheats (8.31 mg/kg), and wholemeal 'Gaja', waxy and blue wheats showed, on average, 21.1, 33.7 and 20.0% lower Zn contents, respectively. A multivariate test of between-subject effects showed that the LAB strain used for fermentation was a significant factor in Cr concentration in wholemeal cereals ($p = 0.007$). On the other hand, wheat variety was a significant factor in Cr, Mn and Cu contents ($p \leq 0.0001$), and LAB strain and wheat variety interaction were significant factors in Cr concentration in wholemeal wheat samples ($p \leq 0.0001$).

Table 5. Macroelement concentrations of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Macroelements, Dry Matter (d.m.)			
	Na, g/100 g	Mg, g/kg	K, g/kg	Ca, g/kg
		Traditional Wheat		
'Gaja'	<0.002	0.682 ± 0.053	2.54 ± 0.22 ^{a,B}	0.210 ± 0.019 ^{a,B}
'Gaja' Pa	0.010 ± 0.002 ^{a,C}	0.638 ± 0.051 ^{a,B}	2.32 ± 0.19 ^{a,C}	0.199 ± 0.018 ^{a,B}
'Gaja' Li.u	0.008 ± 0.001 ^{a,B}	0.669 ± 0.042 ^{a,C}	2.38 ± 0.13 ^{a,B}	0.192 ± 0.019 ^{a,A,B}
'Gaja' La.p	0.007 ± 0.002 ^{a,A}	0.641 ± 0.050 ^{a,C}	2.37 ± 0.21 ^{a,B}	0.189 ± 0.020 ^{a,A}
		Waxy Wheat		
DS8888-3-6	<0.002	0.448 ± 0.045 ^{a,A}	1.98 ± 0.17 ^{a,A}	0.345 ± 0.031 ^{a,C}
DS8888-3-6 Pa	0.013 ± 0.001 ^{a,C}	0.501 ± 0.041 ^{a,A}	2.38 ± 0.23 ^{a,C}	0.310 ± 0.026 ^{a,C}
DS8888-3-6 Li.u	0.011 ± 0.002 ^{a,B}	0.438 ± 0.040 ^{a,A}	2.20 ± 0.18 ^{a,B}	0.321 ± 0.027 ^{a,C}
DS8888-3-6 La.p	0.014 ± 0.001 ^{a,B}	0.509 ± 0.051 ^{a,A,B}	2.37 ± 0.23 ^{a,B}	0.322 ± 0.030 ^{a,C}
		Blue Wheat		
DS8548-7	<0.002	0.510 ± 0.048 ^{a,A}	1.93 ± 0.16 ^{a,A}	0.213 ± 0.018 ^{a,B}
DS8548-7 Pa	0.003 ± 0.001 ^{a,A}	0.511 ± 0.027 ^{a,A}	1.84 ± 0.11 ^{a,A}	0.205 ± 0.014 ^{a,B}
DS8548-7 Li.u	0.002 ± 0.002 ^{a,A}	0.513 ± 0.022 ^{a,A,B}	1.88 ± 0.09 ^{a,A}	0.210 ± 0.016 ^{a,B}
DS8548-7 La.p	0.004 ± 0.001 ^{a,A}	0.534 ± 0.043 ^{a,B}	1.82 ± 0.10 ^{a,A}	0.214 ± 0.012 ^{a,B}
		Purple Wheat		
DS8535-2	<0.002	0.482 ± 0.041 ^{a,A}	1.75 ± 0.16 ^{a,A}	0.173 ± 0.016 ^{a,A}
DS8535-2 Pa	0.006 ± 0.001 ^{a,B}	0.458 ± 0.039 ^{a,A}	1.86 ± 0.20 ^{a,B}	0.162 ± 0.015 ^{a,A}
DS8535-2 Li.u	0.006 ± 0.002 ^{a,A,B}	0.495 ± 0.036 ^{a,A}	1.77 ± 0.14 ^{a,A}	0.163 ± 0.012 ^{a,A}
DS8535-2 La.p	0.006 ± 0.001 ^{a,A}	0.438 ± 0.034 ^{a,A}	1.84 ± 0.11 ^{a,A}	0.162 ± 0.013 ^{a,A}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data are represented as means (n = 3) ± SE. ^{a} Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–C}$ Means with different letters in the column are significantly different between the different varieties of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

Table 6. Essential microelement concentrations of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Essential Microelements, Dry Matter (d.m.)				
	Cr	Mn	Fe	Cu	Zn
	mg/100 g	mg/kg			
		Traditional Wheat			
'Gaja'	<0.010	12.8 ± 1.3 ^{a,B}	24.8 ± 2.1 ^{a,B}	2.19 ± 0.22 ^{a,C}	6.56 ± 0.61 ^{a,A}
'Gaja' Pa	<0.010	11.7 ± 1.2 ^{a,B}	22.4 ± 2.0 ^{a,C}	2.01 ± 0.18 ^{a,C}	6.46 ± 0.59 ^{a,A}
'Gaja' Li.u	<0.010	11.7 ± 1.2 ^{a,B}	25.0 ± 2.3 ^{a}	2.14 ± 0.16 ^{a,C}	6.03 ± 0.42 ^{a,A,B}
'Gaja' La.p	<0.010	11.5 ± 1.2 ^{a,B}	24.8 ± 2.2 ^{a,B}	2.23 ± 0.18 ^{a,D}	6.21 ± 0.42 ^{a,A}
		Waxy Wheat			
DS8888-3-6	0.151 ± 0.014 ^{a}	7.54 ± 0.71 ^{a,A}	15.6 ± 1.4 ^{a,A}	1.14 ± 0.11 ^{a,A,B}	5.51 ± 0.55 ^{a,A}
DS8888-3-6 Pa	0.161 ± 0.007 ^{a}	7.77 ± 0.80 ^{a,A}	14.6 ± 1.3 ^{a,A}	1.27 ± 0.13 ^{a,B}	5.81 ± 0.57 ^{a,A}
DS8888-3-6 Li.u	0.145 ± 0.025 ^{a}	7.69 ± 0.92 ^{a,A}	15.2 ± 1.7 ^{a,A}	1.23 ± 0.12 ^{a,A,B}	5.87 ± 0.42 ^{a,A}
DS8888-3-6 La.p	0.158 ± 0.014 ^{a}	7.71 ± 1.0 ^{a,A}	15.9 ± 1.6 ^{a,A}	1.22 ± 0.19 ^{a,C}	5.74 ± 0.53 ^{a,A}
		Blue Wheat			
DS8548-7	<0.010	15.6 ± 1.3 ^{a,C}	16.2 ± 1.6 ^{a,A}	0.973 ± 0.097 ^{a,A}	6.65 ± 0.63 ^{a,A}
DS8548-7 Pa	<0.010	15.4 ± 1.2 ^{a,C}	15.0 ± 1.1 ^{a,A}	0.926 ± 0.073 ^{a,A,B}	6.73 ± 0.50 ^{a,A}
DS8548-7 Li.u	<0.010	14.1 ± 1.1 ^{a,C}	16.7 ± 1.2 ^{a,A}	0.938 ± 0.074 ^{a,A}	6.38 ± 0.51 ^{a,A,B}
DS8548-7 La.p	<0.010	15.3 ± 1.0 ^{a,C}	16.5 ± 1.2 ^{a,A}	0.921 ± 0.071 ^{a,B}	6.43 ± 0.58 ^{a,A,B}

Table 6. *Cont.*

Wholemeal Wheat Samples	Essential Microelements, Dry Matter (d.m.)				
	Cr	Mn	Fe	Cu	Zn
	mg/100 g	mg/kg			
Purple Wheat					
DS8535-2	<0.010	17.8 ± 1.5 ^{a,C}	16.6 ± 1.7 ^{a,A}	0.805 ± 0.081 ^{a,A}	8.31 ± 0.79 ^{a,B}
DS8535-2 Pa	<0.010	16.1 ± 1.3 ^{a,C}	17.3 ± 1.8 ^{a,A,B}	0.797 ± 0.080 ^{a,A}	7.94 ± 0.75 ^{a,A,B}
DS8535-2 Li.u	<0.010	17.9 ± 1.2 ^{a,D}	16.7 ± 1.5 ^{a,A}	0.775 ± 0.078 ^{a,A}	7.57 ± 0.72 ^{a,B}
DS8535-2 La.p	<0.010	17.4 ± 1.2 ^{a,C}	17.0 ± 1.3 ^{a,A}	0.764 ± 0.070 ^{a,A}	7.54 ± 0.61 ^{a,B}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data are represented as means (n = 3) ± SE. ^{a} Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

Non-essential microelement concentrations of the wholemeal wheat cereal samples (except V, Mo, Ag, Sb, Cs, Ti, Al and Li, whose concentrations were lower than 2, 0.5, 2, 0.5, 2, 2, 5 and 0.050 mg/kg, respectively) are shown in Table 7. Wholemeal 'Gaja' samples showed higher concentrations of As and Rb in comparison with wholemeal waxy, blue and purple wheat flours, in which these microelements were absent. The highest concentration of Sr was found in wholemeal blue wheat samples (2.58 mg/kg), and the wholemeal 'Gaja' samples showed the highest Cd content (0.54 mg/kg). The highest Ba concentration was found in wholemeal blue wheat samples, and the highest Pb content was disclosed in purple wheat wholemeal.

Table 7. Non-essential microelement concentrations of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Non-Essential Microelements, mg/kg (d.m.)					
	As	Rb	Sr	Cd	Ba	Pb
Traditional Wheat						
'Gaja'	0.007 ± 0.001 ^{a}	1.44 ± 0.14 ^{a}	0.963 ± 0.091 ^{a,A}	0.054 ± 0.005 ^{a,C}	2.35 ± 0.24 ^{a,A}	0.012 ± 0.001 ^{a,A}
'Gaja' Pa	0.009 ± 0.002 ^{a}	1.36 ± 0.12 ^{a}	0.976 ± 0.083 ^{a,A}	0.053 ± 0.006 ^{a,D}	2.21 ± 0.19 ^{a,A}	0.014 ± 0.002 ^{a,A}
'Gaja' Li.u	0.008 ± 0.001 ^{a}	1.41 ± 0.10 ^{a}	0.984 ± 0.074 ^{a,A}	0.057 ± 0.004 ^{a,D}	2.33 ± 0.22 ^{a,A}	0.013 ± 0.001 ^{a,A}
'Gaja' La.p	0.008 ± 0.002 ^{a}	1.43 ± 0.11 ^{a}	0.962 ± 0.087 ^{a,A}	0.058 ± 0.005 ^{a,D}	2.36 ± 0.23 ^{a,A}	0.012 ± 0.001 ^{a,A}
Waxy Wheat						
DS8888-3-6	<0.005	<1	1.50 ± 0.13 ^{a,B}	0.029 ± 0.003 ^{a,B}	2.22 ± 0.21 ^{a,A}	<0.010
DS8888-3-6 Pa	<0.005	<1	1.46 ± 0.12 ^{a,A}	0.033 ± 0.003 ^{a,C}	2.45 ± 0.25 ^{a,A,B}	<0.010
DS8888-3-6 Li.u	<0.005	<1	1.42 ± 0.13 ^{a,B}	0.035 ± 0.004 ^{a,C}	2.23 ± 0.22 ^{a,A}	<0.010
DS8888-3-6 La.p	<0.005	<1	1.52 ± 0.14 ^{a,B}	0.034 ± 0.004 ^{a,C}	2.35 ± 0.26 ^{a,A}	<0.010
Blue Wheat						
DS8548-7	<0.005	<1	2.58 ± 0.26 ^{D}	0.021 ± 0.002 ^{a,A}	2.90 ± 0.29 ^{a,B}	<0.010
DS8548-7 Pa	<0.005	<1	2.59 ± 0.20 ^{C}	0.020 ± 0.001 ^{a,B}	2.78 ± 0.26 ^{a,B}	<0.010
DS8548-7 Li.u	<0.005	<1	2.66 ± 0.19 ^{D}	0.022 ± 0.001 ^{a,B}	2.83 ± 0.20 ^{a,B}	<0.010
DS8548-7 La.p	<0.005	<1	2.63 ± 0.21 ^{D}	0.023 ± 0.002 ^{a,B}	2.69 ± 0.25 ^{a,A}	<0.010
Purple Wheat						
DS8535-2	<0.005	<1	2.00 ± 0.20 ^{a,C}	0.019 ± 0.003 ^{a,A}	<2	0.032 ± 0.003 ^{a,B}
DS8535-2 Pa	<0.005	<1	1.97 ± 0.19 ^{a,B}	0.016 ± 0.002 ^{a,A}	<2	0.029 ± 0.002 ^{a,B}
DS8535-2 Li.u	<0.005	<1	1.90 ± 0.14 ^{a,C}	0.017 ± 0.003 ^{a,A}	<2	0.031 ± 0.003 ^{a,B}
DS8535-2 La.p	<0.005	<1	1.87 ± 0.17 ^{a,C}	0.016 ± 0.002 ^{a,A}	<2	0.028 ± 0.003 ^{a,B}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. The data are represented as means (n = 3) ± SE. ^{a} Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

A multivariate test of between-subject effects revealed that the wheat variety was a significant factor on all the analysed non-essential microelement contents ($p \leq 0.0001$) in wholemeal cereals. This finding can lead to the conclusion that different cereal varieties present different capacities to accumulate these elements from the environment.

Cereal grains are an important source of macro- and microelements [78]. Macro- and microelements are essential compounds for human and animal nutrition [79]. The mineral and trace element contents of many raw plant-based materials are known to be related to the cultivar and variety of plant, soil and weather conditions, fertilisers used, etc. [80,81]. The levels of some elements can vary in a large range [78]. From the nutritional standpoint, major attention has been given to the elements Fe, Ca, Cu and Zn [82].

However, other trace elements are also very important, and micronutrient malnutrition afflicts billions of people [83]. According to World Health Organization (WHO), 46% of children (from 5 to 14 years-old) have Fe deficiency anaemia, 48% of pregnant women are Fe deficient [84], and, on average, 200 million people lack essential trace elements [85]. The food industry tries to solve this problem by the addition of nutrients to food, but they do not ensure nutrient absorption from the end product [86]. If nutrient-rich wheat cereal grain can be bred, the problems mentioned above might be solved. It was reported that coloured wheat grains contain numerous nutrients which are beneficial to human health [87–89], and black wheat showed higher contents of iron, zinc, manganese, copper, selenium, magnesium, potassium and phosphorus when compared to traditional ones [90]. Likewise, organic chromium content in black wheat was, on average, four times higher in comparison with traditional ones [90]. Correspondingly, it was reported that Se concentration in purple wheat is higher than in control by, on average, 173.6% [83].

Finally, the determination of the nutrient content in cereals, such as essential elements, is necessary to perceive their dietary intake. Calcium is an element in bone tissues. Potassium and Na play an important role in maintaining osmotic pressure and in the transmission of nervous impulses, and Mg is a cofactor in more than 300 enzymatic reactions. Trace elements (Fe, Cu, Co, Zn, Mn, Mo) are also needed for the proper functioning of the organism but are required in smaller quantities [91–95]. These trace elements are involved in numerous biological processes: Fe is involved in oxygen transport, Co is an element forming part of cobalamin or vitamin B12, and Mo, Mn, Zn or Cu are components in many enzymes [96–98]. This study, considering the importance of cereals in the diet, demonstrates the importance of creating databases of new wheat varieties, which can be a good source of micro- and macroelements in human and animal diets.

3.4. Fatty Acid Composition of Wholemeal Wheat Flour Samples

Fatty acid composition (expressed as a percentage of the total fatty acid content) of the wholemeal wheat cereal samples is given in Table 8. Moreover, the detailed fatty acid profile is given in Supplementary Table S1. A detailed fatty acid profile of the wheat cereal wholemeal samples is in the heatmap of Figure 3.

The highest saturated fatty acids content was found in wholemeal blue wheat flour (38.14% from the total fatty acid content) when comparing the non-fermented samples. Nonetheless, in most of the cases, fermentation reduced the saturated fatty acid content of wholemeal wheat samples—except wholemeal waxy and purple wheat fermented with *Lactiplantibacillus plantarum* and wholemeal blue wheat fermented with *Liquorilactobacillus uvarum*. A multivariate test of between-subjects effects showed that the LAB strain used for fermentation, wheat variety and the interaction of these factors were significant in the saturated fatty acid content of wholemeal wheat cereals ($p \leq 0.0001$).

Table 8. Fatty acid composition (% from the total fatty acid content) of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

Wholemeal Wheat Samples	Fatty Acid Composition, % from the Total Fatty Acid Content					
	SFA	MUFA	PUFA	Omega-3 (ω-3)	Omega-6 (ω-6)	Omega-9 (ω-9)
Traditional Wheat						
'Gaja'	29.85 ± 0.09 ^{c,A}	16.07 ± 0.08 ^{a,B}	54.08 ± 0.25 ^{c,D}	3.22 ± 0.06 ^{a,A}	50.86 ± 0.32 ^{c,C}	16.07 ± 0.11 ^{a,A}
'Gaja' Pa	26.38 ± 0.06 ^{b,C}	17.51 ± 0.05 ^{b,C}	56.11 ± 0.21 ^{d,B}	4.48 ± 0.11 ^{b,C}	51.63 ± 0.29 ^{d,B}	17.51 ± 0.06 ^{b,C}
'Gaja' Li.u	26.31 ± 0.04 ^{b,C}	21.34 ± 0.12 ^{c,D}	52.35 ± 0.17 ^{b,B}	11.85 ± 0.23 ^{d,D}	40.50 ± 0.25 ^{a,A}	21.34 ± 0.14 ^{c,D}
'Gaja' La.p	24.73 ± 0.11 ^{a,A}	24.84 ± 0.14 ^{d,D}	50.44 ± 0.22 ^{a,C}	6.20 ± 0.10 ^{c,D}	44.24 ± 0.23 ^{b,B}	24.84 ± 0.13 ^{d,D}
Waxy Wheat						
DS8888-3-6	34.54 ± 0.12 ^{c,C}	16.54 ± 0.11 ^{b,C}	48.92 ± 0.11 ^{b,C}	3.39 ± 0.09 ^{b,B}	45.53 ± 0.26 ^{b,B}	16.54 ± 0.14 ^{b,B}
DS8888-3-6 Pa	22.26 ± 0.08 ^{b,A}	15.10 ± 0.10 ^{a,A}	62.64 ± 0.23 ^{c,D}	4.78 ± 0.11 ^{c,D}	57.86 ± 0.19 ^{d,D}	15.10 ± 0.13 ^{a,A}
DS8888-3-6 Li.u	21.06 ± 0.14 ^{a,A}	15.23 ± 0.12 ^{a,A}	63.71 ± 0.35 ^{d,D}	8.18 ± 0.14 ^{d,C}	55.52 ± 0.22 ^{c,C}	15.23 ± 0.10 ^{a,A}
DS8888-3-6 La.p	37.73 ± 0.25 ^{d,D}	23.91 ± 0.15 ^{c,C}	38.37 ± 0.28 ^{a,A}	2.17 ± 0.08 ^{a,A}	36.20 ± 0.14 ^{a,A}	23.91 ± 0.19 ^{c,C}
Blue Wheat						
DS8548-7	38.14 ± 0.32 ^{c,D}	16.81 ± 0.22 ^{c,C}	45.04 ± 0.26 ^{b,A}	3.46 ± 0.13 ^{c,B}	41.58 ± 0.21 ^{a,A}	16.81 ± 0.14 ^{c,B}
DS8548-7 Pa	24.77 ± 0.18 ^{a,B}	16.31 ± 0.12 ^{b,B}	58.92 ± 0.33 ^{d,C}	3.80 ± 0.09 ^{d,B}	55.12 ± 0.19 ^{c,C}	16.31 ± 0.12 ^{b,B}
DS8548-7 Li.u	38.97 ± 0.15 ^{d,D}	17.23 ± 0.09 ^{d,B}	43.80 ± 0.19 ^{a,A}	2.53 ± 0.07 ^{a,A}	41.27 ± 0.22 ^{a,B}	17.23 ± 0.15 ^{d,B}
DS8548-7 La.p	33.87 ± 0.12 ^{b,B}	15.12 ± 0.10 ^{a,A}	51.02 ± 0.17 ^{c,D}	2.90 ± 0.14 ^{b,B}	48.11 ± 0.25 ^{b,C}	15.12 ± 0.10 ^{a,A}
Purple Wheat						
DS8535-2	32.01 ± 0.03 ^{c,B}	21.33 ± 0.13 ^{c,A}	46.66 ± 0.15 ^{b,B}	4.71 ± 0.15 ^{c,C}	41.95 ± 0.15 ^{a,A}	21.33 ± 0.11 ^{c,C}
DS8535-2 Pa	31.99 ± 0.14 ^{b,D}	22.38 ± 0.09 ^{d,D}	45.63 ± 0.21 ^{a,A}	2.80 ± 0.10 ^{a,A}	42.83 ± 0.22 ^{b,A}	22.38 ± 0.13 ^{d,D}
DS8535-2 Li.u	21.86 ± 0.12 ^{a,B}	19.16 ± 0.15 ^{b,C}	58.99 ± 0.16 ^{d,C}	3.73 ± 0.09 ^{b,B}	55.26 ± 0.31 ^{d,C}	19.15 ± 0.18 ^{b,C}
DS8535-2 La.p	34.30 ± 0.24 ^{d,C}	17.98 ± 0.13 ^{a,B}	47.72 ± 0.32 ^{c,B}	3.66 ± 0.07 ^{b,C}	44.06 ± 0.14 ^{c,B}	17.98 ± 0.15 ^{a,B}

Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*. SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids. The data are represented as means ($n = 3$) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between with the same LAB strain treated samples) ($p \leq 0.05$).

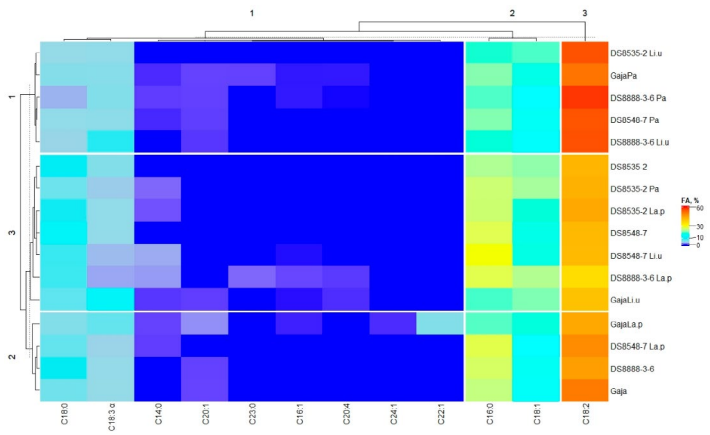

Figure 3. Fatty acid profile of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs). Pa—fermented with *Pediococcus acidilactici*; Li.u—fermented with *Liquorilactobacillus uvarum*; La.p—fermented with *Lactiplantibacillus plantarum*.

Different tendencies of the monounsaturated fatty content were obtained. In addition, when comparing non-fermented samples, the highest content was displayed in wholemeal purple wheat samples (21.33% from the total fatty acids content). However, after fermentation with *Lactiplantibacillus plantarum* and *Liquorilactobacillus uvarum*, the monounsaturated fatty content in wholemeal purple wheat was reduced. Conversely, in samples fermented with *Pediococcus acidilactici*, an increase in monounsaturated fatty acid content was detected. Furthermore, fermentation with any of the tested LAB strains increased monounsaturated fatty acid content in wholemeal 'Gaja' wheat cereal. Moreover, it also increased monounsaturated fatty acid content in wholemeal waxy wheat cereal samples fermented with *Lactiplantibacillus plantarum*, in wholemeal blue wheat cereal samples fermented with *Liquorilactobacillus uvarum* fermented, and, finally, in wholemeal purple wheat cereal samples fermented with *Pediococcus acidilactici*. A multivariate test of between-subjects effects showed that the type of LAB strain used for fermentation and its interaction with the wholemeal wheat variety had a significant effect on the monounsaturated fatty acid content of wholemeal wheat flours ($p = 0.038$).

When analysing polyunsaturated fatty acids in non-fermented samples, the highest content was found in wholemeal 'Gaja' wheat cereal. In turn, in fermented samples, different tendencies were established, and, in most of the cases, fermentation increased the polyunsaturated fatty acid content of wholemeal wheat cereal samples, viz.: in wholemeal 'Gaja', waxy and blue wheat cereal samples fermented with *Pediococcus acidilactici*; in wholemeal waxy and purple wheat cereal samples fermented with *Liquorilactobacillus uvarum*; and in wholemeal blue and purple wheat cereal samples fermented with *Lactiplantibacillus plantarum* fermented. However, analysed factors were not significant in the polyunsaturated fatty acid content of wholemeal cereal samples.

Comparing the omega-3 (ω-3), omega 6 (ω-6) and omega 9 (ω-9) content, fermentation reduced ω-3 content in wholemeal waxy, blue and purple wheat cereal samples fermented with *Lactiplantibacillus plantarum*, as well as in wholemeal blue and purple wheat cereal samples fermented with *Liquorilactobacillus uvarum*, and in wholemeal purple wheat cereal samples fermented with *Pediococcus acidilactici*. A lower content of ω-6 after fermentation was found in wholemeal 'Gaja' and blue wheat cereal samples fermented with *Liquorilactobacillus uvarum*, and in wholemeal 'Gaja' and waxy wheat cereal samples fermented with *Lactiplantibacillus plantarum*. Moreover, fermentation with *Lactiplantibacillus plantarum* reduced ω-9 content in both wholemeal colored wheat samples; fermentation with *Pediococcus acidilactici* and *Liquorilactobacillus uvarum* reduced ω-9 content in wholemeal waxy wheat; and finally, fermentation with *Pediococcus acidilactici* and *Liquorilactobacillus uvarum* was a significant factor on reducing ω-9 content in wholemeal blue and purple wheat samples, respectively. Multivariate test of between-subjects effects showed that the LAB strain used for fermentation and its interaction with wholemeal wheat variety interaction was significant on ω-6 content of wholemeal wheat cereal samples ($p \leq 0.050$). However, analysed factors and their interaction were not significant on ω-3 and ω-9 concentrations of wholemeal cereal samples.

When analysing the content of individual fatty acids in wholemeal wheat samples, the results unveiled that the dominant fatty acids in all wholemeal wheat cereal samples were linoleic acid (C18:2), palmitic acid (C16:0), octadec-9-enoic acid (C18:1 cis, trans), stearic acid (C18:0) and α-linolenic acid (C18:3 α) (Figure 3). Moreover, fermented samples revealed a wider variety of individual fatty acids. In most of the fermented samples, tetradecanoic acid (C14:0) was formed, with the highest content observed in DS8548-7 Li.u wholemeal samples (2.24% of the total fatty acid content). Moreover, six fermented samples showed, in their fatty acid profile, the presence of palmitoleic acid (C16:1), and the highest concentration of C16:1 was found in DS8888-3-6 La.p cereal wholemeal (0.682% from the total fatty acid content). Cis-5,8,11,14-eicosatetraenoic acid (C20:4) accounted for just 4 out of 16 analysed wholemeal samples, and its content ranged from 0.051 to 0.527% of the total fatty acid content (in DS8888-3-6 Pa and DS8888-3-6 La.p, respectively). Cis-13-docosenoic acid (C22:1) (4.88% from the total fat content) and cis-15-tetracosenoic acid

(C24:1) (0.367% from the total fat content) were formed just in 'Gaja'La.p samples. Moreover, tricosanoic acid (C23:0) was just found in 'Gaja'Pa and DS8888-3-6 La.p wholemeal cereal samples (0.628 and 1.17% from the total fatty acid content, respectively). Multivariate test of between-subjects effects showed that the type of LAB strain used for fermentation was a significant factor on the concentrations of C14:0, C16:1, C18:0, C18:2, C20:4, C22:1, C23:0 and C24:1 in wholemeal cereals ($p \leq 0.0001$, $p \leq 0.0001$, $p = 0.034$, $p = 0.023$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, respectively). Moreover, wholemeal wheat variety was a significant factor on the content of C14:0, C16:1, C18:3 α, C20:1, C20:4, C22:1, C23:0 and C24:1 ($p \leq 0.0001$, $p \leq 0.0001$, $p = 0.002$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$ and $p \leq 0.0001$, respectively). However, the interaction of LAB strain and wheat variety was significant on the concentrations of C14:0, C16:1, C18:0, C18:2, C18:3 α, C20:1, C20:4, C22:1, C23:0 and C24:1 in wholemeal wheat samples ($p \leq 0.0001$, $p \leq 0.0001$, $p = 0.004$, $p \leq 0.0001$, $p \leq 0.001$, $p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$, respectively).

It was reported that cereal grains are rich in unsaturated fatty acids as well as are a good source of essential polyunsaturated fatty acids (namely, linoleic and linolenic acids) [99]. Wheat lipids also contain saturated fatty acids [palmitic (20.5%) and stearic acids (1.5%)]. The fatty acids composition of wheat grain makes from a nutritional point of view the cereals balanced foodstuffs [99].

Dominant unsaturated fatty acids in wheat are C18:2, C18:1, C18:3 and C16:1 (which includes two essential ones to know: linoleic and linolenic acids) [99]. Essential fatty acids are involved in many metabolic processes, including cholesterol metabolism [100–102]. The fatty acid profile of wheat is dependent on many factors, including genetics [103], environmental conditions and agronomic practices [100,103,104]. It was reported that durum and hard red wheat have a higher lipid content and distinct fatty acid profile in comparison with soft white wheat [99]. Moreover, cold weather can increase the total lipid concentration and the unsaturated fatty acid content in wheat cereal grains [105]. Bottari et al. reported more than 60 fatty acids in wheat cereal, with even numbers of carbon atoms from C12 to C30 as well as C15 and C17. The major fatty acids were saturated and unsaturated C16 and C18 and, particularly, C16:0, C18:1 and C18:2, which together represented around 90% of the total [106]. However, the main fatty acids in wheat cereals are C16:0, C18:0, C18:1, C18:2 and C18:3, and other fatty acid contents represent, on average, 1–2%. The differences in wheat fatty acid profile reported in different studies are explained by the different genetic, climatic and agronomical conditions, as well as the interaction between genotype × year × treatment as the main factor towards the variability of the fatty acid concentration observed in durum wheat samples [104]. Armanino et al. reported that the fatty acid profile of durum wheat is influenced by the cultivar [103]. In addition, variation in saturated and unsaturated fatty acids within the same wheat cereal grain variety can be obtained, and both biotic and abiotic stresses are related to these differences [105,107]. Finally, the fatty acid composition and level of unsaturation also vary according to the type of cereal, for example, when comparing maize and rye flours [108–112]. Regarding the fatty acid content, this study showed that in addition to the wheat variety, the process of fermentation is a significant factor on most of the fatty acid content in wholemeal wheat cereal grain. Finally, the changes of fatty acid profile during the biotechnological processes should also be taken into consideration to achieve the best technological and nutritional characteristics of the cereal-based end product.

4. Conclusions

Although coloured wheat grain has attracted attention due to its anti-inflammatory, antimicrobial and other desirable biological activities, the reported data, especially about the impact of fermentation, are still scarce. Therefore, this work contributes to the knowledge of the impact of LAB (*Pediococcus acidilactici*, *Liquorilactobacillus uvarum* and *Lactiplantibacillus plantarum*) fermentation on acidity, microbiological and colour traits, fatty and amino acid profiles, gamma-aminobutyric acid and biogenic amine concentrations, macro- and microelement contents in wholemeal cereal grains of 'Gaja' (traditional wheat) and new

breed lines DS8888-3-6 (waxy wheat), DS8548-7 (blue wheat) and DS8535-2 (purple wheat). As our study indicated, due to the good viability of LAB and obtained low pH values in tested samples, all wholemeal wheat varieties were an appropriate substrate for fermentation with selected LAB strains. In most of the samples, essential amino acids and GABA content significantly increased after fermentation, while wholemeal 'Gaja' and waxy wheat samples contained the most GABA (on average, 2.47 μmol/g). The content of macro- and microelements depended on the wheat variety, while most of the fatty acids in wholemeal cereal grains were significantly affected by the fermentation. In addition to the improved nutritional value of fermented wholemeal wheat flours, some safety aspects, such as the formation of biogenic amines, should be pointed out in the production process, as the total levels of biogenic amines in tested samples ranged from 22.7 (in non-fermented DS8888-3-6) to 416 mg/kg (in DS8548-7 fermented with *Lactiplantibacillus plantarum*). The results of this study are beneficial for agri-food and feed industries since it gives information on the effect of fermentation on the final characteristics of cereal-based products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8100563/s1, Supplementary File S1. Field experiments; Supplementary File S2. Analysis of the Amino Acids and Gamma-Aminobutyric Acid; Supplementary File S3. Analysis of the Biogenic Amine Content; Supplementary File S4. Analysis of Macro- and Microelements Content in Grain Wholemeal; Supplementary File S5. Fatty Acid Composition Analysis; Supplementary Table S1. Detailed fatty acids profile of the wheat cereal wholemeal samples; Supplementary Table S2. Images of the wholemeal wheat flour samples (non-fermented flours and fermented baking doughs).

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References

1. Nakamura, T.; Yamamori, M.; Hirano, H.; Hidaka, S.; Nagamine, T. Production of Waxy (Amylose-Free) Wheats. *Mol. Gen. Genet. MGG* **1995**, *248*, 253–259. [CrossRef]
2. Huang, Y.-C.; Lai, H.-M. Noodle Quality Affected by Different Cereal Starches. *J. Food Eng.* **2010**, *97*, 135–143. [CrossRef]
3. Baik, B.-K.; Lee, M.-R. Effects of Starch Amylose Content of Wheat on Textural Properties of White Salted Noodles. *Cereal Chem.* **2003**, *80*, 304–309. [CrossRef]

4. Sasaki, T.; Yasui, T.; Matsuki, J. Effect of Amylose Content on Gelatinization, Retrogradation, and Pasting Properties of Starches from Waxy and Nonwaxy Wheat and Their F1 Seeds. *Cereal Chem.* **2000**, *77*, 58–63. [CrossRef]
5. Blazek, J.; Copeland, L. Pasting and Swelling Properties of Wheat Flour and Starch in Relation to Amylose Content. *Carbohydr. Polym.* **2008**, *71*, 380–387. [CrossRef]
6. Zhang, H.; Zhang, W.; Xu, C.; Zhou, X. Morphological Features and Physicochemical Properties of Waxy Wheat Starch. *Int. J. Biol. Macromol.* **2013**, *62*, 304–309. [CrossRef] [PubMed]
7. Zhou, C.; Sun, Y.; Yao, Y.; Li, H.; He, J. Study of Noodle Quality Based on Protein Properties of Three Wheat Varieties. *J. Food Qual.* **2022**, *2022*, e6383080. [CrossRef]
8. Rausch, K.D.; Singh, V. Crops–Cereals. In *Food Processing Principles and Applications*; Wiley-Blackwell: New York, NY, USA, 2014; pp. 293–304.
9. Song, J.M.; Liu, A.F.; You, M.S.; Li, B.Y.; Wu, X.Y.; Zhan, Z.D.; Liu, G.T. Effects of Waxy Flour Blending on Starch Pasting Properties and Noodle Quality of Non Waxy Flour. *Sci. Agric. Sin.* **2004**, *37*, 1838–1842.
10. Aifeng, L.; Ran, H.; Dungong, C.; Haosheng, L.; Xinyou, C.; Jianmin, S.; Cheng, L.; Jun, G.; Faji, L.; Shengnan, Z.; et al. Applicability of Waxy Wheat Variety for Improving the Quality of Noodle and Steamed Bread. *Int. J. Nutr. Food Sci.* **2021**, *10*, 72. [CrossRef]
11. Gupta, R.; Meghwal, M.; Prabhakar, P.K. Bioactive Compounds of Pigmented Wheat (Triticum Aestivum): Potential Benefits in Human Health. *Trends Food Sci. Technol.* **2021**, *110*, 240–252. [CrossRef]
12. Lachman, J.; Martinek, P.; Kotíková, Z.; Orsák, M.; Šulc, M. Genetics and Chemistry of Pigments in Wheat Grain—A Review. *J. Cereal Sci.* **2017**, *74*, 145–154. [CrossRef]
13. Mbarki, S.; Sytar, O.; Zivcak, M.; Abdelly, C.; Cerda, A.; Brestic, M. Anthocyanins of Coloured Wheat Genotypes in Specific Response to Salstress. *Molecules* **2018**, *23*, 1518. [CrossRef] [PubMed]
14. Paznocht, L.; Kotíková, Z.; Šulc, M.; Lachman, J.; Orsák, M.; Eliášová, M.; Martinek, P. Free and Esterified Carotenoids in Pigmented Wheat, Tritordeum and Barley Grains. *Food Chem.* **2018**, *240*, 670–678. [CrossRef] [PubMed]
15. Sharma, N.; Kumari, A.; Chunduri, V.; Kaur, S.; Banda, J.; Goyal, A.; Garg, M. Anthocyanin Biofortified Black, Blue and Purple Wheat Exhibited Lower Amino Acid Cooking Losses than White Wheat. *LWT* **2022**, *154*, 112802. [CrossRef]
16. Sharma, S.; Chunduri, V.; Kumar, A.; Kumar, R.; Khare, P.; Kondepudi, K.K.; Bishnoi, M.; Garg, M. Anthocyanin Bio-Fortified Colored Wheat: Nutritional and Functional Characterization. *PLoS ONE* **2018**, *13*, e0194367.
17. Sharma, N.; Tiwari, V.; Vats, S.; Kumari, A.; Chunduri, V.; Kaur, S.; Kapoor, P.; Garg, M. Evaluation of Anthocyanin Content, Antioxidant Potential and Antimicrobial Activity of Black, Purple and Blue Colored Wheat Flour and Wheat-Grass Juice against Common Human Pathogens. *Molecules* **2020**, *25*, 5785. [CrossRef]
18. Sharma, S.; Kapoor, P.; Kaur, S.; Kumari, A.; Sharma, N.; Kumar, A.; Chunduri, V.; Garg, M. Changing Nutrition Scenario: Colored Wheat–A New Perspective. In *Physiological, Molecular, and Genetic Perspectives of Wheat Improvement*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 71–88.
19. Sytar, O.; Bośko, P.; Živčák, M.; Brestic, M.; Smetanska, I. Bioactive Phytochemicals and Antioxidant Properties of the Grains and Sprouts of Colored Wheat Genotypes. *Molecules* **2018**, *23*, 2282. [CrossRef]
20. Tian, S.; Chen, Z.; Wei, Y. Measurement of Colour-Grained Wheat Nutrient Compounds and the Application of Combination Technology in Dough. *J. Cereal Sci.* **2018**, *83*, 63–67. [CrossRef]
21. Bartkiene, E.; Bartkevics, V.; Pugajeva, I.; Krungleviciute, V.; Mayrhofer, S.; Domig, K. The Contribution of P. Acidilactici, L. Plantarum, and L. Curvatus Starters and L-(+)-Lactic Acid to the Acrylamide Content and Quality Parameters of Mixed Rye—Wheat Bread. *LWT* **2017**, *80*, 43–50. [CrossRef]
22. Bartkiene, E.; Starkute, V.; Katuskevicius, K.; Laukyte, N.; Fomkinas, M.; Vysniauskas, E.; Kasciukaityte, P.; Radvilavicius, E.; Rokaite, S.; Medonas, D.; et al. The Contribution of Edible Cricket Flour to Quality Parameters and Sensory Characteristics of Wheat Bread. *Food Sci. Nutr.* **2022**. [CrossRef]
23. Bartkiene, E.; Lele, V.; Ruzauskas, M.; Domig, K.J.; Starkute, V.; Zavistanaviciute, P.; Bartkevics, V.; Pugajeva, I.; Klupsaite, D.; Juodeikiene, G.; et al. Lactic Acid Bacteria Isolation from Spontaneous Sourdough and Their Characterization Including Antimicrobial and Antifungal Properties Evaluation. *Microorganisms* **2020**, *8*, 64. [CrossRef] [PubMed]
24. Badaras, S.; Ruzauskas, M.; Gruzauskas, R.; Zokaityte, E.; Starkute, V.; Klupsaite, D.; Mockus, E.; Klementaviciute, J.; Vadopalas, L.; Zokaityte, G.; et al. Different Creep Compound Feed Formulations for New Born Piglets: Influence on Growth Performance and Health Parameters. *Front. Vet. Sci.* **2022**, *9*, 971783. [CrossRef] [PubMed]
25. Bartkiene, E.; Vizbickiene, D.; Bartkevics, V.; Pugajeva, I.; Krungleviciute, V.; Zadeike, D.; Zavistanaviciute, P.; Juodeikiene, G. Application of Pediococcus Acidilactici LUHS29 Immobilized in Apple Pomace Matrix for High Value Wheat-Barley Sourdough Bread. *LWT-Food Sci. Technol.* **2017**, *83*, 157–164. [CrossRef]
26. Bartkiene, E.; Bartkevics, V.; Lele, V.; Pugajeva, I.; Zavistanaviciute, P.; Mickiene, R.; Zadeike, D.; Juodeikiene, G. A Concept of Mould Spoilage Prevention and Acrylamide Reduction in Wheat Bread: Application of Lactobacilli in Combination with a Cranberry Coating. *Food Control* **2018**, *91*, 284–293. [CrossRef]
27. Cai, H.-L.; Zhu, R.-H.; Li, H.-D. Determination of Dansylated Monoamine and Amino Acid Neurotransmitters and Their Metabolites in Human Plasma by Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry. *Anal. Biochem.* **2010**, *396*, 103–111. [CrossRef] [PubMed]

28. Ben-Gigirey, B.; Vieites Baaptista de Sousa, J.M.; Villa, T.G.; Barros-Velazquez, J. Histamine and Cadaverine Production by Bacteria Isolated from Fresh and Frozen Albacore (*Thunnus alalunga*). *J. Food Prot.* **1999**, *62*, 933–939. [CrossRef]
29. Evans, J.D. *Straightforward Statistics for the Behavioral Sciences*; Thomson Brooks/Cole Publishing Co: Belmont, CA, USA, 1996; pp. xxii, 600, ISBN 0-534-23100-4.
30. Kati, K.; Kaisa, P.; Karin, A. Influence and Interactions of Processing Conditions and Starter Culture on Formation of Acids, Volatile Compounds, and Amino Acids in Wheat Sourdoughs. *Cereal Chem.* **2004**, *81*, 598–610. [CrossRef]
31. Zhu, F. Anthocyanins in Cereals: Composition and Health Effects. *Food Res. Int.* **2018**, *109*, 232–249. [CrossRef]
32. Enaru, B.; Dreţcanu, G.; Pop, T.D.; Stănilă, A.; Diaconeasa, Z. Anthocyanins: Factors Affecting Their Stability and Degradation. *Antioxidants* **2021**, *10*, 1967. [CrossRef]
33. Suo, B.; Chen, X.; Wang, Y. Recent Research Advances of Lactic Acid Bacteria in Sourdough: Origin, Diversity, and Function. *Curr. Opin. Food Sci.* **2021**, *37*, 66–75. [CrossRef]
34. De Vuyst, L.; Van Kerrebroeck, S.; Harth, H.; Huys, G.; Daniel, H.-M.; Weckx, S. Microbial Ecology of Sourdough Fermentations: Diverse or Uniform? *Food Microbiol.* **2014**, *37*, 11–29. [CrossRef] [PubMed]
35. Tomičić, Z.; Pezo, L.; Spasevski, N.; Lazarević, J.; Cabarkapa, I.; Tomicic, R. Diversity of Amino Acids Composition in Cereals. *Food Feed Res.* **2022**, *49*, 11–22. [CrossRef]
36. Ferreira, R.R.; Varisi, V.A.; Meinhardt, L.W.; Lea, P.J.; Azevedo, R.A. Are High-Lysine Cereal Crops Still a Challenge? *Braz. J. Med. Biol. Res.* **2005**, *38*, 985–994. [CrossRef] [PubMed]
37. Ramadas, S.; Kumar, T.M.K.; Singh, G.P. *Wheat Production in India: Trends and Prospects*; IntechOpen: London, UK, 2019; ISBN 978-1-78985-450-3.
38. Ufaz, S.; Galili, G. Improving the Content of Essential Amino Acids in Crop Plants: Goals and Opportunities. *Plant Physiol.* **2008**, *147*, 954–961. [CrossRef] [PubMed]
39. Caire-Juvera, G.; Vázquez-Ortíz, F.A.; Higuera-Ciapara, I.; Hernández, G. Composición de aminoácidos, calificación química y digestibilidad. *Nutr. Hosp.* **2013**, *28*, 365–371. [CrossRef]
40. Siddiqi, R.A.; Singh, T.P.; Rani, M.; Sogi, D.S.; Bhat, M.A. Diversity in Grain, Flour, Amino Acid Composition, Protein Profiling, and Proportion of Total Flour Proteins of Different Wheat Cultivars of North India. *Front. Nutr.* **2020**, *7*, 141. [CrossRef]
41. Food and Agriculture Organization of the United Nations (Ed.) *Dietary Protein Quality Evaluation in Human Nutrition: Report of an FAO Expert Consultation, 31 March–April 2011, Auckland, New Zealand*; FAO food and nutrition paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013; ISBN 978-92-5-107417-6.
42. Knežević, D.; Đukić, N.; Madic, M.; Paunović, A.; Zecevic, V. Comparison of Amino Acids Contents in Barley and Wheat. *Res. J. Agric. Sci.* **2007**, *39*, 71–76.
43. Wilson, D.C.; Rafii, M.; Ball, R.O.; Pencharz, P.B. Threonine Requirement of Young Men Determined by Indicator Amino Acid Oxidation with Use of L-[1-13C]Phenylalanine. *Am. J. Clin. Nutr.* **2000**, *71*, 757–764. [CrossRef]
44. Zafar, S.; Naz, N.; Nazir, S.; Abbas, M.; Khan, A.M. Analysis of Selected Amino Acids in Different Varieties of Wheat Available in Punjab, Pakistan. *Chromatogr. Res. Int.* **2014**, *2014*, e867070. [CrossRef]
45. Gheller, M.; Bender, E.; Thalacker-Mercer, A. Safety of Graded-Doses of Histidine in Healthy Adults (P08-062-19). *Curr. Dev. Nutr.* **2019**, *3*, nzz044.P08-062-19. [CrossRef]
46. Moro, J.; Tomé, D.; Schmidely, P.; Demersay, T.-C.; Azzout-Marniche, D. Histidine: A Systematic Review on Metabolism and Physiological Effects in Human and Different Animal Species. *Nutrients* **2020**, *12*, 1414. [CrossRef] [PubMed]
47. Laze, A.; Arapi, V.; Ceca, E.; Gusho, K.; Pezo, L.; Brahushi, F.; Kneževic, D. Chemical Composition and Amino Acid Content in Different Genotypes of Wheat Flour. *Period. Polytech. Chem. Eng.* **2019**, *63*, 618–628. [CrossRef]
48. Jackman, S.R.; Witard, O.C.; Philp, A.; Wallis, G.A.; Baar, K.; Tipton, K.D. Branched-Chain Amino Acid Ingestion Stimulates Muscle Myofibrillar Protein Synthesis Following Resistance Exercise in Humans. *Front. Physiol.* **2017**, *8*, 390. [CrossRef] [PubMed]
49. Jiang, X.; Tian, J.; Hao, Z.; Zhang, W. Protein Content and Amino Acid Composition in Grains of Wheat-Related Species. *Agric. Sci. China* **2008**, *7*, 272–279. [CrossRef]
50. Bandegan, A.; Golian, A.; Kiarie, E.; Payne, R.L.; Crow, G.H.; Guenter, W.; Nyachoti, C.M. Standardized Ileal Amino Acid Digestibility in Wheat, Barley, Pea and Flaxseed for Broiler Chickens. *Can. J. Anim. Sci.* **2011**, *91*, 103–111. [CrossRef]
51. Mayer, R.R.; Cherry, J.H.; Rhodes, D. Effects of Heat Shock on Amino Acid Metabolism of Cowpea Cells 1. *Plant Physiol.* **1990**, *94*, 796–810. [CrossRef]
52. Aurisano, N.; Bertani, A.; Reggiani, R. Anaerobic Accumulation of 4-Aminobutyrate in Rice Seedlings; Causes and Significance. *Phytochemistry* **1995**, *38*, 1147–1150. [CrossRef]
53. Diana, M.; Quílez, J.; Rafecas, M. Gamma-Aminobutyric Acid as a Bioactive Compound in Foods: A Review. *J. Funct. Foods* **2014**, *10*, 407–420. [CrossRef]
54. Di Cagno, R.; De Angelis, M.; Lavermicocca, P.; De Vincenzi, M.; Giovannini, C.; Faccia, M.; Gobbetti, M. Proteolysis by Sourdough Lactic Acid Bacteria: Effects on Wheat Flour Protein Fractions and Gliadin Peptides Involved in Human Cereal Intolerance. *Appl. Environ. Microbiol.* **2002**, *68*, 623–633. [CrossRef]
55. Gänzle, M.G.; Loponen, J.; Gobbetti, M. Proteolysis in Sourdough Fermentations: Mechanisms and Potential for Improved Bread Quality. *Trends Food Sci. Technol.* **2008**, *19*, 513–521. [CrossRef]
56. Gobbetti, M.; Rizzello, C.G.; Di Cagno, R.; De Angelis, M. How the Sourdough May Affect the Functional Features of Leavened Baked Goods. *Food Microbiol.* **2014**, *37*, 30–40. [CrossRef] [PubMed]

57. Paterson, A.; Piggott, J.R. Flavour in Sourdough Breads: A Review. *Trends Food Sci. Technol.* **2006**, *17*, 557–566.
58. Bhanwar, S.; Bamnia, M.; Ghosh, M.; Ganguli, A. Use of Lactococcus Lactis to Enrich Sourdough Bread with γ-Aminobutyric Acid. *Int. J. Food Sci. Nutr.* **2013**, *64*, 77–81. [CrossRef] [PubMed]
59. Diana, M.; Rafecas, M.; Quílez, J. Free Amino Acids, Acrylamide and Biogenic Amines in Gamma-Aminobutyric Acid Enriched Sourdough and Commercial Breads. *J. Cereal Sci.* **2014**, *60*, 639–644. [CrossRef]
60. Okada, T.; Sugishita, T.; Murakami, T.; Murai, H.; Saikusa, T.; Horino, T.; Onoda, A.; Kajimoto, O.; Takahashi, R.; Takahashi, T. Effect of the Defatted Rice Germ Enriched with GABA for Sleeplessness, Depression, Autonomic Disorder by Oral Administration. *Nippon Shokuhin Kagaku Kogaku Kaishi J. Jpn. Soc. Food Sci. Technol.* **2000**, *47*, 596–603. [CrossRef]
61. Rizzello, C.G.; Cassone, A.; Di Cagno, R.; Gobbetti, M. Synthesis of Angiotensin I-Converting Enzyme (ACE)-Inhibitory Peptides and γ-Aminobutyric Acid (GABA) during Sourdough Fermentation by Selected Lactic Acid Bacteria. *J. Agric. Food Chem.* **2008**, *56*, 6936–6943. [CrossRef]
62. Villegas, J.M.; Brown, L.; de Giori, G.S.; Hebert, E.M. Optimization of Batch Culture Conditions for GABA Production by Lactobacillus Brevis CRL 1942, Isolated from Quinoa Sourdough. *LWT-Food Sci. Technol.* **2016**, *67*, 22–26. [CrossRef]
63. Apetrei, I.M.; Apetrei, C. Amperometric Biosensor Based on Diamine Oxidase/Platinum Nanoparticles/Graphene/Chitosan Modified Screen-Printed Carbon Electrode for Histamine Detection. *Sensors* **2016**, *16*, 422. [CrossRef]
64. Biji, K.B.; Ravishankar, C.N.; Venkateswarlu, R.; Mohan, C.O.; Gopal, T.K. Biogenic Amines in Seafood: A Review. *J. Food Sci. Technol.* **2016**, *53*, 2210–2218. [CrossRef]
65. Askar, A.; Treptow, H. *Biogene Amine in Lebensmitteln: Vorkommen, Bedeutung Und Bestimmung*; E. Ulmer: Stuttgart, Germany, 1986; ISBN 3-8001-2132-8.
66. Glória, M.B.A.; Tavares-Neto, J.; Labanca, R.A.; Carvalho, M.S. Influence of Cultivar and Germination on Bioactive Amines in Soybeans (*Glycine max* L. Merril). *J. Agric. Food Chem.* **2005**, *53*, 7480–7485. [CrossRef]
67. Thamm, M.; Scholl, C.; Reim, T.; Grübel, K.; Möller, K.; Rössler, W.; Scheiner, R. Neuronal Distribution of Tyramine and the Tyramine Receptor AmTAR1 in the Honeybee Brain. *J. Comp. Neurol.* **2017**, *525*, 2615–2631. [CrossRef] [PubMed]
68. Izquierdo, C.; Gómez-Tamayo, J.C.; Nebel, J.-C.; Pardo, L.; Gonzalez, A. Identifying Human Diamine Sensors for Death Related Putrescine and Cadaverine Molecules. *PLoS Comput. Biol.* **2018**, *14*, e1005945. [CrossRef] [PubMed]
69. Cardozo, M.; Lima, K.S.; Franca, T.C.; Lima, A.L.S. Aminas Biogênicas: Um Problema de Saúde Pública. *Rev. Virtual Quím.* **2013**, *5*, 149–168.
70. Montet, D.; Ray, R.C. *Fermented Foods, Part I: Biochemistry and Biotechnology*; Taylor & Francis Group: New York, NY, USA, 2020; ISBN 978-0-367-73745-0.
71. Silva, T.M.; Sabaini, P.S.; Evangelista, W.P.; Gloria, M.B.A. Occurrence of Histamine in Brazilian Fresh and Canned Tuna. *Food Control* **2011**, *22*, 323–327. [CrossRef]
72. Oliveira, R.B.A.; Evangelista, W.P.; Sena, M.J.; Gloria, M.B.A. Tuna Fishing, Capture and Post-Capture Practices in the Northeast of Brazil and Their Effects on Histamine and Other Bioactive Amines. *Food Control* **2012**, *25*, 64–68. [CrossRef]
73. Gomes, M.B.; Pires, B.A.D.; Fracalanzza, S.A.P.; Marin, V.A. The Risk of Biogenic Amines in Food. *Cienc. Saude Colet.* **2014**, *19*, 1123. [CrossRef]
74. Gomes Müller, D.; Quadro Oreste, E.; Grazielle Heinemann, M.; Dias, D.; Kessler, F. Biogenic Amine Sensors and Its Building Materials: A Review. *Eur. Polym. J.* **2022**, *175*, 111221. [CrossRef]
75. Dabrowski, W.M.; Sikorski, Z.E. *Toxins in Food*; CRC Press: Boca Raton, FL, USA, 2004; ISBN 0-203-50235-3.
76. U.S. Department of Health and Human Services Food and Drug Administration. *Fish and Fishery Products Hazards and Controls Guidance*; US Department of Health and Human Services: Rockville, MD, USA, 2011.
77. Santos, M.H.S. Biogenic Amines: Their Importance in Foods. *Int. J. Food Microbiol.* **1996**, *29*, 213–231. [CrossRef]
78. Jākobsone, I.; Kantāne, I.; Zute, S.; Jansone, I.; Bartkevičs, V. Macro-Elements and Trace Elements in Cereal Grains Cultivated in Latvia. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2015**, *69*, 152–157. [CrossRef]
79. Underwood, B.A.; Smitasiri, S. Micronutrient Malnutrition: Policies and Programs for Control and Their Implications. *Annu. Rev. Nutr.* **1999**, *19*, 303–324. [CrossRef]
80. Pietola, L.; Salo, T. Response of P, K, Mg and NO3-N Contents of Carrots to Irrigation, Soil Compaction, and Nitrogen Fertilisation. *Agric. Food Sci. Finl.* **2000**, *9*, 319–331. [CrossRef]
81. Hattori, H.; Chino, M. Growth, Cadmium, and Zinc Contents of Wheat Grown on Various Soils Enriched with Cadmium and Zinc. In *Plant Nutrition: Food Security and Sustainability of Agro-Ecosystems through Basic and Applied Research*; Horst, W.J., Schenk, M.K., Bürkert, A., Claassen, N., Flessa, H., Frommer, W.B., Goldbach, H., Olfs, H.-W., Römheld, V., Sattelmacher, B., et al., Eds.; Developments in Plant and Soil Sciences; Springer: Dordrecht, The Netherlands, 2001; pp. 462–463, ISBN 978-0-306-47624-2.
82. Kashian, S.; Fathivand, A.A. Estimated Daily Intake of Fe, Cu, Ca and Zn through Common Cereals in Tehran, Iran. *Food Chem.* **2015**, *176*, 193–196. [CrossRef] [PubMed]
83. Guo, Z.; Zhang, Z.; Xu, P.; Guo, Y. Analysis of Nutrient Composition of Purple Wheat. *Cereal Res. Commun.* **2012**, *41*, 293–303. [CrossRef]
84. Kapur, D.; Nath Agarwal, K.; Kumari Agarwal, D. Nutritional Anemia and Its Control. *Indian J. Pediatr.* **2002**, *69*, 607–616. [CrossRef]
85. Welch, R.M.; Graham, R.D. Breeding for Micronutrients in Staple Food Crops from a Human Nutrition Perspective. *J. Exp. Bot.* **2004**, *55*, 353–364. [CrossRef]

86. Choi, Y.; Jeong, H.-S.; Lee, J. Antioxidant Activity of Methanolic Extracts from Some Grains Consumed in Korea. *Food Chem.* **2007**, *103*, 130–138. [CrossRef]
87. Milder, I.E.; Arts, I.C.; van de Putte, B.; Venema, D.P.; Hollman, P.C. Lignan Contents of Dutch Plant Foods: A Database Including Lariciresinol, Pinoresinol, Secoisolariciresinol and Matairesinol. *Br. J. Nutr.* **2005**, *93*, 393–402. [CrossRef]
88. Hosseinian, F.S.; Beta, T. Saskatoon and Wild Blueberries Have Higher Anthocyanin Contents than Other Manitoba Berries. *J. Agric. Food Chem.* **2007**, *55*, 10832–10838. [CrossRef]
89. Li, W.; Beta, T. Flour and Bread from Black-, Purple-, and Blue-Colored Wheats. In *Flour and Breads and Their Fortification in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 59–67.
90. He, Y.Z.; Ning, J.F. Analysis of Nutrition Composition in the Special Purple Grain Wheat "Qinhei 1" Containing Rich Fe and Zn. *J. Northwest Agric. For. Univ.* **2003**, *31*, 87–90.
91. Blanco, A. *Quimica Biologica/Biological Chemistry*; AbeBooks: Victoria, BC, Canada, 2006; ISBN 13: 9789500204224.
92. Hurley, S.W.; Johnson, A.K. The Biopsychology of Salt Hunger and Sodium Deficiency. *Pflüg. Arch.-Eur. J. Physiol.* **2015**, *467*, 445–456. [CrossRef]
93. Moll, R.; Davis, B. Iron, Vitamin B12 and Folate. *Medicine* **2017**, *45*, 198–203. [CrossRef]
94. Weaver, C.M.; Heaney, R.P. *Calcium and Human Health*, 1st ed.; Humana Press Inc: Totowa, NJ, USA, 2006.
95. EFSA Dietary Reference Values | EFSA. Available online: https://www.efsa.europa.eu/en/topics/topic/dietary-reference-values (accessed on 27 September 2022).
96. Fotedar, A.; Bhasin, J.S.; Chakravarty, A.; Kulkarni, A.; Bhalla, G.; Anwar, F.; Rao, S. Effectiveness of Iron-Fortified Infant Cereals on Hemoglobin Levels of Children Aged 12–24 Months: A Cross-Sectional Study from New Delhi, India. *J. Fam. Med. Prim. Care* **2018**, *7*, 77.
97. Banerjee, P.; Bhattacharya, P. Investigating Cobalt in Soil-Plant-Animal-Human System: Dynamics, Impact and Management. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 2339–2354. [CrossRef]
98. Chasapis, C.T.; Ntoupa, P.-S.A.; Spiliopoulou, C.A.; Stefanidou, M.E. Recent Aspects of the Effects of Zinc on Human Health. *Arch. Toxicol.* **2020**, *94*, 1443–1460. [CrossRef]
99. Rosentrater, K.A.; Evers, A.D. Chapter 4—Chemical Components and Nutrition. In *Kent's Technology of Cereals*, 5th ed.; Rosentrater, K.A., Evers, A.D., Eds.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing: Cambridge, UK, 2018; pp. 267–368, ISBN 978-0-08-100529-3.
100. Lafiandra, D.; Masci, S.; Sissons, M.; Dornez, E.; Delcour, J.; Courtin, C.; Caboni, M.F. Kernel Components of Technological Value. In *Durum Wheat Chemistry and Technology*; AACC International Press: Washington, DC, USA, 2012; pp. 85–124.
101. Russo, G.L. Dietary N-6 and n-3 Polyunsaturated Fatty Acids: From Biochemistry to Clinical Implications in Cardiovascular Prevention. *Biochem. Pharmacol.* **2009**, *77*, 937–946. [CrossRef]
102. Mozaffarian, D.; Wu, J.H.Y. Omega-3 Fatty Acids and Cardiovascular Disease: Effects on Risk Factors, Molecular Pathways, and Clinical Events. *J. Am. Coll. Cardiol.* **2011**, *58*, 2047–2067. [CrossRef]
103. Armanino, C.; De Acutis, R.; Rosa Festa, M. Wheat Lipids to Discriminate Species, Varieties, Geographical Origins and Crop Years. *Anal. Chim. Acta* **2002**, *454*, 315–326. [CrossRef]
104. Beleggia, R.; Platani, C.; Nigro, F.; De Vita, P.; Cattivelli, L.; Papa, R. Effect of Genotype, Environment and Genotype-by-Environment Interaction on Metabolite Profiling in Durum Wheat (*Triticum durum* Desf.) Grain. *J. Cereal Sci.* **2013**, *57*, 183–192. [CrossRef]
105. Nejadsadeghi, L.; Maali-Amiri, R.; Zeinali, H.; Ramezanpour, S.; Sadeghzade, B. Membrane Fatty Acid Compositions and Cold-Induced Responses in Tetraploid and Hexaploid Wheats. *Mol. Biol. Rep.* **2015**, *42*, 363–372. [CrossRef]
106. Bottari, E.; De Acutis, R.; Festa, M.R. On the Lipid Constituents of Wheat of Different Species, Variety, Origin and Crop Year. *Ann. Chim.* **1999**, *89*, 849–862.
107. Upchurch, R.G. Fatty Acid Unsaturation, Mobilization, and Regulation in the Response of Plants to Stress. *Biotechnol. Lett.* **2008**, *30*, 967–977. [CrossRef]
108. Rocha, J.M.; Kalo, P.J.; Malcata, F.X. Fatty acid composition of non-starch and starch neutral lipid extracts of portuguese sourdough bread. *J. Am. Oil Chem. Soc.* **2012**, *89*, 2025–2045. [CrossRef]
109. Rocha, J.M.; Kalo, P.J.; Malcata, F.X. Composition of neutral lipid classes and content of fatty acids throughout sourdough breadmaking. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 294–305. [CrossRef]
110. Rocha, J.M.; Kalo, P.J.; Malcata, F.X. Neutral lipids in free, bound and starch lipid extracts of flours, sourdough and portuguese sourdough bread, determined by NP-HPLC-ELSD. *Cereal Chem.* **2011**, *88*, 400–408. [CrossRef]
111. Rocha, J.M.; Kalo, P.J.; Malcata, F.X. Neutral lipids in non-starch lipid and starch lipid extracts from portuguese sourdough bread. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1138–1149. [CrossRef]
112. Rocha, J.M.; Kalo PJOllilainen, V.; Malcata, F.X. Separation and identification of neutral cereal lipids by normal phase high-performance liquid chromatography, using evaporative light-scattering and electrospray mass spectrometry for detection. *J. Chromatogr. A* **2010**, *1217*, 3013–3025. [CrossRef] [PubMed]

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Comparison Study of Nontreated and Fermented Wheat Varieties 'Ada', 'Sarta', and New Breed Blue and Purple Wheat Lines Wholemeal Flour

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Simple Summary: Nowadays, consumers are looking for higher functional value products that can meet the nutritional requirements of functional compounds. Wheat is the most important crop in the world, and to improve its functional properties, colored wheat has become a very attractive option. However, most of the valuable compounds of the wheat cereal (including colored) are located in the outer layer of the cereal. From this point of view, application of non-refined (wholemeal) flour became very important. The aim of this study was to analyze and compare physico-chemical and microbiological parameters of the nontreated 'Ada', 'Sarta', and new breed blue and purple wholemeal wheat lines with those fermented with lactic acid bacteria (LAB) strains (*Pediococcus acidilactici*, *Liquorilactobacillus uvarum*, *Lactiplantibacillus plantarum*). It was found that all of the used LAB strains are suitable for the tested wheat wholemeal fermentation. However, most of the analyzed parameters were influenced by the wheat variety, the type of LAB used for fermentation, and their interaction. Despite the fact that fermented wheat wholemeal showed a large number of viable LAB, good acidification rates, and a more diverse volatile compound profile. further studies are needed to indicate the technological parameters that lead to the lowest BA formation and mycotoxin degradation.

Abstract: The aim of this study was to analyze and compare the acidity, microbiological, and chromaticity parameters; fatty acid (FA) and volatile compound (VC) profiles; and biogenic amine (BA), macro- and microelement, and mycotoxin concentrations in nontreated 'Ada', 'Sarta', and new breed blue (DS8472-5) and purple (DS8526-2) wheat lines wholemeal (WW) with those fermented with lactic acid bacteria (LAB) possessing antimicrobial/antifungal properties, isolated from spontaneous sourdough: *Pediococcus acidilactici*-LUHS29, *Liquorilactobacillus uvarum*-LUHS245, *Lactiplantibacillus plantarum*-LUHS122). All the fermented WW showed >8.0 $\log_{10}$ CFU/g of LAB count, and the type of LAB was a significant factor in the WW acidity parameters. Phenylethylamine was the predominant BA in WW, and the wheat variety (WV), the type of LAB, and their interaction were significant factors on the BA formation. Despite the fact that some differences in trace element concentrations in WW were obtained, in most of the cases fermentation was not a significant factor in their content. The main FAs in WW were palmitic acid, all-*cis*,*trans*-octadecenoic acid, and linoleic acid. Fermented WW showed a more diverse VC profile; however, the influence of fermentation on deoxynivalenol in WW was varied. Finally, further studies are needed to indicate the technological parameters that would be the most effective for each WV, including the lowest BA formation and mycotoxin degradation.

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1. Introduction

Wheat is the most important food and feed crop in the world [1]. It provides many beneficial nutrients (vitamins B and E; minerals iron and zinc; various phytochemicals, etc.) [2]. The grains of traditional wheat (*T. aestivum*) varieties are amber in color; however, nowadays, colored wheat grain has become a very attractive option because of the higher content of anthocyanins and other phytochemicals [3,4], which provide numerous health benefits [3,5,6]. Also, the antimicrobial activity of these valuable compounds against different Gram-positive and Gram-negative human pathogens could be very promising [7,8]. However, on the other hand, antimicrobial compounds could influence the fermentation process, which is the main technological step in sourdough bread preparation. For this reason, in this study, in addition to the traditional wheat variety ('Ada'), the waxy wheat variety ('Sarta'), and new breed lines of the colored wheat (blue wheat DS8472-5 and purple wheat DS8526-2) for wheat wholemeal (WW) sourdough preparation were tested.

Despite the fact that larger amounts of whole grain products are recommended in the human diet [9–12], there are many challenges associated with the technological and safety characteristics of such types of products. To improve the technological and safety characteristics of WW, fermentation with selected lactic acid bacteria (LAB) strains could be suggested. Also, our previous studies showed that a combination of extrusion and fermentation technologies for wheat bran pre-treatment could be a very attractive solution for the reduction of biogenic amines (BAs) and mycotoxins [13,14].

Overall, sourdough is a mixture consisting mainly of cereal grain flour and water fermented by a heterogeneous microbiota, with predominantly LAB and yeast strains [15,16]. However, characteristics of spontaneous sourdough are dependent on many factors (contamination of flour, environment, etc.). In addition, when WW is used, higher contamination of the outer layer should be taken into consideration. For this reason, the use of selected LAB starter cultures, which possess antifungal and antimicrobial properties and/or are capable of degrading mycotoxins, could be very promising. Also, during the sourdough fermentation process, enzymatic and microbial changes lead to the formation of various volatile compounds (VCs) that improve the overall acceptability of the cereal products [17,18]. However, studies regarding the sourdough VCs are scarce [19]. The acidification properties, microbiological characteristics, and VC production are interesting properties of the sourdough, and the selection of LAB starters has become important to improve the technological properties of cereal products.

Factors affecting sourdough fermentation include microbial diversity, fermentation conditions, and flour composition [20]. In this study, four wheat varieties ('Ada', 'Sarta', new breed lines of the colored wheat known as blue wheat DS8472-5 and purple wheat DS8526-2), and three LAB strains (*Pediococcus acidilactici*, *Liquorilactobacillus uvarum*, *Lactiplantibacillus plantarum*) possessing antimicrobial properties were tested for WW sourdough preparation. The antimicrobial and antifungal properties as well as the metabolic capacity to ferment a large number of carbohydrate sources were taken into consideration in the selection of LAB strains for sourdough preparation [21].

Despite the fact that fermented wheat products are associated with many health benefits [22–26], it should be pointed out that lactofermentation is also associated with the formation of biogenic amines (BAs), which are toxic nitrogenous bases formed by the decarboxylation of amino acids. The tolerance ranges of some BAs have been suggested: histamine, 50–100 mg/kg; tyramine, 100–800 mg/kg; and 2-phenylethylamine, 30 mg/kg [27,28]. Presently, for putrescine and cadaverine, no toxic concentrations were available [29]. However, spermine and spermidine increases the toxicity of histamine and tyramine [30]. For this reason, analysis of the BAs of the prepared sourdoughs in this study was included.

In addition, the fatty acid (FA) profile of the prepared sourdoughs was analyzed. It was reported that hydroxy FAs produced by sourdough LAB exhibit antifungal activity [31], which is related to the increase in the fungal membrane permeability [31]. Also, when sourdough is prepared from cereal bran and/or WW, both the antifungal and mycotoxin degradation properties of the used LAB starters for sourdough preparation are very important. Despite the fact that aflatoxins B1, B2, G1, and G2 are the most important known carcinogenic mycotoxins [16], in addition to regulated mycotoxins, emerging and masking mycotoxins should be controlled. Emerging toxins are a group of mycotoxins for which no regulations exist at the present [32]. The clinical observations in animals in some cases of mycotoxicosis did not correlate with the low mycotoxin content determined in the corresponding feed [33]. The unexpectedly high toxicity has been attributed to undetected conjugated forms of mycotoxins that were possibly hydrolyzed in vivo [32]. Our previous studies showed that cereal pre-treatment by using fermentation with selected LAB strains could lead to the better detoxication of some mycotoxins in vivo [34]. Finally, biocontrol of mycotoxins by LAB could be an important factor to reduce their risks during food fermentation.

The aim of this study was to analyze and compare the acidity, microbiological, and chromaticity parameters; fatty acid (FA) and volatile compound (VC) profiles; and biogenic amine (BA), macro- and microelement, and mycotoxin concentrations in nontreated and fermented (with antimicrobial/antifungal properties possessing LAB strains, isolated from spontaneous sourdough: *Pediococcus acidilactici*-LUHS29, *Liquorilactobacillus uvarum*-LUHS245, *Lactiplantibacillus plantarum*-LUHS122) 'Ada', 'Sarta', and new breed blue (DS8472-5) and purple (DS8526-2) wheat lines WW. Also, this study will contribute to the creation of the database of the grain chemical composition of the new breed wheat lines.

2. Materials and Methods

2.1. Wheat and Lactic Acid Bacteria Strains Used in the Experiments and Wheat Wholemeal Preparation and Fermentation

The principal scheme of the experiment is given in Figure 1. The grains of wheat varieties 'Ada' (traditional wheat), 'Sarta' (waxy wheat), and new breed lines DS8472-5 (blue wheat) and DS8526-2 (purple wheat) were provided by the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (LAMMC, Akademija, Kėdainiai distr., Lithuania). Field trials were conducted during 2020–2021 at the experimental base of the LAMMC in Akademija, Kėdainiai distr., Lithuania (55°39′ N 23°57′ E). Field experiments were designed in four replications (plot size 5 × 1.6 m), and each replication was grown in a separate block, where the field plots were randomly arranged. The trials were conducted under a sustainable growing technology. The soil was light loam Endocalcari-Epihypogleyic Cambisol. Topsoil (0–30 cm) pH was low acid (5.5), close to low in humus (1.6%), high in available phosphorus (251 mg/kg P_2O_5), and moderate in available potassium (175 mg/kg K_2O). Winter wheat was sown with treated seeds at the seed rate of 4.5 million ha^{-1} in the beginning of the 10th of September (2020) after the black fallow. Complex mineral fertilisers ($N_{15}P_{50}K_{100}$) were applied in the entire experimental field before sowing. Nitrogen fertilisers (ammonium nitrate) were applied after resumption of spring vegetation (on the 8 April 2021) and when plants reached the stem elongation stage (on the 4 May 2021). The rate of N_{100+30} was used. Weeds were controlled by the recommended herbicides in the autumn and spring. Yield was harvested on the 21 July 2021. The elevation of the experimental area is 82 m above sea level, belongs to the mid-latitude climate zone in the southwestern subregion of the Atlantic continental forest area. According to the data of the local Dotnuva Meteorological Station (55°23′49.0″ N 23°51′55.0″ E), the climatic conditions were characterized by the long-term (1924–2021) average annual temperature of 6.5 °C and precipitation of 570 mm. Both experiment growing seasons, especially the spring–summer period, were warmer (2.3 °C). In addition, the 2020–2021 growing season was dryer than the long-term average. The autumn of 2020 was warmer and dryer than usual. The winters were quite hard, and thick snow cover protected the

plants from cold damage but provoked intense development of snow mold. The spring meteorological conditions were variable—March was warmer and dryer, April was colder and dryer, and May was extremely cold and wet as precipitation was twice as much. The drought period was recorded from the middle of June to the end of July. June and July were warmer by 3.1 °C and 4.7 °C, respectively. The first part of July was warmer by 6.5 °C; this affected the rapid maturation of plants, which formed small grains. A wheat wholemeal (WW) was prepared by milling wheat grains (moisture content of the wheat was 14%) with Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particles size was 1–2 mm. The moisture content of the grain was determined by using Infratec™ 1241 Grain Analyser (FOSS, Foss Allé DK-3400, Hilleroed, Denmark).

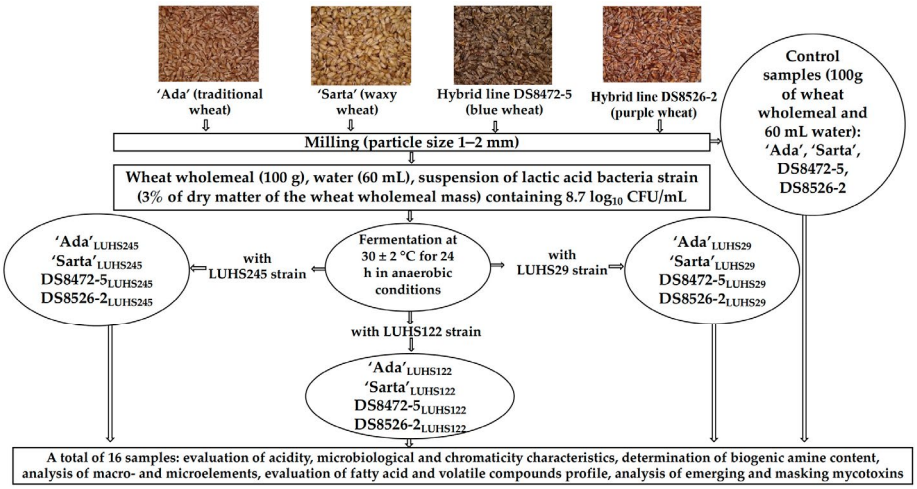

Figure 1. The principal scheme of the experiment (*Pediococcus acidilactici* LUHS29, *Liquorilactobacillus uvarum*—LUHS245, and *Lactiplantibacillus* plantarum—LUHS122).

The LAB strains *Pediococcus acidilactici* LUHS29, *Liquorilactobacillus uvarum*-LUHS245, and *Lactiplantibacillus plantarum*-LUHS122, were used for WW fermentation. The LAB strains were obtained from the Lithuanian University of Health Sciences (Kaunas, Lithuania). Our previous studies showed, that the LUHS122 strain ferment 28, LUHS245—23, and LUHS183—20 out of 47 tested carbohydrates; LUHS122 and LUHS183 showed tolerance to 30, 37, and 45 °C and LUHS122 to 30 and 37 °C; the concentration of viable cells after 2 h incubation at pH 2.5 for LUHS122 was 5.72 ± 0.2 $\log_{10}$ CFU/mL, for LUHS245 was 7.55 ± 0.2 $\log_{10}$ CFU/mL, for LUHS183 was 3.20 ± 0.2 $\log_{10}$ CFU/mL [21].

Before the experiments, the LAB strains were stored at −80 °C (Microbank system, Pro-Lab Diagnostics, Birkenhead, UK) and multiplied in de Man–Rogosa–Sharpe (MRS) broth (Oxoid Ltd., Hampshire, UK) at 30 ± 2 °C for 24 h, before using them in the WW fermentation. The WW, water, and a suspension of LAB strain (3% of dry matter of the WW mass) containing 8.7 $\log_{10}$ CFU/mL were fermented at 30 ± 2 °C for 24 h in a chamber incubator (Memmert GmbH + Co. KG, Schwabach, Germany). For 100 g of WW, 60 mL of water was used. Nonfermented WW samples were analyzed as a control.

2.2. Evaluation of Acidity, Microbiological, and Chromaticity Characteristics of Wheat Wholemeal Samples

The pH of WW was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany). The total titratable acidity (TTA) was evaluated for a 10 g WW sample mixed with 90 mL of water; results were expressed as mL of 0.1 mol/L NaOH solution required to achieve a pH value of 8.2. The LAB count was determined according to the method described by Bartkiene et al. [35]. The chromaticity characteristics of WW were evaluated on the WW surface using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Japan). The results were expressed as the CIE color values L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness).

2.3. Determination of the Biogenic Amine Content in Wheat Wholemeal Samples Nonfermented and Fermented with Different Lactic Acid Bacteria Strains

The extraction and determination of BA in nonfermented and fermented WW followed the procedures developed by Ben-Gigirey et al. (1999) [36]. Perchloric acid (0.4 mol/L, 10 mL) containing a known amount of 1,7-diaminoheptane as an internal standard was added to 3 g of sample, and the mixture was homogenized with Ultra-Turrax apparatus (IKA Labortechnik, Staufen, Germany) and centrifuged at 3000 *g* for 10 min. The residue was extracted again with an equal volume of 0.4 mol/L perchloric acid. Both supernatants were combined, and the final volume was adjusted to 30 mL with 0.4 mol/L perchloric acid. The extract was filtered through Whatman No. 1 paper filter. One mL of extract or standard solution was mixed with 200 mL of 2 mol/L NaOH solution and 300 mL of saturated $NaHCO_3$ solution. A 10 mg/mL solution of 5-(dimethylamino)naphthalene-1-sulphonyl chloride (dansyl chloride) (2 mL) in acetone was added to the mixture and incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 25 mg/L aqueous ammonia solution (100 mL). After incubation at room temperature for 30 min, the mixture was adjusted to 5 mL with acetonitrile. Finally, the mixture was centrifuged at 3000× *g* for 5 min, the supernatant was then filtered through a 0.2 µm filter (Millipore, Bedford, MA, USA) and stored at 25 °C until HPLC analysis.

An Agilent 1200 HPLC system (Carlsbad, CA, USA) equipped with a diode array detector and Chemstation LC software was employed in combination with a Chromolith C_{18} HPLC column (100 mm × 4.6 mm × 4 µm, Merck KGaA/EMD Chemicals, Darmstadt, Germany). Ammonium acetate (0.1 mol/L) and acetonitrile were used as the mobile phases at the flow rate of 0.45 mL/min. The injected sample volume was 10 µL and the amines were monitored at 254 nm wavelength. The detection limits for standard amine solutions were approximately 0.1 mg/kg.

2.4. Analysis of Macro- and Microelements of Wheat Wholemeal Samples Nonfermented and Fermented with Different Lactic Acid Bacteria Strains Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

For macro- and microelement analysis in WW samples, an Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan) and software MassHunter Work Station for ICP-MS, version B.01.01 (Agilent Technologies, Tokyo, Japan) were used. The samples were milled and homogenized (final particle size $\leq$ 150 µm). For the analysis the following chemicals were used: nitric acid (concentration $\geq$ 69.0%), for trace analysis (Saint Quentin-Fallavier, Sigma–Aldrich, Saint Quentin-Fallavier, France), hydrogen peroxide, 30% *w*/*w* (weight/weight), extra pure (Scharlau, Barcelona, Spain), multielement standard solution V for ICP-MS calibration (Sigma–Aldrich, France). Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan), software MassHunter Work Station for ICP-MS, version B.01.01 (Agilent Technologies, Tokyo, Japan) were used for analysis. Sample preparation for ICP-MS analysis was carried out as follows: 0.3 g of the WW was accurately weighed in a microwave vessel; then, 2 mL of de-ionized water, 8 mL of concentrated nitric acid, and 2 mL of concentrated hydrogen peroxide were added. This was left for 2–8 h for reaction stabilization until the formation of bubbles was finished. The vessel was sealed and heated in the microwave system. The following thermal conditions were applied: 150 °C was

reached in approximately 20 min and remained for 30 min, and then 200 °C was reached in approximately 20 min and remained for 30 min for the completion of specific reactions. After cooling (approximately 40 min), the prepared solution was filtered through the filter with a pore size 8–10 µm. The solution was transferred to the 50 mL volumetric flask and filled with water to 50 mL volume. The following operating conditions of Agilent 7700x ICP-MS were used for the analysis of the samples: plasma mode—normal, robust; RF forward power 1300 W; sampling depth 8.00 mm; carrier gas flow 0.6 L/min; dilution gas flow 0.4 L/min; spray chamber temperature 2 °C; extraction lens 1 V; kinetic energy discrimination 3 V.

2.5. Fatty Acid Composition Analysis

The fatty acid (FA) composition of the WW samples was determined using GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph with a mass spectrometer. The fatty acid methyl esters (FAME) concentration was determined using a calibration curve and results were expressed as the percentage of total FAME concentration in the sample. The sample was prepared by homogenizing 1 g of WW sample in 5 mL of 30% (*w*/*v*) NaCl solution. Next, 5 mL of hexane were added. The mixture was shaken on a laboratory shaker for 1 h. The mixture was centrifuged at 4000 rpm. Afterwards 4 mL of the hexane extract were reacted with 300 µL of methylation reagent (2 mol/L of KOH in methanol) by vortexing and shaking using a laboratory shaker for 1 h. The mixture was centrifuged at 4000 rpm and the upper layer was filtered using a 0.22 µm membrane syringe filter and used for the analysis. Capillary Stabilwax-MS column (30 m × 0.25 mm ID × 0.25 µm film thickness. Mass spectrometer operated at full scan mode. Analyte was injected in split mode at 1:60 split ratio. The following parameters were used: MS ion source temperature: 240 °C, MS interface temperature 240 °C, helium (carrier gas) flow: 0.90 mL/min, injector: 240 °C, oven temperature 50 °C (4 min), 10 °C/min to 110 °C (1 min), 15 °C/min to 160 °C (2 min), 25 °C/min to 195 °C (1 min), 2 °C/min to 230 °C (1 min), 2 °C/min to 240 °C (12 min).

2.6. Evaluation of Volatile Compound Profile

The VCs of the WW samples were analyzed by gas chromatography-mass spectrometry (GC-MS). A solid phase microextraction (SPME) device with a Stableflex™ fibre coated with a 50 µm PDMS-DVB-Carboxen™ layer (Supelco, St. Louis, USA) was used for the analysis. A WW sample was weighed and blended with an aqueous NaCl solution (30% *w*/*v*) in a ratio of 1 g of WW to 3 mL of NaCl solution. For headspace extraction, 8 g of prepared sample was transferred to the 20 mL extraction vial, sealed with a polytetrafluoroethylene septum, and thermostatted at 60 °C for 15 min before exposing the fibre in the headspace. The fibre was exposed to the headspace of the vial for 10 min and desorbed in an injector liner for 2 min (splitless injection mode). Prepared samples were analyzed with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph and mass spectrometer. The following conditions were used for the analysis: injector temperature of 250 °C, ion source temperature of 220 °C, interface temperature of 260 °C. Helium was used as the carrier gas at a flowrate of 0.95 mL/min. A Stabilwax-DA capillary column (0.25 mm ID, 0.25 µm film thickness, 30 m length (Restek, Bellefonte, PA, USA)) was used for the analysis. The temperature gradient was programmed from a starting temperature of 40 °C (3 min hold) to 220 °C (6 °C/min) to 250 °C (10 °C/min) (6 min hold). The VCs were identified according to mass spectrum libraries (NIST11, NIST11S, FFNSC2).

2.7. High-Performance Liquid Chromatography Coupled to Triple Quadrupole Mass Spectrometry (HPLC-MS/MS) for Mycotoxin Analysis

2.7.1. Materials and Chemicals

Mycotoxin standards were of at least 95% purity. Standards were supplied by Romer Labs (Tulln, Austria) (Ochratoxin A, Fumonisin B1, Fumonisin B2, T2-Toxin, HT-2 Toxin, Zearalenone, Deoxynivalenol) and Fermentek (Jerusalem, Israel) (Aflatoxin B1). HPLC

grade acetonitrile and methanol (>99% assay), ACS grade formic acid (≥96.0% assay) was used. Ultrapure water (18.2 MΩ × cm) was generated by a Milli-Q system (Millipore, Billerica, MA, USA). QuEChERS buffer–salt extraction kits consisting of magnesium sulphate (4 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g), and disodium hydrogen citrate sesquihydrate (0.5 g) per portion and QuEChERS dSPE kit consisting of magnesium sulphate (900 mg), PSA (primary and secondary amines) (150 mg) and C18E (150 mg) per 15 mL polypropylene tubes were purchased from Phenomenex (Torrance, CA, USA). Unprocessed wheat was used as a blank sample.

2.7.2. Sample Preparation

Samples (2.50 ± 0.01 g) were accurately weighed in 50 mL PP tubes. The quality control (blank) samples were spiked with mycotoxin standard solutions at the appropriate spiking levels. Then water (10 mL), acetonitrile (10 mL), and formic acid (20 µL) were gradually added to the tubes and extraction was started by mixing for 10 min on mechanical shaker. One portion of the QuEChERS buffer salt kit was added to each of the tubes and the extraction was continued for additional 10 min. The obtained mixtures were centrifuged (4500 rpm, 10 min). The supernatants were transferred to 15 mL centrifuge tubes and placed for 15 min at −80 °C in a Heto PowerDry® freeze dryer (Thermo Fisher Scientific, Waltham, MA, USA). After removal, the extracts were immediately centrifuged (4000 rpm, 15 min) at 15 °C, and 500 µL of the upper layer were transferred to 15 mL centrifuge tubes. Then, 6 mL of the remaining extract were transferred to QuEChERS dspe kit, then mixed for 5 min on a mechanical shaker and centrifuged (3000 rpm, 10 min). Next, 3500 µL of supernatant were combined with 500 µL from before and then the combined extracts were evaporated to dryness at 50 °C under a gentle nitrogen stream. The dry residues were reconstructed in 300 µL 0.1% formic acid in water/methanol ($6/4_{v/v}$). Extracts were filtered through 0.22 µm Millipore UltraFree MC centrifuge filters (3900× *g*, 10 min) and transferred into the autosampler for analysis.

2.7.3. Chromatographic Method

The analysis was performed on an UltiMate™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) HPLC coupled with a Thermo Scientific TSQ Quantiva MS/MS detector. The separation was performed on a Kinetex 1.7 µm C18 reversed-phase analytical column (50 × 3.0 mm, 1.7 µm). The autosampler was maintained at 15 °C and the column temperature was 40 °C. The sample injection volume was 15 µL. Ion monitoring was conducted in both positive and negative ion modes and the mass analysis was performed in selected reaction monitoring (SRM) mode. The following instrumental settings were used: spray voltage 3.5 kV (positive ion mode), 2.5 kV (negative ion mode), vaporiser temperature 350 °C, ion transfer temperature 300 °C, sheath gas 55 arbitrary units (arb), auxiliary gas 25 arb, and sweep gas 5 arb. Data processing was performed with TraceFinder software (Thermo Fisher Scientific). More detailed information on parameters of HPLC and MS/MS is provided in Supplementary File S1 Tables S1 and S2.

Six-point calibration curves were constructed using blanks spiked with mycotoxin standard mixtures (1 µg/kg (or LOQ) to 250 µg/kg). The least squares regression method was used for slope construction and calculation of the determination coefficients (R2) of the calibration curves, which were evaluated to a fit of at least 0.99.

2.8. Statistical Analysis

Microbiological results were expressed as the mean (n = 5) ± standard error (SE), and physico-chemical results were expressed as the mean (n = 3) ± standard error (SE). The experiment was performed by preparing three parallel samples for fermentation. In order to evaluate the effects of the different wheat varieties and different LAB strains used for WW fermentation, the data were analyzed by multivariate analysis of variance (ANOVA). To determine the normality the Shapiro–Wilk Test was used, for homoskedasticity evaluation the homoskedasticity test by using SPSS Statistical Package was performed.

The linear Pearson's correlation coefficients were calculated using the statistical package SPSS for Windows (v15.0, SPSS, Chicago, IL, USA). Correlation strength interpretation was performed in accordance with the procedure by Evans (1996) [37]. The results were recognized as statistically significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Acidity, Chromaticity, and Microbiological Characteristics of the Wheat Wholemeal Samples

The acidity, chromaticity, and microbiological characteristics of the WW samples are shown in Table 1. When comparing the acidity parameters (pH and TTA) of the fermented samples, it was established that the type of LAB used for fermentation was a significant factor in sample pH and TTA ($p = 0.0001$ and $p = 0.011$, respectively) (Supplementary File S2 Table S3). However, the wheat variety and the wheat variety * LAB type interaction was not a significant factor in the acidity parameters of the samples. Between the pH and TTA, a negative moderate correlation was found ($r = -0.422$, $p = 0.003$) (Supplementary File S2 Table S4). When comparing the pH of the fermented samples in different wheat variety groups, in 'Ada' and DS8472-5 blue WW, the lowest pH was found after fermentation with the LUHS122 strain (on average, pH 3.97). Also, in the 'Sarta' WW group, the lowest pH was found in the samples fermented with LUHS245 and LUHS122 strains (on average, pH 3.83). Opposite tendencies of the DS8526-2 purple WW were established, and the lowest pH of the DS8526-2 fermented with LUHS29 was obtained (pH 4.24). When comparing the chromaticity characteristics of the samples, the wheat variety * LAB type interaction was a significant factor in the sample redness (a*) ($p = 0.004$) (Supplementary File S2 Table S3); however, significant correlations between the a* and acidity parameters were not found (Supplementary File S2 Table S4). WW lightness (L*) and yellowness (b*) showed moderate positive correlations with sample TTA ($r = 0.578$, $p = 0.0001$ and $r = 0.341$, $p = 0.018$, respectively) (Supplementary File S2 Table S4), and when comparing the sample L* in nontreated and fermented different variety WW groups, different tendencies were established. In the 'Ada' and DS8526-2 purple WW group, fermented samples showed a decrease in the L* coordinates (in comparison with the nonfermented ones, on average, by 8.7% and 14.1%, respectively). Opposite tendencies in the 'Sarta' sample group were found, in which L* coordinates increased after fermentation (in comparison with the nonfermented ones, on average, by 12.6%). The L* of the DS8472-5 blue WW samples was dependent on the LAB strain used for fermentation: the LUHS29 strain decreased the L* (on average, by 14.1%), and fermentation with LUHS245 and LUHS122 increased the L* (on average, by 5.6%). When comparing the a* and b* coordinates of the nontreated and fermented samples, fermentation with all three tested LAB strains reduced the a* and b* parameters of the DS8526-2 purple WW, and the same tendencies were found for the a* coordinates of the fermented 'Sarta' samples. However, fermentation increased the a* coordinates of the DS8472-5 blue WW and b* coordinates of the 'Sarta' samples. The chromaticity parameters of the other fermented samples were varied and depended on the LAB strain used for fermentation and the wheat variety.

Anthocyanin, flavonoids, carotenoids, and some other phenolics are responsible for the different colors in wheat [38]. Anthocyanins are located in the cereal pericarp and account for the blue, purple, or a combination of both colors depending on its concentration [5]. It was reported that the anthocyanin concentration could be reduced during the fermentation process [39]. The yellow color of cereal is related to the presence of carotenoids, and the red color is associated with the presence of phlobaphenes [40]. It was reported that an increase in the carotenoid content can be obtained in fermented doughs, and these changes were explained by an increased mobilization of membrane-associated lipophilic compounds from the cereal matrix; however, this process is strain-specific [41]. In addition, the changes in the lutein and zeaxanthin content in doughs fermented by the different LAB strains are variable and could be due to the higher degradation of this compound during fermentation, probably because of the higher lipid oxidation involving the endogenous lipoxygenase/linoleate system [42]. Also, LAB could possess strong antioxidative effects,

through a decrease in the oxidation-reduction potential of sourdough [43], and, conversely, to reduce oxidation of the color compounds.

Table 1. Acidity, chromaticity, and microbiological characteristics of the wheat wholemeal samples.

Wheat Samples	pH	TTA, °N	Color Coordinates, NBS			LAB Count, log_{10} CFU/g
			L*	a*	b*	
Traditional Wheat Variety						
'Ada'	$6.68 \pm 0.03^{d,D}$	$1.30 \pm 0.02^{a,C}$	$52.8 \pm 1.21^{b,C}$	$5.79 \pm 0.11^{c,D}$	$16.6 \pm 0.13^{c,D}$	$4.41 \pm 0.11^{a,B}$
'Ada'$_{LUHS29}$	$4.29 \pm 0.02^{c,C}$	$4.90 \pm 0.08^{b,B}$	$42.0 \pm 1.10^{a,C}$	$4.27 \pm 0.10^{a,C}$	$8.88 \pm 0.11^{a,C}$	$8.52 \pm 0.15^{b,C}$
'Ada'$_{LUHS245}$	$4.21 \pm 0.01^{b,C}$	$4.80 \pm 0.09^{b,A}$	$50.6 \pm 1.50^{b,C}$	$5.46 \pm 0.16^{b,B}$	$15.7 \pm 0.21^{b,D}$	$8.61 \pm 0.14^{b,A}$
'Ada'$_{LUHS122}$	$3.99 \pm 0.02^{a,B}$	$5.10 \pm 0.15^{c,A}$	$51.9 \pm 0.97^{b,D}$	$5.79 \pm 0.13^{c,D}$	$16.8 \pm 0.19^{c,C}$	$8.72 \pm 0.18^{b,A}$
Waxy Wheat Variety						
'Sarta'	$6.42 \pm 0.03^{c,C}$	$1.00 \pm 0.03^{a,A}$	$40.9 \pm 1.19^{a,A}$	$4.43 \pm 0.09^{c,B}$	$8.10 \pm 0.20^{a,A}$	$4.33 \pm 0.09^{a,A}$
'Sarta'$_{LUHS29}$	$4.19 \pm 0.02^{b,B}$	$4.50 \pm 0.09^{b,A}$	$44.9 \pm 0.94^{b,D}$	$3.85 \pm 0.07^{a,b,A}$	$9.59 \pm 0.14^{b,D}$	$8.11 \pm 0.21^{b,A}$
'Sarta'$_{LUHS245}$	$3.84 \pm 0.01^{a,A}$	$5.50 \pm 0.16^{c,C}$	$46.7 \pm 1.17^{c,B}$	$3.72 \pm 0.11^{a,A}$	$9.89 \pm 0.11^{c,B}$	$8.37 \pm 0.18^{b,A}$
'Sarta'$_{LUHS122}$	$3.81 \pm 0.02^{a,A}$	$5.70 \pm 0.12^{c,B,C}$	$48.9 \pm 1.41^{d,B}$	$3.94 \pm 0.08^{b,A}$	$14.3 \pm 0.13^{d,B}$	$8.64 \pm 0.16^{b,A}$
Blue Wheat—New BreedLine						
DS8472-5	$6.09 \pm 0.02^{d,A}$	$1.0 \pm 0.02^{a,A}$	$47.6 \pm 0.32^{b,B}$	$3.03 \pm 0.07^{c,A}$	$11.3 \pm 0.09^{b,C}$	$4.30 \pm 0.06^{a,A}$
DS8472-5$_{LUHS29}$	$4.07 \pm 0.01^{b,A}$	$5.40 \pm 0.08^{c,C}$	$40.9 \pm 0.27^{a,B}$	$4.08 \pm 0.06^{b,B}$	$6.12 \pm 0.14^{a,B}$	$8.31 \pm 0.12^{b,A,B}$
DS8472-5$_{LUHS245}$	$4.15 \pm 0.02^{c,B}$	$5.10 \pm 0.12^{b,B}$	$50.3 \pm 1.18^{c,C}$	$6.85 \pm 0.16^{d,C}$	$13.8 \pm 0.21^{c,C}$	$8.53 \pm 0.20^{b,c,A}$
DS8472-5$_{LUHS122}$	$3.94 \pm 0.03^{a,B}$	$5.80 \pm 0.04^{d,C}$	$50.6 \pm 0.34^{c,C,D}$	$4.76 \pm 0.07^{c,C}$	$14.0 \pm 0.32^{c,B}$	$8.92 \pm 0.21^{c,A}$
Purple Wheat—New BreedLine						
DS8526-2	$6.18 \pm 0.02^{d,B}$	$1.20 \pm 0.03^{a,B}$	$46.1 \pm 1.32^{c,B}$	$5.12 \pm 0.11^{c,C}$	$9.52 \pm 0.17^{c,B}$	$4.53 \pm 0.09^{a,B}$
DS8526-2$_{LUHS29}$	$4.24 \pm 0.03^{a,B,C}$	$5.00 \pm 0.11^{b,B}$	$38.6 \pm 0.77^{a,A}$	$4.25 \pm 0.12^{b,B,C}$	$5.42 \pm 0.08^{a,A}$	$8.31 \pm 0.11^{b,A,B}$
DS8526-2$_{LUHS245}$	$4.86 \pm 0.04^{c,D}$	$5.90 \pm 0.12^{d,D}$	$40.3 \pm 0.89^{a,b,A}$	$3.58 \pm 0.10^{a,A}$	$5.29 \pm 0.12^{a,A}$	$8.45 \pm 0.17^{b,c,A}$
DS8526-2$_{LUHS122}$	$4.67 \pm 0.02^{b,C}$	$5.60 \pm 0.14^{c,B}$	$39.8 \pm 1.14^{a,A}$	$4.10 \pm 0.09^{b,B}$	$5.86 \pm 0.14^{b,A}$	$8.76 \pm 0.09^{c,A}$

TTA—total titratable acidity; L*—lightness; a*—redness (−a* greenness); b*—yellowness (−b* blueness); LAB—lactic acid bacteria; CFU—colony forming units; LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. The data of physico-chemical parameters are represented as means ($n = 3$) ± SE. The data of lactic acid bacteria count are represented as means ($n = 5$) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of the non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

It was reported that all of the LAB strains belonging to the genera Lactobacillus as well as P. pentosaceus acidified the medium at around pH 3.5 [44]. When comparing the LAB count in the fermented samples, all the fermented WW showed higher than 8.0 log_{10} CFU/g of the LAB number, and a moderate negative correlation between the LAB count and sample pH was established ($r = -0.406$, $p = 0.004$) (Supplementary File S2 Table S4). The main factors that lead to the competitiveness of technological LABs in fermented cereal are fast LAB multiplication in a substrate as well as increases in TTA and decreases in pH [45]. According to other studies, a lower LAB number and acidification rate are obtained in homofermentative LAB fermented cereal in comparison with heterofermentative ones [46]. However, the acidification rate is related to many factors (variety of cereal, type of flour, fermentation temperature, acidification of LAB strain, moisture content, duration of the fermentation process, etc.) [47]. The mechanisms in cereal fermentation are very complex [48]. During fermentation, in addition to biochemical changes that occur in the carbohydrate of the flour, changes in the protein components also take place due to the action of both microorganisms and indigenous flour enzymes [49], as well as endogenous factors in the cereal substrate (free fatty acids, minerals, carbohydrates, nitrogen sources, enzyme activities, etc.) [48]. Also, it should be taken into consideration that the bran fraction contains more minerals and other micronutrients that improve the effectiveness of LAB growth [47]. In addition, the ash has an influence on the buffering capacity of the fermentable substrate; for this reason, it is possible to reach a higher TTA. This study showed that the LAB type is a significant factor in the LAB count in the samples ($p = 0.0001$);

however, the wheat variety and wheat variety * LAB type interaction were not a significant factor in the LAB number in the samples (Supplementary File S2 Table S3).

3.2. Biogenic Amine Content in Wheat Wholemeal Samples

The biogenic amine (BA) content in WW samples is shown in Table 2. Tryptamine (TRY) and spermine (SPRM) were not found in WW; however, all the other BAs identified in the WW samples were significantly influenced by the wheat variety, the type of LAB used for fermentation, and the wheat variety * LAB type interaction (Supplementary File S3 Table S5). Also, between the sample pH and phenylethylamine (PHE), a positive weak correlation was found (r = 0.389, p = 0.006). Also, between the LAB count in the samples and the spermidine (SPRMD) concentration, a positive moderate correlation was established (r = 0.422, p = 0.003) (Supplementary File S3 Table S6). PHE was the predominant BA in the WW samples, and the lowest PHE concentration was found in the DS8526-2 purple WW samples fermented with the LUHS245 strain (26.4 mg/kg). In the other samples, the PHE concentration was from 2.8 to 1.8 times higher (in nontreated 'Ada' WW and in LUHS245 fermented 'Sarta' samples, respectively). Putrescine (PUT) was established in all (nontreated and fermented) 'Ada' and 'Sarta' samples. When comparing the PUT concentration in the 'Ada' samples, the lowest PUT content was found in the LUHS29 fermented 'Ada' WW (20.3 mg/kg). However, in nontreated and in LUHS245 fermented 'Ada' samples, on average, a 40.9% higher PUT concentration was established, and the highest PUT content was formed in the LUHS122 fermented samples (98.1 mg/kg). PUT was not found in DS8472-5 blue WW, and when comparing DS8526-2 purple WW, PUT was formed in just one sample (fermented with the LUHS245 strain) (26.8 mg/kg). Cadaverine (CAD) was not found in waxy wheat 'Sarta' WW and in other wheat varieties WW fermented with the LUHS29 strain. Also, CAD was not established in DS8472-5 blue WW fermented with the LUHS245 strain; however, in DS8526-2 purple WW fermented with the LUHS245 strain, the CAD concentration was, on average, 84.0 mg/kg. In both blue and purple WW fermented with the LUHS122 strain, on average, a CAD concentration of 94.0 mg/kg was found. Histamine (HIS) was found in just two samples (in 'Ada' and 'Sarta' WW fermented with the LUHS122 strain), and tyramine (TYR) was found in one sample (DS8526-2 purple WW fermented with the LUHS122 strain). The spermidine (SPRMD) concentration in WW samples ranged from, on average, 28.0 mg/kg (in nonfermented and LUHS245 fermented 'Ada', in LUHS245 fermented 'Sarta', in LUHS29 and LUHS122 fermented blue WW, and in all purple WW samples) to, on average, 36.3 mg/kg (in LUHS29 fermented 'Ada' WW and in LUHS245 fermented blue WW).

HIS, TYR, PUT, CAD, TYR, agmatine, SPRM, and SPRMD are the main BAs in food and beverages [50,51]. Despite the fact that the LAB has a GRAS (Generally Recognized as Safe) status, these very popular industrial microorganisms are the main BA producers in fermented foods [52]. In addition, it was reported that the pH between 4.0 and 5.5 is optimal for enzymes, forming BAs, activities [50]. Other factors that influence the formation of BAs are bacterial activity, humidity, the concentration of free amino acids, etc.; however, the number of bacterial cells in the substrate does not always correlate with the amount of BAs [53]. Consumption of food containing a high concentration of BAs is implicated in various pharmacological reactions [54], which lead to different types of foodborne diseases [27]. HIS can be formed from histidine, TYR from tyrosine, CAD from lysine, PUT from arginine, and SPRMD and SPRM from arginine and methionine, and their toxicity depends on synergistic effects, e.g., HIS toxicity is enhanced by the presence of CAD, PUT, and TYR [55]. Despite the fact that HIS and TYR are the most toxic BAs [56–59], PUT and CAD, although not toxic themselves, aggravate the adverse effects of HIS and TYR [60]. Until now, the European legislation does not specify a BA threshold; however, the European Food Safety Authority (EFSA) reported a scientific opinion on the risk associated with the formation of BAs in fermented products [29]. Based on the mean content in foods and consumer exposure data, fermented food categories were ranked with respect to HIS and TYR, but other BAs were not included. Finally, further research on BAs in fermented foods

is needed, and, as this study indicated, there is a significant influence of the wheat variety, the type of LAB used for fermentation, and the wheat variety * LAB type interaction on the formation of BAs in fermented WW.

Table 2. Biogenic amine concentration (mg/kg) in wheat wholemeal samples.

Wheat Samples	Biogenic Amine, mg/kg							
	TRY	PHE	PUT	CAD	HIS	TYR	SPRMD	SPRM
Traditional Wheat Variety								
'Ada'	nd	72.8 ± 5.4 ^{b,C}	35.6 ± 2.9 ^{b,A}	nd	nd	nd	23.4 ± 2.2 ^{a,A}	nd
'Ada'$_{LUHS29}$	nd	56.9 ± 3.9 ^{a,b,A}	20.3 ± 1.8 ^{a,A}	nd	nd	nd	36.1 ± 3.3 ^{b,B}	nd
'Ada'$_{LUHS245}$	nd	50.3 ± 3.4 ^{a,B}	33.1 ± 2.5 ^{b,B}	102.3 ± 6.9 ^{a,B}	nd	nd	32.6 ± 3.8 ^{b,A}	nd
'Ada'$_{LUHS122}$	nd	64.1 ± 4.7 ^{b,B,C}	98.1 ± 6.3 ^{c,B}	205.3 ± 9.8 ^{a,B}	35.2 ± 2.6 ^{A}	nd	nd	nd
Waxy Wheat Variety								
'Sarta'	nd	68.8 ± 5.0 ^{b,B}	32.6 ± 2.6 ^{b,A}	nd	nd	nd	nd	nd
'Sarta'$_{LUHS29}$	nd	68.6 ± 5.2 ^{b,B}	28.9 ± 2.1 ^{a,b,B}	nd	nd	nd	nd	nd
'Sarta'$_{LUHS245}$	nd	46.9 ± 3.1 ^{a,B}	26.7 ± 1.9 ^{a,A}	nd	nd	nd	31.9 ± 3.4 ^{A}	nd
'Sarta'$_{LUHS122}$	nd	55.6 ± 3.6 ^{a,A}	26.6 ± 2.1 ^{a,A}	nd	77.4 ± 5.4 ^{B}	nd	nd	nd
Blue Wheat—New BreedLine								
DS8472-5	nd	57.2 ± 3.9 ^{a,b,A}	nd	nd	nd	nd	nd	nd
DS8472-5$_{LUHS29}$	nd	53.6 ± 3.5 ^{a,A}	nd	nd	nd	nd	26.7 ± 3.2 ^{a,A}	nd
DS8472-5$_{LUHS245}$	nd	62.2 ± 4.4 ^{b,C}	nd	nd	nd	nd	36.5 ± 3.8 ^{b,B}	nd
DS8472-5$_{LUHS122}$	nd	68.7 ± 5.1 ^{b,C}	nd	88.0 ± 5.9 ^{A}	nd	nd	31.9 ± 4.1 ^{b,A}	nd
Purple Wheat—New BreedLine								
DS8526-2	nd	58.0 ± 4.3 ^{b,A}	nd	nd	nd	nd	21.4 ± 2.3 ^{a,A}	nd
DS8526-2$_{LUHS29}$	nd	70.4 ± 5.3 ^{c,B}	nd	nd	nd	nd	30.7 ± 3.5 ^{c,A}	nd
DS8526-2$_{LUHS245}$	nd	26.4 ± 2.4 ^{a,A}	26.8 ± 2.7 ^{A}	84.0 ± 5.8 ^{a,A}	nd	nd	27.2 ± 3.1 ^{b,c,A}	nd
DS8526-2$_{LUHS122}$	nd	51.9 ± 3.2 ^{b,A}	nd	99.9 ± 6.5 ^{b,A}	nd	76.0 ± 5.4	26.3 ± 2.1 ^{b,A}	nd

LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain.; TRY—tryptamine; PUTR—putrescine; CAD—cadaverine; HIS—histamine; TYR—tyramine; SPRMD—spermidine; SPRM—spermine; nd—not found. Data are represented as means (n = 3) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$). $^{A–C}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

3.3. Macro- and Microelements of Wheat Wholemeal

Macroelement concentrations in the WW samples are shown in Table 3. When comparing the Na concentration in the WW samples, the Na concentration was higher in all the fermented WW compared to the nontreated WW. Taking into consideration that LAB multiplication was performed in a medium (MRS broth) containing sodium acetate, it could be that the LAB strains were the source of Na in the WW samples. The wheat variety and LAB strain used for fermentation were not significant factors on the Mg and K concentrations in WW; however, for the Ca concentration, the wheat variety was a significant factor (p = 0.002).

Essential microelement concentrations in the WW samples are shown in Table 4. In all the tested samples, the Co concentration was <0.010 mg/kg, the Ni concentration was <0.500 mg/kg, and the Se concentration was <0.200 mg/kg. Also, the Cr concentration was <0.010 mg/100 g in most of the samples except the 'Ada' WW fermented with LUHS122, nontreated and fermented with LUHS29 'Sarta', and nontreated DS8472-5 blue wheat WW. Both the wheat variety and LAB strain used for fermentation were significant factors on the Mn concentration in the WW samples (p = 0.001). When comparing the nonfermented samples, the highest Mn content was found in the DS8526-2 purple WW (15.3 mg/kg), and other varieties showed similar Mn concentrations (on average, 9.77 mg/kg). Also, the wheat variety and LAB strain used for fermentation were significant factors on the Fe concentration in the WW samples (p = 0.003). When comparing the nontreated samples, 'Ada' WW showed the highest Fe concentration (on average, 34.1% higher, in comparison

with 'Sarta', DS8472-5, and DS8526-2 varieties), and Zn concentration in nontreated WW was, on average, 7.27 mg/kg. Wheat variety was a significant factor in the Cu concentration in WW ($p = 0.001$). When comparing nontreated WW samples, the highest Cu concentration was found in 'Ada' WW (1.85 mg/kg) and the lowest in DS8472-5 blue WW (0.763 mg/kg).

Non-essential microelement concentrations in the WW samples are given in Table 5. The wheat variety and LAB strain used for fermentation were significant factors on the Sr concentration in the WW samples ($p = 0.018$ and $p = 0.041$, respectively). When comparing nontreated WW, a higher concentration of Sr in both colored wheat was found in comparison with traditional and waxy wheat (on average, by 33.9%). Also, the wheat variety was a significant factor in the Cd concentration in WW ($p = 0.41$). Pb was found only in 'Ada' WW. Most of the other non-essential microelements in the WW samples were below the detection limits.

Table 3. Macroelement concentrations in wheat wholemeal samples.

Wheat Samples	Macroelements, d.m.			
	Na, g/100 g	Mg, g/kg	K, g/kg	Ca, g/kg
Traditional Wheat Variety				
'Ada'	<0.002	0.662 ± 0.066 ^{a,B}	1.98 ± 0.194 ^{a,B}	0.229 ± 0.023 ^{a,C}
'Ada'$_{LUHS29}$	0.010 ± 0.001 ^{a,B}	0.601 ± 0.060 ^{a,B}	1.91 ± 0.187 ^{a,A}	0.192 ± 0.019 ^{a,B}
'Ada'$_{LUHS245}$	0.007 ± 0.002 ^{a,B}	0.551 ± 0.055 ^{a,A}	1.74 ± 0.170 ^{a,A,B}	0.197 ± 0.018 ^{a,B}
'Ada'$_{LUHS122}$	0.007 ± 0.002 ^{a,A}	0.578 ± 0.058 ^{a,D}	1.79 ± 0.175 ^{a,B}	0.197 ± 0.020 ^{a,C}
Waxy Wheat Variety				
'Sarta'	<0.002	0.555 ± 0.056 ^{c,B}	1.98 ± 0.194 ^{c,B}	0.305 ± 0.031 ^{c,D}
'Sarta'$_{LUHS29}$	0.011 ± 0.002 ^{a,B}	0.607 ± 0.061 ^{c,B}	1.82 ± 0.178 ^{b,c,A}	0.293 ± 0.029 ^{c,C}
'Sarta'$_{LUHS245}$	0.009 ± 0.001 ^{a,B}	0.479 ± 0.048 ^{b,A}	1.52 ± 0.152 ^{b,A}	0.225 ± 0.025 ^{b,B}
'Sarta'$_{LUHS122}$	0.011 ± 0.002 ^{a,A}	0.373 ± 0.037 ^{a,B}	1.15 ± 0.113 ^{a,A}	0.160 ± 0.015 ^{a,B}
Blue Wheat—New BreedLine				
DS8472-5	<0.002	0.401 ± 0.040 ^{a,A}	1.45 ± 0.150 ^{a,A}	0.160 ± 0.014 ^{c,B}
DS8472-5 $_{LUHS29}$	0.008 ± 0.001 ^{a,B}	0.445 ± 0.040 ^{a,A}	1.96 ± 0.200 ^{b,A}	0.148 ± 0.015 ^{a,b,A}
DS8472-5 $_{LUHS245}$	0.009 ± 0.001 ^{a,B}	0.431 ± 0.043 ^{a,A}	1.71 ± 0.170 ^{a,b,A,B}	0.126 ± 0.013 ^{a,A}
DS8472-5 $_{LUHS122}$	0.007 ± 0.002 ^{a,A}	0.456 ± 0.046 ^{a,C}	1.76 ± 0.180 ^{a,b,B}	0.147 ± 0.015 ^{a,b,A,B}
Purple Wheat—New BreedLine				
DS8526-2	<0.002	0.518 ± 0.025 ^{c,B}	1.93 ± 0.190 ^{b,B}	0.146 ± 0.015 ^{a,A}
DS8526-2 $_{LUHS29}$	0.005 ± 0.001 ^{a,A}	0.376 ± 0.038 ^{b,A}	1.96 ± 0.160 ^{b,A}	0.124 ± 0.012 ^{a,A}
DS8526-2 $_{LUHS245}$	0.004 ± 0.001 ^{a,A}	0.500 ± 0.050 ^{c,A}	2.00 ± 0.200 ^{b,B}	0.129 ± 0.013 ^{a,A}
DS8526-2 $_{LUHS122}$	0.006 ± 0.003 ^{a,A}	0.292 ± 0.029 ^{a,A}	1.11 ± 0.110 ^{a,A}	0.134 ± 0.011 ^{a,A}

LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means ($n = 3$) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

Table 4. Essential microelement concentrations in wheat wholemeal samples.

Wheat Samples	Essential Microelements, d.m.							
	Cr, mg/100 g	Mn, mg/kg	Fe, mg/kg	Co, mg/kg	Ni, mg/kg	Cu, mg/kg	Zn, mg/kg	Se, mg/kg
Traditional Wheat Variety								
'Ada'	<0.010	8.21 ± 0.820 ^{c,A}	22.9 ± 2.60 ^{a,B}	<0.010	<0.500	1.85 ± 0.190 ^{a,D}	6.98 ± 0.70 ^{b,A}	<0.200
'Ada'$_{LUHS29}$	<0.010	9.00 ± 0.900 ^{c,A}	28.3 ± 2.80 ^{a,C}	<0.010	<0.500	1.51 ± 0.150 ^{a,C}	7.53 ± 0.75 ^{b,B}	<0.200
'Ada'$_{LUHS245}$	<0.010	5.83 ± 0.580 ^{a,A}	23.2 ± 2.28 ^{a,B}	<0.010	<0.500	1.59 ± 0.160 ^{a,C}	5.51 ± 0.55 ^{a,A}	<0.200
'Ada'$_{LUHS122}$	0.014 ± 0.001	7.81 ± 0.780 ^{b,A}	38.7 ± 3.90 ^{b,C}	<0.010	<0.500	1.61 ± 0.157 ^{a,B}	7.05 ± 0.71 ^{b,B}	<0.200
Waxy Wheat Variety								
'Sarta'	0.047 ± 0.001 ^{a,B}	10.4 ± 1.00 ^{a,b,A}	14.0 ± 1.40 ^{b,A}	<0.010	<0.500	1.24 ± 0.120 ^{b,C}	6.95 ± 0.70 ^{c,A}	<0.200
'Sarta'$_{LUHS29}$	0.052 ± 0.005 ^{a}	13.4 ± 1.30 ^{c,C}	15.4 ± 1.50 ^{b,B}	<0.010	<0.500	1.32 ± 0.130 ^{b,C}	7.14 ± 0.71 ^{c,A}	<0.200
'Sarta'$_{LUHS245}$	<0.010	12.1 ± 1.20 ^{b,c,B,C}	22.0 ± 2.20 ^{c,B}	<0.010	<0.500	1.27 ± 0.128 ^{b,C}	5.23 ± 0.52 ^{b,A}	<0.200
'Sarta'$_{LUHS122}$	<0.010	8.25 ± 0.820 ^{a,A}	5.48 ± 0.550 ^{a,A}	<0.010	<0.500	0.830 ± 0.083 ^{a,A}	3.61 ± 0.36 ^{a,A}	<0.200
Blue Wheat—New BreedLine								
DS8472-5	0.015 ± 0.002 ^{A}	10.7 ± 1.00 ^{a,A}	14.9 ± 1.50 ^{a,A}	<0.010	<0.500	0.763 ± 0.076 ^{a,A}	7.25 ± 0.73 ^{a,A}	<0.200
DS8472-5$_{LUHS29}$	<0.010	13.3 ± 1.30 ^{b,C}	16.6 ± 1.70 ^{a,B}	<0.010	<0.500	0.857 ± 0.086 ^{a,B}	7.05 ± 0.71 ^{a,A}	<0.200
DS8472-5$_{LUHS245}$	<0.010	10.9 ± 1.10 ^{a,B}	13.7 ± 1.40 ^{a,A}	<0.010	<0.500	0.695 ± 0.070 ^{a,A}	6.64 ± 0.66 ^{a,A,B}	<0.200
DS8472-5$_{LUHS122}$	<0.010	14.0 ± 1.40 ^{b,B}	15.6 ± 1.60 ^{a,B}	<0.010	<0.500	0.850 ± 0.085 ^{a,A}	6.70 ± 0.67 ^{a,B}	<0.200
Purple Wheat—New BreedLine								
DS8526-2	<0.010	15.3 ± 1.50 ^{c,B}	16.5 ± 1.70 ^{b,A}	<0.010	<0.500	0.913 ± 0.091 ^{b,B}	7.88 ± 0.79 ^{b,A}	<0.200
DS8526-2$_{LUHS29}$	<0.010	11.1 ± 1.10 ^{b,B}	10.3 ± 1.10 ^{a,A}	<0.010	<0.500	0.692 ± 0.069 ^{a,A}	6.03 ± 0.60 ^{a,A}	<0.200
DS8526-2$_{LUHS245}$	<0.010	13.8 ± 1.40 ^{c,C}	15.9 ± 1.60 ^{b,A}	<0.010	<0.500	0.848 ± 0.085 ^{a,b,A}	7.86 ± 0.79 ^{b,B}	<0.200
DS8526-2$_{LUHS122}$	<0.010	8.71 ± 0.870 ^{a,A}	17.6 ± 1.80 ^{b,B}	<0.010	<0.500	0.728 ± 0.073 ^{a,A}	6.11 ± 0.61 ^{a,B}	<0.200

LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means ($n = 3$) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

Table 5. Non-essential microelement concentrations in wheat wholemeal samples.

Wheat Samples	Non-Essential Macroelements, d.m.													
	As, mg/100 g	V, mg/kg	Rb, mg/kg	Sr, mg/kg	Mo, mg/kg	Ag, mg/kg	Sb, mg/kg	Cs, mg/kg	TI, mg/kg	Cd, mg/kg	Ba, mg/kg	Pb, mg/kg	Al, mg/kg	Li, mg/kg
Traditional Wheat Variety														
'Ada'	<0.050	<2.00	<1.00	1.00 ± 0.100 ^{a,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.038 ± 0.004 ^{b,C}	2.71 ± 0.270 ^{a,A}	0.037 ± 0.002 ^{a}	<5.00	<0.050
'Ada'$_{LUHS29}$	<0.050	<2.00	<1.00	0.949 ± 0.095 ^{a,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.029 ± 0.003 ^{a,B}	2.36 ± 0.240 ^{a,A}	0.032 ± 0.003 ^{a}	<5.00	<0.050
'Ada'$_{LUHS245}$	0.009 ± 0.001 ^{A}	<2.00	<1.00	0.854 ± 0.085 ^{a,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.037 ± 0.004 ^{b,C}	2.27 ± 0.229 ^{a,A}	0.038 ± 0.007 ^{a}	<5.00	<0.050
'Ada'$_{LUHS122}$	<0.050	<2.00	<1.00	0.909 ± 0.091 ^{a,b,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.033 ± 0.003 ^{a,b,D}	2.30 ± 0.230 ^{a,A}	0.034 ± 0.006 ^{a}	<5.00	<0.050
Waxy Wheat Variety														
'Sarta'	0.007 ± 0.003 ^{a,A}	<2.00	<1.00	1.22 ± 0.11 ^{c,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.027 ± 0.003 ^{a,B}	2.30 ± 0.225 ^{a,A}	<0.010	<5.00	<0.050
'Sarta'$_{LUHS29}$	0.011 ± 0.002 ^{a,A}	<2.00	<1.00	1.20 ± 0.12 ^{c,A,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.026 ± 0.004 ^{a,B}	2.38 ± 0.240 ^{a,A}	<0.010	<5.00	<0.050
'Sarta'$_{LUHS245}$	<0.050	<2.00	<1.00	1.02 ± 0.10 ^{b,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.028 ± 0.003 ^{a,B}	2.11 ± 0.210 ^{a,A}	<0.010	<5.00	<0.050
'Sarta'$_{LUHS122}$	0.013 ± 0.003 ^{a,A}	<2.00	<1.00	0.719 ± 0.072 ^{a,A}	<0.500	<2.00	<0.500	<2.00	<2.00	0.022 ± 0.003 ^{a,C}	2.13 ± 0.209 ^{a,A}	<0.010	<5.00	<0.050
Blue Wheat—New BreedLine														
DS8472-5	<0.050	<2.00	<1.00	1.46 ± 0.15 ^{a,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.009 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8472-5$_{LUHS29}$	<0.050	<2.00	<1.00	1.61 ± 0.16 ^{a,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.010 ± 0.002 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8472-5$_{LUHS245}$	<0.050	<2.00	<1.00	1.44 ± 0.14 ^{a,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.009 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8472-5$_{LUHS122}$	<0.050	<2.00	<1.00	1.67 ± 0.17 ^{a,D}	<0.500	<2.00	<0.500	<2.00	<2.00	0.012 ± 0.002 ^{a,B}	<2.00	<0.010	<5.00	<0.050
Purple Wheat—New BreedLine														
DS8526-2	<0.050	<2.00	<1.00	1.90 ± 0.19 ^{b,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.009 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8526-2$_{LUHS29}$	<0.050	<2.00	<1.00	1.45 ± 0.15 ^{a,B}	<0.500	<2.00	<0.500	<2.00	<2.00	0.008 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8526-2$_{LUHS245}$	<0.050	<2.00	<1.00	1.84 ± 0.18 ^{b,C}	<0.500	<2.00	<0.500	<2.00	<2.00	0.009 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050
DS8526-2$_{LUHS122}$	<0.050	<2.00	<1.00	1.32 ± 0.13 ^{a,C}	<0.500	<2.00	<0.500	<2.00	<2.00	0.008 ± 0.001 ^{a,A}	<2.00	<0.010	<5.00	<0.050

LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means ($n = 3$) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$). $^{A–D}$ Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

Wheat grain could be a very important source of micro- and macroelements in the human diet [61]; for this reason, it is very important to have a database of the detailed chemical composition of wheat grains. Also, the concentration of trace elements is a very important factor in wheat-breeding programs focused on obtaining plants resistant to environmental stress [62]. However, the mineral content and their bioavailability can change during the fermentation of cereal [63]. Minerals have a significant role in microorganism growth and their enzyme-excreting properties, as well as the enzyme activity [64]. It was reported that Mn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, K^{+}, and Na^{+} are essential for the nutrient metabolism and enzymatic activity of LABs [65]. From this point of view, they can affect the main processes of the bread preparation, especially sourdough bread production. The colored wheat breed has been reported as a specific micronutrient source, of which the organic Cr content is four times higher than that of common wheat [66]. Also, according to He and Ning, black wheat "Qinhei 1" showed the Fe and Zn contents by 19.2 and 4.1 times higher, respectively, than that of common wheat, and the contents of Mn, Cu, Se, Mg, K, and P also were higher than that of common wheat [67]. It was reported that the Zn, Fe, and Mg content in colored wheat is, on average, from 108.54 to 142.68%, from 8.57 to 42.86%, and from 5.31 to 40.63% higher than that in common wheat, respectively [68]. These results may be related to the lower yield of colored wheat. According to Tian, Chen, and Wei, blue wheat has the highest Ca content [68]. However, published studies on the micro- and macroelements in various wheat varieties are varied. It was reported that the fertilizers that are used are a significant factor in the concentration of these compounds, i.e., fertilizers increases the Fe content in the red wheat variety (68.88 mg/kg) and the Zn content in the common variety (40.43 mg/kg); however, the same fertilizer was not so effective on the Fe and Zn content in the purple wheat variety (Fe—55.59 mg/kg, Zn—38.50 mg/kg) [69]. Finally, the development of the database of new wheat cereal breed lines is very important because of the efficient use of the cereal in food and/or feed industries.

3.4. Fatty Acid Composition of the Wheat Wholemeal Samples

The saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, omega 3, omega 6, and omega 9 composition of the WW samples is shown in Figure 2, and the detailed FA profile is given in Supplementary File S4 Table S7. The main FAs in WW were palmitic acid (C16:0), all-*cis*,*trans*-octadecenoic acid (C18:1), and linoleic acid (C18:2). The type of LAB used for fermentation was a significant factor in the C18:1 content in WW ($p = 0.033$). Also, the wheat variety, LAB type used for fermentation, and their interaction were significant factors on most of the FA content except C18:3 α (alfa linolenic acid) (Supplementary File S4 Table S8). When comparing 'Ada' WW, in all the cases, in nontreated WW and WW fermented with the LUHS122 strain, SFA, MUFA, PUFA, omega 3, omega 6, and omega 9 quantities were similar (on average, 39.95, 23.12, 36.93, 2.11, 34.82, and 23.12%, respectively). However, in WW samples fermented with LUHS29 and LUHS245 strains, SFA, MUFA, and omega 9 were decreased (in comparison with nontreated ones, on average, by 23.4, 17.4, and 17.34%, respectively), and PUFA, omega 3, and omega 6 were increased (in comparison with nontreated ones, on average, by 36.1, 36.0, and 36.3%, respectively). When comparing nontreated and fermented waxy wheat 'Sarta' samples, fermentation with LUHS29 decreases SFA, MUFA, and omega 3 and 9 and increases PUFA and omega 6 in WW. Opposite tendencies (except omega 3) were found in samples fermented with LUHS245 and LUHS122, in which an increase in SFA, MUFA, and omega 9 was found, as well as a decrease in PUFA, omega 3 and 6. When comparing nontreated and fermented DS8472-5 blue WW, all the tested LAB strains showed the same tendency of decreasing SFA, MUFA, and omega 9 and increasing PUFA, omega 3, and omega 6. Different tendencies of the FA in DS8526-2 purple WW were found. All the fermented samples showed lower SFA and higher PUFA and omega 3 content; however, fermentation with LUHS29 and LUHS245 increases MUFA and omega 9, and fermentation with LUHS122 showed opposite tendencies: decrease in MUFA and omega 9 in the WW

samples. Also, fermentation with LUHS29 decreases omega 6 in the purple WW samples and increases omega 6 in comparison with the nonfermented ones.

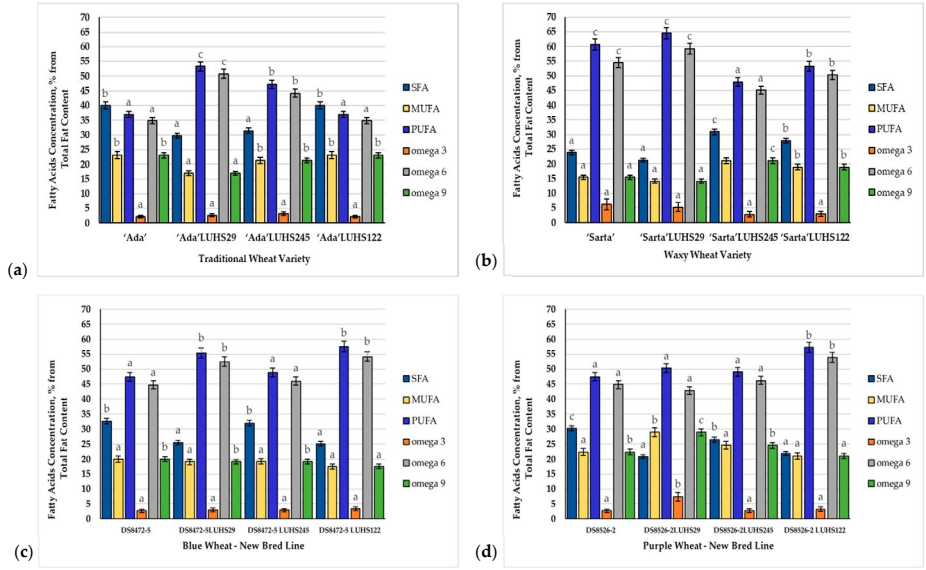

Figure 2. Fatty acid profiles of wheat wholemeal samples: (**a**) Traditional wheat variety fatty acid profile (**b**) Waxy wheat variety fatty acid profile (**c**) Blue wheat—new breed line fatty acid profile (**d**) Purple wheat—new breed line. LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain; SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids. Data are represented as means ($n = 3$) ± SE. $^{a–c}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$).

It was reported that the wheat kernels grains contain, on average, 2.4–3.8% dry basis of lipids [70]. The lipid location by botanical wheat part is followed: on average, 66% of lipids are located in germ, 15% in the bran, and 20% in the endosperm [71]. According to Narducci et al., wheat lipids consist of mostly unsaturated FAs, and linoleic and linolenic FAs are essential [71]. The above mentioned FAs are precursors of prostaglandins and membrane phospholipid molecules in the human body and are involved in regulation processes of the blood lipid [70,72,73]. However, the lipid content in wheat cereal and their composition is related to many factors [70,74,75]. It was reported that cold weather increases the lipid content in wheat [76]. Also, biotic and abiotic stresses have an influence on the FA composition in wheat [77]. In addition, different analytical methods can also be a factor that influences the differences in the FA profile [70,78]. It was reported that in the FA profile of wheat cereal, more than 60 peaks were identified, and the major components were saturated and unsaturated C16 and C18 and particularly C16:0, C18:1, and C18:2; however, C16:0, C18:0, C18:1, C18:2, and C18:3 were mentioned as the most important FAs in durum wheat [70]. The differences in the FA composition in wheat cereal are explained by the differences in genetic characteristics, pedoclimatic conditions, agronomical treatments, and analytical procedures [71]. It was reported that the interaction

of genotype × year × treatment is the main factor that contributes to the variability in the FA in durum wheat [75]. In addition, the differences in SFA and UFA within the same variety could be associated with various kinds of biotic and abiotic stresses [76,77]. Finally, the knowledge of this research will be very useful in updating common and new lines wheat cereal composition databases in general.

3.5. Volatile Compound Profile of the Different Wheat Wholemeal Varieties

Volatile compounds (VCs) that were >5% in at least one WW sample are shown in Figure 3, and the detailed VC profile is given in Supplementary File S5 Table S9. When comparing acetic acid in the WW samples, it was not established in all the nonfermented samples, as well as in both color WW samples fermented with the LUHS245 strain. The odor of acetic acid is described as sharp, pungent, sour, and vinegary. However, in small concentrations, acetic acid is a flavor enhancer [15]. Heterofermentative LAB produces acetic acid, as well as specific esters, and reduces aldehydes to alcohols [79,80]. In purple WW fermented with the LUHS122 strain, 1-butanol was found (8.03% of the total VC). The odor of 1-butanol is described as fusel, oily, sweet, balsamic, and like whiskey. Acetoin, whose odor is described as creamy, dairy, sweet, oily, milky, buttery and like yogurt, was found only in nonfermented samples except purple WW. 1-Pentanol, which possesses a pungent, fermented, bready, yeasty, fusel, winey, and solvent-like odor, was one of the main compounds of nonfermented 'Ada' WW (27.7% of the total VC). Also, 2,3-butanediol (fruity, creamy, buttery odor) was found in all nonfermented WW, and its concentration changed in relation to the LAB strain used for fermentation. When comparing nonfermented samples, hexanal was found only in 'Sarta' WW (29.9% of the total VC); also, after fermentation, this VC was established in LUHS29 fermented 'Ada', 'Sarta', and purple WW (2.67, 6.66, and 5.51% of the total VC, respectively), in LUHS245 strain fermented 'Ada' and 'Sarta', and in LUHS122 strain fermented 'Sarta' WW. The hexanal concentration was 28.8% of the total VC. The odor of hexanal is described as aldehydic, fatty, green, and powerful. It was reported that the VC in the sourdoughs varied in relation with the starter culture used [81]. According to Hammes and Gänzle (1998) and Corona et al. (2016), the main VCs in wheat sourdough are acetic acid, hexanal, and octenal [48,82]. The lipid oxidation is the main factor for hexanal formation [18]. A high concentration of hexanal is undesirable, and the heterofermentative LAB could reduce its negative flavor by converting aldehydes to alcohols [18,83,84]. It was reported that hexanol is present in all fermentations [15]. Ethyl butyrate (sweet, fruity, tutti frutti, lifting, and diffusive odor) was found only in LUHS122 fermented purple WW (19.9% of the total VC). Ethyl lactate (sweet, fruity, acidic, etherial with a brown nuance odor) was found only in LUHS29 fermented 'Ada', 'Sarta', and blue WW (3.61, 5.05, and 5.99% of the total VC, respectively). Butanoic acid (sharp, dairy-like, cheesy, buttery with a fruity nuance odor) was one of the main VCs in LUHS122 fermented purple WW (37.7% of the total VC), while 1-Hexanol (citrus, fresh, floral, oily, sweet odor) was established in most of the samples except in purple WW fermented with the LUHS122 strain. L-lactic acid (sugar, wheat, malt, sweet odor) was found in 'Ada' and purple WW fermented with the LUHS29 strain and in 'Ada', 'Sarta', and blue WW fermented with the LUHS245 strain. More than half of the entire VC profile in blue WW fermented with the LUHS122 strain was 4-methylpentanoic acid (54.8% of the total VC), whose odor is described as pungent cheese. Furthermore, 2-Heptenal (intense green, fatty, oily, with fruity overtones odor) was found in most of the WW samples except in nonfermented 'Sarta', purple WW, and blue WW fermented with the LUHS122 strain. Hexanoix acid, whose odor is described as sour, fatty, sweat, and like cheese, was not formed in nonfermented 'Ada' and both colored WW samples, while 2-Pentylfuran (fruity, green, earthy beany with vegetable like nuances odor) was not established in only two WW samples (blue WW fermented with the LUHS122 strain and nontreated purple WW). Butylbutanoate (fruity, banana, pineapple, green cherry, tropical fruit, ripe fruit, and juicy fruit odor) was typical for purple WW fermented with the LUHS122 strain (17.9% of the total VC). The highest content of hexanoic acid ethyl ester (sweet, fruity, pineapple, waxy, green, banana odor)

was found in LUHS122 fermented blue WW (22.4% of the total VC), and the LUHS245 fermented 'Ada' and blue WW had the highest content of 3-ethyl-2-methyl-1,3-hexadiene (on average, 9.50% of the total VC). Benzeneacetaldehyde (green, sweet, floral, honey, and cocoa odor) was typical for all the fermented samples, and oct-(2E)-enal (fresh, cucumber, fatty, green, herbal, banana, waxy, and green leaf odor) was found in most of the samples except in LUHS29 fermented blue WW. LUHS245 fermented purple WW had the highest content of 4-ethylguaiacol (spicy and clove-like with medicinal, woody, and sweet vanilla nuances odor) (8.27% of the total VC). Deca-(2E,4E)-dien-1-ol was abundant only in 'Sarta' WW fermented with all the tested LAB strains, and a very small amount of this compound was established in nonfermented blue WW. The odor of Deca-(2E,4E)-dien-1-ol is described as fatty and waxy, like white meat chicken and turkey with a slight melon fruity and a dairy nuance. The highest content of 4-vinylguaiacol (sweet, spicy, clove, carnation, phenolic, peppery, smoky, woody, powdery odor) was found in the VC profile of nonfermented purple WW (16.2% of the total VC).

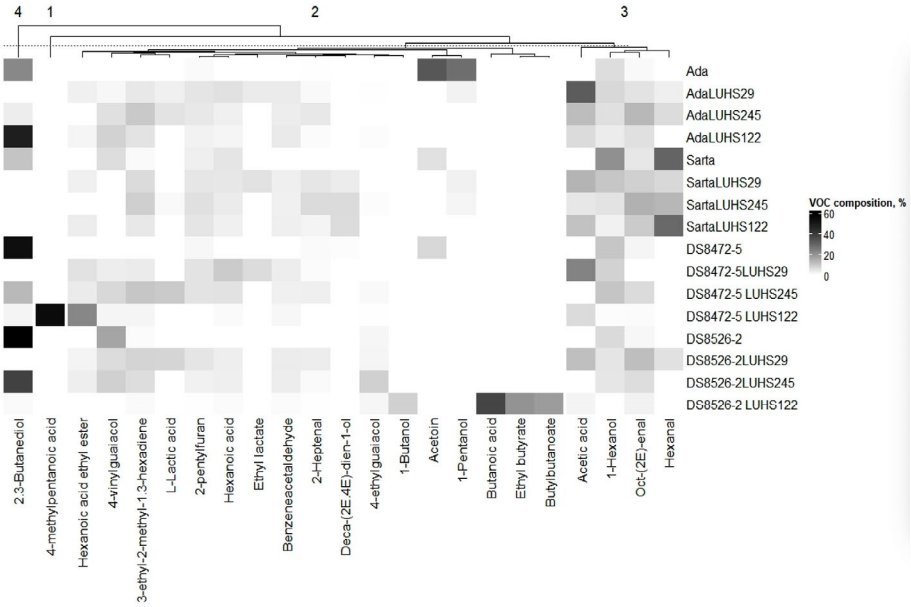

Figure 3. Volatile compounds that are >5% in at least one wheat wholemeal sample (LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means (n = 3)).

VCs that were >1% and <5% in at least one WW sample are shown in Figure 4, and the detailed FA profile is given in Supplementary File S5 Table S10. (E)-2-Nonenal, octanoic acid, ethyl octanoate, nonanoic acid, and dihydro-5-pentyl-2(3H)-furanone were found in all the WW samples. When comparing all WW samples, the highest content of 2,5-dimethylpyrazine, 1-heptanol, 1-octen-3-ol, heptanoic acid, phenethyl alcohol, and dodecanoic acid were found in DS8472-5 blue WW fermented with the LUHS29 strain. The highest content of (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, 4-ethyl-3-nonen-5-yne,

and trans-4,5-epoxy-(E)-2-decenal were found in 'Sarta' WW fermented with the LUHS245 strain. 4-methylhexanoic acid and ethylhydrocinnamate were found only in DS8472-5 blue WW fermented with the LUHS245 strain. The highest content of benzaldehyde was found in DS8526-2 purple WW. Blue WW DS8472-5 fermented with the LUHS245 strain showed the highest 2-nonanone and 4-ethylphenol content, and the nonfermented blue WW showed the highest content of nonanal. Found in only two samples was 4-methyl-1-(pent-4-en-1-yl)-2,3-diazabicyclo [2.2.1] hept-2-ene, and the highest content of this VC in nontreated blue WW was established.

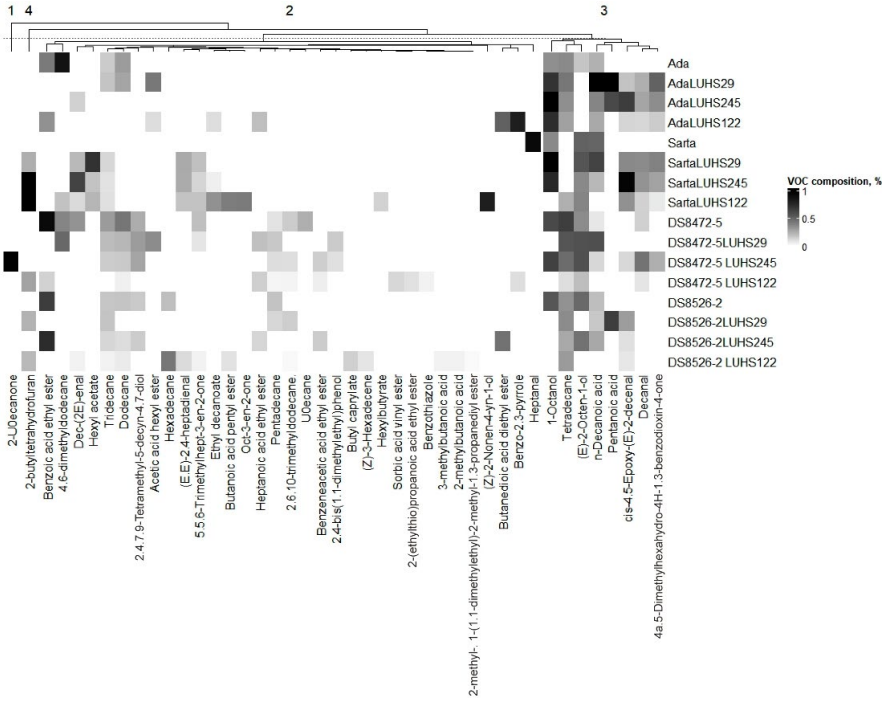

Figure 4. Volatile compounds that are >1% and <5% in at least one wheat wholemeal sample (LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means (n = 3)).

VCs that were <1% in the WW samples are shown in Figure 5, and the detailed VC profile is given in Supplementary File S5 Table S11. Further, 3-methylbutanoic acid, 2-methylbutanoic acid, (Z)-3-hexadecene, butyl caprylate and propanoic acid, and 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester were found in only purple WW fermented with the LUHS122 strain; heptanal was only found in nontreated 'Sarta'; and oct-3-en-2-one, (Z)-2-nonen-4-yn-1-ol, and hexylbutyrate were found only in 'Sarta' WW fermented with LUHS122. Undecane was identified in nontreated blue WW, and 2-(ethylthio)propanoic acid ethyl ester; sorbic acid vinyl ester; and benzothiazole were only found in DS8472-5 blue WW fermented with the LUHS122 strain. It was found that 2,4,7,9-

Tetramethyl-5-decyn-4,7-diol; 2,6,10-trimethyldodecane; 2,4-bis(1,1-dimethylethyl)phenol; pentadecane; and hexadecane were more typical for the colored WW samples (nontreated and fermented ones).

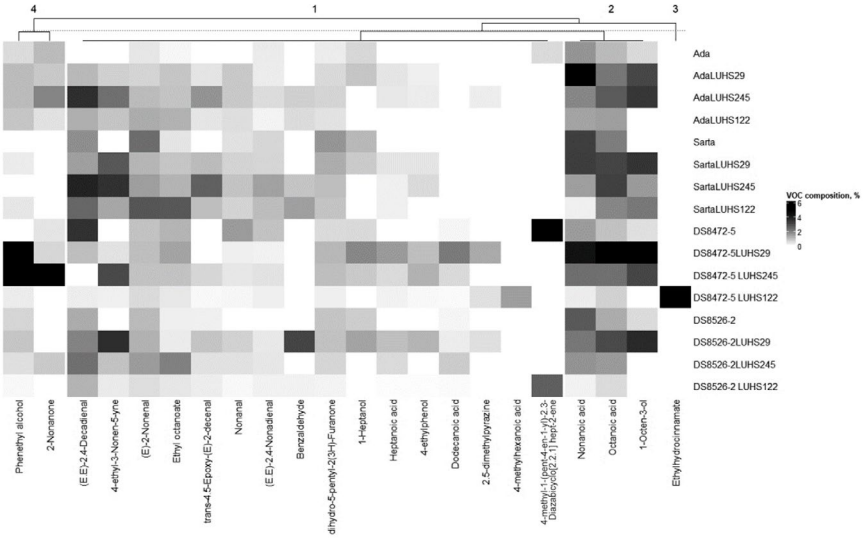

Figure 5. Volatile compounds that are <1% in wheat wholemeal samples (LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. Data are represented as means (n = 3)).

During the WW fermentation, many factors have an influence on VC formation, such as protein hydrolysis due to the cereal proteases at low pH, release of phenolic compounds, synthesis of flavor precursors, etc. [50]. LAB based sourdough has a more diverse VC profile in comparison with yeast-fermented doughs [85], and the most abundant VCs are carboxylic acids, esters, alcohols, ketones, aldehydes, and heterocycles [86]. It should be mentioned that the dominant VC produced by various processes of sourdough fermentation are often reported differently [82,86]. It was reported that the LAB fermentation can also mask bitterness due to the increased bran content in WW in comparison with refined flour, and these changes are related to the degradation of phenolic acids and aldehydes [87]. Sourdough fermentation can also increase the fruitiness [88]. Our results showed that the wheat variety, LAB strain used for fermentation, and their interaction are significant factors on the content of most of the identified VC compounds in the WW samples (Supplementary File S5 Table S12). Finally, according to the obtained results, a desirable VC profile of the wheat sourdoughs could be designed by selecting the appropriate wheat variety as well as LAB strains for its fermentation.

3.6. Changes in the Mycotoxin Concentration in Wheat Wholemeal during Fermentation

The mycotoxin concentration in the WW samples is shown in Table 6. In 'Ada' samples (nontreated and fermented) and nonfermented and LUHS122 strain fermented 'Sarta' samples, all the tested mycotoxin concentrations were below the detection limits. However, in 'Sarta' samples fermented with the LUHS29 and LUHS245 strain, the deoxynivalenol

(DON) concentration was, on average, 19.5 µg/kg. Also, DON was established in all the tested DS8472-5 blue and DS8526-2 purple WW, with the highest DON concentration in samples fermented with LUHS29 (163.0 and 392.3 µg/kg, respectively). In DS8472-5 blue WW samples, after fermentation with all the tested LAB strains, the DON concentration was increased in comparison with the nontreated samples (in samples fermented with LUHS29 by 69.1%, in samples fermented with LUHS245 by 39.0%, and in samples fermented with LUHS122 by 20.3%). Different tendencies of the DON concentration in DS8526-2 purple WW samples were established, and after fermentation with LUHS29 and LUHS245, an increase in DON was found (by 43.0% and 18.0%, respectively); however, after fermentation with LUHS122, a 17.6% lower concentration of DON was established in the WW samples. It was reported that the anti-fungal activity of sourdough LAB is mainly attributed to the inhibitory metabolites produced during fermentation [89] and the synergistic interactions of the LAB metabolites with dough or microbial derived compounds, and this inhibitory effect may even change depending on the type of flour used [90,91]. However, the reduction of mycotoxins by LAB mainly depends on the binding of the toxin to the bacterial cell wall or, more rarely, due to the metabolism of the toxin [92]. Despite the fact that the main role in the removal of mycotoxins is attributed to the bacterial cell-wall components, [93] it was reported that the organic acids and antifungal ingredients such as phenyllactic acid, hydroxyphenyllactic acid, and indole lactic acid produced by the LAB also have anti-aflatoxigenic effects. To summarize, differences in the structure of the bacterial cell wall, the initial concentration of the toxin, the bacterial population, the incubation conditions, the type of the LAB, and probably the types and the amounts of the LAB metabolites are important in this effect [93,94]. Finally, it could be suggested that by changing the duration of the fermentation, moisture content of the fermentable substrate, etc., the results of the mycotoxin concentration in fermented WW could vary. Further research is needed to evaluate the influence of technological parameters on mycotoxin degradation in WW samples.

3.7. The Limitations and Perspectives of the Current Study

Taking into consideration that the functional and/or higher value food market is growing, the new data on the colored wheat chemical composition and its possible changes during fermentation are very important. Fermentation is the main process in bread as well as in cereal beverages preparation, and this study showed that despite all the tested WW and LAB strains being suitable for WW treatment, most of the parameters were related to the cereal variety, LAB strain used, and the interaction of these factors. From this point of view, further studies are needed to evaluate more parameters (i.e., enzyme activities, dietary fibre compounds, amino acids, etc.) and to optimize the fermentation process. Also, it would be very prospective to analyze the new breed lines cereal grains, which growth was performed at the different conditions, because the same conditions could have a different effect on different cereal breed lines grain chemical as well as technological characteristics. As a perspective, the application of fermented WW could be promising for various food products and feed preparation. However, taking into consideration the different technological requirements for different food materials, to produce good sensory properties possessing and safe food products, an optimization process (of the WW fermentation) could be performed specifically, just to obtain desirable properties for specific products.

Table 6. Mycotoxin concentration in wheat wholemeal samples.

Wheat Samples	Mycotoxin Concentration, µg/kg						
	DON	HT2	T2	FB1	FB2	ZEA	OTA
Traditional Wheat Variety							
'Ada' 'Ada'$_{LUHS29}$ 'Ada'$_{LUHS245}$ 'Ada'$_{LUHS122}$	<15	<1.5	<1.5	<15	<15	<3	<1.5
Waxy Wheat Variety							
'Sarta' 'Sarta'$_{LUHS29}$ 'Sarta'$_{LUHS245}$ 'Sarta'$_{LUHS122}$	<15 21.5 ± 2.1 ^{a,B} 17.4 ± 1.8 ^{a,B} <15	<1.5	<1.5	<15	<15	<3	<1.5
Blue Wheat—New BreedLine							
DS8472-5 DS8472-5$_{LUHS29}$ DS8472-5$_{LUHS245}$ DS8472-5$_{LUHS122}$	96.4 ± 4.2 ^{a,A} 163.0 ± 7.8 ^{d,A} 134.0 ± 6.5 ^{c,A} 116.0 ± 9.2 ^{b,A}	<1.5	<1.5	<15	<15	<3	<1.5
Purple Wheat—New BreedLine							
DS8526-2 DS8526-2$_{LUHS29}$ DS8526-2$_{LUHS245}$ DS8526-2$_{LUHS122}$	274.4 ± 9.8 ^{d,B} 392.3 ± 11.3 ^{b,B} 323.9 ± 14.5 ^{c,B} 226.0 ± 11.6 ^{a,B}	<1.5	<1.5	<15	<15	<3	<1.5

LUHS29—fermented with *Pediococcus acidilactici* LUHS29 strain; LUHS245—fermented with *Liquorilactobacillus uvarum* LUHS245 strain; LUHS122—fermented with *Lactiplantibacillus plantarum* LUHS122 strain. DON—deoxynivalenol; HT2—HT2 toxin; T2—T2 toxin; FB1—fumonisin B1; FB2—fumonisin B2; ZEA—zearalenone; OTA—ochratoxin A. Data are represented as means (n = 3) ± SE. $^{a–d}$ Means with different letters in the column are significantly different between the same variety of non-treated and fermented samples ($p \leq 0.05$). ^{A,B} Means with different letters in the column are significantly different between the different variety of samples (between non-treated and between the same LAB strain treated samples) ($p \leq 0.05$).

4. Conclusions

All the fermented WW showed >8.0 $\log_{10}$ CFU/g of LAB count, and the type of LAB was a significant factor in the WW acidity parameters. Phenylethylamine was the predominant BA in WW, and the wheat variety (WV), the type of LAB, and their interaction were significant factors in the BA formation. Despite the fact that some differences in trace element concentrations in WW were obtained, in most of the cases fermentation was not a significant factor in their content. The main FAs in WW were palmitic acid, all-*cis*,*trans*-octadecenoic acid, linoleic acid. Fermented WW showed a more diverse VC profile; however, the influence of fermentation on deoxynivalenol in WW was varied. Finally, further studies are needed to indicate technological parameters that would be the most effective for each WV, including the lowest BA formation and mycotoxin degradation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology11070966/s1, Supplementary File S1. Instrumental parameters for the HPLC-MS/MS analysis of mycotoxins: Table S1. HPLC parameters. Table S2. Analyzed compound retention time, precursor and product m/z. Supplementary File S2. Statistical data of the WW acidity, chromaticity, microbiological characteristics: Table S3. The influence of the wheat variety and type of LAB on the acidity, chromaticity, and microbiological characteristics of the WW samples. Table S4. Pearson correlations between the WW acidity, chromaticity, and microbiological characteristics. Supplementary File S3. Statistical data of the biogenic amines in WW samples: Table S5. The influence of the wheat variety and type of LAB on the biogenic amine content in WW samples. Table S6. Pearson correlations between the WW acidity, chromaticity, and microbiological characteristics. Supplementary File S4. Table S7. Fatty acid profile of the wheat

samples. Table S8. The influence of the wheat variety and type of LAB on the fatty acid content in WW samples. Supplementary File S5. Wheat sample volatile compounds: Table S9. Volatile compounds that are >5% in at least one wheat wholemeal sample. Table S10. Volatile compounds that are >1% and <5% in at least one wheat wholemeal sample. Table S11. Volatile compounds that are <1% in at least one wheat wholemeal sample. Table S12. The influence of the wheat variety and type of LAB on the acidity, chromaticity, and microbiological characteristics of the WW samples.

Author Contributions: Conceptualization, E.B. and V.R.; methodology, V.B., A.B., E.M. and Ž.L.; software, V.S. and E.Z.; validation, V.S, E.Z., D.K. and E.M.; formal analysis, V.S., E.Z., D.K., A.B., and E.M.; investigation, V.R., Ž.L., V.B., E.B., V.S., E.Z., D.K., A.B. and E.M.; resources, E.B., V.B., Ž.L. and V.R.; data curation, V.R., Ž.L., V.B., E.B., V.S., E.Z., D.K., A.B. and E.M.; writing—original draft preparation, E.B., V.S., E.Z., D.K. and V.B.; writing—review and editing, E.B., V.R., Ž.L., V.S., E.Z., D.K., R.G. and V.B.; visualization, V.S. and E.Z.; supervision, E.B. and V.R.; project administration, E.B. All authors have read and agreed to the published version of the manuscript.

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References

1. Reynolds, M.P. *Climate Change and Crop Production*; CABI: Wallingford, UK, 2010; ISBN 978-1-84593-633-4.
2. Shewry, P.R.; Hey, S.J. The Contribution of Wheat to Human Diet and Health. *Food Energy Secur.* **2015**, *4*, 178–202. [CrossRef] [PubMed]
3. Sytar, O.; Bośko, P.; Živčák, M.; Brestic, M.; Smetanska, I. Bioactive Phytochemicals and Antioxidant Properties of the Grains and Sprouts of Colored Wheat Genotypes. *Molecules* **2018**, *23*, 2282. [CrossRef] [PubMed]
4. Calderaro, A.; Barreca, D.; Bellocco, E.; Smeriglio, A.; Trombetta, D.; Lagana, G. Chapter Eight—Colored Phytonutrients: Role and Applications in the Functional Foods of Anthocyanins | Elsevier Enhanced Reader. In *Phytonutrients in Food*, 1st ed.; Woodhead Publishing: Sawston, UK, 2020; pp. 177–195.
5. Garg, M.; Chawla, M.; Chunduri, V.; Kumar, R.; Sharma, S.; Sharma, N.K.; Kaur, N.; Kumar, A.; Mundey, J.K.; Saini, M.K.; et al. Transfer of Grain Colors to Elite Wheat Cultivars and Their Characterization. *J. Cereal Sci.* **2016**, *71*, 138–144. [CrossRef]
6. Wang, C.-J.; Wang, J.-M.; Lin, W.-L.; Chu, C.-Y.; Chou, F.-P.; Tseng, T.-H. Protective Effect of Hibiscus Anthocyanins against Tert-Butyl Hydroperoxide-Induced Hepatic Toxicity in Rats. *Food Chem. Toxicol.* **2000**, *38*, 411–416. [CrossRef]
7. Burdulis, D.; Sarkinas, A.; Jasutienė, I.; Stackevicenė, E.; Nikolajevas, L.; Janulis, V. Comparative Study of Anthocyanin Composition, Antimicrobial and Antioxidant Activity in Bilberry (*Vaccinium Myrtillus* L.) and Blueberry (*Vaccinium Corymbosum* L.) Fruits. *Acta Pol. Pharm.* **2009**, *66*, 399–408.
8. Cisowska, A.; Wojnicz, D.; Hendrich, A.B. Anthocyanins as Antimicrobial Agents of Natural Plant Origin. *Nat. Prod. Commun.* **2011**, *6*, 1934578X1100600136. [CrossRef]
9. Adams, J.; Hofman, K.; Moubarac, J.-C.; Thow, A.M. Public Health Response to Ultra-Processed Food and Drinks. *BMJ* **2020**, *369*, m2391. [CrossRef]
10. Hu, Y.; Ding, M.; Sampson, L.; Willett, W.C.; Manson, J.E.; Wang, M.; Rosner, B.; Hu, F.B.; Sun, Q. Intake of Whole Grain Foods and Risk of Type 2 Diabetes: Results from Three Prospective Cohort Studies. *BMJ* **2020**, *370*, m2206. [CrossRef] [PubMed]
11. Reynolds, A.N.; Akerman, A.P.; Mann, J. Dietary Fibre and Whole Grains in Diabetes Management: Systematic Review and Meta-Analyses. *PLoS Med.* **2020**, *17*, e1003053. [CrossRef]
12. Seal, C.J.; Thielecke, F. Health Benefits and Recommendations for Daily Whole Grain Intake. *Cereal Foods World* **2018**, *63*, 103–106. [CrossRef]
13. Bartkiene, E.; Zokaityte, E.; Lele, V.; Starkute, V.; Zavistanaviciute, P.; Klupsaite, D.; Cernauskas, D.; Ruzauskas, M.; Bartkevics, V.; Pugajeva, I.; et al. Combination of Extrusion and Fermentation with Lactobacillus Plantarum and L. Uvarum Strains for Improving the Safety Characteristics of Wheat Bran. *Toxins* **2021**, *13*, 163. [CrossRef]
14. Zokaityte, E.; Lele, V.; Starkute, V.; Zavistanaviciute, P.; Klupsaite, D.; Bartkevics, V.; Pugajeva, I.; Bērziņa, Z.; Gruzauskas, R.; Sidlauskiene, S.; et al. The Influence of Combined Extrusion and Fermentation Processes on the Chemical and Biosafety Parameters of Wheat Bran. *LWT* **2021**, *146*, 111498. [CrossRef]

15. Lancetti, R.; Sciarini, L.; Pérez, G.T.; Salvucci, E. Technological Performance and Selection of Lactic Acid Bacteria Isolated from Argentinian Grains as Starters for Wheat Sourdough. *Curr. Microbiol.* **2021**, *78*, 255–264. [CrossRef] [PubMed]
16. Sadeghi, A.; Ebrahimi, M.; Mortazavi, S.A.; Abedfar, A. Application of the Selected Antifungal LAB Isolate as a Protective Starter Culture in Pan Whole-Wheat Sourdough Bread. *Food Control* **2019**, *95*, 298–307. [CrossRef]
17. Gobbetti, M.; De Angelis, M.; Di Cagno, R.; Calasso, M.; Archetti, G.; Rizzello, C.G. Novel Insights on the Functional/Nutritional Features of the Sourdough Fermentation. *Int. J. Food Microbiol.* **2019**, *302*, 103–113. [CrossRef]
18. Gänzle, M.G. Enzymatic and Bacterial Conversions during Sourdough Fermentation. *Food Microbiol.* **2014**, *37*, 2–10. [CrossRef]
19. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2017**, *59*, 105–123. [CrossRef]
20. Clarke, C.I.; Arendt, E.K. A Review of the Application of Sourdough Technology to Wheat Breads. *Adv. Food Nutr. Res.* **2005**, *49*, 137–161. [CrossRef]
21. Bartkiene, E.; Lele, V.; Ruzauskas, M.; Domig, K.J.; Starkute, V.; Zavistanaviciute, P.; Bartkevics, V.; Pugajeva, I.; Klupsaite, D.; Juodeikiene, G.; et al. Lactic Acid Bacteria Isolation from Spontaneous Sourdough and Their Characterization Including Antimicrobial and Antifungal Properties Evaluation. *Microorganisms* **2020**, *8*, 64. [CrossRef]
22. Weegels, P.L. The Future of Bread in View of Its Contribution to Nutrient Intake as a Starchy Staple Food. *Plant. Foods Hum. Nutr.* **2019**, *74*, 1–9. [CrossRef]
23. Zhu, F. Staling of Chinese Steamed Bread: Quantification and Control. *Trends Food Sci. Technol.* **2016**, *55*, 118–127. [CrossRef]
24. Ayua, E.O.; Kazem, A.E.; Hamaker, B.R. Whole Grain Cereal Fibers and Their Support of the Gut Commensal Clostridia for Health. *Bioact. Carbohydr. Diet. Fibre* **2020**, *24*, 100245. [CrossRef]
25. Gong, L.; Cao, W.; Chi, H.; Wang, J.; Zhang, H.; Liu, J.; Sun, B. Whole Cereal Grains and Potential Health Effects: Involvement of the Gut Microbiota. *Food Res. Int* **2018**, *103*, 84–102. [CrossRef]
26. Capurso, A.; Capurso, C. The Mediterranean Way: Why Elderly People Should Eat Wholewheat Sourdough Bread-a Little Known Component of the Mediterranean Diet and Healthy Food for Elderly Adults. *Aging Clin. Exp. Res.* **2020**, *32*, 1–5. [CrossRef]
27. Shalaby, A.R. Significance of Biogenic Amines to Food Safety and Human Health. *Food Res. Int.* **1996**, *29*, 675–690. [CrossRef]
28. Taylor, S.L.; World Health Organization. *Histamine Poisoning Associated with Fish, Cheese, and Other Foods*; World Health Organization: Geneva, Switzerland, 1985.
29. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on Risk Based Control of Biogenic Amine Formation in Fermented Foods. *EFSA J.* **2011**, *9*, 2393. [CrossRef]
30. Rodriguez, M.B.R.; da Silva Carneiro, C.; da Silva Feijó, M.; Júnior, C.A.C.; Mano, S.B. Bioactive Amines: Aspects of Quality and Safety in Food. *Food Nutr. Sci.* **2014**, *5*, 138–146. [CrossRef]
31. Black, B.A.; Zannini, E.; Curtis, J.M.; Gänzle, M.G. Antifungal Hydroxy Fatty Acids Produced during Sourdough Fermentation: Microbial and Enzymatic Pathways, and Antifungal Activity in Bread. *Appl. Environ. Microbiol.* **2013**, *79*, 1866–1873. [CrossRef]
32. Ekwomadu, T.I.; Akinola, S.A.; Mwanza, M. Fusarium Mycotoxins, Their Metabolites (Free, Emerging, and Masked), Food Safety Concerns, and Health Impacts. *Int. J. Environ. Res. Public Health* **2021**, *18*, 11741. [CrossRef]
33. Ekwomadu, T.I.; Dada, T.A.; Nleya, N.; Gopane, R.; Sulyok, M.; Mwanza, M. Variation of Fusarium Free, Masked, and Emerging Mycotoxin Metabolites in Maize from Agriculture Regions of South Africa. *Toxins* **2020**, *12*, 149. [CrossRef]
34. Vadopalas, L.; Ruzauskas, M.; Lele, V.; Starkute, V.; Zavistanaviciute, P.; Zokaityte, E.; Bartkevics, V.; Pugajeva, I.; Reinolds, I.; Badaras, S.; et al. Combination of Antimicrobial Starters for Feed Fermentation: Influence on Piglet Feces Microbiota and Health and Growth Performance, Including Mycotoxin Biotransformation in Vivo. *Front. Vet. Sci.* **2020**, *7*, 528990. [CrossRef] [PubMed]
35. Bartkiene, E.; Bartkevics, V.; Pugajeva, I.; Krungleviciute, V.; Mayrhofer, S.; Domig, K. The Contribution of P. Acidilactici, L. Plantarum, and L. Curvatus Starters and L-(+)-Lactic Acid to the Acrylamide Content and Quality Parameters of Mixed Rye—Wheat Bread. *LWT* **2017**, *80*, 43–50. [CrossRef]
36. Ben-Gigirey, B.; Vieites Baaptista de Sousa, J.M.; Villa, T.G.; Barros-Velazquez, J. Histamine and Cadaverine Production by Bacteria Isolated from Fresh and Frozen Albacore (Thunnus Alalunga). *J. Food Prot.* **1999**, *62*, 933–939. [CrossRef] [PubMed]
37. Evans, J.D. *Straightforward Statistics for the Behavioral Sciences*; Thomson Brooks/Cole Publishing Co.: Belmont, CA, USA, 1996; p. 600. ISBN 0-534-23100-4.
38. Kidoń, M.; Grabowska, J. Bioactive Compounds, Antioxidant Activity, and Sensory Qualities of Red-Fleshed Apples Dried by Different Methods. *LWT* **2021**, *136*, 110302. [CrossRef]
39. Gao, L.; Girard, B.; Mazza, G.; Reynolds, A.G. Changes in Anthocyanins and Color Characteristics of Pinot Noir Wines during Different Vinification Processes. *J. Agric. Food Chem.* **1997**, *45*, 2003–2008. [CrossRef]
40. Lachman, J.; Martinek, P.; Kotíková, Z.; Orsák, M.; Šulc, M. Genetics and Chemistry of Pigments in Wheat Grain—A Review. *J. Cereal Sci.* **2017**, *74*, 145–154. [CrossRef]
41. Antognoni, F.; Mandrioli, R.; Potente, G.; Taneyo Saa, D.L.; Gianotti, A. Changes in Carotenoids, Phenolic Acids and Antioxidant Capacity in Bread Wheat Doughs Fermented with Different Lactic Acid Bacteria Strains. *Food Chem.* **2019**, *292*, 211–216. [CrossRef]
42. McDonald, C.E. Lipoxygenase and Lutein Bleaching Activity of Durum Wheat Semolina. *Cereal Chem.* **1979**, *2*, 84–89.
43. Capuani, A.; Behr, J.; Vogel, R.F. Influence of Lactic Acid Bacteria on the Oxidation–Reduction Potential of Buckwheat (Fagopyrum Esculentum Moench) Sourdoughs. *Eur. Food Res. Technol.* **2012**, *235*, 1063–1069. [CrossRef]
44. Stanzer, D.; Ivanuša, I.; Kazazić, S.; Hanousek Čiča, K.; Mrvčić, J. Diversity of Lactic Acid Bacteria on Organic Flours and Application of Isolates in Sourdough Fermentation. *Hrvat. Časopis Za Prehrambenu Tehnol. Biotehnol. I Nutr.* **2017**, *12*, 44–51.

45. De Vuyst, L.; Van Kerrebroeck, S.; Harth, H.; Huys, G.; Daniel, H.-M.; Weckx, S. Microbial Ecology of Sourdough Fermentations: Diverse or Uniform? *Food Microbiol.* **2014**, *37*, 11–29. [CrossRef] [PubMed]
46. Paramithiotis, S.; Chouliaras, Y.; Tsakalidou, E.; Kalantzopoulos, G. Application of Selected Starter Cultures for the Production of Wheat Sourdough Bread Using a Traditional Three-Stage Procedure. *Process. Biochem.* **2005**, *40*, 2813–2819. [CrossRef]
47. Chavan, R.S.; Chavan, S.R. Sourdough Technology—A Traditional Way for Wholesome Foods: A Review. *Compr. Rev. Food Sci. Food Saf.* **2011**, *10*, 169–182. [CrossRef]
48. Hammes, W.P.; Gänzle, M.G. Sourdough Breads and Related Products. In *Microbiology of Fermented Foods*; Wood, B.J.B., Ed.; Springer: Boston, MA, USA, 1998; pp. 199–216. ISBN 978-1-4613-0309-1.
49. Spicher, G. *Baked Goods*; Biotechnology, Food and Feed Productions with Microorganisms; Verlag Chemie: Weinheim, Germany, 1983; Volume 5, pp. 1–80.
50. Silla Santos, M.H. Biogenic Amines: Their Importance in Foods. *Int. J. Food Microbiol.* **1996**, *29*, 213–231. [CrossRef]
51. Visciano, P.; Schirone, M.; Tofalo, R.; Suzzi, G. Biogenic Amines in Raw and Processed Seafood. *Front. Microbiol.* **2012**, *3*, 188. [CrossRef]
52. Özogul, F.; Hamed, I. The Importance of Lactic Acid Bacteria for the Prevention of Bacterial Growth and Their Biogenic Amines Formation: A Review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1660–1670. [CrossRef]
53. Marijan, A.; Džaja, P.; Bogdanović, T.; Škoko, I.; Cvetnić, Ž.; Dobranić, V.; Zdolec, N.; Šatrović, E.; Severin, K. Influence of Ripening Time on the Amount of Certain Biogenic Amines in Rind and Core of Cow Milk Livno Cheese. *Mljekarstvo Časopis Za Unaprjeđenje Proizv. I Prerade Mlijeka* **2014**, *64*, 159–169. [CrossRef]
54. Moret, S.; Smela, D.; Populin, T.; Conte, L.S. A Survey on Free Biogenic Amine Content of Fresh and Preserved Vegetables. *Food Chem.* **2005**, *89*, 355–361. [CrossRef]
55. Mantis, F.; Tsachev, I.; Sabatakou, O.; Burriel, A.; Vacalopoulos, A.; Ramantanis, S. Safety and shelflife of widely distributed vacuum packed, heat treated sausages. *Bulg. J. Vet. Med.* **2006**, *4*, 245–254.
56. Maintz, L.; Novak, N. Histamine and Histamine Intolerance. *Am. J. Clin. Nutr.* **2007**, *85*, 1185–1196. [CrossRef]
57. Hungerford, J.M. Scombroid Poisoning: A Review. *Toxicon* **2010**, *56*, 231–243. [CrossRef] [PubMed]
58. Linares, D.M.; del Rio, B.; Redruello, B.; Ladero, V.; Martin, M.C.; Fernandez, M.; Ruas-Madiedo, P.; Alvarez, M.A. Comparative Analysis of the in Vitro Cytotoxicity of the Dietary Biogenic Amines Tyramine and Histamine. *Food Chem.* **2016**, *197*, 658–663. [CrossRef] [PubMed]
59. Glória, M.B.A. Bioactive Amines. In *Handbook of Food Science, Technology and Engineering*; CRC Press: Boca Raton, FL, USA, 2005.
60. Landete, J.M.; de las Rivas, B.; Marcobal, A.; Muñoz, R. Molecular Methods for the Detection of Biogenic Amine-Producing Bacteria on Foods. *Int. J. Food Microbiol.* **2007**, *117*, 258–269. [CrossRef] [PubMed]
61. Shi, R.; Li, H.; Tong, Y.; Jing, R.; Zhang, F.; Zou, C. Identification of Quantitative Trait Locus of Zinc and Phosphorus Density in Wheat (*Triticum Aestivum* L.) Grain. *Plant Soil* **2008**, *306*, 95–104. [CrossRef]
62. Sieprawska, A.; Filek, M.; Walas, S.; Tobiasz, A.; Mrowiec, H.; Miszalski, Z. Does Micro- and Macroelement Content Differentiate Grains of Sensitive and Tolerant Wheat Varieties? *Acta Physiol. Plant.* **2014**, *36*, 3095–3100. [CrossRef]
63. Verni, M.; Rizzello, C.G.; Coda, R. Fermentation Biotechnology Applied to Cereal Industry By-Products: Nutritional and Functional Insights. *Front. Nutr.* **2019**, *6*, 42. [CrossRef]
64. Foucaud, C.; Francois, A.; Richard, J. Development of a Chemically Defined Medium for the Growth of Leuconostoc Mesenteroides. *Appl. Environ. Microbiol.* **1997**, *63*, 301–304. [CrossRef]
65. Von Wright, A.; Axelsson, L. Lactic Acid Bacteria: An Introduction. In *Lactic Acid Bacteria: Microbiological and Functional Aspects*; CRC Press: Boca Raton, FL, USA, 2011; Volume 4, pp. 1–16.
66. Guo, Z.; Zhang, Z.; Xu, P.; Guo, Y. Analysis of Nutrient Composition of Purple Wheat. *Cereal Res. Commun.* **2012**, *41*, 293–303. [CrossRef]
67. He, Y.Z.; Ning, J.F. Analysis of Nutrition Composition in the Special Purple Grain Wheat "Qinhei 1" Containing Rich Fe and Zn. *J. Northwest A F Univ. Nat. Sci. Ed.* **2003**, *31*, 87–90.
68. Tian, S.; Chen, Z.; Wei, Y. Measurement of Colour-Grained Wheat Nutrient Compounds and the Application of Combination Technology in Dough. *J. Cereal Sci.* **2018**, *83*, 63–67. [CrossRef]
69. Ma, D.; Zhang, J.; Hou, J.; Li, Y.; Huang, X.; Wang, C.; Lu, H.; Zhu, Y.; Guo, T. Evaluation of Yield, Processing Quality, and Nutritional Quality in Different-Colored Wheat Grains under Nitrogen and Phosphorus Fertilizer Application. *Crop. Sci.* **2018**, *58*, 402–415. [CrossRef]
70. Lafiandra, D.; Masci, S.; Sissons, M.; Dornez, E.; Delcour, J.; Courtin, C.; Caboni, M.F. Kernel Components of Technological Value. In *Durum Wheat Chemistry and Technology*, 2nd ed.; Academic Press: Cambridge, MA, USA, 2012; pp. 85–124.
71. Narducci, V.; Finotti, E.; Galli, V.; Carcea, M. Lipids and Fatty Acids in Italian Durum Wheat (Triticum Durum Desf.) Cultivars. *Foods* **2019**, *8*, 223. [CrossRef] [PubMed]
72. Russo, G.L. Dietary N-6 and n-3 Polyunsaturated Fatty Acids: From Biochemistry to Clinical Implications in Cardiovascular Prevention. *Biochem. Pharm.* **2009**, *77*, 937–946. [CrossRef] [PubMed]
73. Mozaffarian, D.; Wu, J.H.Y. Omega-3 Fatty Acids and Cardiovascular Disease: Effects on Risk Factors, Molecular Pathways, and Clinical Events. *J. Am. Coll. Cardiol.* **2011**, *58*, 2047–2067. [CrossRef] [PubMed]
74. Armanino, C.; De Acutis, R.; Rosa Festa, M. Wheat Lipids to Discriminate Species, Varieties, Geographical Origins and Crop Years. *Anal. Chim. Acta* **2002**, *454*, 315–326. [CrossRef]

75. Beleggia, R.; Platani, C.; Nigro, F.; De Vita, P.; Cattivelli, L.; Papa, R. Effect of Genotype, Environment and Genotype-by-Environment Interaction on Metabolite Profiling in Durum Wheat (Triticum Durum Desf.) Grain. *J. Cereal Sci.* **2013**, *57*, 183–192. [CrossRef]
76. Nejadsadeghi, L.; Maali-Amiri, R.; Zeinali, H.; Ramezanpour, S.; Sadeghzade, B. Membrane Fatty Acid Compositions and Cold-Induced Responses in Tetraploid and Hexaploid Wheats. *Mol. Biol Rep.* **2015**, *42*, 363–372. [CrossRef]
77. Upchurch, R.G. Fatty Acid Unsaturation, Mobilization, and Regulation in the Response of Plants to Stress. *Biotechnol. Lett.* **2008**, *30*, 967–977. [CrossRef]
78. Rosicka-Kaczmarek, J.; Mi, K.; Makowski, B. Composition and Functional Properties of Lipid Components from Selected Cereal Grains. *Res. Signpost* **2015**. Available online: https://www.semanticscholar.org/paper/Composition-and-functional-properties-of-lipid-from-Rosicka-Kaczmarek-Mi%C5%9Bkiewicz/207d83d4582d72cf8a545348b50a641f6bfedc09 (accessed on 28 May 2022).
79. Kaseleht, K.; Paalme, T.; Mihhalevski, A.; Sarand, I. Analysis of Volatile Compounds Produced by Different Species of Lactobacilli in Rye Sourdough Using Multiple Headspace Extraction. *Int. J. Food Sci. Technol.* **2011**, *46*, 1940–1946. [CrossRef]
80. Damiani, P.; Gobbetti, M.; Cossignani, L.; Corsetti, A.; Simonetti, M.S.; Rossi, J. The Sourdough Microflora. Characterization of Hetero- and Homofermentative Lactic Acid Bacteria, Yeasts and Their Interactions on the Basis of the Volatile Compounds Produced. *LWT—Food Sci. Technol.* **1996**, *29*, 63–70. [CrossRef]
81. Hansen, B.; Hansen, Å. Volatile Compounds in Wheat Sourdoughs Produced by Lactic Acid Bacteria and Sourdough Yeasts. *Z. Für Lebensm. -Unters. Und-Forsch.* **1994**, *198*, 202–209. [CrossRef]
82. Corona, O.; Alfonzo, A.; Ventimiglia, G.; Nasca, A.; Francesca, N.; Martorana, A.; Moschetti, G.; Settanni, L. Industrial Application of Selected Lactic Acid Bacteria Isolated from Local Semolinas for Typical Sourdough Bread Production. *Food Microbiol.* **2016**, *59*, 43–56. [CrossRef] [PubMed]
83. Czerny, M.; Schieberle, P. Important Aroma Compounds in Freshly Ground Wholemeal and White Wheat FlourIdentification and Quantitative Changes during Sourdough Fermentation. *J. Agric. Food Chem.* **2002**, *50*, 6835–6840. [CrossRef]
84. Vermeulen, N.; Czerny, M.; Gänzle, M.G.; Schieberle, P.; Vogel, R.F. Reduction of (E)-2-Nonenal and (E,E)-2,4-Decadienal during Sourdough Fermentation. *J. Cereal Sci.* **2007**, *45*, 78–87. [CrossRef]
85. Ma, S.; Wang, Z.; Guo, X.; Wang, F.; Huang, J.; Sun, B.; Wang, X. Sourdough Improves the Quality of Whole-Wheat Flour Products: Mechanisms and Challenges—A Review. *Food Chem.* **2021**, *360*, 130038. [CrossRef]
86. Ripari, V.; Gänzle, M.G.; Berardi, E. Evolution of Sourdough Microbiota in Spontaneous Sourdoughs Started with Different Plant Materials. *Int. J. Food Microbiol.* **2016**, *232*, 35–42. [CrossRef]
87. Prückler, M.; Lorenz, C.; Endo, A.; Kraler, M.; Dürrschmid, K.; Hendriks, K.; Soares da Silva, F.; Auterith, E.; Kneifel, W.; Michlmayr, H. Comparison of Homo- and Heterofermentative Lactic Acid Bacteria for Implementation of Fermented Wheat Bran in Bread. *Food Microbiol.* **2015**, *49*, 211–219. [CrossRef]
88. Spaggiari, M.; Ricci, A.; Calani, L.; Bresciani, L.; Neviani, E.; Dall'Asta, C.; Lazzi, C.; Galaverna, G. Solid State Lactic Acid Fermentation: A Strategy to Improve Wheat Bran Functionality. *LWT* **2020**, *118*, 108668. [CrossRef]
89. Messens, W.; De Vuyst, L. Inhibitory Substances Produced by Lactobacilli Isolated from Sourdoughs—a Review. *Int. J. Food Microbiol.* **2002**, *72*, 31–43. [CrossRef]
90. Lavermicocca, P.; Valerio, F.; Evidente, A.; Lazzaroni, S.; Corsetti, A.; Gobbetti, M. Purification and Characterization of Novel Antifungal Compounds from the Sourdough Lactobacillus Plantarum Strain 21B. *Appl. Environ. Microbiol.* **2000**, *66*, 4084–4090. [CrossRef] [PubMed]
91. Ryan, L.A.M.; Zannini, E.; Dal Bello, F.; Pawlowska, A.; Koehler, P.; Arendt, E.K. Lactobacillus Amylovorus DSM 19280 as a Novel Food-Grade Antifungal Agent for Bakery Products. *Int. J. Food Microbiol.* **2011**, *146*, 276–283. [CrossRef] [PubMed]
92. Dalié, D.K.D.; Deschamps, A.M.; Richard-Forget, F. Lactic Acid Bacteria—Potential for Control of Mould Growth and Mycotoxins: A Review. *Food Control.* **2010**, *21*, 370–380. [CrossRef]
93. Guimaraes, J.T.; Silva, E.K.; Ranadheera, C.S.; Moraes, J.; Raices, R.S.; Silva, M.C.; Ferreira, M.S.; Freitas, M.Q.; Meireles, M.A.A.; Cruz, A.G. Effect of High-Intensity Ultrasound on the Nutritional Profile and Volatile Compounds of a Prebiotic Soursop Whey Beverage. *Ultrason. Sonochemistry* **2019**, *55*, 157–164. [CrossRef] [PubMed]
94. Haskard, C.A.; El-Nezami, H.S.; Kankaanpää, P.E.; Salminen, S.; Ahokas, J.T. Surface Binding of Aflatoxin B1 by Lactic Acid Bacteria. *Appl. Environ. Microbiol.* **2001**, *67*, 3086–3091. [CrossRef]

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Influence of different lactic acid bacteria strains and milling process on the solid-state fermented green and red lentils (*Lens culinaris* L.) properties including gamma-aminobutyric acid formation

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The aim of this study was to evaluate the influence of lactic acid bacteria (LAB) strains (*Lactiplantibacillus plantarum* No.122 and *Lacticaseibacillus casei* No.210) and milling process on the solid-state fermented (for 24h, at 30°C) green and red lentils (*Lens culinaris* L.) properties, chiefly pH, LAB viable counts, color coordinates, free amino acid (FAA) profile, γ-aminobutyric acid (GABA) and biogenic amine (BA) concentrations, fatty acid (FA) and volatile compound (VC) profiles. Results showed that both of the tested LAB strains are suitable for the fermentation of lentils: pH of fermented lentils was <4.5 and LAB viable counts >8.0 $\log_{10}$ colony-forming units (CFU)/g. A very strong negative correlation was found ($r=-0.973$, $p\leq0.0001$) between LAB counts and pH of the samples. Also, fermentation and milling process were significant factors toward color coordinates of the lentils. In most of the cases, solid-state fermentation (SSF) increased essential FAA content in lentils; however, some of the non-essential FAA content was reduced. SSF significantly increased GABA concentration in lentils and milling process was a significant factor on GABA content of the samples ($p\leq0.05$). The main BA in lentils was spermidine, and SSF decreased their total BA content (34.8% on average in red lentils and 39.9% on average in green lentils). The main FA in lentils were linoleic and oleic. The main VC in lentils were hexanal, 1-hexanol, hexanoic acid, D-limonene and (E)-2-nonen-1-ol. Furthermore, most of the VC showed significant correlations with pH of lentil samples, LAB counts and FA content. Finally, the LAB strain used for fermentation and the milling process of lentils are significant factors for most of the analyzed parameters in lentil. Moreover, despite the higher GABA concentration found in green non-milled SSF lentils, application of combined milling and SSF is recommended because they showed the lowest BA content in addition to higher essential FAA and GABA concentrations.

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KEYWORDS

lentils (*Lens culinaris* L.), solid-state fermentation, lactic acid bacteria, gamma-aminobutyric acid, biogenic amines

1. Introduction

Lentils (*Lens culinaris* L.; Family: *Fabaceae*) are used as a very valuable stock for human (1) and animal nutrition (2). Nowadays, these plant species are diversified (3) and well known for their rich dietary compositions (4). Depending on the cultivar., lentils can be yellow, orange, red, green, brown or black (5). According to the Food and Agriculture Organization (FAO), the global production of the lentils is primarily cultivated and harvested in Canada and India, which are estimated to be 1.99 and 1.1 million metric tons, respectively (6). There has been growing scientific interest to study lentils as a functional material due to their high biological value, as well as the presence of bioactive compounds (1).

Lentils have low fat content and their fatty acid (FA) fraction comprising 16.7% of saturated, 23.7% of monounsaturated and 58.8% of polyunsaturated FA (7). However, the composition of lentils varies widely, *viz.*: protein from 15.9 to 31.4%, carbohydrates from 43.4 to 74.9%, fat from 0.3 to 3.5%, total fiber content from 5.1 to 26.6% and ash content from 2.2 to 6.4% (8). These variations are explained by plant genetics, agri-ecological factors and production practices, as well as biotic and abiotic stresses (8). According to the nutrient data of US Department of Agriculture, raw lentil contains 24.6% protein, 63.4% carbohydrates, 1.1% fat and 2.7% ash (9). Despite that, in comparison with wheat or rice, lentils are richer in protein and lower in carbohydrates (10).

To increase the functional value, lentils can be fermented. Indeed, fermentation is an effective technology to lead many beneficial characteristics of the fermentable substrate, including higher quantities of phenolic compounds (11, 12) and better antioxidant properties (13, 14). In comparison submerged and solid-state fermentation (SSF), the latter is more sustainable because of the lower water content, smaller fermentation vessels used, in addition to the fact that during SSF various enzymes are excreted in higher concentrations by the existing microorganisms (15). Also, during the fermentation, antinutritional compounds are significantly degraded (11, 16).

Though many studies on modeling, the nutritional and functional characteristics of lentils *via* SSF have been carried out, to the best of our knowledge, changes in free amino acid (FAA) profile, γ-aminobutyric acid (GABA) and biogenic amine (BA) concentrations, fatty acid (FA) and volatile compound (VC) profiles due to SSF with *Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210 and milling process of the fermentable substrate have not been discussed so far. Also, to evaluate an influence of milling process is very important. Particle size can be of significance when biological treatment is applied, because of microorganism nutrients accessibility, technological starters can show different excretion properties of the enzymes, as well as other metabolites, which can lead to different properties of the fermented substrate.

The most abundant amino acid (AA) in lentils is glutamic acid, followed by aspartic acid, arginine, leucine and lysine. However, methionine and tryptophan are limiting AA in lentils (10). Taking into consideration the AA profile of lentils, we hypothesized in this study that SSF of lentils can be a good mean to increase the content of FAA as well as GABA. GABA is a potent bioactive compound which is most commonly produced *via* decarboxylation of glutamate, and lactic acid bacteria (LAB) strains are most widely used for producing GABA-enriched products by fermentation (17). Despite, that most of the production studies on GABA synthesis have been reported by submerged fermentation, SSF is more suitable in cost effective GABA production (10). It was reported that *Limosilactobacillus fermentum*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Limosilactobacillus reuteri*, *Bifidobacterium* spp., *Levilactobacillus brevis*, *Pediococcus acidilactici* and *Latilactobacillus sakei* are the most popular LAB for GABA production (18, 19). Despite LAB fermentation technology has a Generally Regarded As Safe (GRAS) status, there are two main metabolic pathways—chiefly glutamate decarboxylase pathway and putrescine pathway—which are followed by microorganisms in the production of GABA, and during the same pathways BA can be formed (20). In the case of fermented substrates, decarboxylase-producing microorganisms can be used as technological starter cultures (21). From here, on may conclude that the control of BA concentration in the end-product is required.

Under this context, the present research study was carried out to evaluate the influence of two LAB strains (*Lactiplantibacillus plantarum* No.122 and *Lacticaseibacillus casei* No.210) and milling process on the properties (pH, LAB count, color coordinates, FAA profile, GABA and BA concentrations, FA and VC profiles) of green and red lentils (*Lens culinaris* L.) subjected to SSF.

Abbreviations: Ala, Alanine; AA, Amino acid; Arg, Arginine; Asp, Aspartic acid; BA, Biogenic amines; −b*, Blueness; CAD, Cadaverine; CFU, Colony-forming units; MRS, De Man, Rogosa and Sharpe; EU, European Union; FAME, Fatty acid methyl esters; FA, Fatty acids; FAO, Food and Agriculture Organization; FAA, Free amino acids; GABA, γ-aminobutyric acid; GC, Gas chromatographer/phy; GRAS, Generally regarded as safe; Glu, Glutamic acid; Gln, Glutamine; Gly, Glycine; Gr, Green lentils; −a*, Greenness; His, Histidine; Ile, Isoleucine; LAB, Lactic acid bacteria; *Lc. casei*, *Lacticaseibacillus casei*; *Lp. plantarum*, *Lactiplantibacillus plantarum*; Leu, Leucine; L*, Lightness; Lys, Lysine; MS, Mass spectrometer/try; Met, Methionine; MUFA, Monounsaturated fatty acids; PHE, Penylethylamine; Phe, Phenylalanine; PUFA, Polyunsaturated fatty acids; Pro, Proline; PUTR, Putrescine; Re, Red lentils; a*, Redness; SFA, Saturated fatty acids; Ser, Serine; SPME, Solid-phase microextraction; SSF, Solid-state fermentation; SPRMD, Spermidine; SPRM, Spermine; SE, Standard error; Thr;, Threonine; TRP, ryptamine; TYR, Tyramine; Tyr, Tyrosine; UV/VIS, Ultra-violet/visible; Val, Valine; VC, Volatile compounds; b*, Yellowness.

2. Materials and methods

2.1. Characteristics of the lentils, lactic acid bacteria used for fermentation of the lentils and fermentation conditions

Green (variety 'CDC Lemay') and red (variety 'CDC Red Rider') lentils (composition per 100 g of the green lentils: total carbohydrates 48.5 g, protein 24.0 g, fat 1.5 g; composition per 100 g of the red lentils: total carbohydrates 25.0 g, protein 13.0 g, fat 0.7 g) were provided by Ltd. 'Galinta ir partneriai' (Kaunas, Lithuania). To evaluate the influence of the milling process, samples were grounded with a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) to 1–2 mm particle size.

The LAB strains (*Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210) were acquired from the Lithuanian University of Health Sciences collection (Kaunas, Lithuania). Before the experiment, LAB strains were incubated and multiplied in De Man, Rogosa and Sharpe (MRS) broth culture medium (Biolife, Milano, Italy) at 30°C under anaerobic conditions for 24 h. A total of 3 mL of fresh viable LAB grown on MRS broth (average cell concentration of 8.6 $\log_{10}$ CFU mL) were inoculated in 100 g of lentils (lentils/water ratio was 1:1, w/w), where the final densities of the viable LAB strain in the lentils-water mixtures were on average 5.02 (red lentils) and 4.98 (green lentils) $\log_{10}$ CFU/g. Afterwards, the lentil samples were fermented under anaerobic conditions in a chamber incubator without agitation (Memmert GmbH Co. KG, Schwabach, Germany) for 24 h, at 30°C. Non-fermented lentil samples (mixed with water) were analyzed as the control.

The applied experimental design gave rise to a total of 10 samples, chiefly: non-treated (i.e. non-milled and non-fermented) red and green lentils (Re and Gr, respectively); non-milled red and green lentils (solid-state) fermented with No.122 and No. 210 LAB strains (Re_{122}, Re_{210}, Gr_{122}, Gr_{210}, respectively); milled red and green lentils (solid-state) fermented with No.122 and No. 210 LAB strains ($Re_{122milled}$, $Re_{210milled}$, $Gr_{122milled}$, $Gr_{210milled}$, respectively).

Before and after fermentation, the pH, color coordinates, LAB viable counts, FAA profile, GABA and BA concentrations, FA and VC profiles of the lentil samples were analyzed. The experimental design is schematised in Figure 1.

2.2. Analysis of pH, color coordinates, and lactic acid bacteria viable counts in the lentil samples

The pH of lentil samples was evaluated using a pH meter (Inolab 3, Hanna Instruments, Venet, Italy) by inserting the pH electrode into the lentil samples. The color coordinates of the lentil samples were evaluated on the surface using the CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Marunouchi, Tokyo Japan). The LAB viable counts were determined according to the method described by Bartkiene et al. (22).

Evaluation of free amino acid (FAA) profile and gamma-aminobutyric acid (GABA) concentration in the lentil samples.

Sample preparation and dansylation was performed according to the method of Ben-Gigirey et al. (23) with some modifications here described. Homogenizsd sample (~ 1,000 mg) was weighted in a 15 mL sample tube and analytes were extracted with 10 mL of aqueous

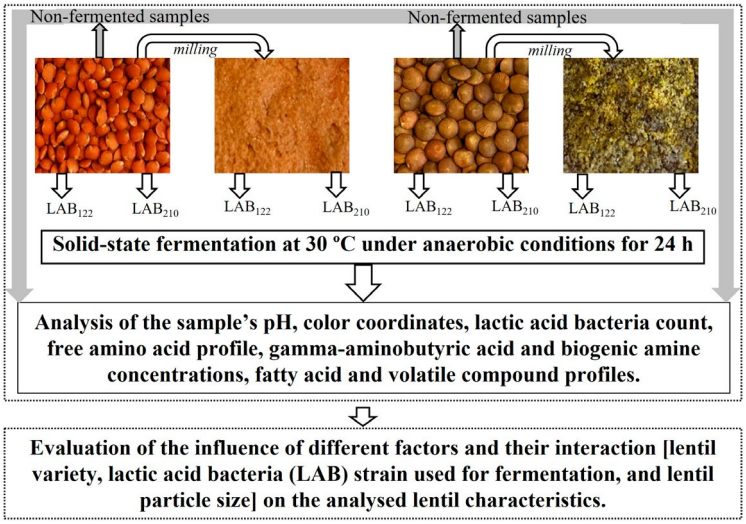

FIGURE 1
Experimental design (LAB—lactic acid bacteria strain; $_{122}$—*Lactiplantibacillus plantarum* strain; $_{210}$—*Lacticaseibacillus casei* strain).

0.1 M HCl solution by shaking for 1 h. Resultant mixture was centrifuged at 4,000 rpm for 10 min. For derivatization, 100 µL of resultant supernatant was diluted to 500 µL with 0.1 M HCl solution. Resultant mixture was alkalinised by adding 40 µL of 2 M NaOH and 70 µL of saturated $NaHCO_3$ solution. Derivatization was performed by adding 1 mL of 10 mg/mL dansyl chloride solution in acetonitrile and heating the resulting mixture at 60°C for 30 min. Reaction mixture was quenched using 50 µl of 25% ammonia solution and filtered through a 0.22 µm membrane filter to the autosampler vial. Concentration of analytes were determined using a Varian ProStar HPLC system (Varian Corp., Palo Alto, California, USA, two ProStar 210 pumps, a ProStar 410 autosampler) and Thermo Scientific LCQ Fleet Ion trap mass detector. For analyte detection, the mass spectrometer operated at positive ionisation single ion monitoring mode and single reaction monitoring mode (for glutamine). Concentration of analytes was determined using the standard addition method by spiking extract with known concentration of analytes. For the separation of derivatives, a Discovery® HS C18 column (150 × 4.6 mm, 5 µm; SupelcoTM Analytical, Bellefonte, Pennsylvania, USA) was used. The mobile phase A was 0.1% formic acid in 5% aqueus acetonitrile and the mobile phase B was 0.1% acetonitrile. A flow-rate of 0.3 mL/min and an injection volume of 10 µL were used for analysis. The analytical gradient of the mobile phase was as follows: 0 to 10 min (linear gradient) 15 to 60% B, 10 to 40 min (linear gradient) 60 to 95% B and 40 to 48 min 95% B, followed by reequilibration for 10 min with 15% B (increased to 0.6 mL/min flow-rate). The limit of quantification (according to lowest concentration of constructed calibration curve) was 0.02 µmol/g.

2.3. Analysis of biogenic amine concentration in the lentil samples

Sample preparation and identification and quantification of the BA—which included tryptamine (TRP), phenylethylamine (PHE), putrescine (PUTR), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPRMD) and spermine (SPRM)—in lentil samples was conducted by following the experimental procedure reported by Ben-Gigirey et al. (24) with some modifications. Briefly, the standard BA solutions were prepared by dissolving known amounts of each BA (including internal standard) in 20 mL of deionised water. The extraction of BA in samples (5 g) was undertaken by using 0.4 mol/l perchloric acid. The derivatization of sample extracts and standards was performed using dansyl chloride solution (10 mg/mL) as reagent. The chromatographic analyses were carried out using a Varian ProStar HPLC system (Varian Corp., Palo Alto, California, USA) with two ProStar 210 pumps, a ProStar 410 auto-sampler, a ProStar 325 UV/VIS Detector and Galaxy software (Agilent, Santa Clara, California, USA) for data processing. For the separation of amines, a Discovery® HS C18 column (150 × 4.6 mm, 5 µm; SupelcoTM Analytical, Bellefonte, Pennsylvania, USA) was used. The eluents of the mobile phase were ammonium acetate (A) and acetonitrile (B) and the elution programme consisted of a gradient system at 0.8 mL/min flow-rate. The detection wavelength was set to 254 nm, the oven temperature was 40°C and samples were injected in 20 µl aliquots. The target compounds were identified based on their retention times in comparison to their corresponding standards.

2.4. Analysis of fatty acid profile in the lentil samples

The extraction of lipids for FA quantification was undertaken with chloroform/methanol (2:1, v/v) and fatty acid methyl esters (FAME) were prepared according to the protocol described by Pérez-Palacios et al. (25). The FA composition of the lentil samples was identified using a gas chromatograph GC-2010 Plus (Shimadzu Europa GmbH, Duisburg, Germany) equipped with a mass spectrometer GCMS-QP2010 (Shimadzu Europa GmbH, Duisburg, Germany). Separation was carried out on a Stabilwax-MS column (30 m length, 0.25 mmID and 0.25 µm df) (Restek Corporation, Bellefonte, US). The mass spectrometer operated at full scan mode and the analyte was injected in split mode at 1:60 split ratio. The following parameters were used: MS ion source temperature: 240°C; MS interface temperature 240°C; helium (carrier gas) flow-rate: 0.90 mL/min; injector: 240°C and oven temperature programme was: 50°C (4 min), 10°C/min to 110°C (1 min), 15°C/min to 160°C (2 min), 2.5°C/min to 195°C (1 min), 2°C/min to 230°C (1 min) and 2°C/min to 240°C (12 min). The individual FAME peaks were identified by comparing their retention times with FAME standards (Merck & Co., Inc., Kenilworth, NJ, USA). The quantification was determined by using the corrected area normalization method.

2.5. Analysis of volatile compound profile in the lentil samples

The VC of the lentil samples were analyzed by gas chromatography–mass spectrometry (GC–MS). A solid-phase microextraction (SPME) device with Stableflex™ fiber coated with a 50 µm PDMS-DVB-Carboxen™ layer (Supelco, USA) was used for analysis. For headspace extraction, 1 g of sample and 10 mL of 1 M phosphate buffer (pH = 3) were transferred to the 20 mL extraction vial, mixed, sealed with a polytetrafluoroethylene septum and thermostated at 60°C for 30 min before exposing the fiber in the headspace. The fiber was exposed to the headspace of the vial for 10 min and desorbed in an injector liner for 2 min (splitless injection mode). Prepared samples were analyzed with a GCMS-QP2010 (Shimadzu, Japan) gas chromatograph coupled with a mass spectrometer. The following conditions were used for analysis: injector temperature 250°C; ion source temperature 220°C and interface temperature 260°C. Helium was used as the carrier gas at 0.65 mL/min flowrate. For separation of VC, a low polarity Rxi®-5MS column (Restek, USA) (length 30 m, coating thickness 0.25 µm, inner diameter of 0.25 mm) was used. The temperature gradient was programmed from starting at 40°C (3 min hold) to 220°C (5°C/min) up to 310°C (15°/min) (6 min hold). The VC were identified according to mass spectrum libraries (NIST11, NIST11S, and FFNSC2). For identification purposes, alkane mix (C8-C20) was analyzed to obtain the retention indexes of unknown compounds.

2.6. Statistical analysis

Statistical analysis was completed using IBM SPSS Statistics for Windows, v28.0.1.0 (142) (SPSS, Chicago, Illinois, USA). The results were expressed as the mean values (for lentil samples $n = 6$;

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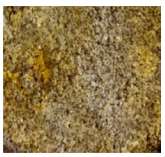
TABLE 1 pH, color coordinates, lactic acid bacteria (LAB) viable counts and images of non-treated and fermented lentils.

Lentil samples	pH after 0h	pH after 24h	Color coordinates, NBS			LAB viable counts, log_{10} CFU/g
			L*	a*	b*	
	Parameters of the red lentil samples					
Re	6.14 ± 0.02^{e}	-	55.90 ± 2.34^{b}	24.04 ± 0.98^{g}	26.40 ± 1.03^{f}	4.21 ± 0.33^{a}
Re_{122}	5.79 ± 0.01^{a,b}	4.21 ± 0.02^{a}	53.19 ± 1.98^{b}	19.59 ± 1.32^{e}	22.79 ± 0.87^{e}	8.04 ± 0.27^{b}
Re_{210}	5.98 ± 0.02^{d}	4.33 ± 0.01^{b}	53.23 ± 1.45^{b}	20.92 ± 1.96^{f}	23.46 ± 0.67^{e}	8.15 ± 0.19^{b}
$Re_{122milled}$	5.75 ± 0.03^{a}	4.31 ± 0.03^{b}	44.18 ± 1.54^{a}	5.92 ± 0.47^{c}	17.51 ± 0.37^{c}	8.01 ± 0.34^{b}
$Re_{210milled}$	5.70 ± 0.02^{a}	4.43 ± 0.02^{c}	45.86 ± 2.01^{a}	6.10 ± 0.52^{c}	18.45 ± 0.38^{d}	8.16 ± 0.29^{b,c}
	Parameters of the green lentil samples					
Gr	6.43 ± 0.02^{f}	-	42.88 ± 1.98^{a}	6.28 ± 0.38^{c}	14.32 ± 0.25^{a}	4.10 ± 0.25^{a}
Gr_{122}	5.81 ± 0.03^{b}	4.37 ± 0.02^{b,c}	58.55 ± 2.05^{b}	16.94 ± 1.30^{d}	25.91 ± 0.58^{f}	8.34 ± 0.28^{b,c}
Gr_{210}	5.88 ± 0.01^{c}	4.40 ± 0.03^{c}	57.98 ± 2.93^{b}	15.93 ± 1.22^{d}	25.83 ± 0.76^{f}	8.37 ± 0.30^{b,c}
$Gr_{122milled}$	5.99 ± 0.01d	4.23 ± 0.01^{a}	52.61 ± 3.27^{b}	1.87 ± 0.11^{b}	18.91 ± 0.64^{d}	8.59 ± 0.26^{b,c}
$Gr_{210milled}$	5.82 ± 0.04b	4.35 ± 0.02^{b}	52.68 ± 2.62^{b}	1.40 ± 0.20^{a}	15.57 ± 0.31^{b}	8.60 ± 0.17^{c}
Images of the non-treated and treated lentils						
						
Re	Re_{122}		Re_{210}	$Re_{122milled}$	$Re_{210milled}$	
						
Gr	Gr_{122}		Gr_{210}	$Gr_{122milled}$	$Gr_{210milled}$	

L* – lightness; a* – redness (−a* greenness); b* – yellowness (−b* blueness); LAB, lactic acid bacteria; NBS, National Bureau of Standards units; CFU, colony forming units; Re, non-treated red lentils; Gr, non-treated green lentils; $_{122}$ – fermented with Lactiplantibacillus plantarum strain; $_{210}$ – fermented with Lacticaseibacillus casei strain; $_{milled}$ – milled lentils. Data are represented as means ($n = 6$) ± SE. -not analyzed; $^{a-g}$Means with different letters in the column are significantly different all sample groups ($p \leq 0.05$).

fermentation was performed two times, and from one substrate 3 samples were taken for analysis) ± standard error (SE). In order to evaluate the effects of different lentil cultivars, different LAB used for fermentation and milling process on the lentil quality parameters, data were analyzed by multivariate analysis of variance and Tukey-HSD tests as post-hoc tests. A linear Pearson's correlation was used to quantify the strength of the relationship between the variables. The results were recognized as statistically significant at $p \leq 0.05$.

3. Results and discussion

pH, color coordinates and lactic acid bacteria (LAB) viable counts in the lentil samples.

The pH, color coordinates, LAB viable counts and images of the lentil samples are given in Table 1. When comparing the red lentil samples, the lowest pH values were reached with Re_{122} samples (4.21), and samples Re_{210} and $Re_{122milled}$ pH was, on average, 4.32. Regarding the green lentil group, the lowest pH values was attained in $Gr_{122milled}$ samples (4.23), whereas in the other samples, the pH was 2.8, 3.3 and 4.0% higher ($Gr_{210milled}$, Gr_{122} and Gr_{210}, respectively). Most of the analyzed factors and their interactions were significant on the pH of the milling process (Supplementary Table S1.1).

In comparison LAB count in all the lentil groups, in all the SSF groups, LAB count was above 8.0 log_{10} CFU/g, and the milling process was not a significant factor on LAB count in lentils (Supplementary Table S1.1). However, between the LAB viable counts and pH, a very strong negative correlation was found ($r = -0.973$, $p \leq 0.0001$).

Mousavi et al. reported that the beverages produced by 100% of lentil and fermented with *Bifidobacterium bifidum* displayed significantly higher acidity values, in comparison with beverages produced with lower content of lentil flours (26). Also, the number of lactobacilli and bifidobacteria (after 24 h of fermentation) in beverages was over 9.0 log_{10} CFU/mL. Another study reported that the *Lp. plantarum* TK9 and *Lacticaseibacillus paracasei* TK1501 strains are

suitable to ferment lentils both under liquid-state and solid-state fermentation conditions and LAB viable counts were higher than 8.0 log_{10} CFU/g (27). Our study showed that despite the low pH values of the fermented samples, viable LAB counts over than 8.0 log_{10} CFU/g were established. This finding can be explained by the high tolerance to acidic conditions of the LAB strains used for fermentation. Our previous studies showed that *Lactiplantibacillus plantarum* 122 and *Lacticaseibacillus casei* 210 strains are versatile technological microorganisms with high-acidity resistance (28). Nevertheless, the adaptation of LAB to low pH conditions depends on the environmental factors and phenotype traits of the strains (29). This can explain different results reported in different studies. Indeed, LAB strain and milling process can be manipulated to obtain the most appropriated characteristics of the end products, including high viable counts of LAB.

Observing the lightness (L*) of red lentil samples, $Re_{122milled}$ and $Re_{210milled}$ showed, on average, 16.8% lower values than non-treated (Re) and non-milled (solid-state) fermented samples (Re_{122} and Re_{210}). In all the cases, fermentation decreased red lentil redness (a*), and the lowest a* coordinates were attained in the milled and fermented samples (i.e., $Re_{122milled}$ and $Re_{210milled}$ samples with a* coordinates of 45.02 NBS, on average). Similar tendencies were found of the red lentil yellowness (b*), and the lowest b* coordinates were found in $Re_{122milled}$ samples (17.51 NBS).

In what concerns to the green lentil group, in all cases the fermentation process increased L* and, in comparison with the non-treated green lentil sample, fermented samples showed, on average, 29.3% higher L* values. However, significant differences on L* coordinate between non-milled- and milled-fermented samples were not observed. The lowest a* coordinate was obtained with the sample $Gr_{210milled}$ (1.40 NBS), and the lowest b* coordinate was displayed by the green lentil control (14.32 NBS). In comparison with the non-treated sample (Gr), b* coordinates of fermented samples were 8.73% (in $Gr_{210milled}$ sample) to 80.6% (in Gr_{122} and Gr_{210} samples) higher.

The color of the product is a very important quality indicator and critical to consumer's sensory acceptance. The main colored compounds in lentils are carotenoids and tocopherols, and lentils are a good source of both (30). It was reported data about carotenoid and tocopherol compositions in ten red and ten green lentils (31). The predominant tocopherol in lentils was γ-tocopherol (96–98% of the total tocopherol content), followed by δ- and α-tocopherols (31). Changes in the color of lentils during the fermentation can be explained by their reaction with organic acids as well as by enzymatic hydrolysis. It was reported that the acidic additive or LAB inoculation can affect the α-tocopherol and β-carotene content of the substrate (32); however, the results from different studies are inconsistent.

The microorganism strains used for fermentation can utilize phytochemicals and lead to their degradation (33, 34). Hubert et al. reported that a decrease in tocopherols can be obtained during the soybean germ lactofermentation (33). Our study showed that significant factors on L* of the lentil samples were the LAB strain used for fermentation and the milling process (Supplementary Table S1.1). Finally, all the analyzed factors showed individually to significantly influence the a* and b* coordinates of lentils, but not all of their interactions were statistically significant (Supplementary Table S1.1).

3.1. Free amino acid profile and gamma-aminobutyric acid concentration in the lentil samples

The content of GABA and FAA in lentil samples is given in Supplementary Table S2.1 and Figure 2.

In most of the cases, fermentation increased the essential FAA content in both type of lentils (red and green lentils), except valine in Re_{210} and histidine in Re_{122}, $Re_{210,}$ $Re_{122milled}$, and $Re_{210milled}$ and Gr_{122}, $Gr_{210,}$ $Gr_{122milled}$ and $Gr_{210milled}$ samples. In comparison with all the tested lentil groups, the highest content of threonine was found in $Gr_{122milled}$ samples (3.13 μmol/g). The highest content of methionine and phenylalanine was found in red lentil samples $Re_{122milled}$ and $Re_{210milled}$ (on average, 0.560 and 1.04 μmol/g, respectively). In comparison with red and green lentil samples, in most of the cases (except Gr_{210} samples), higher concentration of valine was found in red lentil samples and fermentation increased valine content in samples by 2 times on average. The highest concentration of leucine/isoleucine was detected in $Re_{122milled}$ and $Re_{210milled}$, and $Gr_{210milled}$ samples (on average, 4.32 μmol/g). However, the highest amount of lysine was displayed in $Gr_{210milled}$ samples (2.02 μmol/g). Comparing histidine content in lentils, different tendencies were established: in 4 out of 10 analyzed sample groups, SSF decreased histidine content (in Re_{122}, Re_{210}, $Re_{210milled}$, and $Gr_{122milled}$ samples, on average, by 26.4, 32.0, 12.9, and 49.7%, respectively) in comparison with non-fermented ones.

Analyzing the content non-essential FAA in lentil samples, in all the cases, SSF increased serine and tyrosine content in samples in comparison with non-fermented ones. Comparing red lentil groups, SSF increased in every case glycine and alanine concentration (on average, from 1.4 to 2.7 times and from 2.6 to 3.7 times, respectively) and decreased glutamine and arginine (Arg) concentrations (on average, from 1.8 to 1.3 times and from 3.1 to 1.2 times, respectively), in comparison with the control. However, asparagine concentration was lower in both red and green lentils after SSF but $Gr_{210milled}$ samples, in which asparagine content after SSF remained similar to the control group (on average, 4.10 μmol/g). In the majority of the SSF samples, glutamic acid concentration showed a tendency to reduce, except Gr_{122} and Gr_{210} – where, in these last samples, glutamic concentration remained similar (on average, 5.24 μmol/g) and slightly higher (by 31.1%), respectively, in comparison with control ones. Different trends were found in green lentil samples: glycine and alanine contents increased in both milled green lentil samples, however, proline (Pro) content decreased in both green lentil groups (non-milled and milled) fermented with No. 210 LAB strain. Opposite trends were observed in the arginine content in green lentil samples; moreover, arginine concentration increased after SSF with No. 210 strain but decreased after fermentation with No. 122 strain. Yet, in milled green lentil samples fermented with No. 210 strain, arginine concentration was found lower, in comparison with non-fermented ones.

As depicted in the Supplementary Table S1.2, most of the analyzed factors and their interactions were significant on the FAA concentration in lentil samples.

Looking at the GABA concentration in lentil samples, the values were always higher in SSF samples, when compared with non-fermented ones. In non-fermented red lentil samples, GABA was inexistent. However, in non-milled SSF samples with No. 122 and No. 210 strains, GABA content was, on average, 4.53 and 2.91 μmol/g, respectively. Milling process was proved to be a significant factor on

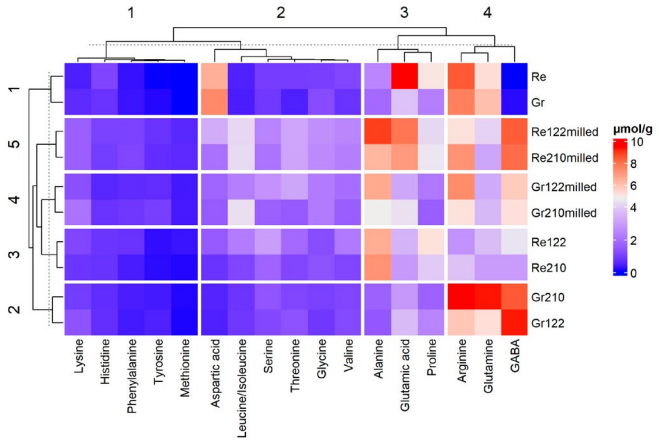


FIGURE 2
Gamma-aminobutyric acid (GABA) and free amino acid (FAA) content in lentil samples (Re—non-treated red lentils; Gr—non-treated green lentils; $_{122}$—fermented with *Lactiplantibacillus plantarum* strain; $_{210}$—fermented with *Lacticaseibacillus casei* strain; $_{milled}$—milled lentils).

GABA content of lentils (Supplementary Table S1.2). Indeed, in milled SSF red lentil samples higher GABA concentration was obtained (on average, by 1.87 and 2.79 times, in SSF with No. 122 and No. 210 strains, respectively). Furthermore, opposite tendencies were found in green lentil samples: non-milled SSF samples contained higher GABA concentration, in comparison with milled – non-fermented ones (by 1.62 and 1.63 times, in SSF with No. 122 and No. 210 strains samples, respectively).

Naturally, different fermentation designs lead to variations in the protein or amino acid profiles of fermentable substrate (35). Several studies reported that the protein concentration increases during fermentation (34, 36). However, antagonist results were also reported (36). These differences may be explained by the loss of dry matter, as a result of the microbial metabolic activities (35). Furthermore, the degradation of protein by microbial starters release FAA to the fermentable substrate (36). On the other hand, fermenting microorganisms can use FAA as a nutritional source (36, 37). Important to note that during fermentation the microorganisms increases the digestibility of plant proteins (36, 38). Also, the combination of fermentation with other processing technologies (for instance, thermal treatment) is more effective in reducing antinutritional factors (35). Pranoto et al. reported that *Lp. plantarum* can breakdown complex proteins, thereby releasing more peptides and FAA (36). As a matter of fact, during the fermentation a simultaneous increase and decrease of FAA in the fermentable substrate can be observed. Therefore, a control of the technological fermentation conditions as well as the quality parameters of the end-product are needed to avoid a loss of protein.

GABA production in fermented material also varied and these variations are related with many factors, including fermentation temperature, pH, substrate composition, process duration, *etc* (39). It was reported that the optimum process temperature for GABA synthesis is 30°C; however, other authors reported that the optimal temperature is 37°C (40). These differences can be related with the strains used for fermentation (28). The synthesis of GABA is catalyzed by the glutamic acid decarboxylase (18, 41, 42), which can be produced by LAB, yeasts and fungi (43–48). Though, decarboxylation of FAA can lead to BA formation. For this reason, this factor should be taken into consideration when selecting the most appropriate fermentation technology.

3.2. Biogenic amine concentration in the lentil samples

The BA concentrations in non-treated and fermented lentil samples are given in Table 2. Biogenic amines tryptamine (TRY), cadaverine (CAD), histamine (HIST) and tyramine (TYR) were not detected in lentil samples, and the main BA in lentil samples was spermidine (SPRMD). In all the cases SSF decreased SPRMD concentration in samples, and the lowest SPRMD content was found in $Gr_{122milled}$ and $Gr_{210milled}$ samples (on average, 108 mg/kg). Separate analyzed factors were significant on SPRMD concentration in lentil samples; conversely, their interactions were not significant on SPRMD formation (Supplementary Table S1.3). Between SPRMD concentration and pH of the samples, moderate positive correlation was found ($r = 0.795$, $p \leq 0.0001$), as well as a strong negative correlation between SPRMD concentration and LAB count was established ($r = -0.810$, $p \leq 0.0001$). In comparison spermine (SPRM) content in samples, all the SSF samples showed lower contents, in comparison with non-fermented ones. Furthermore, the lowest SPRM content was found in $Gr_{122milled}$, $Gr_{210milled}$ and $Re_{210milled}$ samples (on average, 30.3 mg/kg). Likewise SPRMD, all the separate analyzed factors were significant on SPRM concentration

TABLE 2 Biogenic amine (BA) concentration (mg/kg) in non-treated and fermented lentil samples.

Lentil samples	Biogenic amine, mg/kg							
	TRY	PHE	PUT	CAD	HIST	TYR	SPRMD	SPRM
	Parameters of the red lentil samples							
Re	nd	7.43 ± 0.65^{c}	83.9 ± 5.3^{d}	nd	nd	nd	235 ± 15.3^{d}	69.1 ± 4.3^{c}
Re_{122}	nd	5.52 ± 0.32 ^{c}	59.8 ± 3.8^{b,c}	nd	nd	nd	168 ± 9.32^{c}	55.0 ± 4.8^{d}
Re_{210}	nd	5.43 ± 0.41 ^{c}	62.4 ± 4.5^{c}	nd	nd	nd	172 ± 11.2^{c}	55.8 ± 3.9^{d}
$Re_{122milled}$	nd	9.27 ± 0.71^{f}	41.0 ± 2.9^{a}	nd	nd	nd	142 ± 12.5^{b}	36.1 ± 2.9^{b}
$Re_{210milled}$	nd	11.3 ± 0.11^{g}	42.0 ± 3.1^{a}	nd	nd	nd	134 ± 11.8^{b}	33.5 ± 3.2^{a,b}
	Parameters of the green lentil samples							
Gr	nd	9.49 ± 0.76^{f}	82.8 ± 6.8^{d}	nd	nd	nd	204 ± 16.3^{d}	55.2 ± 4.6^{d}
Gr_{122}	nd	6.57 ± 0.54 ^{d}	52.9 ± 3.9^{b}	nd	nd	nd	138 ± 10.5^{b}	42.7 ± 2.7^{c}
Gr_{210}	nd	0.640 ± 0.025^{a}	53.8 ± 4.1^{b}	nd	nd	nd	139 ± 11.4^{b}	43.6 ± 2.6^{c}
$Gr_{122milled}$	nd	4.18 ± 0.29^{b}	39.3 ± 2.5^{a}	nd	nd	nd	108 ± 9.1^{a}	29.2 ± 2.7^{a}
$Gr_{210milled}$	nd	8.69 ± 0.62^{e,f}	41.6 ± 2.9^{a}	nd	nd	nd	108 ± 8.6^{a}	28.2 ± 2.1^{a}

Re, non-treated red lentils; Gr, non-treated green lentils; $_{122}$ – fermented with Lactiplantibacillus plantarum strain; $_{210}$ – fermented with Lacticaseibacillus casei strain; $_{milled}$ – milled lentils. TRY, tryptamine; PHE, phenylethylamine; PUT, putrescine; CAD, cadaverine; HIST, histamine; TYR, tyramine; SPRMD, spermidine; SPRM, spermine; nd, not detected. Data are represented as means ($n = 6$) ± SE. $^{a-g}$Means with different letters in the column are significantly different all sample groups ($p \leq 0.05$).

in lentil samples; however, their interactions were not significant (Supplementary Table S1.3). Between the SPRM concentration and pH of the samples, a moderate positive correlation was found ($r = 0.627$, $p \leq 0.0001$), as well as a strong negative correlation between SPRM concentration and LAB viable counts was recognized ($r = -0.660$, $p \leq 0.0001$). Similar tendencies were established with putrescine (PUT). Particularly, SSF samples constantly showed lower PUT concentration, and the lowest content was found on both samples of red and green milled and fermented with both LAB strains (on average, 41.0 mg/kg). Significant influence on PUT concentration in lentils was obtained with the factors LAB strain used for fermentation and milling process (Supplementary Table S1.3). Also, strong positive correlation was found between the PUT concentration and samples pH ($r = 0.844$, $p \leq 0.0001$), as well as a strong negative correlation between PUT concentration and LAB viable counts was attained ($r = -0.829$, $p \leq 0.0001$). PHE concentration in red lentil samples ranged from 5.48 mg/kg (in Re_{122} and Re_{210} samples) to 11.3 mg/kg (in $Re_{210milled}$ samples), whereas in green lentil samples ranged from 0.640 mg/kg (in Gr_{210} samples) to, on average, 9.09 mg/kg (in Gr and $Gr_{210milled}$ samples). Finally, all the analyzed factors and their interactions were significant on PHE content in lentil samples (Supplementary Table S1.3).

Correlations between GABA and FAA with BA concentration were also analyzed in lentil samples (Table 3). Between the biogenic amine phenylethylamine (PHE) and aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), methionine (Met), phenylalanine (Phe), leucine (Leu)/isoleucine (Ile) and histidine (His) moderate positive correlations were found. However, between PHE and glutamine (Gln) negative moderate correlation was established. The biogenic amine PUT showed negative correlations with serine (Ser), threonine (Thr), glycine, GABA, alanine (Ala), methionine, valine (Val), phenylalanine, leucine/isoleucine, lysine (Lys) and tyrosine (Tyr). Similar tendencies on the correlations between the biogenic amine SPRMD and FAA and GABA were found; however, SPRM showed positive correlation with aspartic acid, proline and histidine, in addition to negative correlations with threonine, glycine, GABA, methionine, valine, phenylalanine, leucine/isoleucine, lysine and tyrosine.

The amino acid arginine can be converted to agmatine or to ornithine from which PUTR is formed during the decarboxylation pathway. The amino acid lysine can be decarboxylised into CAD. Moreover, HIS, TYR, TRYP and PHE can be formed from the amino acids histidine, tyrosine, tryptophan and phenylalanine, respectively. SPRM is formed from SPRMD, which is formed from PUTR, by SPRM synthase and SPRMD synthase, respectively (20). Physiologically, the most important BA are HIST and TYR because of their toxicity (49). These later BA were not found in lentil samples. Usually, plant-based material contain PUTR, SPRM and SPRMD and lower concentrations of HIST (21), and the European Union (EU) established legislative limit values only for HIST in fish (50). However, there are recommendations for PHE (30 mg/kg) in food (51). Indeed, it was reported about the BA present in fermented soybean products (52–56). The presence of BA has been reported in legumes, and the total BA content in lentils was established to be 130 mg/kg, with predominant CAD (57, 58). However, studies about BA content in fermented lentils are very scarce. Overall, fermented vegetables were reported as the group of products in which high quantities of PUTR (264 mg/kg) and CAD (26–35.4 mg/kg) can be formed (59). Finally, taking into consideration that the high concentration of BA (1,000 mg of total BA/kg and 8 mg of HIST) can cause serious health problems (60, 61), the control of the end-product is of foremost importance.

3.3. Fatty acid profile in the lentil samples

The FA concentrations (% from total fat content) in the lentil samples are shown in Table 4 and Figure 3. Its content in red lentil samples ranged from 44.70% (in Re_{122} samples) to, on average, 47.95% (in Re, $Re_{122milled}$ and $Re_{210milled}$ samples), whereas in green lentil samples ranged from, on average, 44.76% (in Gr_{122} and

$Gr_{122milled}$ samples) to, on average, 47.35% (in Gr samples). All the analyzed factors and their interactions were significant on linoleic acid content in lentil samples (Supplementary Table S1.4). Additionally, positive moderate correlation was found between pH and linoleic acid concentration in lentil samples ($r = 0.501$, $p = 0.005$), as well as moderate negative correlation was observed

TABLE 3 Pearson correlations between gamma-aminobutyric acid (GABA), free amino acid (FAA) and biogenic amine (BA) concentration in lentil samples.

Amino acids	Biogenic amines							
	PHE		PUT		SPRMD		SPRM	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Serine	−0.077	0.686	−0.549**	0.002	−0.416*	0.022	−0.353	0.055
Aspartic acid	0.472**	0.009	0.714**	0.0001	0.740**	0.0001	0.516**	0.003
Glutamic acid	0.569**	0.001	0.180	0.342	0.418*	0.021	0.225	0.233
Threonine	0.236	0.210	−0.0720**	0.0001	−0.530**	0.003	−0.608**	0.0001
Glycine	0.497**	0.005	−0.642**	0.0001	−0.487**	0.006	−0.653**	0.0001
Alanine	0.237	0.207	−0.487**	0.006	−0.282	0.131	−0.294	0.115
Proline	0.340	0.066	0.271	0.148	0.527**	0.003	0.517**	0.003
Methionine	0.424*	0.019	−0.752**	0.0001	−0.562**	0.001	−0.664**	0.0001
Valine	0.312	0.093	−0.616**	0.0001	−0.399*	0.029	−0.452*	0.012
Phenylalanine	0.463**	0.010	−0.696**	0.0001	−0.520**	0.003	−0.613**	0.0001
Leu/Ile	0.508**	0.004	−0.722**	0.0001	−0.574**	0.001	−0.685**	0.0001
Lysine	0.328	0.077	−0.794**	0.0001	−0.747**	0.0001	−0.792**	0.0001
Histidine	0.596**	0.001	0.282	0.131	0.528**	0.003	0.378*	0.040
Tyrosine	0.298	0.109	−0.0814**	0.0001	−0.774**	0.0001	−0.869**	0.0001
Glutamine	−0.461*	0.010	0.361	0.050	0.249	0.185	0.266	0.155
Arginine	−0.150	0.429	0.244	0.195	0.189	0.316	0.062	0.744
GABA	−0.132	0.486	−0.761**	0.0001	−0.704**	0.0001	−0.651**	0.0001

PHE, phenylethylamine; PUT, putrescine; SPRMD, spermidine; SPRM, spermine; GABA, gamma-aminobutyric acid; *r*, Pearson correlation; *p*, significance, correlation is significant, when $p \leq 0.05$. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

TABLE 4 Fatty acid (FA) profile in lentil samples.

Lentil samples	Fatty acids				
	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1 *cis,trans*)	Linoleic acid (C18:2)	α-Linolenic acid (C18:3 α)
	Fatty acids concentration, % from total fat content				
	Parameters of the red lentil samples				
Re	12.56 ± 0.23^{a}	1.50 ± 0.14^{d}	26.90 ± 0.29^{a}	47.97 ± 0.35^{c,d}	11.12 ± 0.09^{e}
Re_{122}	15.70 ± 0.20^{d}	1.46 ± 0.12^{d}	30.00 ± 0.21^{d}	44.70 ± 0.33^{a}	8.13 ± 0.36^{a}
Re_{210}	13.52 ± 0.45^{b}	1.39 ± 0.11^{d}	28.40 ± 0.34^{c}	46.35 ± 0.20^{b}	10.33 ± 0.09^{c}
$Re_{122milled}$	13.02 ± 0.28^{a,b}	0.363 ± 0.002^{a}	28.20 ± 0.27^{c}	48.07 ± 0.32^{c,d}	10.36 ± 0.05^{c}
$Re_{210milled}$	13.57 ± 0.14^{b}	0.857 ± 0.023^{c}	26.90 ± 0.22^{a}	47.82 ± 0.53^{c,d}	10.83 ± 0.08^{d}
	Parameters of the green lentil samples				
Gr	13.04 ± 0.29^{a,b}	0.787 ± 0.03^{s}	28.00 ± 0.16^{c}	47.35 ± 0.31^{c}	10.78 ± 0.07^{d}
Gr_{122}	14.43 ± 0.13^{c}	1.31 ± 0.09^{d}	30.81 ± 0.15^{d}	44.47 ± 0.21^{a}	9.02 ± 0.06^{b}
Gr_{210}	14.01 ± 0.31^{c}	1.24 ± 0.08^{d}	27.80 ± 0.11^{b}	46.26 ± 0.22^{b}	10.73 ± 0.08^{d}
$Gr_{122milled}$	14.39 ± 0.13^{c}	0.858 ± 0.021^{c}	31.00 ± 0.18^{e}	45.05 ± 0.35^{a}	8.68 ± 0.32^{a,b}
$Gr_{210milled}$	14.34 ± 0.12^{c}	0.617 ± 0.014^{b}	30.41 ± 0.24^{d}	45.99 ± 0.13^{b}	8.68 ± 0.51^{a,b}

Re, non-treated red lentils; Gr, non-treated green lentils; $_{122}$ – fermented with Lactiplantibacillus plantarum strain; $_{210}$ – fermented with Lacticaseibacillus casei strain; $_{milled}$ – milled lentils; SFA, saturated fatty acids, MUFA – monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data are represented as means ($n = 6$) ± SE. $^{a-e}$Means with different letters in the column are significantly different all sample groups ($p \leq 0.05$).

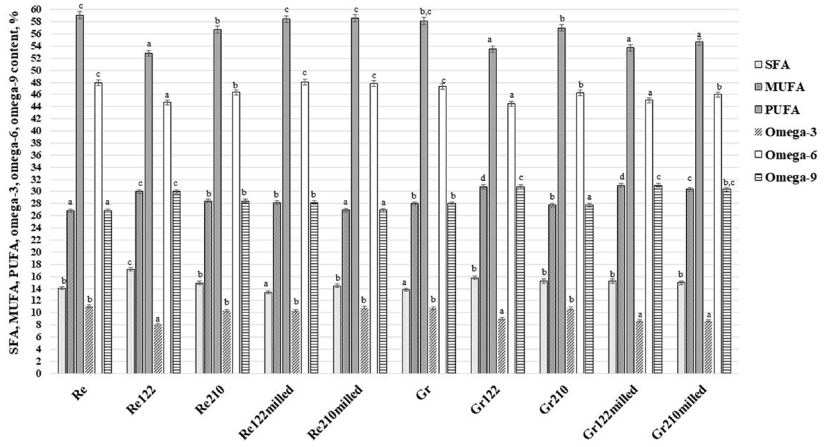


FIGURE 3
Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid, omega-3, omega-6 and omega-9 contents in lentil samples (Re—non-treated red lentils; Gr—non-treated green lentils; $_{122}$—fermented with *Lactiplantibacillus plantarum* strain; $_{210}$—fermented with *Lacticaseibacillus casei* strain; $_{milled}$—milled lentils; $^{a-d}$Means with different letters between the columns are significantly different, when $p \leq 0.05$).

between LAB viable counts and linoleic acid concentration in lentil samples ($r = -0.482$, $p = 0.007$). The following dominant FA in the lentil samples was oleic acid and most of the factors and their interaction had a significant effect on this FA content (Supplementary Table S1.4). Comparing oleic acid content in red and green lentils, one found out, on average, a 3.93% higher content in green lentils. However, different tendencies of the oleic acid content in SSF samples were disclosed. Moreover, when looking among red lentil samples, the content of oleic acid in Re_{122}, Re_{210} and $Re_{122milled}$, increased but in $Re_{210milled}$ remained similar to the control. Comparing green lentil samples, the lowest oleic acid content was found in Gr_{210} samples (on average, 27.80% from total fat content) and the highest in $Gr_{122milled}$ samples (on average, 31.00% from total fat content). In both red and green lentil samples, SSF increased palmitic acid content. In addition, in red lentils the highest palmitic acid content was found in Re_{122} samples (on average, 15.70% from total fat content). Besides, palmitic acid content in all the fermented green lentil samples increased in comparison with the control, and the average content was 14.29%. All the analyzed factors and their interactions were significant on palmitic acid content in lentil samples but ReGr x LAB strain interaction (Supplementary Table S1.4). A negative moderate correlation between pH and palmitic acid concentration in lentil samples was unfolded ($r = -0.573$, $p = 0.001$), as well as a moderate positive correlation was found between LAB viable counts and palmitic acid ($r = 0.606$, $p = 0.0001$). In most of the cases, SSF decreased α-linolenic acid concentration in lentil samples but Gr_{210} samples. α-Linolenic acid concentration in lentils showed moderate positive correlation with pH values ($r = 0.549$, $p = 0.002$), and moderate negative correlation ($r = -0.511$, $p = 0.004$) with LAB viable counts in lentils.

Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, and omega-3, omega-6 and omega-9 fatty acid contents in lentil samples are illustrated in Figure 3. Predominant FA groups in lentils were PUFA and MUFA, and SSF showed the tendency to increase MUFA in both red and green lentils. However, opposite trends of PUFA were obtained and their content in SSF samples was lower in comparison with the controls. Also, SSF increase SFA content in green lentils and, in most of the cases, in red lentil samples but $Re_{122milled}$. Comparing omega-3 content in lentils, in all fermented samples, omega-3 content was lower in comparison with the controls. Similar trends were unfolded with omega-6 content: in most of the fermented samples, omega-6 content was lower but $Re_{122milled}$. Nevertheless, opposite tendencies were disclosed regarding the omega-9 content in lentil samples, and in fermented samples in most of the cases (except Gr_{210} samples) omega-9 content was higher than the controls.

It was reported that the main FA in lentil samples are linoleic, palmitic, oleic and linolenic acids (62). Yet, in lentil samples were detected stearic, *cis* 11 eicosenoic and myristic acids. The PUFA were the major group of FA in lentils, whereas SFA were found in minor concentrations. Also, omega-6:omega-3 ratio in lentils was found to be between 1.67 and 6.65, which is in accordance with the average values described by Paucean et al. for red and green lentils (63).

The changes obtained during the fermentation can be explained by activities of the endogenous enzymes, which are present in legumes, e.g. lipoxygenase utilize unsaturated fatty acids to release volatile compounds—some of which possessing undesirable odors (64). This is especially a problem for legumes with a high proportion of unsaturated fatty acids (>80%). The

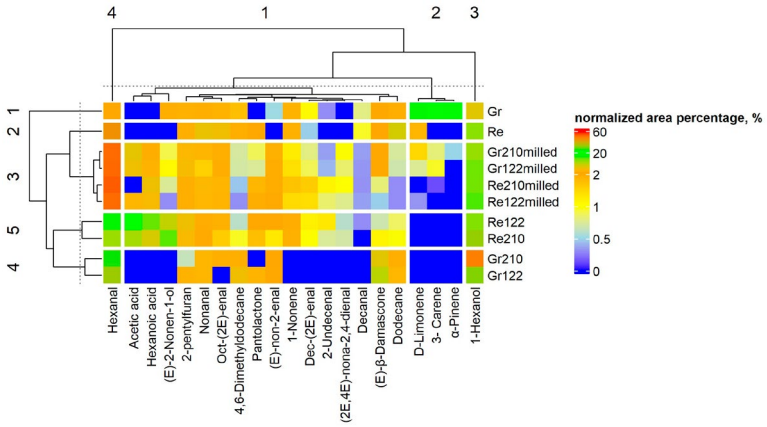


FIGURE 4
Volatile compound (VC) profile (% from the total volatile compounds content) in lentil samples (Re—non-treated red lentils; Gr—non-treated green lentils; $_{122}$—fermented with *Lactiplantibacillus plantarum* strain; $_{210}$—fermented with *Lacticaseibacillus casei* strain; $_{milled}$—milled lentils).

evolution and presence of volatile compounds are discussed further in the next section.

3.4. Volatile compounds profile in the lentil samples

The VC profile in lentil samples (% from the total VC content) is given in Supplementary Table S3.1 and Figure 4. The main VC in lentil samples, which content (% from the total VC content) was at least in one sample higher than 10%, were hexanal, 1-hexanol, hexanoic acid, D-limonene and (E)-2-nonen-1-ol. Hexanal flavor is described as green, fatty, leafy, vegetative, fruity and clean with a woody nuance; 1-hexanol flavor is pungent, ethereal, fusel oil, fruity and alcoholic, sweet and with a green top note; hexanoic acid is sour, fatty, sweat and cheese; D-limonene is citrus, orange, fresh and sweet and (E)-2-nonen-1-ol flavor is described as green, fatty, melon and with an oily tallow nuance (Supplementary Table S3.2). Likewise hexanoic acid in non-fermented green lentils, in non-fermented red lentil samples hexanoic acid and (E)-2-nonen-1-ol were not detected. In non-fermented red and green lentils 13 and 17 VC, respectively, were identified. However, in non-milled and milled SSF with No. 122 strain 18 and 19 VC were found, respectively, as well as in non-milled and milled SSF with No. 210 strain 17 and 18 VC were identified, respectively. Despite that in non-fermented green lentils higher variety of VC was found, in fermented samples (with both tested LAB strains) 9 VC were detected. In opposite to this trend, in milled SSF samples more than two times broader spectrum of VC was formed: 20 VC in $Gr_{122milled}$ and 21 VC in $Gr_{210milled}$ were identified. Analyzed factors were statistically significant on most of the VC content in lentil samples (Supplementary Table S1.5). Correspondingly, most of the VC concentrations showed significant correlations with pH and LAB viable counts of the samples (Table 4). Additionally, some of the VC showed significant correlations with FA content (Supplementary Table S4.1).

It was reported that different cultivars and color of legumes have similar characteristic volatile compound profiles (64). The undesirable odors of legumes are related to the lipoxygenase-catalyzed unsaturated fatty acid oxidation (65, 66), *viz.* volatile terpenes may be formed from degradation of carotenes by either legume lipoxygenases or hydroperoxides generated from autolytic and enzyme-catalyzed lipid oxidation (67). D-limonene is considered as potential discriminant VC in lentils (64). The latter is associated with a citrus and fresh odor (68). Beany and green odors are majorly derived from hexanal and 1-octen-3-ol (65, 66, 69). Previous studies were focused on soybean volatile compounds (65, 70–73) and the experimental data concerning lentil volatile compounds are scarce (Table 5).

The FA composition of legumes in conjunction with their VC were studied. The study conveyed that the large number (13) of terpenes and hexanal sets up the most abundant compounds in lentils (64). In opposite to aldehydes, alcohols, ketones and hydrocarbons, which are products of FA oxidation, terpenes are naturally present in plants (67), and α- and β-pinene are the most common terpenes in legumes (64). Furthermore, nonanal and 2-hexenal were the second and third most abundant VC in legumes (64). These findings are in agreement with our current study. The difference in hexanal abundance was explained by the difference in the content of linoleic and linolenic FA, because they are the precursor for lipoxygenase-catalyzed evolution of hexanal (65). Hydroperoxide lyase isozymes degrade hydroperoxides into isomeric nonenals, including hexanal (74, 75). The latter VC can be further used by enzymes, further generating additional volatile aldehydes (76), which may help to explain the presence of other analogous aldehydes. Also, legumes

TABLE 5 Pearson correlations between pH and lactic acid bacteria (LAB) viable counts with volatile compound (VC) content in lentil samples.

Volatile compounds	pH 24		LAB viable counts	
	r	*p*	*r*	*p*
Acetic acid	−0.446*	0.013	0.359	0.051
Hexanal	−0.322	0.083	0.352	0.057
1-Hexanol	−0.368*	0.045	0.416*	0.022
α-Pinene	0.719**	0.0001	−0.655**	0.000
Hexanoic acid	−0.499**	0.005	0.404*	0.027
2-pentylfuran	−0.190	0.315	0.169	0.372
3- Carene	0.712**	0.0001	−0.647**	0.0001
D-Limonene	0.841**	0.0001	−0.782**	0.0001
Oct-(2E)-enal	0.489**	0.006	−0.503**	0.005
1-Nonene	0.331	0.074	−0.409*	0.025
Pantolactone	−0.227	0.227	0.179	0.344
Nonanal	0.400*	0.029	−0.395*	0.031
(E)-non-2-enal	−0.788**	0.0001	0.837**	0.0001
(E)-2-Nonen-1-ol	−0.170	0.368	0.108	0.569
Dodecane	0.676**	0.0001	−0.661**	0.0001
Decanal	0.841**	0.0001	−0.839**	0.0001
(2E,4E)-nona-2,4-dienal	−0.590**	0.001	0.585**	0.001
Dec-(2E)-enal	−0.054	0.779	0.018	0.923
4,6-Dimethyldodecane	0.205	0.276	−0.152	0.424
2-Undecenal	−0.403*	0.027	0.325	0.080
(E)-β-Damascone	−0.088	0.643	0.175	0.356

LAB, lactic acid bacteria; *r*, Pearson correlation; *p*, significance, correlation is significant, when $p \leq 0.05$. ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

contain alcohol dehydrogenases, which can catalyze the interconversion of aldehydes, alcohols and acids (77), thus possibly explaining the abundance of 1-hexanol. Other groups of VC, like ketones and hydrocarbons, are also derived from both non-enzymatic and enzymatic lipid oxidation (67), however, these VC are more typical in dry beans (78).

4. Conclusion

From this research effort, it can be concluded that both studied LAB strains are suitable for lentil fermentation (pH < 4.5, LAB viable counts >8.0 $\log_{10}$ CFU/g) and solid-state fermentation with these strains increases essential free amino acid content in lentils. However, some of the non-essential FAA content in fermented lentils decreased. Additionally, SSF significantly increases GABA concentration in lentils, and milling process is a significant factor on GABA synthesis in the studied fermentable substrates. Predominant biogenic amine in lentils was SPRMD. In overall, SSF reduces the total BA content in lentils (on average, 34.8% in red lentils and 39.9% in green lentils). The main fatty acids in lentils were linoleic and oleic acids, and SSF showed the trend to increase MUFA and decrease PUFA contents. The main volatile compounds in lentil samples were hexanal, 1-hexanol, hexanoic acid, D-limonene and (E)-2-nonen-1-ol, and most of the VC showed significant correlations with the pH, LAB viable counts and FA of the lentil samples. Finally, LAB strain used for SSF and milling process are statistically significant factors for most of the analyzed parameters, and, despite that in green non-milled SSF lentils, higher GABA concentration was found, for both (green and red) lentils, milling and SSF combination is recommended, because these groups of samples showed the lowest BA content, in addition to high concentrations of essential FAA and GABA.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

EB: conceptualization, resources, supervision, project administration, validation, and data curation. EM, VB, and DK: methodology. EM, EZ, and VS: software and visualization. EM, EZ,

DK, VS, and RR: formal analysis. EM and JR: investigation. EM, DK, VS, RR, and EZ: writing—original draft preparation. JR, VB, and EB: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1118710/full#supplementary-material

References

1. Ganesan K, Xu B. Polyphenol-rich lentils and their health promoting effects. *Int J Mol Sci*. (2017) 18:2390. doi: 10.3390/ijms18112390

2. Mudgal V, Mehta MK, Rane AS. Lentil straw (lens Culinaris): an alternative and nutritious feed resource for kids. *Anim Nutr*. (2018) 4:417–21. doi: 10.1016/j.aninu.2018.04.009

3. Faris MA-IE, Takruri HR, Issa AY. Role of lentils (lens *Culinaris* L.) in human health and nutrition: a review. *Mediterr J Nutr Metab*. (2013) 6:3–16. doi: 10.1007/s12349-012-0109-8

4. Food and Agriculture Organization (FAO) Traditional food plants, FAO: Rome, Italy. Available at: https://www.fao.org/3/W0078E/w0078e12.htm (Accessed November 27, 2022).

5. Xu B, Chang SK. Phenolic substance characterization and chemical and cell-based antioxidant activities of 11 lentils grown in the northern United States. *J Agric Food Chem*. (2010) 58:1509–17. doi: 10.1021/jf903532y

6. FAOSTAT. Statistics. Available at: http://www.fao.org/statistics/en/ (Accessed November 27, 2022).

7. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. Phytosterol, Squalene, Tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods Hum Nutr*. (2007) 62:85–91. doi: 10.1007/s11130-007-0046-8

8. Grusak MA. Nutritional and health-beneficial quality/the lentil—botany, production and uses. *Wallingford Comm Agric Bur*. (2009):368–90. doi: 10.1079/9781845934873.0368

9. US Department of Agriculture FoodData central. Available at: https://fdc.nal.usda.gov/ (Accessed November 27, 2022)

10. Dhull SB, Kinabo J, Uebersax MA. Nutrient profile and effect of processing methods on the composition and functional properties of lentils (lens Culinaris Medik): a review. *Legum Sci*. (2023) 5:e156. doi: 10.1002/leg3.156

11. Bartkiene E, Sakiene V, Bartkevics V, Rusko J, Lele V, Juodeikiene G, et al. Lactofermentation and protein isolation: effects on phenolic compounds and Genistein, antioxidant properties, trypsin inhibitor activity, and protein digestibility. *Eur Food Res Technol*. (2018) 244:1521–31. doi: 10.1007/s00217-018-3066-8

12. Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, et al. Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with Cordyceps Militaris SN-18. *J Funct Foods*. (2014) 10:210–22. doi: 10.1016/j.jff.2014.06.008

13. Dey TB, Chakraborty S, Jain KK, Sharma A, Kuhad RC. Antioxidant Phenolics and their microbial production by submerged and solid state fermentation process: a review. *Trends Food Sci Technol*. (2016) 53:60–74.

14. Magro AEA, Silva LC, Rasera GB, de Castro RJS. Solid-state fermentation as an efficient strategy for the biotransformation of lentils: enhancing their antioxidant and Antidiabetic potentials. *Bioresour Bioprocess*. (2019) 6:1–9. doi: 10.1186/s40643-019-0273-5

15. Dhull SB, Punia S, Kidwai MK, Kaur M, Chawla P, Purewal SS, et al. Solid-state fermentation of lentil (lens *Culinaris* L.) with Aspergillus Awamori: effect on phenolic compounds, mineral content, and their bioavailability. *Legume Sci*. (2020) 2:37. doi: 10.1002/leg3.37

16. Soetan KO, Oyewole OE. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: a review. *Afr J Food Sci*. (2009) 3:223–32.

17. Grewal J. Gamma-Aminobutyric acid (GABA): a versatile bioactive compound. *Eur J Mol Clin Med*. (2020) 7:3068–75.

18. Diana M, Quílez J, Rafecas M. Gamma-Aminobutyric acid as a bioactive compound in foods: a review. *J Funct Foods*. (2014) 10:407–20. doi: 10.1016/j.jff.2014.07.004

19. Li H, Qiu T, Huang G, Cao Y. Production of gamma-Aminobutyric acid by lactobacillus Brevis NCL912 using fed-batch fermentation. *Microb Cell Factories*. (2010) 9:85. doi: 10.1186/1475-2859-9-85

20. Sarkadi LS. Amino acids and biogenic amines as food quality factors. *Pure Appl Chem*. (2019) 91:289–300. doi: 10.1515/pac-2018-0709

21. Sarkadi LS. Biogenic amines In: *Process-Induced Food Toxicants*. eds. R. H. Stadler and D. R. Lineback John Wiley & Sons, Ltd (2008). Hoboken, NJ, 321–361.

22. Bartkiene E, Starkute V, Katuskevicius K, Laukyte N, Fomkinas M, Vysniauskas E, et al. The contribution of edible cricket flour to quality parameters and sensory characteristics of wheat bread. *Food Sci Nutr*. (2022) 10:4319–30. doi: 10.1002/fsn3.3024

23. Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. Histamine and Cadaverine production by bacteria isolated from fresh and frozen albacore (Thunnus Alalunga). *J Food Prot*. (1999) 62:933–9. doi: 10.4315/0362-028X-62.8.933

24. Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. Changes in biogenic amines and microbiological analysis in albacore (Thunnus Alalunga) muscle during frozen storage. *J Food Prot*. (1998) 61:608–15. doi: 10.4315/0362-028X-61.5.608

25. Pérez-Palacios T, Ruiz-Carrascal J, Solomando JC, Antequera T. Strategies for enrichment in ω-3 fatty acids aiming for healthier meat products. *Food Rev Int*. (2019) 35:485–503. doi: 10.1080/87559129.2019.1584817

26. Mousavi M-H, Gharekhani M, Alirezalu K, Roufegarinejad L, Azadmard-Damirchi S. Production and characterization of nondairy gluten-free fermented beverage based on buckwheat and lentil. *Food Sci Nutr*. (2021) 11:1342–53. doi: 10.1002/fsn3.3170

27. Chen K, Gao C, Han X, Li D, Wang H, Lu F. Co-fermentation of lentils using lactic acid bacteria and bacillus Subtilis Natto increases functional and antioxidant components. *J Food Sci*. (2021) 86:475–83. doi: 10.1111/1750-3841.15349

28. Bartkiene E, Lele V, Ruzauskas M, Domig KJ, Starkute V, Zavistanaviciute P, et al. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. *Microorganisms*. (2020) 8:64. doi: 10.3390/microorganisms8010064

29. Papadimitriou K, Pratsinis H, Nebe-von-Caron G, Kletsas D, Tsakalidou E. Acid tolerance of streptococcus Macedonicus as assessed by flow Cytometry and single-cell sorting. *Appl Environ Microbiol*. (2007) 73:465–76. doi: 10.1128/AEM.01244-06

30. Zhang B, Peng H, Deng Z, Tsao R. Phytochemicals of lentil (lens Culinaris) and their antioxidant and anti-inflammatory effects. *J Food Bioact*. (2018) 1:93–103. doi: 10.31665/JFB.2018.1128

31. Zhang B, Deng Z, Tang Y, Chen P, Liu R, Ramdath DD, et al. Fatty acid, carotenoid and Tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities. *Food Chem*. (2014) 161:296–304. doi: 10.1016/j.foodchem.2014.04.014

32. Liu QH, Shao T, Bai YF. The effect of Fibrolytic enzyme, lactobacillus Plantarum and two food antioxidants on the fermentation quality, alpha-Tocopherol and Beta-carotene of high moisture Napier grass silage ensiled at different temperatures. *Anim Feed Sci Technol*. (2016) 221:1–11. doi: 10.1016/j.anifeedsci.2016.08.020

33. Hubert J, Berger M, Nepveu F, Paul F, Daydé J. Effects of fermentation on the phytochemical composition and antioxidant properties of soy germ. *Food Chem*. (2008) 109:709–21. doi: 10.1016/j.foodchem.2007.12.081

34. El Hag ME, El Tinay AH, Yousif NE. Effect of fermentation and Dehulling on starch, Total polyphenols, Phytic acid content and in vitro protein digestibility of pearl millet. *Food Chem*. (2002) 77:193–6. doi: 10.1016/S0308-8146(01)00336-3

35. Nkhata SG, Ayua E, Kamau EH, Shingiro J-B. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Sci Nutr*. (2018) 6:2446–58. doi: 10.1002/fsn3.846

36. Pranoto Y, Anggrahini S, Efendi Z. Effect of natural and lactobacillus Plantarum fermentation on in-vitro protein and starch Digestibilities of sorghum flour. *Food Biosci*. (2013) 2:46–52. doi: 10.1016/j.fbio.2013.04.001

37. Osman MA. Effect of traditional fermentation process on the nutrient and Antinutrient contents of pearl millet during preparation of Lohoh. *J Saudi Soc Agric Sci*. (2011) 10:1–6. doi: 10.1016/j.jssas.2010.06.001

38. Bartkiene E, Sakiene V, Bartkevics V, Juodeikiene G, Lele V, Wiacek C, et al. Modulation of the nutritional value of lupine Wholemeal and protein isolates using submerged and solid-state fermentation with Pediococcus Pentosaceus strains. *Int J Food Sci Technol*. (2018) 53:1896–905. doi: 10.1111/ijfs.13775

39. Sahab NRM, Subroto E, Balia RL, Utama GL. γ-Aminobutyric acid found in fermented foods and beverages: current trends. *Heliyon*. (2020) 6:e05526. doi: 10.1016/j.heliyon.2020.e05526

40. Ohmori T, Tahara M, Ohshima T. Mechanism of gamma-Aminobutyric acid (GABA) production by a lactic acid bacterium in yogurt-sake. *Process Biochem*. (2018) 74:21–7. doi: 10.1016/j.procbio.2018.08.030

41. Wu C-H, Hsueh Y-H, Kuo J-M, Liu S-J. Characterization of a potential probiotic lactobacillus Brevis RK03 and efficient production of γ-Aminobutyric acid in batch fermentation. *Int J Mol Sci*. (2018) 19:143. doi: 10.3390/ijms19010143

42. Hong J, Kim K-J. Crystal structure of γ-Aminobutyrate aminotransferase in complex with a PLP-GABA adduct from Corynebacterium Glutamicum. *Biochem Biophys Res Commun*. (2019) 514:601–6. doi: 10.1016/j.bbrc.2019.04.194

43. Han S-M, Lee J-S. Production and its anti-hyperglycemic effects of γ-Aminobutyric acid from the wild yeast strain Pichia Silvicola UL6-1 and Sporobolomyces Carnicolor 402-JB-1. *Mycobiology*. (2017) 45:199–203. doi: 10.5941/MYCO.2017.45.3.199

44. Li H, Cao Y. Lactic acid bacterial cell factories for gamma-Aminobutyric acid. *Amino Acids*. (2010) 39:1107–16. doi: 10.1007/s00726-010-0582-7

45. Rai AK, Pandey A, Sahoo D. Biotechnological potential of yeasts in functional food industry. *Trends Food Sci Technol*. (2019) 83:129–37. doi: 10.1016/j.tifs.2018.11.016

46. Kim D-H, Dasagrandhi C, Park S-K, Eom S-H, Huh M-K, Mok J-S, et al. Optimization of gamma-Aminobutyric acid production using sea tangle extract by lactic acid bacterial fermentation. *Lwt*. (2018) 90:636–42. doi: 10.1016/j.lwt.2018.01.011

47. Liao W-C, Wang C-Y, Shyu Y-T, Yu R-C, Ho K-C. Influence of preprocessing methods and fermentation of adzuki beans on γ-Aminobutyric acid (GABA) accumulation by lactic acid bacteria. *J Funct Foods*. (2013) 5:1108–15. doi: 10.1016/j.jff.2013.03.006

48. Kumar S, Punekar NS. The metabolism of 4-Aminobutyrate (GABA) in fungi. *Mycol Res*. (1997) 101:403–9. doi: 10.1017/S0953756296002742

49. Ladero V, Calles-Enriquez M, Fernandez M, Alvarez MA. Toxicological effects of dietary biogenic amines. *Curr Nutr Food Sci*. (2010) 6:145–56. doi: 10.2174/157340110791233256

50. Directive E. Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. *JL*. (1991) 268:0015–34.

51. ten Brink B, Damink C, Joosten HMLJ, Huis in 't veld JHJ. Occurrence and formation of biologically active amines in foods. *Int J Food Microbiol*. (1990) 11:73–84. doi: 10.1016/0168-1605(90)90040-C

52. Kung H-F, Tsai Y-H, Wei C-I. Histamine and other biogenic amines and histamine-forming bacteria in miso products. *Food Chem*. (2007) 101:351–6. doi: 10.1016/j.foodchem.2005.12.057

53. Shukla S, Park H-K, Kim J-K, Kim M. Determination of biogenic amines in Korean traditional fermented soybean paste (Doenjang). *Food Chem Toxicol*. (2010) 48:1191–5. doi: 10.1016/j.fct.2010.01.034

54. Tsai Y-H, Chang S-C, Kung H-F. Histamine contents and histamine-forming bacteria in Natto products in Taiwan. *Food Control*. (2007) 18:1026–30. doi: 10.1016/j.foodcont.2006.06.007

55. Yongmei L, Xiaohong C, Mei J, Xin L, Rahman N, Mingsheng D, et al. Biogenic amines in Chinese soy sauce. *Food Control*. (2009) 20:593–7. doi: 10.1016/j.foodcont.2008.08.020

56. Guan R-F, Liu Z-F, Zhang J-J, Wei Y-X, Wahab S, Liu D-H, et al. Investigation of biogenic amines in Sufu (Furu): a Chinese traditional fermented soybean food product. *Food Control*. (2013) 31:345–52. doi: 10.1016/j.foodcont.2012.10.033

57. Simon-Sarkadi L, Holzapfel W-H. Biogenic amines and microbial quality of sprouts. Z. Für Lebensm.-Unters. *Forsch*. (1995) 200:261–5. doi: 10.1007/BF01187516

58. Skowronek F, Simon-Sarkadi L, Holzapfel WH. Hygienic status and biogenic amine content of Mung bean sprouts. *Zeitschrift für Untersuchung der Lebensmittel-Untersuchung und -Forschung*. (1998) 207:97–100. doi: 10.1007/s002170050301

59. EFSA Scientific opinion on risk based control of biogenic amine formation in fermented foods | EFSA. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/2393 (Accessed July 26, 2022)

60. Sivamaruthi BS, Kesika P, Chaiyasut C. A narrative review on biogenic amines in fermented fish and meat products. *J Food Sci Technol*. (2021) 58:1623–39. doi: 10.1007/s13197-020-04686-x

61. Erdag D, Merhan O, Yildiz B. Biochemical and pharmacological properties of biogenic amines. *Biog Amines*. (2018) 8:1–14. doi: 10.5772/intechopen.81569

62. Lastras C, Revilla I, González-Martín MI, Vivar-Quintana AM. Prediction of fatty acid and mineral composition of lentils using near infrared spectroscopy. *J Food Compos Anal*. (2021) 102:104023. doi: 10.1016/j.jfca.2021.104023

63. Socaci SA, Dulf FV, Alexa E, Man SMM, An AE, Muste S. Folic acid, minerals, amino-acids, fatty acids and volatile compounds of green and red lentils. Folic acid content optimization in wheat-lentils composite flours. *Chem Cent J*. (2018) 12:88. doi: 10.1186/s13065-018-0456-8

64. Khrisanapant P, Kebede B, Leong SY, Oey I. A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. *Foods*. (2019) 8:651. doi: 10.3390/foods8120651

65. MacLeod G, Ames J, Betz NL. Soy flavor and its improvement. *Crit Rev Food Sci Nutr*. (1988) 27:219–400. doi: 10.1080/10408398809527487

66. Rackis JJ, Sessa DJ, Honig DH. Flavor Problems of Vegetable Food Proteins. *J Am Oil Chem Soc*. (1979) 56:262–71. doi: 10.1007/BF02671470

67. Azarnia S, Boye JI. *Flavour Compounds in Legumes: Chemical and Sensory Aspects*. New York: Prog. Food Sci. Technol. Nova Science Publishers (2011).

68. Mosciano G, Pohero W, Michalski J, Holmgren C, Young D. Organoleptic characteristics of flavor materials. *Perfum Flavorist*. (2000) 25:71–4. doi: 10.1097/00006205-200025020-00005

69. Roland WS, Pouvreau L, Curran J, van de Velde F, de Kok PM. Flavor aspects of pulse ingredients. *Cereal Chem*. (2017) 94:58–65. doi: 10.1094/CCHEM-06-16-0161-FI

70. Zhang Y, Guo S, Liu Z, Chang SK. Off-flavor related volatiles in soymilk as affected by soybean variety, grinding, and heat-processing methods. *J Agric Food Chem*. (2012) 60:7457–62. doi: 10.1021/jf3016199

71. Li Y-Q, Zhang W-N. Effects of pulsed electric fields on volatile constituents of soymilk. *Agro Food Ind Hi-Tech*. (2012) 23:36–7.

72. Achouri A, Boye JI, Zamani Y. Soybean variety and storage effects on soymilk flavour and quality. *Int J Food Sci Technol*. (2008) 43:82–90. doi: 10.1111/j.1365-2621.2006.01393.x

73. Min S, Yu Y, Yoo S, Martin SS. Effect of soybean varieties and growing locations on the flavor of soymilk. *J Food Sci*. (2005) 70:C1–C11. doi: 10.1111/j.1365-2621.2005.tb09009.x

74. Olias JM, Rios JJ, Valle M, Zamora R, Sanz LC, Axelrod B. Fatty acid Hydroperoxide Lyase in germinating soybean seedlings. *J Agric Food Chem*. (1990) 38:624–30. doi: 10.1021/jf00093a009

75. Matoba T, Hidaka H, Kitamura K, Kaizuma N, Kito M. Contribution of Hydroperoxide Lyase activity to N-Hexanal formation in soybean. *J Agric Food Chem*. (1985) 33:856–8. doi: 10.1021/jf00065a022

76. Liu K. *Soybeans: Chemistry, Technology, and Utilization* Springer (2012). Berlin p.

77. Gomes J, Jadrić S, Winterhalter M, Brkić S. Alcohol dehydrogenase Isoenzymes in chickpea cotyledons. *Phytochemistry*. (1982) 21:1219–24. doi: 10.1016/0031-9422(82)80114-3

78. Oomah BD, Liang LS, Balasubramanian P. Volatile compounds of dry beans (Phaseolus Vulgaris L.). *Plant Foods Hum Nutr*. (2007) 62:177–83. doi: 10.1007/s11130-007-0059-3

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Changes in Chemical Composition of Lentils, Including Gamma-Aminobutyric Acid and Volatile Compound Formation during Submerged and Solid-State Fermentation with *Pediococcus acidilactici*

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Abstract: The aim of this study was to evaluate and compare the characteristics of non-treated and fermented [via submerged (SMF) and solid-state (SSF) fermentation using *Pediococcus acidilactici*] lentils (*Lens culinaris*) grown either in pure stands (L) or relay intercropped with winter rye (LR). It was observed that the lentils were suitable substrate for lacto-fermentation. Most of the free amino acid concentrations increased in lentils after both fermentations. The highest concentration of γ-aminobutyric acid was found in SSF LR samples. However, fermentation led to higher biogenic amines (BA) content in lentils. The most abundant fatty acid in lentils was C18:2. SSF lentils showed more complex volatile compound (VC) profiles (with between nine and seventeen new VCs formed), whereas, in SMF samples, between two and five newly VCs were formed. When comparing lentil grown types, L contained significantly higher concentrations of Na, K, Ca, P, Mn, and Se, while LR contained significantly higher concentrations of Fe and Ni. To sum up, fermentation with lactic acid bacteria (LAB) contributed to the improved biological value of lentils; still, the quantity of BA needs to be considered. Further investigations into the *P. acidilactici* metabolism of certain compounds (such as phenolic and antinutritional compounds) in lentils during fermentation ought to be carried out.

Keywords: amino acids; biogenic amines; fatty acids; fermentation; gamma-aminobutyric acid; trace elements; volatile compounds; lentils

1. Introduction

Cultivated lentil (*Lens culinaris*), one of the ancient crops, is highly important due to its high protein content (20–25%), diverse and rich profile of essential microelements (iron, zinc, copper, manganese, and phosphorus), and significant amounts of B group vitamins [thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and folic acid (B9)] [1]. Lentil landraces contain a diverse profile of carbohydrates, which consists of

slowly digestible starch, prebiotic dietary fibre (such as raffinose family oligosaccharides and fructooligosaccharides), and sugar alcohols [2,3]. Moreover, leguminous crops contain various phytochemicals with beneficial properties, such as the ability to promote or inhibit enzymatic reactions, provide antioxidant properties, and inhibit harmful gut bacteria [4,5].

The popularity of lentil crops in the food and feed industries is increasing, as the worldwide production rate has grown two-fold, up to 6.54 million metric tons in 2020 [3]. Although lentils are not as popular in Europe as in America, our traditions and older research show that the cultivation of lentils in Lithuania could be advanced: we could breed more suitable varieties and apply sustainable farming technologies to their cultivation [6]. Researchers are investigating various mixed cropping systems of legume with cereals by improving the cycling and uptake of nutrients. Mixed cropping demonstrates a yield advantage in different climatic conditions, confirming the suitability of cereal and legume intercropping in North and Central Europe [7]. Its potential mechanisms and effects consist of competition (niche differentiation, resource use sharing, and weed control), diversity (pest and disease control), facilitation (physical support and the excretion of N and allelochemicals), and associated diversity (habitats for natural predators; litter diversity enhances soil microbial diversity) [8]. The mixed cropping technique is intended to provide long soil cover with living cover crop and its maintenance after the main crop is harvested may protect the soil against erosion, provide the next crop with nitrogen, prevent nitrate leaching, and improve plant nutrition and production quality [9,10].

However, its digestive properties are limited, due to various antinutritional factors [11,12]. Fermentation is often described as classic, simple, and low-cost bioprocessing method that helps to increase a product's shelf-life and improve its nutritional and organoleptic properties by reducing undesirable compounds and supplementing the food matrix with essential amino acids and vitamins [13,14]. Moreover, fermentation can reduce various antinutritional factors, such trypsin inhibitors, tannins, and phytic acid [13] and can improve the food matrix's digestibility by promoting the hydrolysis of proteins [15].

The fermentation of various matrices can be performed under submerged (SMF) and solid-state fermentation (SSF) conditions [16,17]. On one hand, SMF is defined as a type of process wherein the anaerobic or partially anaerobic fermentation of a substrate is performed in the presence of free water, also known as a liquid medium [18]. On the other hand, SSF is defined as a type of process wherein fermentation occurs on a solid matrix in the absence of free water [18,19]. The SMF process is fairly popular in the industry for producing various fermentation products, due to its ease of control over the fermentation process, shorter fermentation duration, consistent productivity, and the ease of the purification of the products [18,19]. Nevertheless, SSF has advantages over SMF, such as a higher productivity, lower energy expenditures, simpler instrumentation, lower catabolic repression, a lower risk of contamination, and the better microbial growth and production of secondary metabolites [18–21].

Although the fermentation of legumes provides numerous benefits to their nutritional profile, it should be emphasized that fermentation is often associated with the formation of exogenous biogenic amines (BAs) [22,23]. Although BAs are essential to some biological functions, such as the synthesis of hormones or alkaloids, the excess consumption of exogenous BA can lead to various food poisoning symptoms, such as headaches, vomiting, diarrhoea, dizziness, nausea, or pseudo-allergic reactions [24,25]. For this reason, BA control in fermented products is of the utmost importance.

Previous studies have reported changes in nutritional profile, phenolic compounds, antioxidant properties, and the antidiabetic potential of lentils during fungal solid-state fermentation [26–29]. It was also reported that the phenolic content, protein quality, and digestibility of lentil proteins were improved using water kefir seed fermentation [30].

Several studies have been conducted on lentil fermentation with lactic acid bacteria (LAB). The study of Chen et al. [31] revealed that the solid-state co-fermentation of lentils using LAB and *Bacillus subtilis* natto increased the antioxidant activity and LAB proliferation in the fermented lentil products. Torino et al. [14] used liquid and solid-state

fermentations with *Lactobacillus plantarum* and *Bacillus subtilis* to produce water-soluble fermented lentil extracts with antioxidant and antihypertensive properties. The study of De Pasquale et al. [32] on the lactic acid fermentation of gelatinized yellow and red lentil flour was carried out in order to analyse the alterations in total phenolic compounds, protein digestibility, antinutritional factors (tannins, trypsin inhibitor activity, phytic acid content, etc.), and antioxidant capacity. Another study was carried out to examine the impact of sonication or precooking and fermentation with *Lactobacillus plantarum* and *Pediococcus acidilactici* on the antinutritional factors of several legume flours, including lentil [13]. Budryn et al. [33] examined the impact of fermentation with *Lactobacillus casei* on increasing the content of isoflavonoids and microbiological safety in lentils. The above-mentioned previous studies on lentil fermentation varied regarding the type of initial lentil raw material used, the microorganisms used, and the examined characteristics, mainly antioxidant and antinutritional, of the fermented product. Therefore, to the best of our knowledge, this study is the first to use the differently grown lentil variety 'Danaja' for fermentation with a newly isolated *Pediococcus acidilactici* strain, in order to compare the changes in compounds such as biogenic amines, volatile compounds, fatty acids, and γ-aminobutyric acid in the obtained products.

The aim of this study was to analyse and compare the physical, chemical, and microbiological characteristics [chromaticity parameters, pH, BAs, volatile compounds (VCs), fatty (FA) and free amino (FAA) acids, γ-aminobutyric acid (GABA) concentration, and lactic acid bacteria (LAB) viable count] of non-treated and fermented (via SMF and SSF with *Pediococcus acidilactici* strain) lentils grown either in pure stands (L) or relay intercropped with winter rye (LR).

2. Materials and Methods

2.1. Lentil and Lactic Acid Bacteria Strains Used for Fermentation

The grains of lentil variety 'Danaja' (*Lens culinaris* Medik.) were provided by the Lithuanian Research Centre for Agriculture and Forestry (LAMMC, Akademija, Kėdainiai distr., Lithuania). Field trials were conducted during the 2022 vegetation period at the experimental base of the LAMMC (55°39′ N 23°57′ E). The field experiment was designed in four replications (plot size 3 × 20 m), which were randomly arranged. The trial was conducted under organic growing conditions. The soil of the experimental site was a loamy *Endocalcaric Epigleyic Cambisol* (Drainic, Loamic) CM-can.glp-dr.lo. The characteristics of the soil arable layer (0–25 cm) were as follows: pH 7.2, humus content 2.7%, total nitrogen 117 mg/kg, available phosphorus (P_2O_5) 51 mg/kg, and available potassium (K_2O) 68 mg/kg. Lentil was grown either in pure stands (L) or was relay intercropped with winter rye (LR), which involves growing a second crop after harvesting the first (see supplementary file: Figure S1). Both crops were planted in May in the same plot. The lentils were harvested in September. Winter rye was the cover crop between the lentil rows in the sowing year and was left over the winter period as a cereal for grain harvest.

The lentils (moisture content 14%) were milled with a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particle size was 1–2 mm and were then mixed with water: for submerged (SM) conditions, the lentil/water ratio was 1:5 (weight/weight, *w*/*w*), and, for solid-state (SS) conditions, the lentil/water ratio was 1:1 (*w*/*w*). In total, the following twelve sample groups were obtained: four control groups—LR and L control groups for SM and SS conditions (C_{LR}SM, C_{LR}SS, C_LSM, and C_LSS); four groups fermented for 24 h under SM and SS conditions (SMF_{LR}24, SSF_{LR}24, SMF_L24, and SSF_L24); and four groups fermented for 48 h under SM and SS conditions (SMF_{LR}48, SSF_{LR}48, SMF_L48, and SSF_L48). The principal scheme of the experiment is given in Figure 1.

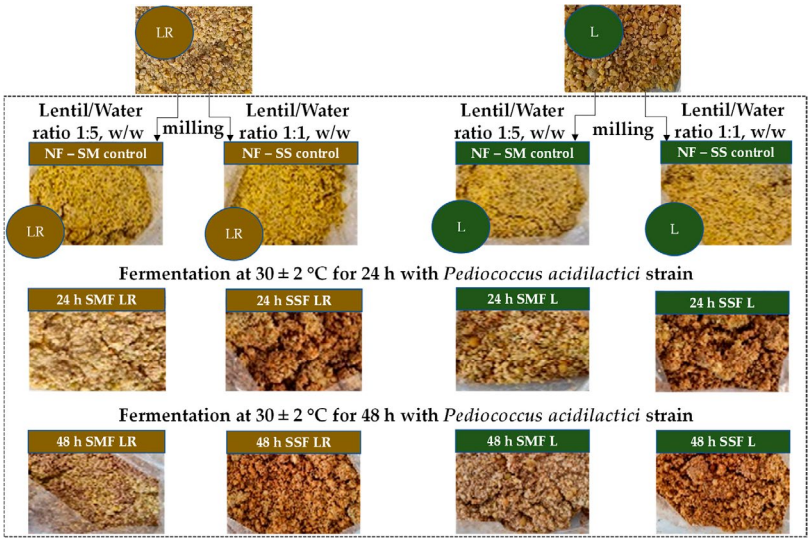

Figure 1. The principal scheme of the experiment (L—lentil obtained from pure stands; LR—lentil obtained from relay incorporated with winter rye; NF—non-fermented; SM—submerged conditions, SS—solid-state conditions; SMF—submerged fermented; SSF—solid-state fermented).

The *Pediococcus acidilactici* strain, previously isolated from spontaneous fermented cereal, was used for lentil fermentation [34]. Previous studies disclosed that the *Pediococcus acidilactici* strain is able to ferment various carbohydrate- and protein-rich matrices and multiply rapidly, reaching 3.20 ± 0.1 $\log_{10}$ CFU/mL within 2 h [34,35].

Before the experiments, the *Pediococcus acidilactici* strain was stored at −80 °C (Microbank system, Pro-Lab Diagnostics, Birkenhead, UK) and multiplied in de Man–Rogosa–Sharpe (MRS) broth (Oxoid Ltd., Hampshire, UK) at 30 ± 2 °C for 24 h, before using it for lentil fermentation. Milled lentil, water, and a suspension of *Pediococcus acidilactici* strain [3% of dry matter (d.m.) of the lentil mass, containing 8.7 log10 CFU/mL] was fermented at 30 ± 2 °C for 24 and 48 h in a chamber incubator (Memmert GmbH + Co. KG, Schwabach, Germany). For 100 g of lentils, 500 mL and 100 mL of water was added for submerged fermentation and solid-state fermentation, respectively. Non-fermented samples (mixed with water) were analysed as a control.

2.2. Evaluation of Acidity and the Microbiological and Chromaticity Characteristics of Lentil Wholemeal Samples

The pH of the lentil samples was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany). The LAB viable counts were determined according to the method described by Bartkiene et al. [36]. The chromaticity characteristics of lentil samples were evaluated on the samples' surfaces using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Tokyo, Japan). The results were expressed as the CIE colour values L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness).

2.3. Analysis of Free Amino Acids in Lentil Wholemeal Samples

Sample preparation and dansylation were performed according to the method of Hua-Lin Cai et al., with some modifications [37]. A homogenous sample (~1 g) was

weighed into a 50 mL tube, and analytes were extracted with 10 mL of aqueous 0.1 M HCl solution, by blending the mixture with a laboratory blender and shaking for 30 min. The resultant mixture was centrifuged at 4000 rpm for 10 min. For derivatization, 100 µL of resultant supernatant was diluted to 500 µL with 0.1 M HCl solution. The resultant mixture was made alkaline by adding 40 µL of 2 M NaOH and 70 µL of saturated $NaHCO_3$ solution. Derivatization was performed by adding 0.5 mL of 20 mg/mL dansyl chloride solution in acetonitrile and heating the resulting mixture at 60 °C for 30 min. The reaction mixture was cooled down to room temperature, centrifuged at 12 k rotations per minute (rpm) for 5 min, at room temperature, and filtered with a 0.20 µm membrane filter (Chromafil®Xtra PA-20/25, nylon, Düren, Germany) into the auto-sampler vial. The concentration of analytes was determined using the Varian ProStar high-performance liquid chromatography (HPLC) system (Varian Corp., Palo Alto, CA, USA), which included two ProStar 210 pumps, a ProStar 410 autosampler, and a Thermo Scientific LCQ Fleet Ion trap mass detector (Thermofisher Scientific, San Jose, CA, USA). For analyte detection, the mass spectrometer was operated in the positive ionisation consecutive reaction monitoring mode, for specific ions which corresponded to derivatized analyte. The concentration of analyte was determined from the calibration curve, which was obtained by derivatizing analytes at different concentrations. For the separation of derivatives, a Discovery® HS C18 column (150 mm × 4.6 mm-ϕ, 5 µm-ϕ particle size; SupelcoTM Analytical, Bellefonte, PA, USA) was used. The mobile phase A was 0.1% formic acid in 5% aqueous acetonitrile and phase B was 0.1% in acetonitrile. A 10 µL injection volume was used for analyses. The analytical gradient was as follows: 0 to 1.0 min, 15 to 50% B, (linear gradient, flow rate 1 mL/min); 1.0 to 3.0 min (flow-rate 1 mL/min); 3.0 to 3.5 min (flow-rate 1.0 to 0.3 mL/min); 3.5 to 10 min (linear gradient), 50 to 70% B; 10 to 15.0 min (linear gradient), 70 to 90% B; 15 to 30 min, 90 to 95% B; and followed by column re-equilibration for 11 min with 15% B (flow-rate increased to 0.8 mL/min).

2.4. Determination of the Biogenic Amines Content

The extraction and determination of BAs in non-fermented and fermented lentil samples followed the procedures developed by Ben-Gigirey et al. [38]. Perchloric acid (0.4 mol/L, 10 mL) containing a known amount of 1,7-diaminoheptane, as an internal standard (ISTD), was added to 3 g of sample, and the mixture was homogenized with Ultra-Turrax apparatus (IKA Labortechnik, Staufen, Germany) and centrifuged at 3000× *g* for 10 min at room temperature. The residue was extracted again with an equal volume of 0.4 mol/L perchloric acid. Both supernatants were combined, and the final volume was adjusted to 30 mL with 0.4 mol/L perchloric acid. The extract was filtered through a Whatman No. 1 paper filter. One millilitre of extract or standard solution was mixed with 200 mL of 2 mol/L NaOH solution and 300 mL of saturated $NaHCO_3$ solution. Two millilitres of 10 mg/mL solution of 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) in acetone was added to the mixture and incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 100 mL of 25 mg/L aqueous ammonia solution. After incubation at room temperature for 30 min, the mixture was adjusted to 5 mL with acetonitrile. Finally, the mixture was centrifuged at 3000× *g* for 5 min at room temperature; the supernatant was then filtered through a 0.2 µm filter (Millipore, nylon, Bedford, MA, USA) and stored at 25 °C until HPLC analysis. An Agilent 1200 HPLC system (Carlsbad, CA, USA) equipped with a diode array detector (DAD) and Chemstation liquid chromatography (LC) software (version B.04.03) was employed in combination with a Chromolith C_{18} HPLC column (diode array detector (DAD) 100 mm × 4.6 mm-ϕ × 4 µm-ϕ particle size, Merck KGaA/EMD Chemicals, Darmstadt, Germany). Ammonium acetate (0.1 mol/L) and acetonitrile were used as the mobile phases at the flow-rate of 0.45 mL/min. The injected sample volume was 10 µL and the biogenic amines were monitored at 254 nm wavelength (λ). The detection limits for standard amine solutions were approximately 0.1 mg/kg.

2.5. Fatty Acid Composition Analysis

The fatty acid composition of the lentil samples was determined using a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph with a mass spectrometer (GC-MS). The fatty acid methyl ester (FAME) concentration was determined using a calibration curve, and the results were expressed as percentage of total FAME concentration in fat. Samples were prepared by extracting 1 g of the homogenized sample with 10 mL of extraction solution [chloroform–methanol, 2:1 (volume/volume, v/v)] by shaking for 1 h. Afterwards, the mixture was centrifuged at 4000 rpm for 5 min at room temperature, and 5 mL of the organic phase was washed with 1 mL of 0.9% aqueous sodium chloride solution. The resulting mixture was centrifuged at 4000 rpm for 5 min at room temperature, and 1 mL of organic phase was evaporated using nitrogen gas. The resulting residue was dissolved in 1 mL of hexane and reacted with 70 µL of methylation reagent (2 mol/L of KOH in methanol) by vortexing and shaking using a laboratory shaker for 30 min. The mixture was centrifuged at 13.2 k rpm for 5 min at room temperature and the upper layer was used for the analysis. We used a Capillary Stabilwax-MS column (30 m $\times$ 0.25 mm-ϕ internal diameter (ID) $\times$ 0.25 µm-ϕ film thickness). The mass spectrometer operated at full scan mode. The analyte was injected in split mode at a 1:60 split ratio. The following parameters were used: MS ion source temperature: 240 °C; MS interface temperature: 240 °C; helium (carrier gas) flow-rate: 0.90 mL/min; injector: 240 °C; oven temperature program: 50 °C (4 min), 10 °C min^{-1} to 110 °C (1 min), 15 °C min^{-1} to 160 °C (2 min), 2.5 °C min^{-1} to 195 °C (1 min), 2 °C min^{-1} to 230 °C (1 min), and 2 °C min^{-1} to 240 °C (12 min).

2.6. Evaluation of Volatile Compound Profiles

The VCs of samples were analysed using gas chromatography–mass spectrometry. A solid-phase microextraction (SPME) device with Stableflex™ fibre, coated with a 50 µm-ϕ PDMS-DVB-Carboxen™ layer (Supelco, USA), was used for analyses. For the headspace extraction of the samples, 1 g of sample and 12 mL of 1 M phosphate buffer (pH = 3) were transferred to the 20 mL extraction vial, mixed, sealed with a polytetrafluoroethylene septum, and thermostatted at 60 °C for 30 min before exposing the fibre in the headspace. The fibre was exposed to the headspace of the vial for 10 min and desorbed in an injector liner for 2 min (splitless injection mode). The prepared samples were analysed with a GCMS-QP2010 (Shimadzu, Japan) gas chromatograph and mass spectrometer. The following conditions were used for analysis: injector temperature 250 °C; ion source temperature 220 °C; and interface temperature 260 °C. Helium was used as a carrier gas at 0.65 mL/min flow-rate. For the separation of VCs, a low polarity Rxi®-5MS column (Restek, Centre County, PA, USA) (length 30 m, coating thickness 0.25 µm-ϕ, inner diameter of 0.25 mm-ϕ) was used. The temperature gradient was programmed from starting at 40 °C (3 min hold) to 220 °C (5 °C/min) up to 310 °C (15°/min) (6 min hold). The VCs were identified according to mass spectrum libraries (National Institute of Technology spectral library (NIST11) and Flavors and Fragrances of Natural and Synthetic Compounds 2 spectral library (FFNSC2)). For identification purposes, alkane mixes (C8–C20) were analysed to obtain the retention indices of unknown compounds.

2.7. Analysis of Micro- and Macro-Elements Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

For macro- and micro-element analysis in lentil samples, an Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan) and a software Mass Hunter Work Station for inductively coupled plasma mass spectrometry (ICP-MS), version B.01.01 (Agilent Technologies, Japan) were used. The samples were milled and homogenised (final particle size $\leq$ 150 µm). For the analysis, the following chemicals were used: nitric acid (concentration $\geq$ 69.0%) for trace analysis (Sigma-Aldrich, Paris, France); hydrogen peroxide, 30% w/w, extra pure (Scharlau, Sentmenat, Spain); and multielement standard solution V for ICP-MS calibration (Sigma-Aldrich, France). An Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan), with the software Mass Hunter Work Station for ICP-MS, version B.01.01 (Agilent

Technologies, Japan), was used for analyses. The sample preparation for ICP-MS analysis was carried out as follows: in a microwave vessel, we accurately weighed 0.3 g of the homogenized sample, 2 mL of deionized water, 8 mL of concentrated nitric acid, and 2 mL of concentrated hydrogen peroxide, which were added together, and we waited for 2–8 h for reaction stabilization, until the formation of bubbles was finished. The vessel was sealed and heated in the microwave system. The following thermal conditions were applied: a 150 °C temperature was reached in approximately 20 min and remained for 30 min and then, 200 °C was reached in approximately 20 min and maintained for 30 min for the completion of specific reactions. After cooling (approximately 40 min), the prepared solution was filtered through the filter with a pore size 8–10 µm-ϕ. The solution was transferred to a 50 mL volumetric flask and filled with deionized water to a 50 mL volume. The following operating conditions of the Agilent 7700x ICP-MS were used for the analysis of the samples: plasma mode—normal, robust; RF forward power 1300 W; sampling depth 8.00 mm; carrier gas-flow 0.6 L/min; dilution gas-flow 0.4 L/min; spray chamber temperature 2 °C; extraction lens 1 V; and kinetic energy discrimination 3 V.

2.8. Statistical Analysis

Microbiological and physicochemical results were expressed as the mean (n = 3) ± standard error (SE). The experiment was performed by preparing three parallel samples for fermentation, and one sample was taken from each for analysis. In order to evaluate the effects of the different factors (viz. growing conditions of lentil: pure stands and relay incorporated with winter rye; different fermentation conditions; and different durations of the fermentation), the data were analysed using multivariate analysis of variance (ANOVA). A Tukey HSD (honest significant difference) post hoc test was conducted to detect differences among sample groups. To determine the normality, the Shapiro–Wilk test was used; for homoskedasticity evaluation, the homoskedasticity test, using the SPSS Statistical Package (v15.0, SPSS, Chicago, IL, USA), was performed. The linear Pearson's correlation coefficients were calculated using the statistical package SPSS for Windows (v15.0, SPSS, Chicago, IL, USA). Correlation strength interpretation was performed according to the method described by Evans (1996) [39]. The results were recognized as statistically significant at $p \leq 0.05$. Heatmap visualization and analysis was carried out using the R statistical programming software package "ComplexHeatmap" (version 2.14.0). Bar plot visualization was carried out using the "tidyverse" package (version 2.0.0).

3. Results and Discussion

3.1. Acidity and Microbiological and Chromaticity Characteristics of Lentil Wholemeal Samples

The pH values, chromaticity characteristics, and LAB viable counts of lentil samples are presented in Table 1. Comparing the pH values of non-fermented LR and L sample groups (SM and SS), higher pH values were observed in LR sample groups. However, after 24 and 48 h of SMF, the opposite tendencies were established; i.e., L samples showed lower pH values than LR groups. SSF conditions were more effective in reducing the pH values of L samples; however, when comparing SSF L and LR sample groups, lower pH values were attained in L sample groups (after 24 and 48 h of SSF). In both sample groups (LR and L) under both conditions (SMF and SSF), the pH values decreased between 24 and 48 h of fermentation.

Despite the pH differences in non-fermented samples (between C_{LR}SM and C_{L}SM; C_{LR}SS and C_{L}SS), significant differences in LAB viable counts were found only between C_{LR}SM and C_{L}SM samples. Contrasting non-fermented samples (C_{LR}SM, C_{LR}SS, C_{L}SM, and C_{L}SS), LAB viable counts were, on average, 4.67 $\log_{10}$ CFU/g. In comparison, for LAB numbers in different lentil sample groups (LR and L) with the same treatment, significant differences were also inexistent, and, in both sample groups (SMF and SSF), higher LAB viable counts were detected after 48 h of fermentation, with an average value of 8.23 $\log_{10}$ CFU/g. The analysed factors (type of fermentation and fermentation duration) and the interaction of growing conditions * fermentation duration were significant ($p = 0.005$,

$p < 0.001$, and $p = 0.003$, respectively) for the LAB viable counts in lentil samples (see supplementary file: Table S1). Also, a strong negative correlation ($r = -0.886$, $p < 0.001$) between lentil pH values and LAB numbers was found (see supplementary file: Table S2).

Table 1. Acidity, microbiological, and chromaticity characteristics in lentil wholemeal samples.

Lentil Samples	Sample Groups	pH	LAB Viable Counts, log_{10} CFU/g	Colour Coordinates, NBS		
				L*	a*	b*
LR	$C_{LR}SM$	$6.12 \pm 0.03^{b,E}$	$4.80 \pm 0.23^{b,A}$	$68.0 \pm 0.31^{a,E}$	$1.85 \pm 0.11^{b,B}$	$31.0 \pm 0.29^{a,D}$
	$C_{LR}SS$	$6.03 \pm 0.01^{b,D}$	$4.60 \pm 0.19^{a,A}$	$67.9 \pm 0.22^{a,E}$	$1.78 \pm 0.12^{b,B}$	$31.3 \pm 0.23^{a,D}$
	$SMF_{LR}24$	$5.20 \pm 0.03^{a,C}$	$7.98 \pm 0.21^{b,BC}$	$63.2 \pm 0.27^{a,C}$	$1.08 \pm 0.07^{a,A}$	$23.5 \pm 0.41^{a,C}$
	$SMF_{LR}48$	$4.36 \pm 0.02^{a,A}$	$8.27 \pm 0.22^{a,C}$	$61.6 \pm 0.43^{b,B}$	$2.96 \pm 0.14^{a,C}$	$21.6 \pm 0.36^{b,B}$
	$SSF_{LR}24$	$5.22 \pm 0.03^{b,C}$	$7.63 \pm 0.23^{a,B}$	$64.0 \pm 0.25^{a,D}$	$1.19 \pm 0.08^{a,A}$	$24.3 \pm 0.25^{a,C}$
	$SSF_{LR}48$	$4.72 \pm 0.02^{b,B}$	$8.14 \pm 0.19^{a,BC}$	$50.9 \pm 0.19^{a,A}$	$5.34 \pm 0.21^{a,D}$	$15.6 \pm 0.21^{a,A}$
L	$C_{L}SM$	$6.02 \pm 0.01^{a,E}$	$4.42 \pm 0.15^{a,A}$	$69.6 \pm 0.25^{b,E}$	$1.01 \pm 0.06^{a,A}$	$34.7 \pm 0.37^{b,E}$
	$C_{L}SS$	$5.94 \pm 0.03^{a,D}$	$4.87 \pm 0.24^{a,A}$	$69.4 \pm 0.39^{b,E}$	$1.08 \pm 0.05^{a,A}$	$34.5 \pm 0.41^{b,E}$
	$SMF_{L}24$	$5.58 \pm 0.02^{b,C}$	$7.61 \pm 0.20^{a,BC}$	$67.7 \pm 0.26^{b,D}$	$1.98 \pm 0.12^{b,B}$	$32.3 \pm 0.32^{b,D}$
	$SMF_{L}48$	$4.45 \pm 0.02^{b,A}$	$8.41 \pm 0.19^{a,D}$	$58.8 \pm 0.32^{a,B}$	$5.12 \pm 0.23^{b,C}$	$18.6 \pm 0.09^{a,B}$
	$SSF_{L}24$	$4.71 \pm 0.03^{a,B}$	$7.30 \pm 0.22^{a,B}$	$66.8 \pm 0.42^{b,C}$	$1.68 \pm 0.14^{b,B}$	$29.7 \pm 0.14^{b,C}$
	$SSF_{L}48$	$4.46 \pm 0.01^{a,B}$	$8.10 \pm 0.18^{a,CD}$	$54.5 \pm 0.29^{b,A}$	$7.03 \pm 0.25^{b,D}$	$17.6 \pm 0.11^{b,A}$

C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours). L*—brightness or (-) darkness; a*—redness or (-) greenness; b*—yellowness or (-) blueness; NBS—National Bureau of Standards units; LAB—lactic acid bacteria; CFU—colony-forming units. Data are represented as means (n = 3) ± standard errors (SE). ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–E}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

The fermentation of leguminous crops can provide probiotic benefits in foods [13]. Moreover, it was reported that SSF is more commonly used for various biological conversion of substrates, because of its higher efficiency [40]. Our study showed that all the analysed factors (type of fermentation, growing conditions, and fermentation duration) and their interactions were significant ($p < 0.001$) on the pH values of lentil samples (see supplementary file: Table S1). The results also showed that, in all of the fermented lentil samples, LAB viable counts were higher than 6.0 log_{10} CFU/g; such a number of desirable microorganisms may ensure the probiotic properties of foodstuffs, if the strains used for fermentation are probiotics (which is the case here).

The carbohydrate metabolism of *P. acidilactici* strain was studied in 47 different carbon sources, and this strain demonstrated fermentation activity for 20 out of 47 tested carbohydrates [34]. Additionally, the optimal growth temperature for this strain was 30 °C. This characteristic can explain the differences in the fermentation activity of the *P. acidilactici* strain reported in the study of Byanju et al. [13], who found that, for *P. acidilactici* (provided by Lallemand Animal Nutrition–North America, Milwaukee, WI, USA), the exponential growth in lentil flour was observed between 6 and 24 h in SMF conditions. In addition, the pH decreased significantly during the first 24 h of fermentation, and the microbial population attained the highest value during this time period [13].

Throughout fermentation, the low pH of the substrate can damage both the non-desirable and desirable microorganism's cell wall and cell membrane [41]. LAB adaptation to low pH conditions depends on their phenotype characteristics and other conditions under which cells are exposed to stress [42]. The molecular mechanisms of LAB adaptation and habituation to stress may overlap to a certain degree, but they are not completely identical [34]. This may explain the different results reported in distinct LAB species for the fermentation effectiveness parameters. However, the pH values and the number of LAB are the main characteristics of fermentation efficiency. This study showed that *P. acidilactici*

is a suitable strain for lentil fermentation; furthermore, a 48 h fermentation duration was shown to be most suitable for lentil fermentation with this particular LAB strain, because statistically significant lower pH and higher LAB viable counts after 48 h of SMF and SSF were obtained, when compared with 24 h fermented samples.

In comparison, chromaticity characteristics of the non-fermented lentils (LR samples: C_{LR}SM and C_{LR}SS) showed, respectively, on average, 1.03 and 10.6% lower brightness (L*) and yellowness (b*), as well as, on average, 42.3% higher redness (a*), in comparison with L samples. In all cases, the same tendencies were found: with an increasing duration of fermentation, the L* and b* values of the lentils decreased and the a* values increased. Lentil L*, a*, and b* values showed statistically significant correlations with pH and LAB viable counts (with pH: r = 0.763, $p < 0.001$; r = −0.679, $p < 0.001$; and r = 0.839, $p < 0.001$, respectively; with LAB viable counts: r = −0.710, $p < 0.001$; r = 0.552, $p < 0.001$; and r = −0.799, $p < 0.001$, respectively). Analysed factors (fermentation conditions, lentil growing conditions, and fermentation duration) and their interactions were statistically significant ($p < 0.001$) for the colour coordinate values of lentil samples, except for the interaction between growing conditions, type of fermentation, and fermentation durations on a* values (see supplementary file: Table S1).

The colour of the lentil is a key quality parameter due to its impact on the acceptability of the legume products. The lentil colour ranges from light tan to dark brown, and the latter is often referred as being a lower quality product. Furthermore, a lighter colour can be linked to a loss of nutrients or other secondary metabolites, such as polyphenolic compounds [2]. Our study discovered that the changes in lentil colour can be found during the fermentation process. It was reported that the anthocyanins can be hydrolysed by bacterial β-glucosidase enzymes and converted to small phenolic compounds [43]. Likewise, other flavonoid pigments (flavonols, flavones isoflavones, etc.) may be degraded by bacterial enzymes [43]. Excreted bacterial enzymes break glycosidic bonds to form aglycones and, then, the latter, after prolonged fermentation, may be further be converted into smaller phenolic compounds [43,44]. Moreover, yellow to orange pigments—carotenoids [45]—can degrade under LAB fermentation conditions, producing volatile low-molecular weight carotenoid derivatives, such as β-ionone and β-damascenone [46]. Finally, further studies are needed to explain, in detail, the colour changes in lentils during fermentation but we found correlations between the lentil colour characteristics and the main fermentation parameters (pH and LAB viable counts)—exposing that the changes occurring during lentil fermentation are very complex and should be taken into consideration during its processing.

3.2. Free Amino Acid Profile and γ-Aminobutyric Acid (GABA) Concentration in Lentil Wholemeal Samples

The non-essential free amino acid concentrations in lentil samples are presented in Table 2, and GABA concentrations in lentil samples are presented in Figure 2. The fermentation process proved to be a significant factor of reduction in arginine, asparagine, glutamine, and aspartic acid concentrations in most of the cases, while glutamic acid, glycine, alanine, and proline concentrations in fermented lentil samples were significantly higher in most cases, in comparison to respective control samples. Moreover, serine concentration in SMF samples (SMF_{LR}24, SMF_{LR}48, SMF_{L}24, and SMF_{L}48) was significantly lower, while its concentration in SSF samples (SSF_{LR}24, SSF_{LR}48, SSF_{L}24, and SSF_{L}48) was significantly higher than the respective control sample groups. Additionally, SMF_{L}24, SMF_{L}48, SSF_{L}24, and SSF_{L}48 samples contained significantly lower concentration of GABA, while its concentration was significantly higher in most of SMF and SSF LR samples, in comparison with the respective control samples.

Table 2. Concentration of non-essential free amino acids (FAAs) in lentil wholemeal samples.

Lentil Samples	Arginine	Glutamine	Asparagine	Glutamic Acid	Serine	Aspartic Acid	Glycine	Alanine	Proline	Tyrosine
	µmol/g									
LR										
$C_{LR}SM$	1.86 ± 0.028 ^{a,B}	0.943 ± 0.042 ^{a,B}	1.60 ± 0.019 ^{a,BC}	3.42 ± 0.085 ^{b,A}	0.819 ± 0.057 ^{a,B}	nd	1.50 ± 0.091 ^{a,A}	3.62 ± 0.069 ^{a,A}	0.750 ± 0.027 ^{a,A}	0.476 ± 0.039 ^{a,A}
$C_{LR}SS$	2.28 ± 0.11 ^{a,C}	1.96 ± 0.086 ^{a,E}	1.84 ± 0.085 ^{a,CD}	4.16 ± 0.134 ^{b,B}	1.03 ± 0.012 ^{a,B}	0.738 ± 0.027 ^{a,B}	1.91 ± 0.023 ^{a,C}	6.69 ± 0.176 ^{a,C}	0.990 ± 0.043 ^{a,B}	0.679 ± 0.009 ^{b,BC}
$SMF_{LR}24$	nd	0.172 ± 0.014 ^{a,A}	1.17 ± 0.026 ^{a,A}	4.85 ± 0.073 ^{a,C}	0.128 ± 0.017 ^{a,A}	0.879 ± 0.052 ^{a,C}	1.58 ± 0.027 ^{a,AB}	5.09 ± 0.078 ^{a,B}	0.760 ± 0.053 ^{a,A}	0.635 ± 0.011 ^{b,B}
$SMF_{LR}48$	nd	0.178 ± 0.01 ^{a,A}	1.33 ± 0.023 ^{a,AB}	5.00 ± 0.165 ^{a,C}	nd	1.08 ± 0.076 ^{a,D}	1.73 ± 0.026 ^{a,B}	5.23 ± 0.077 ^{a,B}	0.960 ± 0.042 ^{a,B}	0.627 ± 0.013 ^{a,B}
$SSF_{LR}24$	0.75 ± 0.041 ^{a,A}	1.70 ± 0.059 ^{b,D}	1.97 ± 0.175 ^{a,D}	4.73 ± 0.120 ^{a,C}	1.44 ± 0.090 ^{a,C}	0.439 ± 0.012 ^{a,A}	2.26 ± 0.057 ^{a,D}	8.43 ± 0.652 ^{b,D}	1.37 ± 0.092 ^{a,C}	0.613 ± 0.011 ^{b,B}
$SSF_{LR}48$	nd	1.11 ± 0.039 ^{a,C}	1.90 ± 0.138 ^{a,D}	6.02 ± 0.074 ^{a,D}	1.67 ± 0.171 ^{a,D}	0.949 ± 0.036 ^{a,C}	2.67 ± 0.108 ^{a,E}	9.01 ± 0.755 ^{a,D}	1.56 ± 0.032 ^{a,D}	0.753 ± 0.053 ^{a,C}
L										
C_LSM	2.99 ± 0.152 ^{b,A}	1.35 ± 0.027 ^{b,B}	3.63 ± 0.043 ^{b,B}	2.14 ± 0.051 ^{a,A}	0.893 ± 0.077 ^{a,B}	nd	1.62 ± 0.093 ^{a,A}	3.90 ± 0.09 ^{b,A}	0.752 ± 0.072 ^{a,A}	0.501 ± 0.042 ^{a,A}
C_LSS	4.35 ± 0.31 ^{b,B}	2.00 ± 0.112 ^{a,C}	4.68 ± 0.300 ^{b,C}	2.73 ± 0.029 ^{a,A}	1.25 ± 0.089 ^{b,C}	nd	2.21 ± 0.080 ^{b,BC}	8.32 ± 0.09 ^{b,D}	1.11 ± 0.020 ^{b,B}	0.634 ± 0.014 ^{a,B}
SMF_L24	nd	0.694 ± 0.007 ^{b,A}	3.14 ± 0.201 ^{b,A}	4.95 ± 0.163 ^{a,B}	0.269 ± 0.003 ^{b,A}	1.39 ± 0.016 ^{b,B}	1.88 ± 0.036 ^{b,AB}	5.90 ± 0.393 ^{b,B}	0.890 ± 0.065 ^{b,A}	0.595 ± 0.009 ^{a,B}
SMF_L48	nd	0.708 ± 0.051 ^{b,A}	3.23 ± 0.038 ^{b,AB}	5.67 ± 0.311 ^{b,BC}	0.427 ± 0.021 ^{a,A}	1.57 ± 0.103 ^{b,C}	2.01 ± 0.117 ^{b,B}	6.29 ± 0.162 ^{b,BC}	1.15 ± 0.053 ^{b,B}	0.632 ± 0.031 ^{a,B}
SSF_L24	nd	1.20 ± 0.053 ^{a,B}	3.41 ± 0.237 ^{b,AB}	5.10 ± 0.411 ^{a,B}	1.34 ± 0.094 ^{a,C}	0.928 ± 0.035 ^{b,A}	2.05 ± 0.173 ^{a,B}	7.02 ± 0.246 ^{a,C}	1.44 ± 0.015 ^{a,C}	0.495 ± 0.022 ^{a,A}
SSF_L48	nd	1.25 ± 0.016 ^{b,B}	3.53 ± 0.049 ^{b,AB}	6.15 ± 0.437 ^{a,C}	1.74 ± 0.031 ^{a,D}	1.60 ± 0.044 ^{b,C}	2.51 ± 0.191 ^{a,C}	8.23 ± 0.711 ^{a,D}	1.70 ± 0.085 ^{b,D}	0.818 ± 0.019 ^{a,C}

C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours); nd—not detected. ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–E}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

L control samples (C_LSM and C_LSS) contained significantly higher concentrations of arginine, asparagine, and alanine than the LR control samples. Meanwhile, LR control samples ($C_{LR}SM$ and $C_{LR}SS$) contained significantly higher concentrations of glutamic acid, in comparison with L control samples. Arginine concentration in almost all fermented samples was reduced to undetectable levels. L SMF samples (SMF_L24 and SMF_L48) contained significantly higher concentrations of glutamine, asparagine, serine, aspartic acid, glycine, alanine, proline, and GABA, in comparison to LR SMF samples ($SSF_{LR}24$ and $SSF_{LR}48$), while glutamic acid was significantly higher only in SMF_L48 samples. Moreover, L SSF samples (SSF_L24 and SSF_L48), in comparison with $SSF_{LR}24$ and $SSF_{LR}48$, contained significantly higher concentrations of asparagine and aspartic acid, while glutamine and proline showed significantly higher concentrations only in SSF_L48 samples. Furthermore, significantly higher concentrations of GABA were observed in LR SSF samples ($SSF_{LR}24$ and $SSF_{LR}48$), in comparison with SSF_L24 and SSF_L48, while only $SSF_{LR}24$ samples contained significantly higher concentrations of glutamine, alanine, and tyrosine.

Correlations between FAA and GABA concentrations and pH and LAB count are presented in supplementary file: Table S4. Only glutamine and arginine showed positive correlations with samples' pH ($r = 0.784$, $p < 0.001$ and $r = 0.479$, $p = 0.003$, respectively) and negative correlations with LAB count ($r = -0.892$, $p < 0.001$ and $r = -0.593$, $p < 0.001$, respectively). On the other hand, glutamic acid, aspartic acid, glycine, alanine, proline, and tyrosine showed negative correlations with lentil samples' pH ($r = -0.828$, $p < 0.001$; $r = -0.758$, $p < 0.001$; $r = -0.431$, $p = 0.009$; $r = -0.360$, $p = 0.031$; $r = -0.608$, $p < 0.001$; and

r = −0.442, $p = 0.007$, respectively) and negative correlations with LAB count (r = 0.869, $p < 0.001$; r = 0.794, $p < 0.001$; r = 0.369, $p = 0.027$; r = 0.329, $p = 0.050$; r = 0.472, $p = 0.004$; and r = 0.433, $p = 0.008$, respectively).

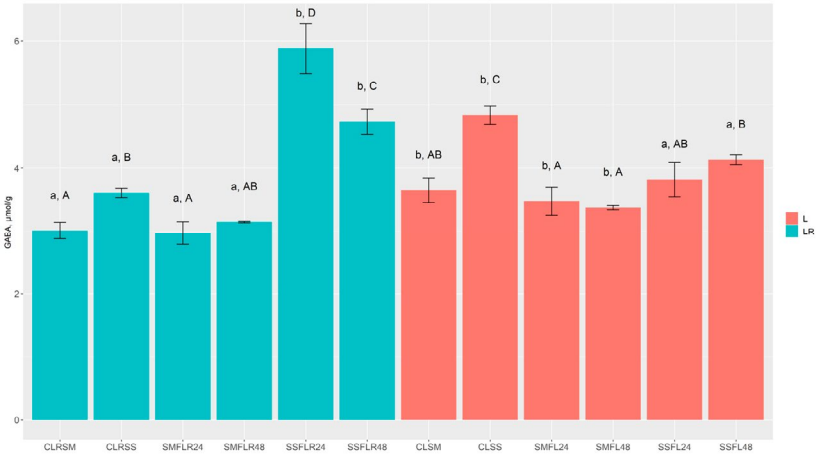

Figure 2. Gamma-aminobutyric acid (GABA) concentrations (µmol/g) in non-treated and fermented lentil wholemeal samples. C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, w/w); SS—submerged conditions (lentils/water ratio 1:1, w/w); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours). ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–D}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

The significance of analysed factors and their interactions on FAA and GABA concentrations are presented in supplementary file: Table S3. The growing conditions factor was statistically significant for almost all nonessential FAA concentrations, except for glycine and alanine. The type of fermentation factor was statistically significant for all nonessential FAA concentrations. The fermentation duration factor was significant for almost all FAA concentrations, except for asparagine. The interaction of growing conditions * type of fermentation was significant for almost all nonessential FAAs, except for glutamic acid, aspartic acid, proline, and tyrosine. The interaction of growing conditions * fermentation duration was statistically significant for arginine, glutamine, serine, GABA, and tyrosine, while the interaction of type of fermentation * fermentation duration was significant for almost all nonessential FAAs, except for asparagine, alanine, and proline. Moreover, the interaction of growing conditions * type of fermentation * fermentation duration was statistically significant for arginine, glutamine, GABA, and tyrosine.

The essential free amino acid concentrations in lentils are depicted in Table 3. All of the fermented samples contained significantly higher concentrations of threonine, valine, phenylalanine, and leucine/isoleucine, while methionine and lysine were significantly higher for most of the fermented sample groups, in comparison to the respective control groups. However, histidine concentrations were not significantly higher in fermented sample groups, in comparison to respective control groups.

Table 3. Concentrations of essential free amino acids (FAA) in lentil wholemeal samples.

Lentil Samples	Threonine	Valine	Methionine	Phenylalanine	Leucine/ Isoleucine	Lysine	Histidine
	µmol/g						
	LR						
$C_{LR}SM$	1.15 ± 0.091 ^{a,A}	1.080 ± 0.086 ^{a,A}	0.115 ± 0.008 ^{a,A}	0.484 ± 0.040 ^{a,A}	1.27 ± 0.019 ^{a,A}	1.69 ± 0.118 ^{a,A}	0.62 ± 0.019 ^{a,A}
$C_{LR}SS$	1.46 ± 0.028 ^{a,B}	1.5 ± 0.039 ^{a,B}	0.195 ± 0.025 ^{a,C}	0.632 ± 0.012 ^{a,B}	1.77 ± 0.036 ^{b,B}	2.04 ± 0.031 ^{a,B}	0.86 ± 0.019 ^{a,C}
$SMF_{LR}24$	1.39 ± 0.099 ^{a,B}	1.76 ± 0.112 ^{a,B}	0.16 ± 0.002 ^{a,B}	0.697 ± 0.020 ^{a,B}	2.75 ± 0.173 ^{a,CD}	1.80 ± 0.094 ^{a,AB}	0.54 ± 0.012 ^{a,A}
$SMF_{LR}48$	1.47 ± 0.116 ^{a,B}	1.65 ± 0.008 ^{a,B}	0.152 ± 0.008 ^{a,B}	0.685 ± 0.034 ^{a,B}	2.56 ± 0.108 ^{a,C}	1.98 ± 0.070 ^{a,B}	0.58 ± 0.017 ^{a,A}
$SSF_{LR}24$	2.1 ± 0.061 ^{a,C}	2.3 ± 0.145 ^{a,C}	0.112 ± 0.002 ^{a,A}	0.848 ± 0.075 ^{a,C}	3.09 ± 0.244 ^{a,D}	2.77 ± 0.086 ^{a,C}	0.86 ± 0.067 ^{b,C}
$SSF_{LR}48$	2.43 ± 0.046 ^{a,D}	2.98 ± 0.148 ^{a,D}	0.222 ± 0.003 ^{a,C}	0.912 ± 0.024 ^{a,C}	4.39 ± 0.150 ^{a,E}	2.90 ± 0.134 ^{a,C}	0.73 ± 0.063 ^{a,B}
	L						
$C_{L}SM$	1.21 ± 0.098 ^{a,A}	1.122 ± 0.103 ^{a,A}	0.168 ± 0.014 ^{b,BC}	0.428 ± 0.032 ^{a,A}	1.22 ± 0.101 ^{a,A}	1.721 ± 0.113 ^{a,A}	0.58 ± 0.022 ^{a,AB}
$C_{L}SS$	1.59 ± 0.023 ^{b,B}	1.53 ± 0.030 ^{a,B}	0.219 ± 0.005 ^{a,DE}	0.638 ± 0.018 ^{a,B}	1.61 ± 0.055 ^{a,B}	2.24 ± 0.041 ^{b,B}	1.01 ± 0.043 ^{b,D}
$SMF_{L}24$	1.69 ± 0.117 ^{b,BC}	1.94 ± 0.046 ^{b,C}	0.176 ± 0.012 ^{a,BC}	0.680 ± 0.051 ^{a,BC}	2.69 ± 0.037 ^{a,C}	1.93 ± 0.014 ^{b,A}	0.55 ± 0.036 ^{a,A}
$SMF_{L}48$	1.93 ± 0.047 ^{b,CD}	2.05 ± 0.061 ^{b,C}	0.199 ± 0.002 ^{b,CD}	0.769 ± 0.025 ^{b,CD}	3.48 ± 0.026 ^{b,D}	2.73 ± 0.031 ^{b,C}	0.64 ± 0.009 ^{a,B}
$SSF_{L}24$	2.14 ± 0.173 ^{a,D}	2.36 ± 0.122 ^{a,D}	0.141 ± 0.017 ^{b,A}	0.834 ± 0.050 ^{a,D}	3.38 ± 0.044 ^{a,D}	2.78 ± 0.189 ^{a,C}	0.73 ± 0.039 ^{a,C}
$SSF_{L}48$	2.52 ± 0.155 ^{a,E}	3.33 ± 0.051 ^{b,E}	0.254 ± 0.021 ^{b,E}	1.093 ± 0.052 ^{b,E}	5.47 ± 0.164 ^{b,E}	3.38 ± 0.130 ^{b,D}	0.73 ± 0.022 ^{a,C}

C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours). ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–E}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

L SMF samples ($SMF_{L}24$ and $SMF_{L}48$) contained significantly higher concentrations of threonine, valine, and lysine, in comparison to $SMF_{LR}24$ and $SMF_{LR}48$, respectively, while methionine, phenylalanine, and leucine/isoleucine contained significantly higher concentrations only in $SMF_{L}48$ samples. Furthermore, L SSF samples ($SSF_{L}24$ and $SSF_{L}48$), in comparison with $SSF_{LR}24$ and $SSF_{LR}48$, respectively, contained significantly higher concentrations of methionine, while valine, phenylalanine, leucine/isoleucine, and lysine concentrations were significantly higher only in $SSF_{L}48$ samples. Moreover, histidine concentration in $SSF_{LR}24$ samples was significantly higher than in the $SSF_{L}24$ samples.

Threonine, valine, phenylalanine, leucine/isoleucine, and lysine showed negative correlations with pH ($r = -0.670$, $p < 0.001$; $r = -0.709$, $p < 0.001$; $r = -0.740$, $p < 0.001$; $r = -0.808$, $p < 0.001$; and $r = -0.661$, $p < 0.001$, respectively) and positive correlations with LAB count ($r = 0.609$, $p < 0.001$; $r = 0.693$, $p < 0.001$; $r = 0.714$, $p < 0.001$; $r = 0.783$, $p < 0.001$; and $r = 0.537$, $p < 0.001$, respectively).

Growing conditions, the type of fermentation, and fermentation duration factors were statistically significant for all essential FAAs, except histidine. The interaction of growing conditions * type of fermentation was significant for threonine, leucine/isoleucine, lysine, and histidine, while the interaction of growing conditions * fermentation duration was statistically significant for all essential FAAs, except for threonine and methionine. Moreover, the interaction of type of fermentation * fermentation duration was significant for all essential FAAs, except for lysine.

Microbial protease enzymes can effectively break down protein to produce polypeptides and free amino acids [22]. Moreover, bacteria can produce essential and non-essential amino acids by utilizing various products from the glycolysis pathway [47]. Therefore, most the changes in free amino acid composition after fermentation can be linked to the activity of bacterial proteases and other metabolic processes. The different proteolytic activities of LAB are the primary cause of the variation in the degree of protein hydrolysis [22]. Varied proteolytic activities and the ability to produce extracellular amino acids was reported for *Pediococcus acidilactici* and *Pediococcus pentosaceus* [48].

Furthermore, it has been noted that SSF, in opposite to SMF, is often associated with higher yields of fermentation products and, despite the exact reasoning not being well understood, in the literature, it is often associated with SSF conditions being similar to the natural conditions [49–51].

Arginine consumption during fermentation can be explained by BA production [52]. Asparagine and glutamine reduction could be explained by the activity of bacterial asparaginases and glutaminases in converting to aspartic and glutamic acid, respectively [53]. Glutamic acid is regarded as an intermediary amino acid that is often involved as an amino group donor for aminotransferases, while it is also consumed by the bacteria to produce GABA via decarboxylation [54]. Some species of LAB, e.g., *Lactobacillus* and *Leuconostoc*, with glutamate decarboxylase activity, also contribute to GABA production [55]. GABA is an important compound towards the inhibition of neurotransmitters, and, when implemented into the diet, can reduce sleeplessness, depression, and anxiety, enhance immunity, regulate blood pressure, etc. [54,55].

3.3. The Concentration of Biogenic Amines in Lentil Wholemeal Samples

Biogenic amine concentration in lentil samples is tabulated in Table 4. Tryptamine (TRIP), cadaverine (CAD), histamine (HIS), and tyramine (TYR) were not detected in any lentil groups. Phenylethylamine (PHE) was found only in $SMF_{LR}24$ samples. In most of the cases, fermented samples contained higher concentrations of other detected biogenic amines—chiefly, putrescine (PUT), spermidine (SPRMD), and spermine (SPRM)—in comparison to the respective sample control groups. In this regard, total BA concentration in L and LR SMF and SSF sample groups were significantly higher in than the respective control sample groups.

Table 4. Biogenic amine (BA) concentration in lentil wholemeal samples.

Lentil Samples		TRIP	PHE	PUT	CAD	HIS	TYR	SPRMD	SPRM	Total BA Content
								mg/kg		
LR	$C_{LR}SM$	nd	nd	nd	nd	nd	nd	$75.8 \pm 5.06^{a,A}$	$13.6 \pm 1.1^{a,A}$	$89.4 \pm 6.2^{a,A}$
	$C_{LR}SS$	nd	nd	$14.6 \pm 0.12^{a,A}$	nd	nd	nd	$110.1 \pm 8.53^{a,C}$	$20.7 \pm 1.6^{a,B}$	$145.4 \pm 10.3^{a,B}$
	$SMF_{LR}24$	nd	$4.9 \pm 0.41^{a,A}$	$36.2 \pm 2.14^{a,B}$	nd	nd	nd	$87.8 \pm 6.87^{a,B}$	$15.2 \pm 1.2^{a,A}$	$144.1 \pm 10.6^{a,B}$
	$SMF_{LR}48$	nd	nd	$41.8 \pm 3.12^{a,C}$	nd	nd	nd	$84.7 \pm 5.42^{a,A,B}$	$13.8 \pm 1.1^{a,A}$	$140.3 \pm 9.6^{a,B}$
	$SSF_{LR}24$	nd	nd	$32.2 \pm 2.81^{a,B}$	nd	nd	nd	$129 \pm 8.23^{b,D}$	$25.3 \pm 1.6^{b,C}$	$186.5 \pm 12.6^{a,C}$
	$SSF_{LR}48$	nd	nd	$37.2 \pm 2.89^{a,BC}$	nd	nd	nd	$116.7 \pm 7.29^{b,CD}$	$22.4 \pm 1.8^{a,BC}$	$176.3 \pm 12.0^{a,C}$
L	$C_{L}SM$	nd	nd	nd	nd	nd	nd	$87.3 \pm 5.32^{b,A}$	nd	$87.3 \pm 5.3^{a,A}$
	$C_{L}SS$	nd	nd	$22.1 \pm 1.83^{b,A}$	nd	nd	nd	$117.1 \pm 8.36^{a,C}$	$23.9 \pm 1.5^{a,C}$	$163.1 \pm 11.7^{a,B}$
	$SMF_{L}24$	nd	nd	$79.5 \pm 5.45^{b,B}$	nd	nd	nd	$96.5 \pm 7.21^{a,AB}$	$16.4 \pm 1.3^{a,A}$	$192.4 \pm 14.0^{b,BC}$
	$SMF_{L}48$	nd	nd	$97.4 \pm 6.84^{b,C}$	nd	nd	nd	$99.6 \pm 6.81^{b,B}$	$17.9 \pm 1.6^{b,AB}$	$214.9 \pm 15.3^{b,C}$
	$SSF_{L}24$	nd	nd	$71.3 \pm 5.42^{b,B}$	nd	nd	nd	$103.4 \pm 8.25^{a,C}$	$19.5 \pm 1.7^{a,B}$	$194.2 \pm 1.1^{a,BC}$
	$SSF_{L}48$	nd	nd	$77.2 \pm 6.12^{b,B}$	nd	nd	nd	$101.5 \pm 7.46^{a,BC}$	$19.3 \pm 1.8^{a,AB}$	$198 \pm 15.4^{a,C}$

C—control samples (non-fermented); L—lentil obtained from pure stands; LR—lentil obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours). TRIP—tryptamine; PHE—phenylethylamine; PUT—putrescine; CAD—cadaverine; HIS—histamine; TYR—tyramine; SPRMD—spermidine; SPRM—spermine; BA—biogenic amines. nd—not detected. ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–D}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

All factors and their interactions were statistically significant on PHE concentration in lentil samples (see supplementary file: Table S6). Moreover, all factors and the growing conditions * type of fermentation interaction were statistically significant for PUT concentration. Almost all factors and the interactions thereof, except type of fermentation, were statistically significant for SPRMD concentration. The type of fermentation, the growing conditions * type of fermentation interaction, and the growing conditions * fermentation

duration interaction were statistically significant for SPRM concentration in lentil samples. The correlation analysis (see supplementary file: Table S5) discovered that only PUT and SPRM possessed strong and weak positive correlations with LAB viable counts (r = 0.761, $p < 0.001$ and r = 0.331, $p = 0.049$, respectively), while only PUT showed a significant strong positive correlation with the pH of the lentils (r = 0.719, $p < 0.001$). Strong and weak negative correlations of PUT with arginine and glutamine (r = −0.686, $p < 0.001$ and r = −0.341, $p = 0.042$, respectively) were established. Moreover, strong positive correlations of PUT with glutamic and aspartic acids, valine, phenylalanine, leucine/isoleucine, and lysine (r = 0.734, $p < 0.001$; r = 0.881, $p < 0.001$; r = 0.614, $p < 0.001$; r = 0.639, $p < 0.001$; r = 0.697, $p < 0.001$; and r = 0.583, $p < 0.001$, respectively), and moderate positive correlations with threonine, glycine, and proline (r = 0.594, $p < 0.001$; r = 0.349, $p = 0.037$; and r = 0.468, $p = 0.004$, respectively) were found. SPRMD showed moderate positive correlations with GABA and tyrosine (r = 0.424, $p = 0.010$; and r = 0.534, $p < 0.001$, respectively). Also, a weak positive correlation of SPRMD with methionine (r = 0.356, $p = 0.033$) was observed. Strong positive correlations were found between SPRM and alanine, proline, glycine, phenylalanine, and histidine (r = 0.787, $p < 0.001$; r = 0.604, $p < 0.001$; r = 0.644, $p < 0.001$; r = 0.610, $p < 0.001$; and r = 0.668, $p < 0.001$, respectively). Furthermore, moderate positive correlations between SPRM and glutamic acid, serine, threonine, GABA, leucine/isoleucine, tyrosine, valine, and lysine (r = 0.461, $p = 0.005$; r = 0.430, $p = 0.009$; r = 0.563, $p < 0.001$; r = 0.566, $p < 0.001$; r = 0.425, $p = 0.010$; r = 0.466, $p = 0.004$; r = 0.520, $p = 0.001$; and r = 0.575, $p < 0.001$, respectively) were established. Yet, a weak positive correlation between SPRM and glutamine (r = 0.354, $p = 0.034$) was observed.

Aromatic and heterocyclic biogenic amines (TRIP, PHE, HIS, and TYR) are produced from the respective amino acids (i.e., tryptophan, phenylalanine, histidine, and tyrosine, respectively) by decarboxylation with enzymes that are produced by bacteria [52]. Certain LAB, e.g., *Lactococci*, *Pediococcus*, and *Lactobacilli*, which are decarboxylase-positive, may result in biogenic amine synthesis during fermentation processes [56]. Some strains of *Pediococcus* spp. have been found to accumulate cadaverine and tyramine in alcoholic beverages at low levels [52]. The aliphatic amine PUT can be formed from arginine via multiple pathways, e.g., arginine decarboxylation to agmatine, which is converted to PUT via agmatine deaminase and PUT carbamoyl transferase; arginine deaminization to citrulline and conversion to PUT via ornithine carbamoyl transferase; arginine conversion to ornithine via arginase/arginine ureohydrolase and conversion to PUT via ornithine decarboxylase; and decarboxylation to agmatine and conversion to PUT via agmatinase/agmatine ureohydrolase. CAD is formed from lysine via lysine decarboxylase [57]. SPRMD is formed from PUT via spermidine synthase and SPRM is formed from SPRMD via spermine synthase [58]. The Food and Drug Administration (FDA) of the United Sates of America (USA) and the Food and Agriculture Organization (FAO) of the United Nations (UN), with the World Health Organization (WHO), proposed a maximum acceptable histamine limit of 50 mg/kg in fish and food products [59]. On the other hand, according to a rat model, the no observed adverse effect level (NOAEL) was set at 2000 ppm for tyramine, putrescine, and cadaverine, 1000 ppm for spermidine, and 200 ppm for spermine [60].

3.4. Fatty Acid Composition in Lentil Wholemeal Samples

The results of fatty acid profiles in lentil samples are presented in Table 5. Linoleic acid (C18:2) was the most abundant fatty acid in all of the lentil samples, ranging, on average, from 41.3 to 50.3%. The least abundant fatty acid detected in all samples was saturated stearic acid (C18:0), ranging, on average, from 1.63 to 3.29%.

Table 5. Fatty acid (FA) profiles in lentil wholemeal samples.

		C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0	C20:1
		Fatty Acids Concentration, % of Total Fat Content							
LR	C_{LR}SM	nd	$17.5 \pm 1.1^{a,AB}$	$1.9 \pm 0.21^{a,A}$	$18.0 \pm 0.32^{a,B}$	$48.1 \pm 0.43^{a,A}$	$14.6 \pm 1.23^{a,AB}$	nd	nd
	C_{LR}SS	nd	$18.6 \pm 1.0^{b,B}$	$2.39 \pm 0.13^{b,B}$	$16.9 \pm 1.20^{a,AB}$	$47.3 \pm 3.77^{a,A}$	$14.8 \pm 1.04^{a,AB}$	nd	nd
	SMF_{LR}24	nd	$15.3 \pm 1.2^{a,A}$	$1.9 \pm 0.10^{a,A}$	$15.8 \pm 0.71^{a,A}$	$51.2 \pm 1.07^{b,A}$	$15.8 \pm 0.83^{b,B}$	nd	nd
	SMF_{LR}48	nd	$17.0 \pm 0.56^{b,A}$	$2.46 \pm 0.14^{b,B}$	$16.5 \pm 0.45^{a,AB}$	$48.0 \pm 2.21^{a,A}$	$16.0 \pm 0.67^{b,B}$	nd	nd
	SSF_{LR}24	nd	$18.3 \pm 0.29^{a,B}$	$2.38 \pm 0.22^{b,B}$	$18.0 \pm 0.51^{a,B}$	47.4 ± 1.34 a,^{A}	$14.0 \pm 0.33^{b,A}$	nd	nd
	SSF_{LR}48	nd a	$18.0 \pm 0.35^{a,B}$	$2.56 \pm 0.09^{a,B}$	$17.6 \pm 0.68^{a,B}$	$47.4 \pm 1.05^{a,A}$	$14.0 \pm 0.48^{b,A}$	$0.33 \pm 0.03^{a,A}$	nd
L	C_LSM	nd	$16.3 \pm 0.29^{a,B}$	$1.89 \pm 0.38^{a,AB}$	$19.2 \pm 0.69^{b,AB}$	$48.2 \pm 1.10^{a,B}$	$14.4 \pm 0.82^{a,B}$	nd	nd
	C_LSS	nd	$16.2 \pm 0.58^{a,B}$	$1.63 \pm 0.28^{a,A}$	$19.8 \pm 1.12^{b,AB}$	$48.4 \pm 3.82^{a,B}$	$14.0 \pm 0.65^{a,B}$	nd	nd
	SMF_L24	2.63 ± 0.23^{A}	$20.3 \pm 1.25^{b,D}$	$3.29 \pm 0.24^{b,C}$	$20.4 \pm 0.68^{b,B}$	$41.3 \pm 2.23^{a,A}$	$12.1 \pm 0.41^{a,A}$	nd	nd
	SMF_L48	nd	$14.1 \pm 0.54^{a,A}$	$1.83 \pm 0.21^{a,AB}$	$19.0 \pm 0.63^{b,A}$	$50.3 \pm 2.28^{a,B}$	$14.8 \pm 0.43^{a,B}$	nd	nd
	SSF_L24	nd	$17.9 \pm 0.42^{a,C}$	$1.94 \pm 0.15^{a,AB}$	$20.3 \pm 1.30^{b,AB}$	$46.6 \pm 2.40^{a,B}$	$12.6 \pm 0.55^{a,A}$	nd	$0.671 \pm 0.03^{a,A}$
	SSF_L48	nd	$17.5 \pm 0.45^{a,C}$	$2.31 \pm 0.28^{a,B}$	$21.2 \pm 1.11^{b,B}$	$46.4 \pm 3.31^{a,AB}$	$12.6 \pm 0.48^{a,A}$	nd	nd

C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid state conditions; 24, 48—duration of fermentation (in hours). C14:0—tetradecanoic acid; C16:0—palmitic acid; C18:0—stearic acid; C18:1—9-octadecenoic acid; C18:2—linoleic acid; C18:3 α—α-linolenic acid; C20:0—eicosanoic acid; C20:1—*cis*-11-eicosenoic acid. nd—not detected. ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–D}$ Mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

Palmitic acid's (C16:0) relative concentration in the SMF and SSF LR groups (SMF_{LR}24, SMF_{LR}48, SSF_{LR}24, and SSF_{LR}48) was not significantly different from the respective control groups, while SMF_L24, SSF_L24, and SSF_L48 samples contained significantly higher percentages of C16:0, in comparison with respective control. Relative concentrations of C18:0 in SMF and SSF LR samples were not significantly different from the respective control groups (except in SMF_{LR}48 samples). Moreover, C18:0 relative concentrations in SMF and SSF L samples were significantly higher only in the SMF_{LR}48, SMF_L24, and SSF_L48 samples, in comparison to respective control groups. Only the SMF_{LR}24 samples contained significantly lower relative percentages of 9-octadecenoic acid (C18:1), in comparison with the respective control group, while other fermented samples did not show significant differences. Similarly, the C18:1 concentration in fermented L samples did not show significant differences when compared to the respective control groups. Furthermore, only SMF_L24 samples contained a significantly different relative concentration of C18:2, in comparison to the respective controls. α-linolenic acid's (C18:3 α) relative concentration in SMF and SSF LR samples was not different from the respective controls. On the other hand, its relative concentrations in SMF_L24, SMF_{LR}24, SSF_L24, and SSF_L48 sample groups were lower than those in the respective controls. Tetradecanoic acid (C14:0), eicosanoic acid (C20:0), and *cis*-11-eicosenoic acid (C20:1) were only detected in fermented sample groups. The factor of growing conditions was statistically significant in almost all the content of fatty acids in lentils but C16:0 and C18:0 (see supplementary file: Table S8). The type of fermentation was a statistically significant factor for almost all fatty acids content except C18:0 and C18:2. Fermentation duration was a statistically significant factor for most fatty acids content except C18:0, C18:1, and C18:2. The factor interactions (growing conditions * type of fermentation and type of fermentation * fermentation duration) were shown to be statistically significant for the most of the fatty acids, but not for C18:1 and C18:2, while the growing conditions * fermentation duration factor's interaction was only not statistically significant for C18:1 content in lentils. Furthermore, the interaction of the factors growing conditions * type of fermentation * fermentation duration was statistically significant for all fatty acid content in lentils. A weak positive correlation was found between C18:0 concentration and LAB viable counts (r = 0.363, p = 0.030) in lentils (see supplementary file: Table S7).

Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids and omega-3, omega-6, and omega-9 fatty acid concentrations (% of the total fat content) of lentils are displayed in Table 6. As expected, the predominant groups of fatty acids in lentils were PUFA and omega-6. Only L fermented samples often showed significantly higher SFA contents than in the respective control groups. Other fatty acid groups were not significantly different, in comparison to the non-treated and fermented lentil groups.

Table 6. Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, and omega-3, omega-6, and omega-9 contents in lentil wholemeal samples.

Lentil Samples		SFA	MUFA	PUFA	Omega-3	Omega-6	Omega-9
		Concentration, % of Total Fat Content					
LR	$C_{LR}SM$	$19.4 \pm 1.67^{a,AB}$	$18.0 \pm 1.38^{a,A}$	$62.6 \pm 2.88^{a,A}$	$14.6 \pm 0.58^{a,A}$	$48.1 \pm 4.48^{a,A}$	$18.0 \pm 1.45^{a,B}$
	$C_{LR}SS$	$20.9 \pm 2.04^{b,B}$	$16.9 \pm 0.87^{a,A}$	$62.2 \pm 5.11^{a,A}$	$14.8 \pm 1.12^{a,AB}$	$47.3 \pm 3.14^{a,A}$	$16.9 \pm 0.783^{a,AB}$
	$SMF_{LR}24$	$17.2 \pm 0.72^{a,A}$	$15.8 \pm 1.11^{a,A}$	$67.1 \pm 2.21^{b,A}$	$15.8 \pm 0.84^{b,AB}$	$51.2 \pm 3.10^{b,A}$	$15.8 \pm 0.67^{a,A}$
	$SMF_{LR}48$	$19.5 \pm 0.76^{b,B}$	$16.5 \pm 0.71^{a,A}$	$64.0 \pm 3.08^{a,A}$	$16.0 \pm 0.50^{b,B}$	$48.0 \pm 1.54^{a,A}$	$16.5 \pm 0.89^{a,AB}$
	$SSF_{LR}24$	$20.7 \pm 1.26^{a,B}$	$18.0 \pm 1.64^{a,A}$	$61.3 \pm 5.72^{a,A}$	$14.0 \pm 1.20^{a,A}$	$47.4 \pm 3.65^{a,A}$	$18.0 \pm 0.88^{a,B}$
	$SSF_{LR}48$	$20.9 \pm 0.75^{a,B}$	$17.6 \pm 1.26^{a,A}$	$61.4 \pm 5.42^{a,A}$	$14.0 \pm 1.06^{a,A}$	$47.4 \pm 2.14^{a,A}$	$17.6 \pm 1.47^{a,AB}$
L	$C_{L}SM$	$18.2 \pm 1.29^{a,AB}$	$19.2 \pm 0.77^{a,A}$	$62.6 \pm 4.67^{a,AB}$	$14.4 \pm 1.05^{a,B}$	$48.2 \pm 1.83^{a,B}$	$19.2 \pm 1.76^{a,A}$
	$C_{L}SS$	$17.8 \pm 0.70^{a,AB}$	$19.8 \pm 0.84^{b,AB}$	$62.4 \pm 2.96^{a,B}$	$14.0 \pm 0.61^{a,B}$	$48.4 \pm 3.55^{a,AB}$	$19.8 \pm 0.91^{b,A}$
	$SMF_{L}24$	$26.2 \pm 0.93^{b,C}$	$20.4 \pm 0.78^{b,AB}$	$53.4 \pm 5.23^{a,A}$	$12.1 \pm 0.89^{a,A}$	$41.3 \pm 3.74^{a,A}$	$20.4 \pm 1.23^{b,A}$
	$SMF_{L}48$	$15.9 \pm 1.55^{a,A}$	$19.0 \pm 1.15^{b,A}$	$65.1 \pm 5.62^{a,B}$	$14.8 \pm 0.53^{a,B}$	$50.3 \pm 4.26^{a,B}$	$19.0 \pm 1.84^{a,A}$
	$SSF_{L}24$	$19.9 \pm 1.00^{a,B}$	$21.0 \pm 0.65^{b,B}$	$59.2 \pm 4.67^{a,AB}$	$12.5 \pm 0.56^{a,A}$	$46.6 \pm 4.17^{a,AB}$	$21.0 \pm 1.30^{b,A}$
	$SSF_{L}48$	$19.8 \pm 1.49^{a,B}$	$21.2 \pm 1.29^{b,AB}$	$59.0 \pm 4.82^{a,AB}$	$12.6 \pm 1.12^{a,AB}$	$46.4 \pm 2.86^{a,AB}$	$21.2 \pm 1.99^{b,A}$

C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, *w*/*w*); SS—submerged conditions (lentils/water ratio 1:1, *w*/*w*); SMF—fermented under submerged conditions; SSF—fermented under solid-state conditions; 24, 48—duration of fermentation (in hours). SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids. ^{a,b} Mean values denoted with different letters indicate significantly different values between the different lentil sample groups (LR and L) with the same treatment (SM, SF, SMF24, SMF48, SSF24, and SSF48); $^{A–C}$ mean values denoted with different letters indicate significantly different values between different treatments (SM, SF, SMF24, SMF48, SSF24, and SSF48) within the same group (LR or L). Results are statistically significant when $p \leq 0.05$.

It has been reported that C16:0, C18:0, C18:1, C18:2, and C18:3 α are the main fatty acids detected in lentils (*Lens culinaris*). The major fatty acid detected in lentil samples was C18:2, as noted in the literature, and, furthermore, driving PUFA were described to be most abundant group of fatty acids in lentils [5,61,62]. Most of the established differences observed between the L and LR sample groups (fermented and non-fermented) can be explained by differences in type of cultivar, genotype, or growing conditions [63,64].

3.5. Volatile Compound Profile in Lentil Wholemeal Samples

The volatile compound profiles (% of the total identified VC content) are presented in Figure 3 (see supplementary file: Table S11). The most abundant VCs (the total area percentage of which was at least 10% in one of the sample groups) were 1-hexanol, hexanal, hexanoic acid, β-damascenone, pentadecanal, 11-hexadecyn-1-ol, 14-methyl-8-hexadecenal, and eugenol. The most abundant VCs presented as various classes of chemical compounds: alcohols (1-hexanol and 11-hexadecyn-1-ol), aldehydes (hexanal, pentadecanal, and 14-methyl-8-hexadecenal), organic acids (hexanoic acid), ketones (β-damascenone), and allylbenzenes (eugenol). Most of the identified VCs with lower abundances (i.e., an area percentage under 10%) in lentils are aldehydes. Furthermore, in almost all cases, identified VCs with lower abundances in the sample control groups had significantly higher abundances in fermented sample groups. Moreover, the complexity of VC composition in the SSF fermented sample groups (for example, there were 30, 35, 28, and 39 VCs detected in the $SSF_{LR}24$, $SSF_{L}24$, $SSF_{LR}48$, and $SSF_{L}48$ samples, respectively) was higher than in the respective control groups (i.e., 25 and 22 VCs were detected in the $C_{LR}SS$ and $C_{L}SS$ samples, respectively). In addition, the $SSF_{LR}24$ and $SSF_{LR}48$ sample groups contained nine newly formed VCs, while the $SSF_{L}24$ and $SSF_{L}48$ samples contained thirteen and seventeen

newly formed VCs, respectively. Moreover, between 38.5 and 55.6% of newly formed VCs detected in SSF samples were aldehydes. However, in SMF sample groups, only three, five, and two newly formed VCs were detected in the $SMF_{LR}24$, $SMF_{L}24$, and $SMF_{L}48$ sample groups, while, in the $SMF_{LR}48$ sample group, the VC profile was similar to the non-fermented samples. Most of the SMF samples contained VCs belonging to aldehydes and alkanes for the $SMF_{LR}24$ and $SMF_{L}24$ sample groups, while alcohols and ketones were the largest newly formed VC classes in the $SMF_{L}48$ sample group.

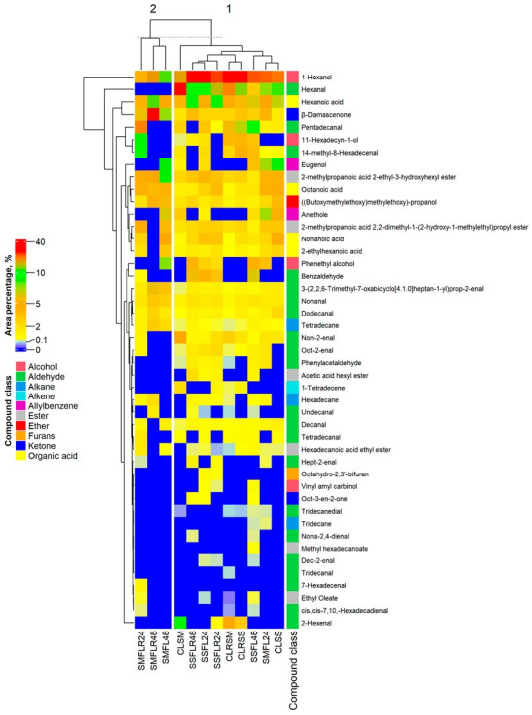

Figure 3. Volatile compound (VC) profile (% of the total volatile compounds content) in lentils. C—control samples (non-fermented); L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; SM—submerged conditions (lentils/water ratio 1:5, w/w); SS—submerged conditions (lentils/water ratio 1:1, w/w); SMF—fermented under submerged conditions; SSF—fermented under solid state conditions; 24, 48—duration of fermentation (in hours).

Most of VCs detected in lentils are known to be associated with specific flavour and aroma characteristics. The 1-hexanol is described having light apple, sweet, fruity, ethereal, and herbal flavour notes with herbal and oily aroma characteristics [65,66]. Some of the most abundant aldehydes—hexanal and pentadecanal—have similar flavour and aroma traits and are described as fatty, grassy, fresh, and green [65–67]. Organic acids, such as hexanoic acid, are described as having sour, fatty, and cheesy flavours and aromas [65]. β-Damascenone contributes to woody, fruity, stewed apple, and sweet aroma and flavour notes [65]. Eugenol is known to contribute to flavour and aroma with clove, spicy, and honeylike notes [65].

The aliphatic aldehydes and ketones, such as hexanal or oct-3-en-2-one, are associated with fatty acid oxidation [68]. The detected aromatic aldehydes and alcohols, benzaldehyde phenylacetaldehyde and phenylethyl alcohol, showed a statistically significant correlation with the aromatic amino acid phenylalanine (see supplementary file: Table S11). Aromatic aldehydes and alcohols are associated with the catabolism of aromatic amino acids; in this case, phenylalanine [69]. The respective amino acid is converted to α-keto acid by the respective aminotransferase, which can be converted to aldehyde by decarboxylases [53,69]. Furthermore, the respective aldehyde can be oxidised to form carboxylic acids or reduced to form alcohols [53,68]. The ester formation has four main biosynthesis pathways, as follows: alcohol and acid conversion to ester via esterase; hemiacetal, which is formed via spontaneous reaction between aldehyde and alcohol, with dehydrogenation via its dehydrogenases; ketone conversion to ester via Baeyer–Villiger monooxygenases; and alcohol and acyl-CoA conversion to ester via alcohol acyltransferases [70]. The production of hydrocarbons, such as tetradecane, tridecane, or 1-tetradecene, is associated with fatty acid catabolism [71]. Finally, all the factors and their interactions were statistically significant for most of the VCs in lentils (see supplementary file: Table S12).

3.6. Micro- and Macro-Elements Concentration in Lentil Wholemeal Samples

The concentrations of micro- and macro-elements in lentil samples are presented in Table 7. Almost all concentrations of macro-elements were significantly higher in L samples, in comparison with LR ones, except magnesium. Moreover, of all essential micro-elements, only phosphorus, manganese, and selenium concentrations in the L sample group were significantly higher than in the LR group. Moreover, iron and nickel concentrations in the LR samples were significantly higher than in the L samples.

Lentils are defined as a leguminous crop, containing significant amounts of macro-elements and essential micro-elements, such as phosphorus, potassium, iron, and zinc [72,73]. Furthermore, the concentration of essential microelements, such as iron, is high enough for the addition of lentils in a diet to prevent iron deficiency anaemia [5]. The other minerals that are plentiful in lentils are crucial for important biological functions in humans [74]. These functions include, but are not limited to, the following: muscle and nerve functioning (Mg and Ca); cell growth, function, and energy production (P); electrolyte balance and muscle contraction (Na); the metabolism of fat and carbohydrates (Cr); the structural components of many enzymes (Mn, Zn, and Cu); etc. [74,75].

According to various sources in the literature, the concentrations of various essential microelements can vary within the ranges of 2000–5300 mg/kg, 0.12–0.60 mg/kg, 10.5–29.0 mg/kg, 49–81.4 mg/kg, 1.1–1.8 mg/kg, 9.4–14.3 mg/kg, 36.7–64.2 mg/kg, and 0.18–1.6 mg/kg for phosphorus, chromium, manganese, iron, nickel, copper, zinc, and selenium, respectively [1,72,76–78]. The mineral composition of lentils can be affected by differences in growing conditions (e.g., phosphorus and other mineral fertilization, water availability, and environmental conditions), plant origin (e.g., genotype and cultivar), or other parameters (e.g., harvesting time) [73,79–83].

However, the aforementioned micronutrients have their bioavailability restrained due to the existing antinutrient factors (phytates, oxalates, and phenolic compounds) [72,84]. Mineral bioavailability can be enhanced by employing certain strategies, such as genetic manipulation, dehulling, soaking, enzymatic treatment, or fermentation [5,72,84,85]. The bioavailability enhancement of minerals via fermentation can be explained by the disruption of plant cell wall structures, production of phytase enzymes, or even the activation of endogenous phytase enzymes, due to the existing low pH environment [26,86,87].

Table 7. Micro- and macro-element concentrations in lentil wholemeal samples.

	LR	L
	Macro-elements, mg/kg d.m. (dry matter)	
23 Na	7.12 ± 0.45 ^{A}	21.0 ± 1.85 ^{B}
24 Mg	676 ± 32.1 ^{A}	726 ± 56.3 ^{A}
39 K	4118 ± 115 ^{A}	4925 ± 136 ^{B}
44 Ca	510 ± 49.1 ^{A}	747 ± 57.3 ^{B}
	Essential micro-elements, mg/kg d.m. (dry matter)	
31 P	3406 ± 123 ^{A}	3899 ± 142 ^{B}
52 Cr	0.405 ± 0.39 ^{A}	0.405 ± 0.028 ^{A}
55 Mn	8.96 ± 0.52 ^{A}	13.9 ± 0.93 ^{B}
56 Fe	116 ± 9.35 ^{B}	96.3 ± 7.41 ^{A}
59 Co	0.059 ± 0.04 ^{A}	0.064 ± 0.007 ^{A}
60 Ni	1.43 ± 0.11 ^{B}	0.893 ± 0.09 ^{A}
63 Cu	7.29 ± 0.62 ^{A}	7.12 ± 0.41 ^{A}
66 Zn	22.0 ± 1.93 ^{A}	24.6 ± 1.84 ^{A}
78 Se	nd	0.014 ± 0.002 ^{A}
	Non-essential micro-elements, mg/kg d.m. (dry matter)	
7 Li	nd	nd
9 Be	nd	nd
11 B	2.48 ± 0.12 ^{A}	6.62 ± 0.58 ^{B}
27 Al	88.3 ± 1.69 ^{B}	25.3 ± 1.32 ^{A}
47 Ti	3.19 ± 0.25 ^{B}	0.767 ± 0.052 ^{A}
51 V	nd	nd
71 Ga	nd	nd
75 As	0.019 ± 0.002 ^{A}	0.017 ± 0.002 ^{A}
85 Rb	7.22 ± 0.52 ^{A}	6.33 ± 0.29 ^{A}
88 Sr	2.65 ± 0.22 ^{A}	3.02 ± 0.30 ^{A}
95 Mo	2.09 ± 0.19 ^{A}	1.84 ± 0.17 ^{A}
105 Pd	nd	nd
107 Ag	nd	nd
111 Cd	nd	nd
118 Sn	nd	nd
121 Sb	nd	nd
133 Cs	nd	nd
137 Ba	0.865 ± 0.023 ^{A}	1.49 ± 0.11 ^{B}
201 Hg	nd	nd
208 Pb	0.177 ± 0.012 ^{B}	0.036 ± 0.003 ^{A}
115 In	nd	nd
195 Pt	nd	nd
205 Tl	nd	nd
209 Bi	nd	nd
238 U	nd	nd

L—lentils obtained from pure stands; LR—lentils obtained from relay incorporated with winter rye; ^{A,B} mean values denoted with different letters indicate significantly different values between the LR and L of lentil samples. Results are statistically significant when $p \leq 0.05$. nd—not detected.

4. Conclusions

This study showed that the *Pediococcus acidilactici* strain was suitable for the fermentation of lentils, once the LAB count in all fermented lentil samples was higher than 7.00 $\log_{10}$ CFU/g and a strong negative correlation between pH and LAB viable counts in lentils (r = −0.886, $p < 0.001$) was established. The analysed factors (growing conditions, type of fermentation, and fermentation duration) were statistically significant in most of the lentils' chromaticity parameters. Solid-state fermented (SSF) lentils showed a more complex volatile compound profile (between nine and seventeen new VCs were formed), whereas, in submerged fermented samples (SMF), between two and five newly formed

VCs were detected. Comparing lentils grown in pure stands (L) and lentils grown in relay intercropped with winter rye (LR), L contained significantly higher concentrations of Na, K, and Ca and the essential microelements P, Mn, and Se, while LR contained significantly higher concentrations of the essential microelements Fe and Ni. The most of free amino acid concentrations, as well as the total biogenic amine content, increased in lentils after SMF and SSF. The most abundant fatty acid in lentils was C18:2 (which ranged from 41.3 to 50.3% of total FA content). The SSF LR samples contained the most GABA, while all the analysed factors and their interactions were statistically significant for GABA concentration in lentils. This research effort showed that fermentation is a useful biotechnology for increasing the biological value of lentils; however, biogenic amine concentration should be taken into consideration. Overall, this study will contribute to the creation of a database on the micro- and macro-elements composition of new varieties of lentils, grown either in pure stands or relay intercropped with winter rye.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods13081249/s1. Table S1. Significance of analysed factors (different growing conditions of lentils, different fermentation conditions, and different durations of fermentation) and their interaction in lentil wholemeal pH, chromaticity, and lactic acid bacteria (LAB) viable counts. Table S2. Pearson correlations between the pH, chromaticity, and lactic acid bacteria (LAB) viable counts in lentil wholemeal samples. Table S3. Significance of analysed factors (different growing conditions of lentils, different fermentation conditions, and different durations of fermentation) and their interaction in lentil wholemeal free amino acids (FAA) and γ-aminobutyric acid (GABA). Table S4. Pearson correlations between the free amino acids (FAA) and γ-aminobutyric acid (GABA) and pH and lactic acid bacteria (LAB) viable counts in lentil whole samples. Table S5. Pearson correlations between biogenic amines (BA) and free amino acids (FAA), γ-aminobutyric acid (GABA), pH, and lactic acid bacteria (LAB) viable counts in lentil whole samples. Table S6. Significance of analysed factors (different growing conditions of lentils, different fermentation conditions, and different durations of fermentation) and their interaction in biogenic amine (BA) lentil wholemeal concentration. Table S7. Pearson correlations between fatty acids (FA) and pH and lactic acid bacteria (LAB) viable counts in lentil wholemeal samples. Table S8. Significance of analysed factors (different growing conditions of lentils, different fermentation conditions, and different durations of fermentation) and their interaction in fatty acid (FA) lentil wholemeal composition. Table S9. Volatile compound (VC) profile (% of the total volatile compounds content) in lentil whole samples. Table S10. Pearson correlations between volatiles (VCs) and pH and lactic acid bacteria (LAB) viable counts, as well as biogenic amines (BAs) and fatty acid (FA) composition, in lentil whole samples. Table S11. Pearson correlations between volatiles and free amino acids (FAA) and γ-aminobutyric acid (GABA). Table S12. Significance of analysed factors (different growing conditions of lentils, different fermentation conditions, and different durations of fermentation) and their interaction in volatile compound (VC) lentil wholemeal composition. Figure S1. Image of the relay intercropped lentil with winter rye (LR).

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Abbreviations

9-octadecenoic acid (C18:1); α-linolenic acid (C18:3 α); analysis of variance (ANOVA); biogenic amines (BA); brightness/darkness (L*/−L*); cadaverine (CAD); cis-11-eicosenoic acid (C20:1); colony-forming units (CFU); de Man–Rogosa–Sharpe (MRS) agar; diode array detector (DAD); dry matter (d.m.); eicosanoic acid (C20:1); fatty acid methyl esters (FAME); fatty acids (FAs); folic acid (vitamin B9); Food and Agriculture Organization (FAO); Food and Drug Administration (FDA); free amino acids (FAA); γ-aminobutyric acid (GABA); gas chromatographer(ry)/mass spectrometer, gas chromatography(/ph) mass spectrometry(/ter) (GC-MS); high-performance liquid chromatography (HPLC); histamine (HIS); inductively coupled plasma mass spectrometry (ICP-MS); internal diameter (ID); internal standard (ISTD); lactic acid bacteria (LAB); lentils grown in pure stands (L); lentils grown in relay intercropped with winter rye (LR); linoleic acid (C18:2); monounsaturated fatty acids (MUFA); National Bureau of Standards (NBS); niacin (vitamin B3); no observed adverse effect level (NOAEL); non-fermented (NF); palmitic acid (C16:0); pantothenic acid (vitamin B5); phenylethylamine (PHE); polyunsaturated fatty acids (PUFA); putrescine (PUT); pyridoxine (vitamin B6); redness/greenness (a*/−a*); riboflavin (vitamin B2); saturated fatty acids (SFA); solid-phase microextraction (SPME); solid-state (SS); solid-state fermentation (SSF); spermidine (SPRMD); spermine (SPRM); standard error (SE); stearic acid (C18:0); submerged (SM); submerged fermentation (SMF); tetradecanoic acid (C14:0); thiamine (vitamin B1); tryptamine (TRIP); tyramine (TYR); United Nations (UN); United States of America (USA); volatile compounds (VCs); volume by volume (*v*/*v*); wavelength (λ); weight by weight (*w*/*w*); World Health Organization (WHO); yellowness/blueness (b*/−b*).

References

1. Karaköy, T.; Erdem, H.; Baloch, F.S.; Toklu, F.; Eker, S.; Kilian, B.; Özkan, H. Diversity of Macro- and Micronutrients in the Seeds of Lentil Landraces. *Sci. World J.* **2012**, *2012*, e710412. [CrossRef]
2. Kaale, L.D.; Siddiq, M.; Hooper, S. Lentil (*Lens culinaris* Medik.) as Nutrient-Rich and Versatile Food Legume: A Review. *Legume Sci.* **2023**, *5*, e169. [CrossRef]
3. Johnson, N.; Johnson, C.R.; Thavarajah, P.; Kumar, S.; Thavarajah, D. The Roles and Potential of Lentil Prebiotic Carbohydrates in Human and Plant Health. *Plants People Planet* **2020**, *2*, 310–319. [CrossRef]
4. Zhang, B.; Deng, Z.; Ramdath, D.D.; Tang, Y.; Chen, P.X.; Liu, R.; Liu, Q.; Tsao, R. Phenolic Profiles of 20 Canadian Lentil Cultivars and Their Contribution to Antioxidant Activity and Inhibitory Effects on α-Glucosidase and Pancreatic Lipase. *Food Chem.* **2015**, *172*, 862–872. [CrossRef]
5. Li, M.; Xia, M.; Imran, A.; de Souza, T.S.P.; Barrow, C.; Dunshea, F.; Suleria, H.A.R. Nutritional Value, Phytochemical Potential, and Biological Activities in Lentils (*Lens culinaris* Medik.): A Review. *Food Rev. Int.* **2023**, 1–31. [CrossRef]
6. Kažemėkas, O. Lęšių Veislės, "Diskiai" Ir, "Smėlinukai". *Mokslo Darb. Zemdirb.* **1999**, *65*, 171–175.
7. Jensen, E.S.; Carlsson, G.; Hauggaard-Nielsen, H. Intercropping of Grain Legumes and Cereals Improves the Use of Soil N Resources and Reduces the Requirement for Synthetic Fertilizer N: A Global-Scale Analysis. *Agron. Sustain. Dev.* **2020**, *40*, 5. [CrossRef]
8. Hauggaard-Nielsen, H.; Jørnsgaard, B.; Kinane, J.; Jensen, E.S. Grain Legume-Cereal Intercropping: The Practical Application of Diversity, Competition and Facilitation in Arable and Organic Cropping Systems. *Renew. Agric. Food Syst. Print Ed.* **2008**, *23*, 3–12. [CrossRef]
9. Zampieri, M.; Weissteiner, C.J.; Grizzetti, B.; Toreti, A.; van den Berg, M.; Dentener, F. Estimating Resilience of Crop Production Systems: From Theory to Practice. *Sci. Total Environ.* **2020**, *735*, 139378. [CrossRef] [PubMed]
10. Lamichhane, J.R.; Alletto, L.; Cong, W.-F.; Dayoub, E.; Maury, P.; Plaza-Bonilla, D.; Reckling, M.; Saia, S.; Soltani, E.; Tison, G.; et al. Relay Cropping for Sustainable Intensification of Agriculture across Temperate Regions: Crop Management Challenges and Future Research Priorities. *Field Crops Res.* **2023**, *291*, 108795. [CrossRef]
11. Joshi, M.; Timilsena, Y.; Adhikari, B. Global Production, Processing and Utilization of Lentil: A Review. *J. Integr. Agric.* **2017**, *16*, 2898–2913. [CrossRef]
12. Aryee, A.N.A.; Boye, J.I. Comparative Study of the Effects of Processing on the Nutritional, Physicochemical and Functional Properties of Lentil. *J. Food Process. Preserv.* **2017**, *41*, e12824. [CrossRef]
13. Byanju, B.; Hojilla-Evangelista, M.P.; Lamsal, B.P. Fermentation Performance and Nutritional Assessment of Physically Processed Lentil and Green Pea Flour. *J. Sci. Food Agric.* **2021**, *101*, 5792–5806. [CrossRef]
14. Torino, M.I.; Limón, R.I.; Martínez-Villaluenga, C.; Mäkinen, S.; Pihlanto, A.; Vidal-Valverde, C.; Frias, J. Antioxidant and Antihypertensive Properties of Liquid and Solid State Fermented Lentils. *Food Chem.* **2013**, *136*, 1030–1037. [CrossRef]

15. Alrosan, M.; Tan, T.-C.; Koh, W.Y.; Easa, A.M.; Gammoh, S.; Alu'datt, M.H. Overview of Fermentation Process: Structure-Function Relationship on Protein Quality and Non-Nutritive Compounds of Plant-Based Proteins and Carbohydrates. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 7677–7691. [CrossRef] [PubMed]
16. Mockus, E.; Zokaityte, E.; Starkute, V.; Klupsaite, D.; Ruibys, R.; Rocha, J.M.; Bartkevics, V.; Bartkiene, E. Influence of Different Lactic Acid Bacteria Strains and Milling Process on the Solid-State Fermented Green and Red Lentils (*Lens culinaris* L.) Properties Including Gamma-Aminobutyric Acid Formation. *Front. Nutr.* **2023**, *10*, 1118710. [CrossRef] [PubMed]
17. Liu, X.; Kokare, C. Chapter 11—Microbial Enzymes of Use in Industry. In *Biotechnology of Microbial Enzymes*; Brahmachari, G., Ed.; Academic Press: Cambridge, MA, USA, 2017; pp. 267–298. ISBN 978-0-12-803725-6.
18. Kapoor, M.; Panwar, D.; Kaira, G.S. Chapter 3—Bioprocesses for Enzyme Production Using Agro-Industrial Wastes: Technical Challenges and Commercialization Potential. In *Agro-Industrial Wastes as Feedstock for Enzyme Production*; Dhillon, G.S., Kaur, S., Eds.; Academic Press: San Diego, CA, USA, 2016; pp. 61–93. ISBN 978-0-12-802392-1.
19. López-Gómez, J.P.; Venus, J. Potential Role of Sequential Solid-State and Submerged-Liquid Fermentations in a Circular Bioeconomy. *Fermentation* **2021**, *7*, 76. [CrossRef]
20. Srivastava, N.; Srivastava, M.; Ramteke, P.W.; Mishra, P.K. Chapter 23—Solid-State Fermentation Strategy for Microbial Metabolites Production: An Overview. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Gupta, V.K., Pandey, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 345–354. ISBN 978-0-444-63504-4.
21. Singhania, R.R.; Patel, A.K.; Soccol, C.R.; Pandey, A. Recent Advances in Solid-State Fermentation. *Biochem. Eng. J.* **2009**, *44*, 13–18. [CrossRef]
22. Emkani, M.; Oliete, B.; Saurel, R. Effect of Lactic Acid Fermentation on Legume Protein Properties, a Review. *Fermentation* **2022**, *8*, 244. [CrossRef]
23. Senanayake, D.; Torley, P.J.; Chandrapala, J.; Terefe, N.S. Microbial Fermentation for Improving the Sensory, Nutritional and Functional Attributes of Legumes. *Fermentation* **2023**, *9*, 635. [CrossRef]
24. Spano, G.; Russo, P.; Lonvaud-Funel, A.; Lucas, P.; Alexandre, H.; Grandvalet, C.; Coton, E.; Coton, M.; Barnavon, L.; Bach, B.; et al. Biogenic Amines in Fermented Foods. *Eur. J. Clin. Nutr.* **2010**, *64*, S95–S100. [CrossRef]
25. Wójcik, W.; Łukasiewicz, M.; Puppel, K. Biogenic Amines: Formation, Action and Toxicity—A Review. *J. Sci. Food Agric.* **2021**, *101*, 2634–2640. [CrossRef]
26. Dhull, S.B.; Punia, S.; Kidwai, M.K.; Kaur, M.; Chawla, P.; Purewal, S.S.; Sangwan, M.; Palthania, S. Solid-State Fermentation of Lentil (*Lens culinaris* L.) with Aspergillus Awamori: Effect on Phenolic Compounds, Mineral Content, and Their Bioavailability. *Legume Sci.* **2020**, *2*, e37. [CrossRef]
27. Sánchez-García, J.; Asensio-Grau, A.; García-Hernández, J.; Heredia, A.; Andrés, A. Nutritional and Antioxidant Changes in Lentils and Quinoa through Fungal Solid-State Fermentation with Pleurotus Ostreatus. *Bioresour. Bioprocess.* **2022**, *9*, 51. [CrossRef]
28. Asensio-Grau, A.; Calvo-Lerma, J.; Heredia, A.; Andrés, A. Enhancing the Nutritional Profile and Digestibility of Lentil Flour by Solid State Fermentation with Pleurotus Ostreatus. *Food Funct.* **2020**, *11*, 7905–7912. [CrossRef]
29. Magro, A.E.A.; Silva, L.C.; Rasera, G.B.; de Castro, R.J.S. Solid-State Fermentation as an Efficient Strategy for the Biotransformation of Lentils: Enhancing Their Antioxidant and Antidiabetic Potentials. *Bioresour. Bioprocess.* **2019**, *6*, 38. [CrossRef]
30. Alrosan, M.; Tan, T.-C.; Mat Easa, A.; Gammoh, S.; Alu'datt, M.H. Effects of Fermentation on the Quality, Structure, and Nonnutritive Contents of Lentil (*Lens Culinaris*) Proteins. *J. Food Qual.* **2021**, *2021*, e5556450. [CrossRef]
31. Chen, K.; Gao, C.; Han, X.; Li, D.; Wang, H.; Lu, F. Co-Fermentation of Lentils Using Lactic Acid Bacteria and Bacillus Subtilis Natto Increases Functional and Antioxidant Components. *J. Food Sci.* **2020**, *86*, 475–483. [CrossRef] [PubMed]
32. De Pasquale, I.; Pontonio, E.; Gobbetti, M.; Rizzello, C.G. Nutritional and Functional Effects of the Lactic Acid Bacteria Fermentation on Gelatinized Legume Flours. *Int. J. Food Microbiol.* **2020**, *316*, 108426. [CrossRef]
33. Budryn, G.; Klewicka, E.; Grzelczyk, J.; Gałązka-Czarnecka, I.; Mostowski, R. Lactic Acid Fermentation of Legume Seed Sprouts as a Method of Increasing the Content of Isoflavones and Reducing Microbial Contamination. *Food Chem.* **2019**, *285*, 478–484. [CrossRef]
34. Bartkiene, E.; Lele, V.; Ruzauskas, M.; Domig, K.J.; Starkute, V.; Zavistanaviciute, P.; Bartkevics, V.; Pugajeva, I.; Klupsaite, D.; Juodeikiene, G.; et al. Lactic Acid Bacteria Isolation from Spontaneous Sourdough and Their Characterization Including Antimicrobial and Antifungal Properties Evaluation. *Microorganisms* **2020**, *8*, 64. [CrossRef]
35. Bartkiene, E.; Bartkevics, V.; Rusko, J.; Starkute, V.; Bendoraitiene, E.; Zadeike, D.; Juodeikiene, G. The Effect of Pediococcus Acidilactici and Lactobacillus Sakei on Biogenic Amines Formation and Free Amino Acid Profile in Different Lupin during Fermentation. *LWT* **2016**, *74*, 40–47. [CrossRef]
36. Bartkiene, E.; Bartkevics, V.; Pugajeva, I.; Krungleviciute, V.; Mayrhofer, S.; Domig, K. The Contribution of *P. Acidilactici*, *L. Plantarum*, and *L. Curvatus* Starters and L-(+)-Lactic Acid to the Acrylamide Content and Quality Parameters of Mixed Rye—Wheat Bread. *LWT* **2017**, *80*, 43–50. [CrossRef]
37. Cai, H.-L.; Zhu, R.-H. Determination of Dansylated Monoamine and Amino Acid Neurotransmitters and Their Metabolites in Human Plasma by Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry. *Anal. Biochem.* **2010**, *396*, 103–111. [CrossRef]
38. Ben-Gigirey, B.; Vieites Baptista de Sousa, J.M.; Villa, T.G.; Barros-Velazquez, J. Histamine and Cadaverine Production by Bacteria Isolated from Fresh and Frozen Albacore (*Thunnus alalunga*). *J. Food Prot.* **1999**, *62*, 933–939. [CrossRef]

39. Evans, J.D. *Straightforward Statistics for the Behavioral Sciences*; Thomson Brooks/Cole Publishing Co.: Belmont, CA, USA, 1996; p. xxii, 600. ISBN 0-534-23100-4.
40. Garrido-Galand, S.; Asensio-Grau, A.; Calvo-Lerma, J.; Heredia, A.; Andrés, A. The Potential of Fermentation on Nutritional and Technological Improvement of Cereal and Legume Flours: A Review. *Food Res. Int.* **2021**, *145*, 110398. [CrossRef]
41. Rocha, J.M. *Microbiological and Lipid Profiles of Broa: Contributions for the Characterization of a Traditional Portuguese Bread (Perfil Microbiologico e Lipidico da Broa: Contribuições para a Caracterização de um Pão Tradicional Português)*; School of Agriculture University of Lisbon (ISA): Lisboa, Portugal, 2011; pp. 1–708.
42. Papadimitriou, K.; Pratsinis, H.; Nebe-von-Caron, G.; Kletsas, D.; Tsakalidou, E. Acid Tolerance of Streptococcus Macedonicus as Assessed by Flow Cytometry and Single-Cell Sorting. *Appl. Environ. Microbiol.* **2007**, *73*, 465–476. [CrossRef] [PubMed]
43. Leonard, W.; Zhang, P.; Ying, D.; Adhikari, B.; Fang, Z. Fermentation Transforms the Phenolic Profiles and Bioactivities of Plant-Based Foods. *Biotechnol. Adv.* **2021**, *49*, 107763. [CrossRef] [PubMed]
44. Yang, F.; Chen, C.; Ni, D.; Yang, Y.; Tian, J.; Li, Y.; Chen, S.; Ye, X.; Wang, L. Effects of Fermentation on Bioactivity and the Composition of Polyphenols Contained in Polyphenol-Rich Foods: A Review. *Foods* **2023**, *12*, 3315. [CrossRef]
45. Rodriguez-Amaya, D.B. Natural Food Pigments and Colorants. *Curr. Opin. Food Sci.* **2016**, *7*, 20–26. [CrossRef]
46. Mapelli-Brahm, P.; Barba, F.J.; Remize, F.; Garcia, C.; Fessard, A.; Khaneghah, A.M.; Sant'Ana, A.S.; Lorenzo, J.M.; Montesano, D.; Meléndez-Martínez, A.J. The Impact of Fermentation Processes on the Production, Retention and Bioavailability of Carotenoids: An Overview. *Trends Food Sci. Technol.* **2020**, *99*, 389–401. [CrossRef]
47. Shimizu, K.; Matsuoka, Y. Feedback Regulation and Coordination of the Main Metabolism for Bacterial Growth and Metabolic Engineering for Amino Acid Fermentation. *Biotechnol. Adv.* **2022**, *55*, 107887. [CrossRef] [PubMed]
48. Toe, C.J.; Foo, H.L.; Loh, T.C.; Mohamad, R.; Abdul Rahim, R.; Idrus, Z. Extracellular Proteolytic Activity and Amino Acid Production by Lactic Acid Bacteria Isolated from Malaysian Foods. *Int. J. Mol. Sci.* **2019**, *20*, 1777. [CrossRef] [PubMed]
49. Kumar, V.; Ahluwalia, V.; Saran, S.; Kumar, J.; Patel, A.K.; Singhania, R.R. Recent Developments on Solid-State Fermentation for Production of Microbial Secondary Metabolites: Challenges and Solutions. *Bioresour. Technol.* **2021**, *323*, 124566. [CrossRef] [PubMed]
50. López-Gómez, J.P.; Manan, M.A.; Webb, C. Chapter 7—Solid-State Fermentation of Food Industry Wastes. In *Food Industry Wastes*, 2nd ed.; Kosseva, M.R., Webb, C., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 135–161, ISBN 978-0-12-817121-9.
51. Pandey, A. Solid-State Fermentation. *Biochem. Eng. J.* **2003**, *13*, 81–84. [CrossRef]
52. Barbieri, F.; Montanari, C.; Gardini, F.; Tabanelli, G. Biogenic Amine Production by Lactic Acid Bacteria: A Review. *Foods* **2019**, *8*, 17. [CrossRef] [PubMed]
53. Fernandez, M.; Zuniga, M. Amino Acid Catabolic Pathways of Lactic Acid Bacteria. *Crit. Rev. Microbiol.* **2006**, *32*, 155–183. [CrossRef] [PubMed]
54. Yogeswara, I.B.A.; Maneerat, S.; Haltrich, D. Glutamate Decarboxylase from Lactic Acid Bacteria—A Key Enzyme in GABA Synthesis. *Microorganisms* **2020**, *8*, 1923. [CrossRef] [PubMed]
55. Cui, Y.; Miao, K.; Niyaphorn, S.; Qu, X. Production of Gamma-Aminobutyric Acid from Lactic Acid Bacteria: A Systematic Review. *Int. J. Mol. Sci.* **2020**, *21*, 995. [CrossRef] [PubMed]
56. Yazgan, H.; Kuley, E.; Güven Gökmen, T.; Regenstein, J.M.; Özogul, F. The Antimicrobial Properties and Biogenic Amine Production of Lactic Acid Bacteria Isolated from Various Fermented Food Products. *J. Food Process. Preserv.* **2021**, *45*, e15085. [CrossRef]
57. Benkerroum, N. Biogenic Amines in Dairy Products: Origin, Incidence, and Control Means. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 801–826. [CrossRef]
58. Kalac, P.; Krausová, P. A Review of Dietary Polyamines: Formation, Implications for Growth and Health and Occurrence in Foods. *Food Chem.* **2005**, *90*, 219–230. [CrossRef]
59. Costa, M.P.; Rodrigues, B.L.; Frasao, B.S.; Conte-Junior, C.A. Chapter 2—Biogenic Amines as Food Quality Index and Chemical Risk for Human Consumption. In *Food Quality: Balancing Health and Disease*; Holban, A.M., Grumezescu, A.M., Eds.; Handbook of Food Bioengineering; Academic Press: Cambridge, MA, USA, 2018; pp. 75–108. ISBN 978-0-12-811442-1.
60. Naila, A.; Flint, S.; Fletcher, G.; Bremer, P.; Meerdink, G. Control of Biogenic Amines in Food—Existing and Emerging Approaches. *J. Food Sci.* **2010**, *75*, R139–R150. [CrossRef] [PubMed]
61. Paucean, A.; Moldovan, O.P.; Mureșan, V.; Socaci, S.A.; Dulf, F.V.; Alexa, E.; Man, S.M.; Mureșan, A.E.; Muste, S. Folic Acid, Minerals, Amino-Acids, Fatty Acids and Volatile Compounds of Green and Red Lentils. Folic Acid Content Optimization in Wheat-Lentils Composite Flours. *Chem. Cent. J.* **2018**, *12*, 88. [CrossRef] [PubMed]
62. Khrisanapant, P.; Kebede, B.; Leong, S.Y.; Oey, I. A Comprehensive Characterisation of Volatile and Fatty Acid Profiles of Legume Seeds. *Foods* **2019**, *8*, 651. [CrossRef] [PubMed]
63. Zaeem, M.; Nadeem, M.; Pham, T.H.; Ashiq, W.; Ali, W.; Gillani, S.S.M.; Moise, E.; Elavarthi, S.; Kavanagh, V.; Cheema, M.; et al. Corn-Soybean Intercropping Improved the Nutritional Quality of Forage Cultivated on Podzols in Boreal Climate. *Plants* **2021**, *10*, 1015. [CrossRef]
64. Zhang, B.; Deng, Z.; Tang, Y.; Chen, P.; Liu, R.; Ramdath, D.D.; Liu, Q.; Hernandez, M.; Tsao, R. Fatty Acid, Carotenoid and Tocopherol Compositions of 20 Canadian Lentil Cultivars and Synergistic Contribution to Antioxidant Activities. *Food Chem.* **2014**, *161*, 296–304. [CrossRef] [PubMed]

65. Rajendran, S.; Silcock, P.; Bremer, P. Flavour Volatiles of Fermented Vegetable and Fruit Substrates: A Review. *Molecules* **2023**, *28*, 3236. [CrossRef] [PubMed]
66. Starowicz, M.; Rostek, D.; Lenkiewicz, M.; Yaneva, T.G.; Wronkowska, M. Profile of Volatile Organic Compounds (VOCs) Produced by Selected *Lactobacillus* Strains in Buckwheat Substrates. *J. Cereal Sci.* **2023**, *109*, 103588. [CrossRef]
67. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2016**, *59*, 105–123. [CrossRef]
68. Chen, Q.; Kong, B.; Han, Q.; Xia, X.; Xu, L. The Role of Bacterial Fermentation in Lipolysis and Lipid Oxidation in Harbin Dry Sausages and Its Flavour Development. *LWT* **2017**, *77*, 389–396. [CrossRef]
69. Ardö, Y. Flavour Formation by Amino Acid Catabolism. *Biotechnol. Adv.* **2006**, *24*, 238–242. [CrossRef] [PubMed]
70. Kruis, A.J.; Bohnenkamp, A.C.; Patinios, C.; van Nuland, Y.M.; Levisson, M.; Mars, A.E.; van den Berg, C.; Kengen, S.W.M.; Weusthuis, R.A. Microbial Production of Short and Medium Chain Esters: Enzymes, Pathways, and Applications. *Biotechnol. Adv.* **2019**, *37*, 107407. [CrossRef] [PubMed]
71. Collins, Y.F.; McSweeney, P.L.H.; Wilkinson, M.G. Lipolysis and Free Fatty Acid Catabolism in Cheese: A Review of Current Knowledge. *Int. Dairy J.* **2003**, *13*, 841–866. [CrossRef]
72. Benayad, A.; Aboussaleh, Y. Mineral Composition of Lentils: Physiological Functions, Antinutritional Effects, and Bioavailability Enhancement. *J. Food Qual.* **2021**, *2021*, e5515654. [CrossRef]
73. Türk, Z. Effects of Different Harvesting Times on Protein Content and Mineral Nutrient Values of Some Lentil (Lens Culinaris) Cultivars Seeds. *J. Plant Nutr.* **2020**, *43*, 563–577. [CrossRef]
74. Gharibzahedi, S.M.T.; Jafari, S.M. The Importance of Minerals in Human Nutrition: Bioavailability, Food Fortification, Processing Effects and Nanoencapsulation. *Trends Food Sci. Technol.* **2017**, *62*, 119–132. [CrossRef]
75. Gupta, U.C.; Gupta, S.C. Sources and Deficiency Diseases of Mineral Nutrients in Human Health and Nutrition: A Review. *Pedosphere* **2014**, *24*, 13–38. [CrossRef]
76. Ray, H.; Bett, K.; Tar'an, B.; Vandenberg, A.; Thavarajah, D.; Warkentin, T. Mineral Micronutrient Content of Cultivars of Field Pea, Chickpea, Common Bean, and Lentil Grown in Saskatchewan, Canada. *Crop Sci.* **2014**, *54*, 1698–1708. [CrossRef]
77. Tziouvalekas, M.; Tigka, E.; Kargiotidou, A.; Beslemes, D.; Irakli, M.; Pankou, C.; Arabatzi, P.; Aggelakoudi, M.; Tokatlidis, I.; Mavromatis, A.; et al. Seed Yield, Crude Protein and Mineral Nutrients of Lentil Genotypes Evaluated across Diverse Environments under Organic and Conventional Farming. *Plants* **2022**, *11*, 3328. [CrossRef]
78. Cabrera, C.; Lloris, F.; Giménez, R.; Olalla, M.; López, M.C. Mineral Content in Legumes and Nuts: Contribution to the Spanish Dietary Intake. *Sci. Total Environ.* **2003**, *308*, 1–14. [CrossRef]
79. Johnson, S.E.; Lauren, J.G.; Welch, R.M.; Duxbury, J.M. A comparison of the effects of micronutrients seed priming and soil fertilization on the mineral nutrition of chickpea (Cicer Arietinum), lentil (Lens Culinaris), rice (Oryza Sativa) and wheat (Triticum Aestivum) in Nepal. *Exp. Agric.* **2005**, *41*, 427–448. [CrossRef]
80. Chen, C.; Etemadi, F.; Franck, W.; Franck, S.; Abdelhamid, M.T.; Ahmadi, J.; Mohammed, Y.A.; Lamb, P.; Miller, J.; Carr, P.M.; et al. Evaluation of Environment and Cultivar Impact on Lentil Protein, Starch, Mineral Nutrients, and Yield. *Crop Sci.* **2022**, *62*, 893–905. [CrossRef]
81. Bansal, R.; Bana, R.S.; Dikshit, H.K.; Srivastava, H.; Priya, S.; Kumar, S.; Aski, M.S.; Kumari, N.K.P.; Gupta, S.; Kumar, S. Seed Nutritional Quality in Lentil (*Lens culinaris*) under Different Moisture Regimes. *Front. Nutr.* **2023**, *10*, 1141040. [CrossRef] [PubMed]
82. Kumar, J.; Thavarajah, D.; Kumar, S.; Sarker, A.; Singh, N.P. Analysis of Genetic Variability and Genotype × Environment Interactions for Iron and Zinc Content among Diverse Genotypes of Lentil. *J. Food Sci. Technol.* **2018**, *55*, 3592–3605. [CrossRef] [PubMed]
83. Gunes, A.; Inal, A.; Kadioglu, Y.K. Determination of Mineral Element Concentrations in Wheat, Sunflower, Chickpea and Lentil Cultivars in Response to P Fertilization by Polarized Energy Dispersive X-Ray Fluorescence. *X-Ray Spectrom.* **2009**, *38*, 451–462. [CrossRef]
84. Cilla, A.; Barberá, R.; López-García, G.; Blanco-Morales, V.; Alegría, A.; Garcia-Llatas, G. 7—Impact of Processing on Mineral Bioaccessibility/Bioavailability. In *Innovative Thermal and Non-Thermal Processing, Bioaccessibility and Bioavailability of Nutrients and Bioactive Compounds*; Barba, F.J., Saraiva, J.M.A., Cravotto, G., Lorenzo, J.M., Eds.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing: Thorston, UK, 2019; pp. 209–239. ISBN 978-0-12-814174-8.
85. Duraiswamy, A.; Sneha, A.N.M.; Jebakani, K.S.; Selvaraj, S.; Pramitha, J.L.; Selvaraj, R.; Petchiammal, K.I.; Kather Sheriff, S.; Thinakaran, J.; Kumar, P.R. Genetic Manipulation of Anti-Nutritional Factors in Major Crops for a Sustainable Diet in Future. *Front. Plant Sci.* **2023**, *13*, 1070398. [CrossRef] [PubMed]
86. Adebo, J.A.; Njobeh, P.B.; Gbashi, S.; Oyedeji, A.B.; Ogundele, O.M.; Oyeyinka, S.A.; Adebo, O.A. Fermentation of Cereals and Legumes: Impact on Nutritional Constituents and Nutrient Bioavailability. *Fermentation* **2022**, *8*, 63. [CrossRef]
87. Samtiya, M.; Aluko, R.E.; Puniya, A.K.; Dhewa, T. Enhancing Micronutrients Bioavailability through Fermentation of Plant-Based Foods: A Concise Review. *Fermentation* **2021**, *7*, 63. [CrossRef]

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Comparative metabolomic assessment of bioconverted traditional and black wheat via the in vitro piglet digestion model

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Colored wheat is gaining significant attention in the food and feed industry due to its components' potential health benefits. As weaning piglets experience major discomfort in their early life, the quality and bioavailability of the feed nutrients become crucial for their survival. Fermentation of the feed aims to be a cheap and efficient stage of the feed production, as it is able to overcome major shortcomings of the cereals. The aim of this study was to investigate the differences in metabolites between traditional (variety "Herkus") and black (variety "DS8558-1") (DS) wheat cereals (both non-pre-treated and fermented with *Lactiplantibacillus plantarum* strain) using an in vitro swine digestion model with weaning piglets' faecal microbiota. The following in vitro digestion and fermentation parameters were evaluated: free and total amino acids concentration in the digesta; total amino acids in the undigested residue and bioconverted/control wheat wholemeal substrate; short-chain fatty acids (SCFAs), volatile organic compounds (VOCs), free amino acids (FAAs), biogenic amines (BAs) and other small non-volatile metabolites in the in vitro fermentation samples. The in vitro digestibility and fermentability were significantly altered by bioconversion, while the substrate factor was significant only for the in vitro fermentability. The in vitro digestion of some of the non-essential and essential (valine, phenylalanine, leucine/isoleucine, histidine) AAs was significantly affected by both factors. Lysine digestibility was affected only by bioconversion. The bioconversion significantly affected the content of SCFAs, such as 2-methylpropanoic, butanoic, 3-methylbutanoic and pentanoic acids in in vitro fermentation (IVF) samples. The substrate was significant for all the analysed SCFAs, except propanoic and hexanoic acids. Benzenacetic, dihydrocinnamic and 4-hydroxyhydrocinnamic acids were significantly upregulated in DS IVF samples. *P*-cresol was upregulated in the bioconverted IVF samples. Both of the factors were significant for *p*-cresol concentrations in the IVF samples. The γ-aminobutyric acid (GABA) production in the IVF samples was significantly affected by both factors. Lactate concentrations in the IVF samples were affected only by the substrate factor. These results highlight the significance of bioconversion for the overall digestibility of wheat wholemeal substrates as well as changes in the production of biologically relevant metabolites for piglets.

Keywords In vitro digestion, Metabolomics, Volatile organic compounds, Colored wheat, In vitro fermentation, Piglet microbiota

Abbreviations

DS	DS8558-1
SCFA	short-chain fatty acid
VOC	volatile organic compound

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FAA	free amino acid
BA	biogenic amine
IVF	in vitro fermentation
SSF	solid state fermentation
LAB	lactic acid bacteria
GRAS	"generally recognised as safe"
QPS	"qualified presumption of safety"
GABA	γ-aminobutyric acid
MRS	Man–Rogosa–Sharpe broth
SPME	solid-phase microextraction
BSTFA	N, O-bis(trimethylsilyl)trifluoroacetamide)
ANOVA	analysis of variance
Tukey HSD	Tukey's honest significant difference
PCA	principle component analysis
PLS-DA	partial least square discriminant analysis
VIP	variable importance in projection
DS_F	DS8558-1 sample fermented with *Lactiplantibacillus plantarum*
DS_NF	DS8558-1 control sample
Herkus_F	Herkus sample fermented with *Lactiplantibacillus plantarum*
Herkus_NF	Herkus control sample
AA	amino acid
IVD	in vitro digestion
TBC	total bacteria count

Wheat continues to be a major cereal grain for human and animal consumption, as various products and byproducts, such as wheat bran, wheat middlings, millrun or red dogs, are used in livestock feeding[1–3]. Coloured wheat is gaining significant attention in nutrition, as anthocyanins, which lie inside the pericarp and aleurone layers, provide additional antioxidative and antimicrobial properties, help in the prevention of cardiovascular diseases, inflammation, diabetes, obesity and improve cognitive functionality[4–7]. Moreover, it has been established that polyphenols, including anthocyanins, can modulate bacterial communities in the gut[8]. Anthocyanins can also impair the digestibility of starch in the food matrix, which can reliably reduce the blood glucose levels of the host[9]. Often, anthocyanins are associated with ocular improvement by myopia inhibition, reduction of eye fatigue, improving vision adaptation and increasing ocular blood flow, which prevents retinal degeneration[6,10]. The majority of these anthocyanins are various derivatives of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, which include singular or multiple glycosylations and acylations[6,11]. Many in vitro studies report significant degradation of anthocyanins in the food matrix, mostly in the intestinal digestion phase[12–14]. As the gastric digestion phase allows rapid absorption of anthocyanins without significant hydrolysis due to favourable conditions, a serious degradation of those compounds in the intestinal phase is observed due to a relatively high pH and high expression of metabolic enzymes that degrade anthocyanins[14,15].

Also, wheat cereal contains various antinutritional factors, such as inhibitors of protease enzymes, tannins and phytates that affect the digestibility of feed, intestinal mucus secretion, and bioavailability of important minerals[16–18]. Evidently, fermentation helps to solve these issues by reducing antinutritional factor concentrations, improving digestion via breakdown of the feed matrix, and providing probiotics for the host's gut[16,19,20]. Solid state fermentation (SSF) is proven to be an attractive method for bioprocessing feed, as it allows the use of any nutrient-rich substrate, doesn't require sterile conditions, demands low consumption of water, allows for easy recovery of products and requires smaller and less complex equipment for processing[21,22].

Lactic acid bacteria (LAB) are a diverse group of gram-positive microorganisms that are widely applied in industrial processing, as they are equipped with a wide range of carbohydrate-degrading enzymes, can improve organoleptic properties, produce a wide range of bioactive metabolites, suppress food spoilage, and improve shelf-life[23,24]. *Lactiplantibacillus plantarum* is able to ferment a wide range of carbohydrates, inhibit pathogens, tolerate very low pH, and produce significant amounts of bioactive compounds, such as neurotransmitters and bacteriocins[25–28]. *Lp. plantarum* is recognised as "generally recognised as safe" (GRAS) by the Food and Drug Administration and has the status of "qualified presumption of safety" (QPS) by the European Food Safety Authorities[24].

As genetic selection and modern intensive farming systems significantly increased the average litter size of sows, piglets nowadays suffer from weaning stress, which is associated with certain changes in microbiota as piglets go through a gradient change from liquid to solid feed[29–31]. As such, dietary changes drastically modify the gut microbiome, which can eventually lead to impaired digestion, post-weaning diarrhea, reduced gut mucosal integrity, or even increase the risk of gastrointestinal tract diseases[32–34]. Wheat-based products are widely used in swine feeding due to their high starch content and noteworthy abundance of essential amino acids[3].

The aim of this study was to investigate the differences in metabolites (amino acids (AAs), GABA, biogenic amines (BAs), volatile organic compounds (VOCs), short-chain fatty acids (SCFAs) and other metabolites) between traditional (variety "Herkus") and black (variety "DS8558-1") wheat cereals (both non-pre-treated and fermented with *Lactiplantibacillus plantarum* strain) using an in vitro swine digestion model with weaning piglets' faecal microbiota.

Materials and methods

Wheat cereals and lactic acid bacteria strain used for fermentation

The winter wheat (*Triticum aestivum* L.) cereal varieties 'Herkus DS' (traditional wheat), and new bred varieties DS8558-1 (black wheat) were provided by the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (LAMMC, Akademija, Kėdainiai distr., Lithuania). A detailed description of the growing conditions is described in the supplementary methods file.

The LAB strain *Lactiplantibacillus plantarum* was obtained from the Lithuanian University of Health Sciences (Kaunas, Lithuania) and used for the wholemeal wheat flour fermentation. The *Lp. plantarum* characteristics are described by Bartkiene et al.[35]. Before the experiments, the LAB strains stored at −80 °C (Microbank system, Pro-Lab Diagnostics, Birkenhead, UK) were activated and multiplied in de Man–Rogosa–Sharpe (MRS) broth (Oxoid Ltd., Hampshire, UK) at (30 ± 2) °C for 24 h, under aerobiosis, before use in the wholemeal flour fermentation. Wheat cereal was milled with a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particle size was 1–2 mm. The wholemeal wheat flours, water (1:1 w/w) and a suspension of LAB strain (3% of dry matter of the wholemeal flour) containing 8.7 $\log_{10}$ CFU/mL were fermented at (30 ± 2) °C for 24 h. The non-fermented wholemeal samples were analysed as a control. The experimental scheme is provided in Fig. 1.

Evaluation of acidity and microbiological characteristics of wheat wholemeal and the in vitro fermentation samples

The analysis of total titratable acidity, pH and microbiological characteristics was performed directly after fermentation, while the rest of the samples were vacuum packed and frozen at − 18 °C until further analysis of other compounds. Detailed protocol is provided in the supplementary methods file.

In vitro digestion protocol of wheat wholemeal samples

The procedure of the in vitro digestion protocol was based on the INFOGEST static in vitro digestion protocol with some modifications[36]. Full procedure with modifications is described in the supplementary methods file. The resulting undigested residues were filtered, washed with ethanol and acetone, and dried in a laboratory oven until a stable mass was achieved.

In vitro fermentation protocol of the in vitro digestion residues

The residue from the in vitro digestion phase was subsequently subjected to colonic fermentation, using an inoculum of fresh faecal samples from healthy piglet donors of 42 days of age. The protocol used for the experiment was based on the protocol described in Burillo et al. (2021)[37] with some modifications. Detailed procedure is described in the supplementary methods file. Resulting unfermented residues were filtered, washed with water, and dried in a laboratory oven until a stable mass was achieved.

2.5 Free and Hydrolysed Amino Acid Analysis of the Bioconverted/Control Wholemeal, Digested Wholemeal Supernatant and Residue Samples.

Analysis protocol of free amino acids and hydrolysates of corresponding samples is described in the supplementary methods file. In brief, free amino acids were extracted with 0.1 M HCl solution, while the hydrolysis of samples was carried out using 6 M HCl solution, containing 0.1% phenol, at 110 ℃ for 23 h. The derivatization and chromatographic analysis protocol were carried out according to Hua-Lin Cai et al. (2010)[38] with some modifications.

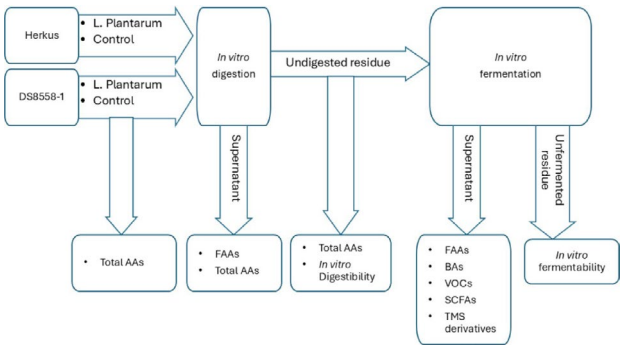

Fig. 1. Schematic representation of the experiment. AA - amino acid, FAA - free amino acid, BA - biogenic amine, VOC - volatile organic compound, SCFA - short-chain fatty acid, TMS - trimethylsilyl.

Volatile organic acid analysis of the in vitro fermentation samples

The VOCs of the IVF samples were analysed using gas chromatography–mass spectrometry. A solid-phase microextraction (SPME) device with Stableflex™ fibre, coated with a 50 µm-ϕ Carboxen™- PDMS layer (Supelco, USA), was used for headspace sampling. The prepared samples were analysed with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph and mass spectrometer. A detailed description of the analysis is provided in the supplementary methods file.

Analysis of free amino acids and biogenic amines in the in vitro fermentation samples

Concentrations of amino acids were determined using the GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph with a mass spectrometer. Samples were diluted and derivatized using isobutylchloroformate. A detailed description is provided in the supplementary methods file.

Analysis of short-chain fatty acids in the in vitro fermentation samples

For SCFA analysis in IVF samples, 100 µL the of sample was spiked with 100 µL 90 mM 3-methylvaleric acid and diluted to 1 mL with 0.1 M HCl solution. The resulting mixture was centrifuged at 13,200 RPM for 5 min, and the resulting supernatant was used for the analysis. The prepared samples were analysed with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph and mass spectrometer. The following conditions were used for analysis: injector temperature 150 °C; injection volume 0.2 µL; ion source temperature 200 °C; and interface temperature 230 °C. The mass spectrometer was operating in single ion monitoring mode. Helium was used as a carrier gas at a 0.86 mL/min flow-rate. For the separation of VOCs, Stabilwax-Da column (Restek, USA) (length 30 m, coating thickness 0.25 µm-ϕ, inner diameter of 0.25 mm-ϕ) was used. The temperature gradient was programmed from starting at 100 °C (0.5 min hold) to 200 °C (5 °C/min, 3 min hold) up to 250 °C (15°/min, 3 min hold).

Untargeted analysis of small non-volatile and semi volatile metabolites in the in vitro fermentation samples

Untargeted analysis of small non-volatile and semi-volatile metabolites was accomplished by diluting samples with methanol and performing derivatization using methoxyamine hydrochloride in pyridine and subsequent silylation with *N*, *O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% of trimethylchlorosilane. The prepared samples were analysed with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph and mass spectrometer. A detailed protocol is provided in the supplementary methods file.

Statistical analysis

Microbiological and physicochemical results were expressed as the mean ($n = 3$) ± standard deviations. Statistical calculations were carried out using R statistical programming software packages. In order to evaluate the effects of the different factors, the data were analysed using analysis of variance (ANOVA), and Tukey HSD (Tukey's honest significant difference) post hoc test was conducted to detect differences among sample groups. To determine the normality and homoskedasticity, the Shapiro–Wilk test and Levene test were performed, using "stats" (version 4.4.2) and "car" (version 3.1-3.1) packages. The bioconversion (fermented/nonfermented) and substrate (Herkus/DS) were chosen for factor analysis. The linear Pearson's correlation coefficients were calculated and visualized using "psych" (version 2.5.6) and "corrplot" (version 0.95) packages. The results were recognized as statistically significant at $p < 0.05$. The heatmap visualization and analysis were carried out using the R statistical programming software package "ComplexHeatmap" (version 2.25.2). The principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and variable importance in projection (VIP) were carried out using the "mixOmics" package (version 6.26.0). Volcano plot analysis and visualizations were carried out using the "EnhancedVolcano" package (version 1.24.0). Missing values for the volcano plot analysis were filled using the half minimum method.

Results

Acidity and microbiological characteristics of wheat wholemeal samples

The results of the acidity and microbiological characteristics are presented in Table 1. The LAB count in fermented wheat wholemeal samples (Herkus_F and DS_F) was significantly higher, while the pH was significantly lower than in control samples (Herkus_NF and DS_NF). Moreover, titratable acidity was significantly higher in

Measurement Sample	pH	TTA°	LAB Count, $\log_{10}$ CFU/g
DS_F	3.943 ± 0.09 c	9.067 ± 0.153 b	8.503 ± 0.015 a
DS_NF	5.643 ± 0.04 b	2.383 ± 0.076 c	4.407 ± 0.035 b
Herkus_F	3.950 ± 0.03 c	9.900 ± 0.10 a	8.483 ± 0.07 a
Herkus_NF	5.943 ± 0.045 a	1.917 ± 0.076 d	4.423 ± 0.042 b

Table 1. LAB count, pH and titratable acidity of wheat wholemeal samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, TTA° - total titratable acidity, LAB – lactic acid bacteria. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

fermented wheat wholemeal samples. In addition, Herkus_F titratable acidity was the highest of all sample groups.

Total amino acid composition of wheat wholemeal samples

The results of the total AA composition of wheat wholemeal samples are presented in Table 2. Overall, most AAs and total AA concentrations were not significantly different between fermented samples and corresponding control samples. Leucine/isoleucine was significantly higher in Herkus_F samples in comparison to Herkus_NF. DS samples contained significantly higher concentrations of arginine (16.5%), glutamic acid (38.9%), threonine (37.5%), glycine (29.3%), proline (49.0%), phenylalanine (45.4%), leucine/isoleucine (41.5%), cystine (31.1%), and total AA (35.7%), in comparison to Herkus sample groups. The bioconversion was a significant factor for leucine/isoleucine, cystine, and lysine concentrations, while the substrate was a significant factor only for leucine/isoleucine (supplementary file results Table 1). Moreover, the bioconversion and substrate factor interaction was significant for all amino acids (supplementary file results Table 1).

Total and free amino acids in the in vitro digestion samples

Table 3 describes the analysis results of the FAAs in the in vitro digestion (IVD) samples. The substrate-containing samples (DS_F, DS_NF, Herkus_F, Herkus_NF) showed significantly higher concentrations of arginine (51.2%), glutamine (82.2%), GABA (84.2%), proline (55.6%), alanine (38.3%), threonine (37.2%), valine (51.5%), methionine (66.6%), phenylalanine (57.5%), leucine/isoleucine (53.5%), histidine (45.5%), and total AA (46.5%). Only the fermented samples contained significantly higher amounts of glycine (20.3%), in comparison to the blank IVD samples. Lysine concentrations were significantly higher in all the substrate IVD samples, except DS_NF, in comparison to the blank IVD samples. Glutamic acid was significantly higher only in the Herkus_F samples. The fermented sample groups contained significantly higher concentrations of GABA (90.0%) and proline (124.5%), and significantly lower concentrations of glutamine (15.6%), in comparison to the nonfermented sample groups. The bioconversion was a significant factor for glutamine, glutamic acid, glycine, GABA, and proline, while the substrate was significant for glutamine, GABA, proline, tryptophan, and cystine (supplementary file results Table 2).

The results of total AA in IVD samples are presented in Table 4. The DS samples contained significantly higher concentrations of leucine/isoleucine (26.8%) and tyrosine (36.1%), in comparison to the Herkus sample groups. The bioconversion was a significant factor only for concentrations of glycine, while the substrate was a significant factor for alanine, glutamic acid, threonine, proline, valine, methionine, phenylalanine, leucine/isoleucine, tyrosine, and the total AAs in the IVD samples (supplementary file results Table 3).

The total AA analysis results of the undigested IVD residue hydrolysates in the undigested IVD residues are presented in Table 5. Significantly higher concentrations of threonine were found in the fermented sample groups, in comparison to the corresponding control sample groups. While glutamic acid (37.2%), glycine (28.6%), alanine (40.0%), proline (43.3%) and total AA (32.5%) concentrations were significantly higher in the Herkus_F samples, in comparison to the Herkus_NF samples. The bioconversion factor was significant for all amino acid concentrations, except serine, valine, phenylalanine, and leucine/isoleucine, while the substrate was significant for glutamic and aspartic acids, threonine, proline, histidine and total AAs (supplementary file results

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	0.406 ± 0.021 a	0.408 ± 0.001 a	0.348 ± 0.014 b	0.351 ± 0.0003 b
Glutamic acid	2.539 ± 0.024 a	2.616 ± 0.08 a	1.820 ± 0.11 b	1.892 ± 0.067 b
Serine	0.354 ± 0.029 a	0.363 ± 0.004 a	0.291 ± 0.043 a	0.288 ± 0.009 a
Aspartic acid	0.313 ± 0.028 a	0.309 ± 0.004 a	0.259 ± 0.035 a	0.258 ± 0.01 a
Threonine	0.225 ± 0.007 a	0.222 ± 0.001 a	0.167 ± 0.001 b	0.158 ± 0.008 b
Glycine	0.287 ± 0.017 a	0.300 ± 0.004 a	0.221 ± 0.017 b	0.233 ± 0.009 b
Alanine	0.281 ± 0.019 a	0.268 ± 0.018 ab	0.227 ± 0.004 ab	0.217 ± 0.01 b
Proline	1.657 ± 0.032 a	1.746 ± 0.133 a	1.166 ± 0.028 b	1.118 ± 0.035 b
Valine	0.285 ± 0.018 a	0.298 ± 0.022 a	0.231 ± 0.009 a	0.224 ± 0.024 a
Methionine	0.068 ± 0.01 a	0.074 ± 0.011 a	0.043 ± 0.005 a	0.054 ± 0.008 a
Phenylalanine	0.411 ± 0.009 a	0.415 ± 0.001 a	0.312 ± 0.039 b	0.256 ± 0.013 b
Leucine/Isoleucine	0.961 ± 0.01 a	0.951 ± 0.039 a	0.741 ± 0.01 b	0.610 ± 0.045 c
Cystine	0.100 ± 0.002 a	0.115 ± 0.009 a	0.072 ± 0.008 b	0.092 ± 0.001 ab
Lysine	0.219 ± 0.005 a	0.207 ± 0.007 a	0.197 ± 0.013 ab	0.164 ± 0.007 b
Histidine	0.158 ± 0.0004 a	0.154 ± 0.008 a	0.128 ± 0.011 ab	0.110 ± 0.015 b
Tyrosine	0.305 ± 0.005 ab	0.325 ± 0.027 a	0.268 ± 0.003 ab	0.265 ± 0.002 b
Total amino acids	8.569 ± 0.162 a	8.771 ± 0.204 a	6.492 ± 0.161 b	6.289 ± 0.149 b

Table 2. Total amino acids (g/100 g) in wheat wholemeal samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results ($n = 2$) are statistically significant when $p < 0.05$.

Sample	Blank	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	256.07 ± 45.47 b	516.38 ± 21.86 a	555.04 ± 52.03 a	498.5 ± 38.1 a	511.08 ± 50.52 a
Glutamine	125.42 ± 14.98 d	620.58 ± 38.21 bc	743.76 ± 33.13 a	550.15 ± 44.78 c	643.03 ± 12.1 b
Asparagine	91.68 ± 10.66 a	108.02 ± 17.88 a	119.33 ± 15.03 a	107.33 ± 14.43 a	116.27 ± 4.34 a
Glutamic acid	148.27 ± 20.21 b	179.17 ± 31.51 ab	154.32 ± 14.26 ab	200.29 ± 6.42 a	176.21 ± 6.96 ab
Serine	86.67 ± 19.04 b	105.98 ± 16.73 ab	115.58 ± 17.1 ab	121.84 ± 7.11 ab	131.76 ± 12.31 a
Aspartic acid	80.78 ± 15.16 a	100.41 ± 13.68 a	96.32 ± 4.46 a	97.8 ± 25.32 a	92.25 ± 27.5 a
Threonine	75.48 ± 15.36 b	113.36 ± 1.05 a	126.39 ± 3.07 a	111.17 ± 12.71 a	119.71 ± 14.81 a
Glycine	119.53 ± 8.71 b	142.96 ± 9.39 a	131.19 ± 5.11 ab	144.6 ± 6.3 a	131.21 ± 5.55 ab
Alanine	107.99 ± 10.55 b	182.25 ± 17.75 a	170.87 ± 13.84 a	188.12 ± 6.62 a	169.92 ± 11.81 a
GABA	2.35 ± 0.38 d	20.04 ± 2.61 b	9.84 ± 0.97 c	30.31 ± 2.13 a	16.67 ± 0.75 b
Proline	5.23 ± 0.53 d	43.18 ± 2.28 a	19.75 ± 0.74 c	37.67 ± 1.21 b	16.27 ± 1.16 c
Tryptophan	109.84 ± 19.42 b	139.03 ± 22.56 ab	139.77 ± 20.77 ab	177.04 ± 11.48 a	180.3 ± 10.41 a
Valine	111.74 ± 21.08 b	194.45 ± 10.56 a	216.43 ± 19.32 a	205.53 ± 27.18 a	231.06 ± 8.66 a
Methionine	29.61 ± 7.21 b	90.62 ± 9.74 a	88.88 ± 19.66 a	88.64 ± 13.94 a	91.62 ± 1.87 a
Phenylalanine	146.36 ± 11 b	361.03 ± 68.06 a	371.05 ± 75.86 a	341.43 ± 16.29 a	341.82 ± 21.89 a
Leucine/Isoleucine	368.89 ± 36.36 b	811.09 ± 143.11 a	770.37 ± 68.86 a	778.27 ± 80.87 a	849.98 ± 8.59 a
Cystine	26.9 ± 6.47 a	22.49 ± 1.24 a	24.76 ± 2.67 a	27.53 ± 3.04 a	29.19 ± 0.99 a
Lysine	275.72 ± 27.84 b	389.8 ± 55.09 a	372.03 ± 58.1 ab	390.22 ± 16.74 a	385.61 ± 15.83 a
Histidine	64.39 ± 8.3 b	108.36 ± 17.97 a	116.67 ± 15.02 a	109.4 ± 3.97 a	120.37 ± 7.66 a
Tyrosine	225.97 ± 26.93 a	344.35 ± 62.79 a	323.46 ± 65.94 a	253.47 ± 34.53 a	302.83 ± 55.96 a
Total AAs	2458.9 ± 304.92 b	4593.52 ± 492.63 a	4665.82 ± 360.1 a	4459.33 ± 176.09 a	4657.16 ± 93.63 a

Table 3. Free amino acids concentrations (mg/L) in the in vitro digestion samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, GABA – γ-aminobutyric acid. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Sample	Blank	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	409.06 ± 7.97 b	885.5 ± 14.4 a	916.4 ± 50.8 a	847.6 ± 116.5 a	718 ± 84.8 a
Glutamic acid	739.93 ± 54.82 b	3538.6 ± 93.7 a	3544.2 ± 257.1 a	2901.4 ± 289.8 a	2882.2 ± 363.5 a
Serine	405.67 ± 39.43 b	828.8 ± 80.9 a	821.8 ± 125.8 a	731.2 ± 89.9 a	820.3 ± 131.6 a
Aspartic acid	770.47 ± 1.61 b	1130.7 ± 92.7 a	1074.8 ± 75.4 a	1066.7 ± 90.3 a	1073 ± 127.9 a
Threonine	294.74 ± 32.06 b	594.4 ± 28 a	555.9 ± 33.6 a	521 ± 50.7 a	517.5 ± 9.7 a
Glycine	531.04 ± 9.81 c	904.6 ± 46 a	850.8 ± 45.4 ab	863.3 ± 63.9 ab	780.7 ± 11.9 b
Alanine	284.86 ± 9.84 b	580.3 ± 34.6 a	582.4 ± 21.1 a	565.4 ± 54.9 a	524.4 ± 32.4 a
Proline	557.23 ± 84.24 c	2633.8 ± 71.6 ab	2786.5 ± 147.3 a	1953.2 ± 158.1 ab	2009 ± 44.7 ab
Valine	323.4 ± 41.59 b	801.1 ± 77.6 a	776.2 ± 29.8 a	657.8 ± 58.6 a	697.4 ± 69.8 a
Methionine	46.17 ± 14.99 b	182.8 ± 2.9 a	178.4 ± 11.2 a	131.7 ± 35.5 a	133.0 ± 10.0 a
Phenylalanine	238.39 ± 30.83 d	699.8 ± 18 ab	757.8 ± 79.5 a	554.3 ± 64.9 c	596.9 ± 29.5 bc
Leucine/Isoleucine	677.95 ± 17.53 d	1813.9 ± 73.1 ab	1910.7 ± 205.9 a	1458 ± 131.1 c	1479.8 ± 40.4 bc
Cystine	128.95 ± 17.88 b	238.2 ± 7.5 a	237.4 ± 10.2 a	240.2 ± 19.8 a	225.5 ± 22.3 a
Lysine	383.14 ± 41.34 b	642.3 ± 40.4 a	693.5 ± 55.4 a	666.1 ± 25.3 a	664.7 ± 51.1 a
Histidine	126.63 ± 5.06 b	345.1 ± 16.9 a	355.6 ± 35.8 a	306.9 ± 31.8 a	326 ± 31.4 a
Tyrosine	359.5 ± 28.31 c	771.7 ± 28.9 a	789.9 ± 22.8 a	506 ± 109 bc	641.2 ± 81.6 ab
Total AAs	6304.3 ± 436.89 c	16667.3 ± 244.8 a	16893.7 ± 586.7 a	14010.4 ± 1183.7 b	14156.7 ± 886.7 b

Table 4. Total amino acids (mg/L) in the in vitro digestion samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
Arginine	0.524 ± 0.036 a	0.527 ± 0.029 a	0.549 ± 0.039 a	0.420 ± 0.025 b
Glutamic acid	1.452 ± 0.128 a	1.33 ± 0.065 a	1.332 ± 0.086 a	0.971 ± 0.144 b
Serine	0.495 ± 0.114 a	0.415 ± 0.035 a	0.430 ± 0.049 a	0.337 ± 0.051 a
Aspartic acid	0.931 ± 0.136 a	0.677 ± 0.038 b	0.782 ± 0.089 ab	0.560 ± 0.076 b
Threonine	0.333 ± 0.036 a	0.216 ± 0.02 bc	0.241 ± 0.007 b	0.154 ± 0.029 c
Glycine	0.493 ± 0.039 ab	0.435 ± 0.046 b	0.526 ± 0.018 a	0.409 ± 0.028 b
Alanine	0.432 ± 0.019 a	0.345 ± 0.023 ab	0.434 ± 0.054 a	0.310 ± 0.042 b
Proline	0.960 ± 0.045 a	0.880 ± 0.008 a	0.934 ± 0.052 a	0.652 ± 0.058 b
Valine	0.429 ± 0.036 a	0.390 ± 0.033 a	0.406 ± 0.051 a	0.390 ± 0.046 a
Methionine	ND	ND	ND	ND
Phenylalanine	0.361 ± 0.026 a	0.322 ± 0.024 a	0.336 ± 0.059 a	0.312 ± 0.029 a
Leucine/Isoleucine	0.860 ± 0.078 a	0.746 ± 0.065 a	0.850 ± 0.19 a	0.684 ± 0.032 a
Cystine	ND	ND	ND	ND
Lysine	0.502 ± 0.084 a	0.392 ± 0.031 ab	0.437 ± 0.031 ab	0.331 ± 0.051 b
Histidine	0.126 ± 0.026 a	0.092 ± 0.011 a	0.131 ± 0.018 a	ND b
Tyrosine	0.572 ± 0.017 a	0.507 ± 0.058 a	0.597 ± 0.047 a	0.503 ± 0.044 a
Total AA	8.477 ± 0.146 a	7.274 ± 0.182 ab	7.984 ± 0.728 a	6.025 ± 0.635 b

Table 5. Total amino acids (g/100 g) in the in vitro digestion undigested samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, ND – not detected. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Sample	DS_F	DS_NF	Herkus_F	Herkus_NF
In vitro digestibility, % DM	83.91 ± 0.49 a	78.79 ± 0.65 b	84.79 ± 0.53 a	77.59 ± 0.49 b
Total AA digested, %	91.88 ± 0.18 a	91.07 ± 0.49 ab	90.57 ± 0.61 ab	88.96 ± 1.41 b
In vitro fermentability, % DM	42.70 ± 0.24 c	48.11 ± 1.27 b	49.56 ± 1.60 b	57.51 ± 0.83 a
Total in vitro digestibility, % DM	90.78 ± 0.31 b	89.00 ± 0.22 c	92.33 ± 0.25 a	90.48 ± 0.21 b

Table 6. In vitro digestibility and fermentability of wheat wholemeal samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, AA – amino acids, DM – dry matter. a-c Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

Table 4). The interaction of the factors was significant for arginine, proline, and histidine concentrations in the IVD residue samples.

In vitro digestibility and fermentability parameters of wheat wholemeal samples

Table 6 contains digestibility and fermentability parameters of the IVD/IVF samples. The in vitro digestibility in the fermented samples was significantly higher than in the respective control samples. Moreover, the highest AA digestibility was observed in the DS_F samples, while the lowest digestion was observed in the Herkus_NF sample groups. The in vitro fermentability in the fermented sample groups was significantly lower than in the corresponding control sample groups. Moreover, Herkus_NF samples had the highest in vitro fermentability results, while the DS_F samples had the lowest in vitro fermentability results. Almost all of the individual AA digestibility was not significantly different in the bioconverted samples, in comparison to the control sample groups (supplementary results file Table 5). Only arginine in the DS samples and valine, phenylalanine, cystine, histidine and tyrosine in the Herkus samples were significantly different between the respective control and the bioconverted sample groups. The bioconversion was a significant factor for all the in vitro digestibility and fermentability parameters, except glutamic acid, serine, aspartic acid, threonine, glycine, and alanine (supplementary results file Table 6). While substrate was a significant factor for all the parameters, except for the in vitro digestibility, arginine, glutamic acid, serine, aspartic acid, threonine, and lysine. The factors' interaction was significant for the in vitro digestibility, valine, phenylalanine, and histidine.

Free amino acids and biogenic amines analysis results of the in vitro fermentation samples

The FAA and BA analysis results are presented in Table 7. The Herkus samples contained significantly higher concentrations of glycine, valine, leucine, serine, proline, aspartic acid, glutamic acid, methionine, phenylalanine, histidine, tryptophan and tyrosine in comparison to the blank samples after 24 h and the DS sample groups. The Herkus samples contained significantly higher amounts of glycine (124.1%), valine (1652.9%), leucine (1481.3%), serine, proline (1073.9%), aspartic acid (265.7%), glutamic acid (2190.9%), methionine (191.6%), phenylalanine

Sample	B0	Bl	DS_F	DS_NF	Herkus_F	Herkus_NF
Alanine	111.88 ± 4.66 bc	1.67 ± 0.83 d	109.08 ± 15.95 c	153.72 ± 18.52 abc	175.08 ± 21.55 ab	186.69 ± 39.09 a
Glycine	38.5 ± 2.58 b	5.33 ± 0.71 c	8.21 ± 3.10 c	56.14 ± 9.18 ab	71.79 ± 2.72 a	72.43 ± 15.44 a
Valine	102.54 ± 7.4 a	9.19 ± 1.76 cd	2.38 ± 0.15 d	1.48 ± 0.09 d	27.5 ± 3.73 bc	40.16 ± 19.76 b
Leucine	231.41 ± 9.75 a	9.92 ± 1.61 c	2.30 ± 0.35 c	2.02 ± 0.33 c	30.89 ± 2.23 b	37.42 ± 15.89 b
Isoleucine	74.52 ± 1.76 a	8.09 ± 1.16 c	5.18 ± 0.05 c	4.21 ± 0.67 c	5.96 ± 0.63 c	41.65 ± 23.42 b
GABA	ND c	ND c	43.99 ± 4.89 c	303.61 ± 105.18 b	385.28 ± 50.94 b	627.96 ± 76.96 a
Serine	64.98 ± 18.96 a	ND c	ND c	ND c	27.74 ± 0.61 b	33.89 ± 3.34 b
Threonine	46.97 ± 14.42 a	ND c	14.62 ± 1.06 bc	11.61 ± 0.44 bc	25.63 ± 1.47 ab	39.74 ± 16.41 a
Proline	24.72 ± 0.05 bc	25.3 ± 5.04 bc	2.66 ± 0.81 c	55.02 ± 13.02 b	331.75 ± 13.89 a	345.38 ± 17.43 a
Asparagine	ND	ND	ND	ND	ND	ND
Aspartic acid	26.41 ± 3.31 a	5.2 ± 0.2 b	8.85 ± 0.12 b	7.86 ± 4.50 b	35.14 ± 8.24 a	25.97 ± 8.73 a
Phenylethylamine	1.82 ± 0.13 a	ND b	ND b	ND b	ND b	ND b
Glutamic acid	55.44 ± 19.23 c	23.65 ± 10.04 c	13.83 ± 0.48 c	9.9 ± 2.31 c	358.27 ± 50.2 a	185.35 ± 13.91 b
Methionine	42.06 ± 11.43 ab	9.25 ± 0.24 c	11.22 ± 0.26 c	16.21 ± 5.24 c	31.9 ± 3.88 b	48.07 ± 7.06 a
Phenylalanine	81.00 ± 30.34 bc	9.47 ± 3.27 c	6.40 ± 0.78 c	104.99 ± 32.53 b	290.37 ± 19.63 a	254.68 ± 53.52 a
Putrescine	ND c	ND c	116.45 ± 6.63 b	107.92 ± 12.68 b	160.29 ± 16.51 a	107.16 ± 11.54 b
Cadaverine	5.38 ± 0.21 c	ND c	63.20 ± 6.41 b	60.03 ± 5.48 b	89.72 ± 4.15 a	60.04 ± 8.06 b
Histamine	ND c	ND c	23.30 ± 1.97 b	23.95 ± 1.15 b	37.95 ± 4.78 a	26.25 ± 5.58 b
Tryptamine	ND c	ND c	5.69 ± 0.06 b	5.76 ± 0.75 b	18.13 ± 3.29 a	6.39 ± 0.63 b
Lysine	57.6 ± 11.12 a	ND d	39.63 ± 0.06 bc	27.11 ± 0.29 c	42.86 ± 3.88 ab	33.09 ± 8.83 bc
Tyramine	ND b	ND b	4.25 ± 0.04 b	7.42 ± 3.10 b	39.39 ± 19.1 a	10.89 ± 4.31 b
Histidine	374.98 ± 34.88 a	ND c	ND c	ND c	336.51 ± 6.93 ab	291.12 ± 52.2 b
Tryptophan	31.93 ± 2.72 b	ND d	ND d	11.50 ± 3.50 c	42.99 ± 2.92 a	33.24 ± 4.17 b
Tyrosine	74.11 ± 25.18 a	5.96 ± 0.34 b	28.54 ± 0.17 b	11.93 ± 5.00 b	87.42 ± 18.64 a	66.24 ± 4.56 a

Table 7. Free amino acids and biogenic amines analysis results (mg/L) in the in vitro fermentation samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, Bl – fermentation blank at 48 h, B0 – fermentation blank at 0 h. a-d Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

(389.3%), histidine, tryptophan (562.9%) and tyrosine (279.7%), in comparison to the DS sample groups. The bioconversion was significant for almost all the FAAs and BAs, except for alanine, valine, leucine, threonine, aspartic acid, phenylalanine, tyramine, histidine and tryptophan, while the substrate was significant for all analytes, except for lysine (supplementary results file Table 7). The interaction of those factors was significant for all analytes, except for alanine, valine, leucine, GABA, threonine, aspartic acid, methionine, lysine, histidine, and tyrosine.

Volatile organic compounds analysis results of the in vitro fermentation samples

The VOCs analysis results are presented in the supplementary results file Table 8 and depicted in the heatmap in Fig. 2. VOCs data were Pareto-scaled, and hierarchical clustering was performed for the individual analytes. Various alkanes (tridecane, octadecane, hexadecane, dodecane, tetradecane), aromatic and heterocyclic compounds (benzothiazole, 2-phenoxyethanol, naphthalene, skatole, 4-ethylphenol, and 2,4-ditertbutylphenol), organic acids and alcohols (nonanoic acid, 4-methylpentanoic acid, and 3-hexadecanol) and several aldehydes (hexadecanal, nonanal) were significantly lower in almost all the IVF DS and Herkus samples, in comparison to the blank after 24 h IVF samples. Moreover, various aliphatic and aromatic esters (propyl pentanoate, hexyl n-valerate, pentyl levulinate), aldehydes (oct-(2E)-enal, pentadecanal, and tetradecanal), various aromatic compounds (*p*-cresol, indole, hydrocinnamic acid, and benzeneacetic acid), and hexanoic acid were the highest in the DS_F samples. The bioconversion factor was significant for 31 out of 53 VOCs, while the substrate was significant for 36 out of 53 VOCs in the IVF samples (supplementary results Table 9). While the factors' interaction was significant for 6 organic acids (propanoic, 2-methylpropanoic, butanoic, 3-methylbutanoic, pentanoic and hexanoic acids), 3 aromatic compounds (indole, hydrocinnamic acid, and *p*-cresol), pentyl levulinate, tetradecanal, and octadecane in the IVF samples.

PLS-DA and VIP analysis results are depicted in Fig. 3. Statistical analysis was performed on Pareto-scaled data. PLS-DA plots show clear separation between the fermented and the nonfermented samples (Fig. 3-a), as well as the different substrates (Fig. 3-c). For the first model, VIP analysis showed that isophorone, mesityl oxide, acetone, pentyl levulinate, methyl thioacetate, aliphatic organic acids (pentanoic, butanoic, nonanoic, octanoic, 2-methylpropanoic, and 3-methylbutanoic acids), alkanes (hexadecane, tridecane, octadecane, and dodecane), aldehydes (pentadecanal, hexadecanal, dodecanal, and decanal), and aromatic compounds (*p*-cresol, isocreosol, 2-phenoxyethanol, and 2,4-ditertbutylphenol) were contributing the most to the differences between the fermented and nonfermented samples. While various aromatic compounds (acetophenone, benzeneacetic

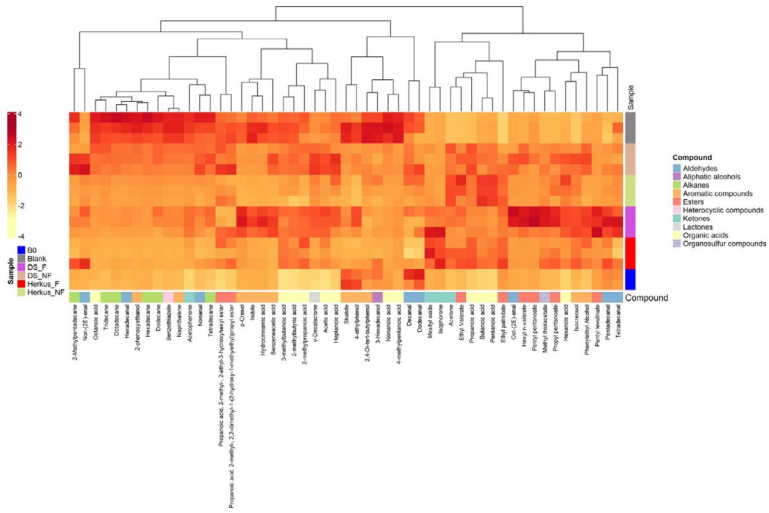

Fig. 2. Heatmap of volatile organic compounds in the in vitro fermentation samples.

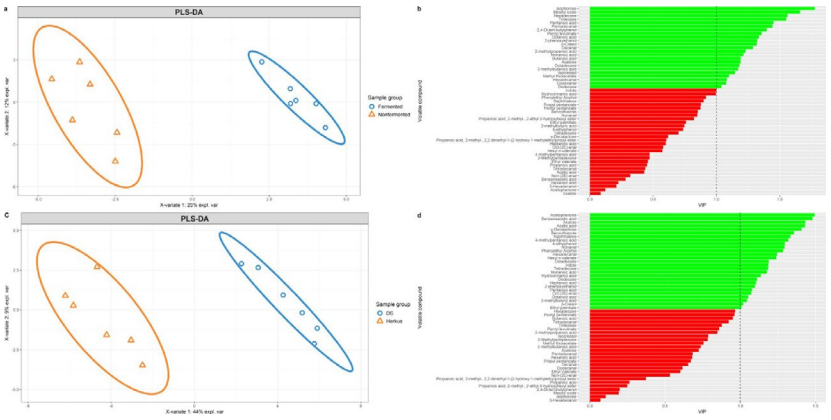

Fig. 3. PLS-DA and VIP plots of the volatile organic compounds of the in vitro fermentation samples. **a**, **c** – PLS-DA analysis plots, where categorical values are bioconversion and substrate; **b**, **d** – VIP plots of the respective PLS-DA analyses.

acid, hydrocinnamic acid, naphthalene, 4-ethylphenol, phenylethyl alcohol, 2-phenoxyethanol, and *p*-cresol), indoles (skatole and indole), aliphatic organic acids (acetic, 4-methylpentanoic, nonanoic, pentanoic, octanoic, 2-methylbutyric, and heptanoic acids), alkanes and aliphatic aldehydes (nonanal, hexadecanal, octadecane, tetradecane, dodecane, and oct-(2E)-enal), γ – decalactone, benzothiazole, hexyl n-valerate, and ethyl palmitate had the highest VIP values for the Herkus – DS PLS-DA model.

Additionally, volcano plots of the VOCs in IVF samples were constructed to depict differences between fermented – nonfermented (Fig. 4-a) and Herkus – DS sample groups (Fig. 4-b). The analysis results depict significantly higher concentrations of *p*-cresol, mesityl oxide, isophorone, and pentyl levulinate in fermented IVF samples. While the comparison between the Herkus and DS sample groups outlines significantly higher

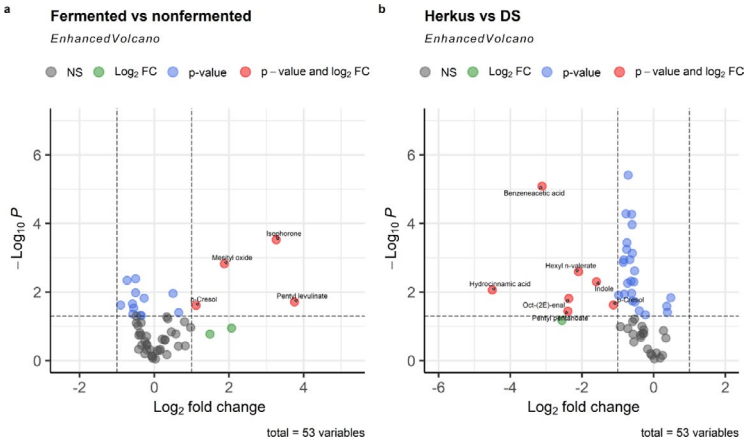

Fig. 4. Volcano plots of the volatile organic compounds of the in vitro fermentation samples.

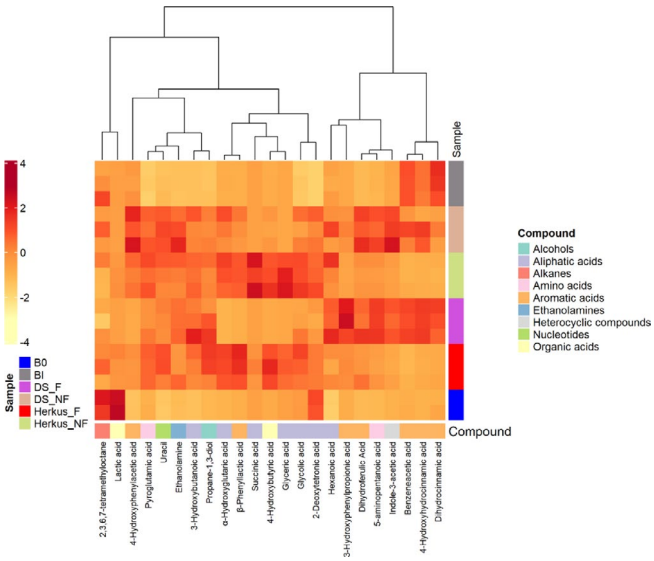

Fig. 5. Heatmap of in vitro fermentation untargeted analysis results.

concentrations of benzenacetic acid, hexyl n-valerate, hydrocinnamic acid, indole, *p*-cresol, oct-(2E)-enal, and pentyl pentanoate in the DS samples.

Untargeted metabolomics analysis results of the in vitro fermentation samples

The IVF untargeted metabolomics analysis results are presented in the supplementary results file Table 11. The heatmap visualization and hierarchical clustering analysis are depicted in Fig. 5. The IVF untargeted metabolomics data were Pareto-scaled and hierarchical clustering was performed for the individual analytes.

Almost all of the identified metabolites' concentrations were significantly higher in the substrate-fermented (DS_F, DS_NF, Herkus_NF, and Herkus_F) samples, in comparison to the blank after 24 h IVF samples. The bioconversion was a significant factor for succinic, glyceric, dihydrocinnamic, 2-deoxytetronic, pyroglutamic, α-hydroxyglutaric, 4-hydroxyphenylacetic, 3-hydroxyphenylpropionic and indole-3-acetic acid concentrations (supplementary results, Table 12). While the substrate was a significant factor for lactic acid, glycolic acid, 4-hydroxybutyric acid, ethanolamine, benzeneacetic acid, succinic acid, glyceric acid, dihydrocinnamic acid, 2-deoxytetronic acid, pyroglutamic acid, α-hydroxyglutaric acid, β-phenyllactic acid, 5-aminopentanoic acid, 3-hydroxyphenylpropionic acid, 4-hydroxyhydrocinnamic acid, dihydroferulic acid, and indole-3-acetic acid in the IVF samples. The interaction of the aforementioned factors was significant for succinic acid, glyceric acid, uracil, dihydrocinnamic acid, α-hydroxyglutaric acid, β-phenyllactic acid, 5-aminopentanoic acid, 4-hydroxyphenylacetic acid, and 3-hydroxyphenylpropionic acid in the IVF samples.

The PLS-DA and VIP analysis results are presented in Fig. 6. The IVF metabolites between the Herkus and the DS sample groups (Fig. 6-c) show clear separation of sample groups, while the fermented and nonfermented sample groups (Fig. 6-a) demonstrate some overlap of the sample groups. The VIP analysis results depict that 4-hydroxyphenylacetic acid, 2-deoxytetronic acid, 3-hydroxyphenylpropionic acid, pyroglutamic acid, propane-1,3-diol, succinic acid, dihydrocinnamic acid, ethanolamine, and α-hydroxyglutaric acid were the most contributing compounds for the fermented – nonfermented PLS-DA model. While 5-aminopentanoic acid, 4-hydroxybutyric acid, indole-3-acetic acid, benzeneacetic acid, glyceric acid, β-phenyllactic acid, 4-hydroxyhydrocinnamic acid, lactic acid, dihydroferulic acid, α-hydroxyglutaric acid, dihydrocinnamic acid, glycolic acid, and succinic acid had the most contribution to the Herkus – DS PLS-DA model.

The volcano plots of the fermented-nonfermented sample groups (Fig. 7-a) show significantly lower concentrations of 4-hydroxyphenylacetic acid, while having significantly higher concentrations of 3-hydroxyphenylpropionic acid in the fermented IVF samples. Glyceric, β-phenyllactic, 4-hydroxybutyric, succinic, lactic, and α-hydroxyglutaric acids were significantly higher in the Herkus sample groups, in comparison to the DS sample groups (Fig. 7-b). While 5-aminopentanoic acid, indole-3-acetic acid, benzenacetic acid, 4-hydroxycinnamic acid, dihydroferulic acid, dihydrocinnamic acid and 3-hydroxyphenylpropionic acid concentrations were significantly higher in the DS sample groups.

The PCA analysis results of the VOCs and untargeted metabolites in the IVF samples are presented in Fig. 8. PCA results of the VOCs in the IVF samples show clear separation between the fermented vs. nonfermented and the Herkus vs. DS sample groups (Fig. 8-a, b). While PCA of the untargeted metabolites showed clear separation between the Herkus and DS sample groups, there was no clear distinction between the fermented and nonfermented samples of untargeted metabolites (Fig. 8-c, d).

Short-chain fatty acids analysis results of the in vitro fermentation samples

The analysis results of SCFA are presented in Table 8. Acetic acid concentrations were the highest in the DS sample groups. The highest concentrations of butanoic and pentanoic acids were observed in the Herkus_NF sample group. The bioconversion factor was significant for the concentrations of 2-methylpropanoic, butanoic, 3-methylbutanoic and pentanoic acids, while the substrate was a significant factor for almost all of the SCFAs, except for propanoic and hexanoic acids (supplementary results file Table 9). The interaction of both factors was significant for propanoic, butanoic, pentanoic, and hexanoic acids in the IVF samples.

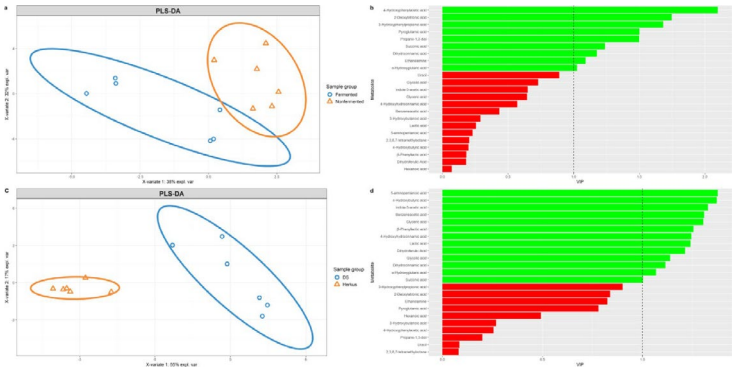

Fig. 6. PLS-DA and VIP plots of the untargeted metabolites of the in vitro fermentation samples. **A**, **c** – PLS-DA analysis plots, where categorical values are bioconversion and substrate; **b**, **d** – VIP plots of the respective PLS-DA analyses.

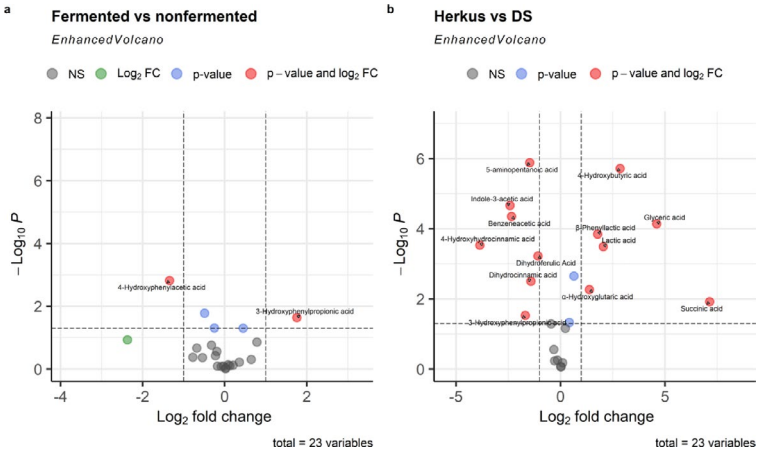

Fig. 7. Volcano plots of the untargeted metabolites of the in vitro fermentation samples.

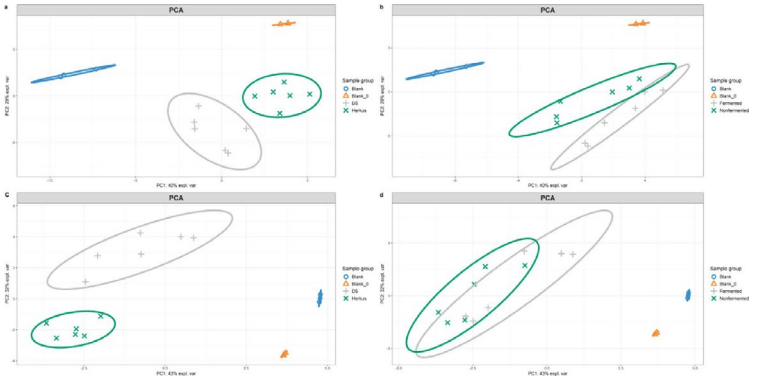

Fig. 8. PCA plots of the in vitro fermentation samples. **a**, **b** - PCA plots of volatile organic compounds of the in vitro fermentation samples; **c**, **d** - PCA plots of untargeted metabolomics results of the in vitro fermentation samples.

Discussion

All of the bioconverted wheat wholemeal samples had pH levels between 3.9 and 4.0. While its optimal range was exceeded, various LAB strains can tolerate pH up to 3.7[39]. Furthermore, *L. plantarum* showed good survivability at low pH, reaching over 8 $\log_{10}$ CFU/g values[40].

Most of the AAs and total AAs in the bioconverted wheat wholemeal samples weren't significantly altered. Similar trends in the crude protein content were observed in spontaneous SSF fermentations of wheat wholemeal as well[41]. While significant increases in crude protein in the SSF fermented samples are also observed whenever the fermentation process exceeds 24 h[42].

While the total FAA concentrations in all the IVD samples did not differ significantly between substrate digested sample groups, certain amino acids, such as glutamine, GABA and proline, were significantly different. Those differences can be attributed to *Lp. plantarum* activity in the wheat wholemeal samples as these bacteria produce various protein-degrading enzymes (proteases and peptidases) as well as the GABA-producing enzyme glutamic acid decarboxylase, which converts glutamic acid into GABA[43,44]. The results of the IVD hydrolysates

Sample	B0	Bl	DS_F	DS_NF	Herkus_F	Herkus_NF
Acetic acid	6.161 ± 0.142 c	34.44 ± 10.526 b	86.466 ± 7.905 a	74.829 ± 8.17 a	47.43 ± 12.029 b	50.394 ± 0.989 b
Propanoic acid	2.06 ± 0.094 c	8.083 ± 0.167 c	39.326 ± 2.663 b	51.636 ± 3.226 a	51.274 ± 7.01 a	46.858 ± 1.619 ab
2-methylpropanoic acid	0.232 ± 0.032 d	2.609 ± 0.063 b	3.261 ± 0.135 a	2.777 ± 0.18 ab	2.616 ± 0.195 b	1.951 ± 0.295 c
Butanoic acid	1.307 ± 0.041 d	11.167 ± 0.266 c	23.707 ± 0.585 b	23.462 ± 2.133 b	23.829 ± 4.016 b	38.35 ± 3.545 a
3-methylbutanoic acid	0.253 ± 0.025 c	3.823 ± 0.078 ab	4.495 ± 0.102 a	4.154 ± 0.168 a	3.982 ± 0.237 ab	3.253 ± 0.661 b
Pentanoic acid	0.376 ± 0.03 e	6.458 ± 0.187 d	12.311 ± 0.726 c	16.404 ± 0.194 b	16.6 ± 1.744 b	26.023 ± 1.878 a
4-methylpentanoic acid	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Hexanoic acid	< 0.1 c	0.085 ± 0.02 c	0.752 ± 0.064 a	0.525 ± 0.155 ab	0.342 ± 0.116 bc	0.711 ± 0.166 a
Heptanoic acid	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Table 8. Short-chain Fatty Acid concentrations (mmol/L) in the in vitro fermentation samples. DS – wheat variety DS8558-1, F - sample fermented with *Lactiplantibacillus plantarum*, NF – control sample, Bl – fermentation blank at 48 h, B0 – fermentation blank at 0 h. a-e Mean values denoted with different letters indicate significantly different values between the different sample groups. Results are statistically significant when $p < 0.05$.

mostly did not differ significantly between the bioconverted and control samples. While we found significant differences in the essential (threonine, methionine, valine, phenylalanine, leucine/isoleucine), non-essential (alanine, glutamic acid, proline, tyrosine) and total amino acid concentrations between the Herkus and DS samples. The IVD residue analysis reveals that fermentation significantly altered the digestibility of some of the non-essential (arginine, proline, tyrosine) and essential (valine, phenylalanine, leucine/isoleucine, lysine, histidine) amino acids. The in vitro digestibility and total AA digestibility were significantly affected by the bioconversion as well. However, the substrate was a significant factor for the digestion of the essential amino acids, such as valine, phenylalanine, isoleucine/leucine and histidine, some of the non-essential amino acids (glycine, proline, tyrosine), as well as total AA digestibility, the in vitro fermentability and the total IVD digestibility. The differences between the in vitro digestibility parameters due to the bioconversion can be attributed to the increased proteolytic activity in the fermented samples, as bacterial proteolytic enzymes are excreted into the sample matrix[45,46]. Certain differences between the substrate digestibility are expected, as cultivars do have an effect on the ileal digestibility of protein in vitro and in vivo[47,48]. The in vitro digestibility values of the individual AAs varied between 75.0 and 94.5%, as similar values(77–94.7%) were reported in arious in vivo and in vitro experiments[48–50]. Interestingly, lysine had one of the lowest digestibility as reported in previous studies, as most of it lies in the aleurone fraction[48,51]. Thus, bioconversion could be used to improve its bioavailability as well as other essential amino acids, such as phenylalanine, histidine, leucine/isoleucine, and valine, due to bacterial proteolytic activity.

Biogenic amines are products of amino acid degradation in the gut microbiome. Most of the BAs, such as histamine, tyramine and phenylethylamine, are produced by a single-step decarboxylation of amino acids via respective decarboxylase enzymes[52,53]. Putrescine can be produced via the ornithine decarboxylase (single step) or arginine decarboxylase and agmatine deiminase (multiple steps using arginine)[52,53]. We found that all of the factors and their interactions were significant for putrescine, cadaverine, histamine and tryptamine, while only substrate and both factors' interaction were significant for tyramine concentration in the IVF samples (supplementary results file Table 7). Certainly, BAs are often produced by bacteria to regulate the pH or even have immunoregulation properties for the host[53–55]. While BAs play a significant role in the animal gut and even other organs, such as the brain, acting as neurotransmitters, the putrefactive fermentation inside the gut can lead to a significant increase in those compounds, leading to serious complications[53,56,57]. Some of those complications include injury of the intestinal mucosa, headaches, and digestive disorders[58,59]. Certainly, the elevated levels of BAs are associated with such problems; they are often a clinical expression of pathogens, especially when the majority of the BAs in the piglet colon are of bacterial origin[57,60]. Moreover, significant negative correlations between putrescine, cadaverine, histamine and total bacteria count (TBC) were found (supplementary results file Fig. 5), confirming the link between the faecal microbiota and its products. Though no significant correlations between the BAs and LAB count were found. This could suggest some mechanism of bacterial community modulation via the BAs, as certain negative correlations with other bacterial communities were observed in the literature[61].

We found significant differences in propanoic, pentanoic, nonanoic and 2-methylpropanoic acids between the fermented and nonfermented samples, which were consistent between the methods (VOC analysis and SCFA analysis). Also, these compounds were among the most important compounds in the VIP analysis results. Additionally, most of the SCFAs, such as acetic, 4-methylpentanoic, nonanoic, pentanoic, octanoic, 2-methylbutyric and heptanoic acids were significantly different between the different substrates, as we found in the VIP analysis from the VOCs results, and from the factor analysis of the SCFAs and the VOCs. The SCFAs are important products of the indigestible polysaccharide fermentation, which can regulate gut metabolism, provide food for intestinal epithelial cells and maintain a favourable environment for the beneficial bacteria by controlling the population of pathogens[56,62]. The branched-chain SCFAs, on the other hand, are produced by the amino acid fermentation in the colon[56,62]. Thus, significant negative correlations between certain amino acids (valine, leucine, isoleucine, serine, threonine and histidine) and 2-methylpropanoic, 2-methylbutyric and 3-methylbutanoic acids in the IVF samples were established as well (supplementary results file Fig. 3). Also, the

possibility of certain modulation of bacterial colonies was observed, as significant negative correlations were established between the SCFAs and TBC and LAB count values (supplementary results file Fig. 2). Interestingly, significant negative correlations were found between the non-branched SCFAs (butanoic, propanoic, pentanoic and hexanoic acids) and TBC, while branched-chain SCFAs (2-methylpropanoic and 3-methylbutanoic acids) and acetic acid were significantly negatively correlated with the LAB count. Branched-chain SCFAs production is often associated with high protein diet and is mainly produced by *Bacteroides* and *Clostridium*[63]. These correlations might indicate certain microbiota modulation, as various correlations between different bacterial genera, especially *Bacteroides* and *Clostridium*, were observed in the literature[64,65].

Certain aliphatic aldehydes and alkanes were significantly contributing to the differences between the fermented vs. nonfermented (pentadecanal, hexadecanal, dodecanal, hexadecane, tridecane, octadecane, dodecane) and the Herkus vs. DS (nonanal, hexadecanal, octadecane, tetradecane, dodecane, oct-(2E)-enal) groups, which can be associated with differences in lipid metabolism in the sample groups[66–68]. Indoles were significant for distinguishing between the Herkus and DS IVF samples, as we found indole from the VOCs and indole-3-acetic acid from the untargeted metabolites downregulated in the Herkus sample group. We found significant negative correlations between the indoles and tryptophan (supplementary results file Figs. 3 and 4), as indoles are associated with bacterial degradation of tryptophan[69].

P-cresol is a product of tyrosine and phenylalanine degradation in the gut, mostly by *Escherichia coli*, *Clostridium sporogenes* and *Clostridium difficile*, which is a protein-binding uremic toxin[70]. Other phenolic compounds, such as 4-ethylphenol, are also associated with tyrosine and phenylalanine catabolism by *Clostridium* species[71]. While it's found in various fermented food products, such as wine, its metabolites at high concentrations in blood can cross the blood-brain barrier and cause serious damage to the host's organism[72,73]. Other products of bacterial phenolic amino acid degradation include 3-hydroxyphenylpropionic acid via the deamination of tyrosine, benzenacetic acid via the oxidative or non-oxidative decarboxylation of phenylalanine, 4-hydroxyhydrocinnamic acid via the catabolism of tyrosine, β-phenyllactic acid via the metabolism of phenylalanine[74–77]. While many of those fermentation products, such as 4-hydroxyhydrocinnamic acid, benzenacetic acid, 3-hydroxyphenylpropionic acid, hydrocinnamic acid (aka dihydrocinnamic acid or benzenepropanoic acid), dihydroferulic acid, can also be attributed to phenolic acids, such as ferulic and caffeic acid, catabolised by the gut microbiome[78–80]. We found benzenacetic, dihydrocinnamic, dihydroferulic and 4-hydroxyhydrocinnamic acids upregulated in the DS sample group (Fig. 7). Significant negative correlations between benzenacetic acid, dihydrocinnamic acid, 4-hydroxyhydrocinnamic acid and phenolic amino acids (tyrosine and phenylalanine) (supplementary results file Figs. 3 and 4), suggesting their origin to be of the amino acid catabolism. While 3-hydroxyphenylpropionic acid was significantly upregulated in the fermented samples (Fig. 7-a) and in the DS samples (Fig. 7-b) as well. It may be a product of phenolic acid degradation, as we failed to find significant correlations with phenolic amino acids (supplementary results file Fig. 4). The sole product of phenolic amino acids, β-phenyllactic acid, was upregulated in the Herkus sample group (Fig. 7). It was also significantly correlated with the TBC (supplementary results file Fig. 2), possibly modulating the bacterial community, as its modulating properties were established in the literature[81]. We found that cresol was upregulated in the fermented sample group and downregulated in the Herkus sample group (Fig. 4). We found significant upregulation of 4-hydroxyphenylacetic acid, a precursor to *p*-cresol, in the non-fermented sample group (Fig. 7). This suggests certain inhibition of its conversion into *p*-cresol in the non-fermented samples. The factors were significant for both *p*-cresol and 4-ethylphenol, while factor interaction was significant only for cresol. The significant negative correlations were established with phenylalanine and tyrosine, as well as other amino acids (supplementary results file Fig. 3). Moreover, the potential modulation of specific bacterial communities was indicated by the significant correlation observed between the *p*-cresol and LAB counts, a relationship that has also been reported in the literature[70].

While 2,4-ditertbutylphenol is considered an exogenous compound, several species of bacteria do produce it in significant amounts[82,83]. Other possible exogenous compounds include 2-phenoxyethanol, as a preservative, naphthalene, as a polycyclic aromatic compound[84,85]. Phenylethyl alcohol could be exogenous, as it is used as a preservative or in perfumes due to its pleasant aroma, while its origins can be linked to yeast activity[86]. Other phenolic metabolites, such as isocreosol, were detected in urine[87]. Acetophenone is associated with the bacterial catabolism of cinnamic acid, and γ-decalactone can be produced by LAB via fatty acid metabolism[88,89].

Acetone production can be attributed to solventogenic clostridia, producing other compounds such as butanol, acetic acid and lactic acid[90,91]. VIP analysis results showed that acetone was significant for the fermented – nonfermented PLS-DA model, as bioconversion of the substrate can reduce complex carbohydrates into smaller fragments, possibly so that other bacteria can more easily compete with clostridia as, it is able to ferment complex carbohydrates[92,93].

Aliphatic organic acids, such as 2-deoxytetronic acid, succinic acid (the intermediate of carbohydrate fermentation to propionate and lysine degradation), α-hydroxyglutaric acid, 5-aminopentanoic acid (metabolites of lysine degradation by *Escherichia coli*), and glyceric acid are produced by bacteria in the gut[94–97]. Pyroglutamic acid is another typical product of the LAB fermentation[98]. Propane-1,3-diol production is associated with carbohydrate fermentation of various *Clostridium*, *Salmonella*, *Bacteroides* strains, and others[99]. Lactic acid is a classic metabolite of carbohydrate fermentation by the LAB[56,100]. Interestingly, succinic acid and α-hydroxyglutaric acid were upregulated in the Herkus samples, while 5-aminopentanoic acid was upregulated in the DS samples. This could suggest possible inhibition of 5-aminopentanoic acid conversion to glutarate semialdehyde via 4-aminobutyrate-2-oxoglutarate transaminase and subsequently to succinate[95]. While the same enzyme can be used in *E. Coli* to catabolize GABA via the GABA shunt mechanism[101–104]. We found positive correlations between the GABA and lysine metabolites (supplementary results file Fig. 4), suggesting that the abundance of substrate reduces the GABA shunt activity[101–104]. Glyceric and lactic acid were also upregulated in the Herkus samples (Fig. 7). While certainly lactic acid is mainly produced by carbohydrate

fermentation in the gut microbiome, it is also produced via branched-chain amino acid catabolism to pyruvate and subsequently to lactate by auxotrophic bacteria[105]. We found significant positive correlations between valine, leucine and isoleucine and lactic acid (supplementary results file Fig. 4), suggesting the absence of auxotrophic bacterial activity. Glyceric acid, although a common product of carbohydrate and glycerol fermentation, may exert detrimental effects at elevated concentrations, as it can act as a metabolic toxin[106].

Conclusions

Lp. plantarum strain is effective for the SSF of wheat cereals, while the total AA composition of bioconverted substrates was not significantly altered for the most part; the in vitro digestibility parameters were altered, notably increasing in vitro total digestibility and some essential and non-essential AAs as well. The differences between different wheat substrates for the in vitro digestibility were also established. The significantly higher concentrations of GABA were observed in the bioconverted IVD samples. The production of most of the BAs was significantly affected by both factors (bioconversion and substrate) and their interaction. The majority of the SCFA production was significantly different due to the substrate factor, while the bioconversion of the substrate affected all of the SCFAs, except acetic acid. Also, propionic acid production was affected by both of the analysed factors. Indole compounds were downregulated in the Herkus samples. We found significant differences in aromatic amino acids and other phenolic acid degradation products between the substrates. Acetone production was significantly affected by the bioconversion of the substrate. Both factors were significant for GABA production in the IVF samples. Substrate was a significant factor for the LAB count. The PCA of the VOCs showed separation between groups, while the untargeted metabolomics results only showed separation between the bioconverted sample groups. The bioconversion and the substrate had significant effects on the major in vitro digestibility and fermentability characteristics, as well as the overall metabolome in the simulated colon fermentation. Therefore, noting substrate selection and its bioprocessing importance for piglets' health and nutrition.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

1. Stas, E. B. et al. Nutritional guide to feeding wheat and wheat co-products to swine: A review. *Transl. Anim. Sci.* **8**, txae106 (2024).
2. Shewry, P. R. & Hey, S. J. The contribution of wheat to human diet and health. *Food Energy Secur.* **4**(3), 178–202 (2015).
3. Rosenfelder, P., Eklund, M. & Mosenthin, R. Nutritive value of wheat and wheat by-products in pig nutrition: A review. *Anim. Feed Sci. Technol.* **185** (3), 107–125 (2013).
4. Padhy, A. K., Kaur, P., Singh, S., Kashyap, L. & Sharma, A. Colored wheat and derived products: Key to global nutritional security. *Crit. Rev. Food Sci. Nutr.* **64**(7), 1894–1910 (2024).
5. Francavilla, A. & Joye, I. J. Anthocyanins in whole grain cereals and their potential effect on health. *Nutrients* **12** (10), 2922 (2020).
6. Saini, P. et al. Bioactive compounds, nutritional benefits and food applications of colored wheat: a comprehensive review. *Crit. Rev. Food Sci. Nutr.* **61** (19), 3197–3210 (2021).
7. Garg, M. et al. Rising demand for healthy foods-anthocyanin biofortified colored wheat is a new research trend. *Front. Nutr.* **9**, 878221 (2022).
8. Li, J. et al. Bilberry anthocyanin extract promotes intestinal barrier function and inhibits digestive enzyme activity by regulating the gut microbiota in aging rats. *Food Funct.* **10** (1), 333–343 (2019).
9. Miao, L. et al. Structural changes of rice starch and activity inhibition of starch digestive enzymes by anthocyanins retarded starch digestibility. *Carbohydr. Polym.* **261**, 117841 (2021).
10. Khoo, H. E., Azlan, A., Tang, S. T. & Lim, S. M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* **61**(1), 1361779 (2017).
11. Lachman, J., Martinek, P., Kotíková, Z., Orsák, M. & Šulc, M. Genetics and chemistry of pigments in wheat grain – A review. *J. Cereal Sci.* **74**, 145–154 (2017).
12. Liu, Y. et al. Stability and absorption of anthocyanins from blueberries subjected to a simulated digestion process. *Int. J. Food Sci. Nutr.* **65**(4), 440–448 (2014).
13. Han, F. et al. Digestion and absorption of red grape and wine anthocyanins through the gastrointestinal tract. *Trends Food Sci. Technol.* **83**, 211–224 (2019).
14. Sun, D., Huang, S., Cai, S., Cao, J. & Han, P. Digestion property and synergistic effect on biological activity of purple rice (*Oryza sativa* L.) anthocyanins subjected to a simulated gastrointestinal digestion in vitro. *Food Res. Int.* **78**, 114–123 (2015).
15. Gui, H. et al. Current knowledge of anthocyanin metabolism in the digestive tract: absorption, distribution, degradation, and interconversion. *Crit. Rev. Food Sci. Nutr.* **63** (22), 5953–5966 (2023).
16. Samtiya, M., Aluko, R. E. & Dhewa, T. Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Prod. Process. Nutr.* **2**(1), 6 (2020).
17. Myrie, S. B., Bertolo, R. F., Sauer, W. C. & Ball, R. O. Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine. *J Anim Sci* **86**(3), 609–619 (2008).
18. Ram, S., Narwal, S., Gupta, O. P., Pandey, V. & Singh, G. P. in *4 - Anti-nutritional factors and bioavailability: approaches, challenges, and opportunities.* 101–128 (eds Gupta, O. P., Pandey, V., Narwal, S., Sharma, P., Ram, S. & Singh, G. P.) (Woodhead Publishing, 2020).
19. Soemarie, Y. B., Milanda, T. & Barliana, M. I. Fermented foods as probiotics: A review. *J. Adv. Pharm. Tech. Res.* **12** (4), 335–339 (2021).
20. Álvarez, A. et al. Solid-state fermentation as a biotechnological tool to reduce antinutrients and increase nutritional content in legumes and cereals for animal feed. *Fermentation* **11**(7), 359 (2025).
21. Kalaiselvan, P. et al. Solid-State Fermentation (SSF)-A Sustainable Future Technology in Aquafeeds? *Front. Mar. Sci.* **12**, 1669719 (2025).
22. Sadh, P. K., Duhan, S. & Duhan, J. S. Agro-industrial wastes and their utilization using solid state fermentation: A review. *Bioresour. Bioprocess.* **5**(1), 1–15 (2018).

23. Abedin, M. M. et al. Lactic acid bacteria in the functional food industry: biotechnological properties and potential applications. *Crit. Rev. Food Sci. Nutr.* **64** (29), 10730–10748 (2024).
24. Behera, S. S., Ray, R. C. & Zdolec, N. *Lactobacillus plantarum* with functional properties: An approach to increase safety and shelf-life of fermented foods. *Biomed. Res. Int.* **2018**(1), 9361614 (2018).
25. Bartkiene, E. et al. Lactobacillus plantarum LUHS135 and paracasei LUHS244 as functional starter cultures for the food fermentation industry: Characterisation, mycotoxin-reducing properties, optimisation of biomass growth and sustainable encapsulation by using dairy by-products. *LWT* **93**, 649–658 (2018).
26. Turchi, B. et al. Preliminary evaluation of probiotic potential of *Lactobacillus plantarum* strains isolated from Italian food products. *World J. Microbiol. Biotechnol.* **29**(10), 1913–1922 (2013).
27. Park, S. J. et al. Enhanced production of γ-aminobutyric acid (GABA) using *Lactobacillus plantarum* EJ2014 with simple medium composition. *LWT* **137**, 110443 (2021).
28. da Silva Sabo, S., Vitolo, M., González, J. M. D. & Oliveira, R. Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. *Food Res. Int.* **64**, 527–536 (2014).
29. Huting, A. M., Middelkoop, A., Guan, X. & Molist, F. Using nutritional strategies to shape the gastro-intestinal tracts of suckling and weaned piglets. *Animals* **11**(2), 402 (2021).
30. Tang, X., Xiong, K., Fang, R. & Li, M. Weaning stress and intestinal health of piglets: A review. *Front. Immunol.* **13**, 1042778 (2022).
31. Blavi, L. et al. Management and feeding strategies in early life to increase piglet performance and welfare around weaning: A review. *Animals* **11** (2), 302 (2021).
32. Gresse, R. et al. Gut microbiota dysbiosis in postweaning piglets: Understanding the keys to health. *Trends Microbiol.* **25**(10), 851–873 (2017).
33. Jayaraman, B. & Nyachoti, C. M. Husbandry practices and gut health outcomes in weaned piglets: A review. *Anim. Nutr.* **3**(3), 205–211 (2017).
34. Li, Y. et al. Weaning stress perturbs gut microbiome and its metabolic profile in piglets. *Sci. Rep.* **8**(1), 18068 (2018).
35. Bartkiene, E. et al. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. *Microorganisms* **8** (1), 64 (2019).
36. Brodkorb, A. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. (2019).
37. Pérez-Burillo, S. et al. An in vitro batch fermentation protocol for studying the contribution of food to gut microbiota composition and functionality. *Nat. Protoc.* **16**(7), 3186–3209 (2021).
38. Cai, H., Zhu, R. & Li, H. Determination of dansylated monoamine and amino acid neurotransmitters and their metabolites in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry. *Anal. Biochem.* **396** (1), 103–111 (2010).
39. Sionek, B., Szydłowska, A., Trząskowska, M. & Kołożyn-Krajewska, D. The impact of physicochemical conditions on lactic acid bacteria survival in food products. *Fermentation* **10** (6), 298 (2024).
40. Mockus, E. et al. The potential of traditional 'Gaja'and new breed lines of waxy, blue and purple wheat in wholemeal flour fermentation. *Fermentation* **8** (10), 563 (2022).
41. Islam, S. et al. Exploring the effects of spontaneous and solid-state fermentation on the physicochemical, functional and structural properties of whole wheat flour (*Triticum aestivum* L.). *Innov. Food Sci. Emerg. Technol.* **97**, 103798 (2024).
42. Feng, X., Ng, K., Ajlouni, S., Zhang, P. & Fang, Z. Effect of solid-state fermentation on plant-sourced proteins: A review. *Food Rev. Int.* **40**(9), 2580–2617 (2024).
43. Cui, Y., Miao, K., Niyaphorn, S. & Qu, X. Production of gamma-aminobutyric acid from lactic acid bacteria: A systematic review. *Int. J. Mol. Sci.* **21** (3), 995 (2020).
44. Reffai, Y. M. & Fechtali, T. A critical review on the role of lactic acid bacteria in sourdough nutritional quality: Mechanisms, potential, and challenges. *Appl Microbiol* **5**(3), 74 (2025).
45. Ayyash, M. et al. In vitro investigation of bioactivities of solid-state fermented lupin, quinoa and wheat using Lactobacillus spp. *Food Chem.* **275**, 50–58 (2019).
46. Yin, Y. et al. Protein degradation in wheat sourdough fermentation with *Lactobacillus plantarum* M616. *Interdiscip. Sci. Comput. Life Sci.* **7**(2), 205–210 (2015).
47. García-Pérez, P. et al. The functional implications of high-amylose wholegrain wheat flours: An in vitro digestion and fermentation approach combined with metabolomics. *Food Chem.* **418**, 135959 (2023).
48. Zhao, J. et al. Determination of chemical composition, energy content, and amino acid digestibility in different wheat cultivars fed to growing pigs. *J. Anim. Sci.* **97**(2), 714–726 (2019).
49. Son, J. & Kim, B. G. Nutrient digestibility of soybean meal products based on in vitro procedures for pigs. *Agriculture* **13** (8), 1631 (2023).
50. Rowan, A. M., Moughan, P. J., Wilson, M. N., Maher, K. & Tasman-Jones, C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *Br. J. Nutr.* **71**(1), 29–42 (1994).
51. Brouns, F., Hemery, Y., Price, R. & Anson, N. M. Wheat aleurone: Separation, composition, health aspects, and potential food use. *Crit. Rev. Food Sci. Nutr.* **52**(6), 553–568 (2012).
52. Barbieri, F., Montanari, C., Gardini, F. & Tabanelli, G. Biogenic amine production by lactic acid bacteria: A review. *Foods* **8** (1), 17 (2019).
53. Sudo, N. Biogenic amines: Signals between commensal microbiota and gut physiology. *Front. Endocrinol.* **10**, 504 (2019).
54. Pugin, B. et al. A wide diversity of bacteria from the human gut produces and degrades biogenic amines. *Microb. Ecol. Health Dis.* **28**(1), 1353881 (2017).
55. Mao, S., Huo, W., Liu, J., Zhang, R. & Zhu, W. In vitro effects of sodium bicarbonate buffer on rumen fermentation, levels of lipopolysaccharide and biogenic amine, and composition of rumen microbiota. *J. Sci. Food Agric.* **97**(4), 1276–1285 (2017).
56. Vasquez, R., Oh, J. K., Song, J. H. & Kang, D. Gut microbiome-produced metabolites in pigs: A review on their biological functions and the influence of probiotics. *J. Anim. Sci. Technol.* **64**(4), 671 (2022).
57. Kiarie, E., Slominski, B. A. & Nyachoti, C. M. Tissue fatty acid profiles, plasma biochemical characteristics and cecal biogenic amines in piglets fed diets containing flaxseed and carbohydrase enzymes. *Livest. Sci.* **121**(1), 1–6 (2009).
58. Carnino, B. B. et al. Feeding weanling piglets for optimal health and performance: What can we learn from research on complex diets?. *Nutr. Res. Rev.* **38**(2), 1–70 (2025).
59. Ma, Y., Han, X., Fang, J. & Jiang, H. Role of dietary amino acids and microbial metabolites in the regulation of pig intestinal health. *Anim. Nutr.* **9**, 1–6 (2022).
60. Tan, B., Xiao, D., Wang, J. & Tan, B. The roles of polyamines in intestinal development and function in piglets. *Animals* **14** (8), 1228 (2024).
61. Chen, J. et al. Exploration of the roles of microbiota on biogenic amines formation during traditional fermentation of *Scomber japonicus*. *Front. Microbiol.* **13**, 1030789 (2022).
62. Martin-Gallausiaux, C., Marinelli, L., Blottière, H. M., Larraufie, P. & Lapaque, N. SCFA: Mechanisms and functional importance in the gut. *Proc. Nutr. Soc.* **80**(1), 37–49 (2021).
63. Rios-Covian, D. et al. An overview on fecal branched short-chain fatty acids along human life and as related with body mass index: associated dietary and anthropometric factors. *Front. Microbiol.* **11**, 973 (2020).

64. Gojda, J. & Cahova, M. Gut microbiota as the link between elevated BCAA serum levels and insulin resistance. *Biomolecules* **11** (10), 1414 (2021).
65. Bourdeau-Julien, I. et al. The diet rapidly and differentially affects the gut microbiota and host lipid mediators in a healthy population. *Microbiome* **11** (1), 26 (2023).
66. Fu, W. et al. Hydrocarbons, the advanced biofuels produced by different organisms, the evidence that alkanes in petroleum can be renewable. *Appl. Microbiol. Biotechnol.* **99** (18), 7481–7494 (2015).
67. Choi, Y. J. & Lee, S. Y. Microbial production of short-chain alkanes. *Nature* **502** (7472), 571–574 (2013).
68. Chang, X. et al. Gut Microbiota and Lipid Metabolism: Impacts on Lamb Flavor Precursors. *Compr. Rev. Food Sci. Food Saf.* **24**(6), e70307 (2025).
69. Tennoune, N., Andriamihaja, M. & Blachier, F. Physiologie de la Nutrition et du Comportement Alimentaire (PNCA (UMR 0914)), AgroParisTech-Université Paris-Saclay-Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE). Production of indole and indole-related compounds by the intestinal microbiota and consequences for the host: the good, the bad, and the ugly. *Microorganisms* **10** (5), 930 (2022).
70. Zhang, Y. et al. Unraveling p-cresol: From biosynthesis to biological and biochemical activities. *Front. Pharmacol.* **16**, 1665421 (2025).
71. Hsiao, E. Y. et al. Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders. *Cell* **155** (7), 1451–1463 (2013).
72. Kridelbaugh, D., Hughes, S., Allen, T. & Doerner, K. C. Production of 4-ethylphenol from 4-hydroxycinnamic acid by *Lactobacillus* sp. isolated from a swine waste lagoon. *J. Appl. Microbiol.* **109**(1), 190–198 (2010).
73. Day, F., O'Sullivan, J. & Pook, C. 4-Ethylphenol—fluxes, metabolism and excretion of a gut microbiome derived neuromodulator implicated in autism. *Front. Mol. Biosci.* **10**, 1267754 (2023).
74. Zhu, Y. et al. Two distinct gut microbial pathways contribute to meta-organismal production of phenylacetylglutamine with links to cardiovascular disease. *Cell Host Microbe* **31**(1), 18-32. e9 (2023).
75. Chen, L. et al. Modulating phenylalanine metabolism by *L. acidophilus* alleviates alcohol-related liver disease through enhancing intestinal barrier function. *Cell Biosci* **13**(1), 24 (2023).
76. Lambert, M. A. & Moss, C. W. Production of p-hydroxyhydrocinnamic acid from tyrosine by *Peptostreptococcus anaerobius*. *J. Clin. Microbiol.* **12**(2), 291–293 (1980).
77. Xiong, X., Liu, D., Wang, Y., Zeng, T. & Peng, Y. Urinary 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-hydroxyphenylacetic acid, and 3-Hydroxyhippuric acid are elevated in children with autism spectrum disorders. *BioMed Res. Int.* **2016**(1), 9485412 (2016).
78. Koistinen, V. M. et al. Metabolite Pattern Derived from Lactiplantibacillus plantarum—Fermented Rye Foods and In Vitro Gut Fermentation Synergistically Inhibits Bacterial Growth. *Mol. Nutr. Food Res.* **66** (21), 2101096 (2022).
79. Leonard, W., Zhang, P., Ying, D. & Fang, Z. Hydroxycinnamic acids on gut microbiota and health. *Compr. Rev. Food Sci. Food Saf.* **20** (1), 710–737 (2021).
80. Vollmer, M. et al. Chlorogenic acid versus amaranth's caffeoylisocitric acid – Gut microbial degradation of caffeic acid derivatives. *Food Res. Int.* **100**, 375–384 (2017).
81. Kim, D. et al. Phenyllactic acid modulates the gut microbiota, enhances intestinal health, and alleviates physical frailty in aging mice. *Eur. J. Pharmacol.* **985**, 177105 (2024).
82. Zhao, F., Wang, P., Lucardi, R. D., Su, Z. & Li, S. Natural sources and bioactivities of 2, 4-di-tert-butylphenol and its analogs. *Toxins* **12**(1), 35 (2020).
83. Guan, W. et al. Impact of 2,4-di-tert-butylphenol on pancreatic lipase activity in emulsions: Multispectral, molecular docking, and in vitro digestion analysis. *Food Chem.* **470**, 142730 (2025).
84. Mohapatra, B. & Phale, P. S. Microbial degradation of naphthalene and substituted naphthalenes: Metabolic diversity and genomic insight for bioremediation. *Front. Bioeng. Biotechnol.* **9**, 602445 (2021).
85. Grecka, K. & Szweda, P. Synergistic effects of propolis combined with 2-phenoxyethanol and antipyretics on the growth of *Staphylococcus aureus*. *Pharmaceutics* **13**(2), 215 (2021).
86. Mitri, S. et al. Bioproduction of 2-phenylethanol through yeast fermentation on synthetic media and on agro-industrial waste and by-products: A review. *Foods* **11** (1), 109 (2022).
87. Wagenstaller, M. & Buettner, A. Characterization of odorants in human urine using a combined chemo-analytical and human-sensory approach: A potential diagnostic strategy. *Metabolomics* **9**(1), 9–20 (2013).
88. Lee, S. M., Lim, H. J., Chang, J. W., Hurh, B. & Kim, Y. Investigation on the formations of volatile compounds, fatty acids, and γ-lactones in white and brown rice during fermentation. *Food Chem.* **269**, 347–354 (2018).
89. Hilton, M. D. & Cain, W. J. Bioconversion of cinnamic acid to acetophenone by a pseudomonad: Microbial production of a natural flavor compound. *Appl. Environ. Microbiol.* **56**(3), 623–627 (1990).
90. Ezeji, T., Milne, C., Price, N. D. & Blaschek, H. P. Achievements and perspectives to overcome the poor solvent resistance in acetone and butanol-producing microorganisms. *Appl. Microbiol. Biotechnol.* **85** (6), 1697–1712 (2010).
91. Datta, R. & Zeikus, J. Modulation of acetone-butanol-ethanol fermentation by carbon monoxide and organic acids. *Appl. Environ. Microbiol.* **49** (3), 522–529 (1985).
92. Yang, Z. et al. *Clostridium* as microbial cell factory to enable the sustainable utilization of three generations of feedstocks. *Bioresour. Technol.* **361**, 127656 (2022).
93. Skrede, G. et al. Effects of lactic acid fermentation on wheat and barley carbohydrate composition and production performance in the chicken. *Anim. Feed Sci. Technol.* **105**(1), 135–148 (2003).
94. Yan, Y. et al. Gut microbiota and metabolites of α-synuclein transgenic monkey models with early stage of Parkinson's disease. *NPJ Biofilms Microbiomes* **7**(1), 69 (2021).
95. Knorr, S. et al. Widespread bacterial lysine degradation proceeding via glutarate and l-2-hydroxyglutarate. *Nat. Commun.* **9**(1), 5071 (2018).
96. Wei, Y., Ma, X., Zhao, J., Wang, X. & Gao, C. Succinate metabolism and its regulation of host-microbe interactions. *Gut Microbes* **15**(1), 2190300 (2023).
97. Wei, Y. et al. Encapsulated mixture of methyl salicylate and tributyrin modulates intestinal microbiota and improves growth performance of weaned piglets. *Microorganisms* **9** (6), 1342 (2021).
98. De Luca, L. et al. Addition of glutamine to milk during fermentation by individual strains of lactic acid bacteria and the effects on pyroglutamic and butyric acid. *J. Food Compos. Anal.* **130**, 106175 (2024).
99. Tao, Y. et al. A comprehensive review on microbial production of 1, 2-propanediol: Micro-organisms, metabolic pathways, and metabolic engineering. *Biotechnol. Biofuels* **14**(1), 216 (2021).
100. Abedi, E. & Hashemi, S. M. B. Lactic acid production – Producing microorganisms and substrates sources-state of art. *Heliyon* **6**(10), e04974 (2020).
101. Strandwitz, P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* **1693**, 128–133 (2018).
102. Otaru, N. et al. GABA production by human intestinal Bacteroides spp.: prevalence, regulation, and role in acid stress tolerance. *Front. Microbiol.* **12**, 656895 (2021).
103. Zhuang, K. et al. Transcriptomic response to GABA-producing *Lactobacillus plantarum* CGMCC 1.2437^{T} induced by L-MSG. *PloS one* **13**(6), e0199021 (2018).

104. Quillin, S. J., Tran, P. & Prindle, A. Potential roles for gamma-aminobutyric acid signaling in bacterial communities. *Bioelectricity* **3** (2), 120–125 (2021).
105. Starke, S. et al. Amino acid auxotrophies in human gut bacteria are linked to higher microbiome diversity and long-term stability. *ISME J.* **17** (12), 2370–2380 (2023).
106. Du, J. et al. Gut microbiota dysbiosis and metabolic perturbations of bile/glyceric acids in major depressive disorder with IBS comorbidity. *Mbio* https://doi.org/10.1128/mbio.02447-25 (2025).

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Declarations

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The authors declare no competing interests.

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