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Lina Laučienė

ANALYSIS OF CHANGES IN THE COMPOSITION OF DAIRY FAT TO DEVELOP THE COMPUTER PROGRAM FOR SCREENING OF FATTY ACIDS IN RAW MILK

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Scientific Supervisor

Prof. Dr. Loreta Šernienė (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002).

Dissertation will be defended at the Veterinary Research Council of Lithuanian University of Health Sciences:

Chairperson

Prof. Dr. Rasa Želvytė (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002).

Members:

Dr. Irena Klimienė (Lithuanian University of Health Sciences, Agricultural Sciences, Veterinary – A 002);

Prof. Dr. Gintarė Zaborskienė (Lithuanian University of Health Sciences, Agricultural Sciences, Animal Sciences – A 003);

Assoc. Prof. Dr. Milda Keršienė (Kaunas University of Technology, Technological Sciences, Chemical Engineering – T 005);

Dr. Nils Kristian Afseth (Norwegian Institute of Food, Fisheries and Aquaculture Research, Agricultural Sciences, Veterinary – A 002).

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Address: Tilžės 18, LT-47181 Kaunas, Lithuania.

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Lina Laučienė

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Mokslinė vadovė

prof. dr. Loreta Šernienė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002).

Disertacija bus ginama Lietuvos sveikatos mokslų universiteto Veterinarijos mokslo krypties taryboje:

Pirmininkė

prof. dr. Rasa Želvytė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002).

Nariai:

dr. Irena Klimienė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, veterinarija – A 002);

prof. dr. Gintarė Zaborskienė (Lietuvos sveikatos mokslų universitetas, žemės ūkio mokslai, gyvūnų mokslai – A 003);

doc. dr. Milda Keršienė (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija – T 005);

dr. Nils Kristian Afseth (Norvegijos maisto, žuvininkystės ir akvakultūros tyrimų institutas, žemės ūkio mokslai, veterinarija – A 002).

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CONTENTS

LIST OF ABREVATIONS

ALA	–	C18:3n3; α -linolenic acid; (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid
AI	–	atherogenicity index
BP	–	batch pasteurization
CID	–	chronic inflammatory diseases
CLA	–	C18:2c9t11; conjugated linoleic acid; (9Z,11E)-Octadeca-9,11-dienoic acid
CVD	–	cardiovascular disease
DFA	–	desirable (hypocholesterolemic) fatty acids
DFSQ	–	Department of Food Safety and Quality
EFSA	–	European Food Safety Authority
FA	–	fatty acid/fatty acids
FAME	–	fatty acid methyl esters
FFA	–	free fatty acids
GC-MS	–	gas chromatography-mass spectrometry
h/H	–	hypocholesterolemic/hypercholesterolemic ratio
HDL	–	high-density lipids
HTST	–	high temperature short time
LA	–	C18:2n6; linoleic acid; (9Z,12Z)-octadeca-9,12-dienoic acid
LAB	–	lactic acid bacteria
LCMTL	–	Lithuanian Central Milk Testing Laboratory
LCFA	–	long-chain fatty acids
LDL	–	low-density lipid
LUHS	–	Lithuanian University of Health Sciences
LQI	–	lipid quality indices
LTLT	–	low temperature long time
MAP	–	modified atmosphere packaging
MCFA	–	middle-chain fatty acids
MFGM	–	milk fat globule membrane
MUFA	–	monounsaturated fatty acids
PUFA	–	polyunsaturated fatty acids
SCFA	–	short-chain fatty acids
SFA	–	saturated fatty acids
sn	–	stereospecific numbering
TG	–	triacylglycerols
TI	–	thrombogenicity index
VA	–	18:1 t11; vaccenic acid; (11E)-Octadec-11-enoic acid
UF	–	ultrafiltration

UFA – unsaturated fatty acids
UHT – ultra high temperature
C4:0 – butyric acid; butanoic acid
C6:0 – caproic acid; hexanoic acid
C8:0 – caprylic acid; octanoic acid
C10:0 – capric acid; decanoic acid
C11:0 – undecylic acid; undecanoic acid
C12:0 – lauric acid; dodecanoic acid
C13:0 – tridecylic acid; tridecanoic acid
C14:0 – myristic acid; tetradecanoic acid
C14:1 – myristoleic acid; (9Z)-Tetradec-9-enoic acid
C15:0 – pentadecylic acid; pentadecanoic acid
C16:0 – palmitic acid; hexadecanoic acid
C16:1 – palmitoleic acid; (9Z)-Hexadec-9-enoic acid
C17:0 – margaric acid; heptadecanoic acid
C18:0 – stearic acid, octadecanoic acid
C18:1c9 – oleic acid; (9Z)-Octadec-9-enoic acid
C18:1t9 – elaidic acid; (E)-octadec-9-enoic acid
C18:2n6 – LA; linoleic acid; (9Z,12Z)-octadeca-9,12-dienoic acid
C18:3n3 – ALA; α -linolenic acid; (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid
C20:0 – arachidic acid; eicosanoic acid
C21:0 – heneicosylic acid; heneicosanoic acid

INTRODUCTION

Milk lipids are composed of 70% saturated, 25% monounsaturated, and 5% polyunsaturated fatty acids [1]. Their composition and content can be adjusted by changing the feed composition using special ruminant fat-containing feed additives to manipulate milk fat content and changing the properties of dairy products [2]. The consequences of these processes are twofold. On the one hand, such milk makes it easy to produce dairy products that meet consumer expectations (e.g., the spreadability of natural butter) [3]. On the other hand, with the popularity of cow's feed additives, dairy products are no longer in line with many of the norms and recommendations found in the literature in their fat composition [4–6].

Consumers are increasingly demanding the product's naturalness, authenticity, and quality. Nevertheless, product food fraud is a serious problem today. According to Canja et al. (2016), falsification involves all kinds of food [7]. Milk and dairy products are among the leading food categories of food contamination. The abundant adulterants of milk fats are vegetable oils (soybean, sunflower, groundnut, coconut, palm, and peanut oil) and animal fat (cow tallow and pork lard) [8]. The falsification of milk fat is another reason that may lead to changes in the composition of fatty acids.

Today, some countries, due to low quality, misleading consumer information, and possible falsification of dairy products, set regulatory requirements for the content of fatty acids both for domestic production and for imported dairy products [9]. Non-compliance with national production with export regulations may constitute a severe obstacle to export.

The assortment of dairy products in Lithuania is continuously expanding; therefore, the internal market is flooded. Manufacturers are looking for new markets for their products, in many cases highlighting the problem of non-compliance with foreign product quality requirements. The composition of milk fat in dairy products is particularly relevant in this regard. One of the reasons for the reduced export of Lithuanian produce to foreign markets is the non-compliance of the content of fatty acids in the final milk products with the updated requirements of food legislation.

On the other hand, non-compliance of Lithuanian dairy products with the standards of other countries may be influenced by many external (cow feeding ration composition, dairy farm management, rearing type, milking, season) or internal/biological (breed, dairy cow individuality, lactation period, parity and stage) factors [5, 10–14]. All of these factors can vary significantly from country to country; hence, the same dairy product produced in different countries can vary in composition.

Nevertheless, it is also essential to keep in mind the effect of the production process when analyzing possible causes. We still lack information on whether all of the raw milk fatty acids are transferred to an end product, whether their composition changes, or whether a significant reduction of a particular fatty acid during dairy processing is possible.

Scientific novelty

There are very few studies on the impact of the technological process on fatty acids profile of dairy products conducted under commercial settings. Usually, researchers analyze the impact of the technological process on fatty acids profile under laboratory conditions [15–17] or the change in the fatty acid profile during the production of traditional dairy products [18–21].

It is also difficult to compare the effect of the technological processes on the composition of the fatty acids due to differences in heat treatment regimes, other technological parameters, equipment, raw material composition, the fat content of the final product, etc. in different countries and dairy plants.

To date, no research has been carried out in Lithuania to determine the profile of fatty acids in the technological dairy chain covering every major step of the technological process and to analyze the relationship between the raw material, end product, and by-product under the commercial settings. Thus, this makes this case study relevant and new.

As this study result, a prototype computer program was developed and tailored for the specific dairy processor's needs. This tool enables the screening of the fatty acids composition of procured cow's milk data, provided by Lithuanian Central Milk Testing Laboratory (LCMTL), according to the respective period, region, or selected raw milk producers. This program can help dairy processors to produce export products of the desired (standard) fatty acid composition and become more competitive in both domestic and foreign markets.

The aim and objectives

This thesis aimed to evaluate the effect of season, processing, and storage on dairy fatty acids composition in processed milk to develop the computer program prototype for fatty acid screening in the procured raw milk.

Objectives of the study:

1. To perform a retrospective analysis of major fatty acids content in procured Lithuanian cow's raw milk samples routinely analyzed in Lithuanian Central Milk Testing Laboratory in 2016–2017.

2. To evaluate and analyze the effect of season on the full profile of milk fatty acids and fat lipid quality indices in the bulk tank raw milk samples collected from dairy plants in 2018–2019.

3. To evaluate and analyze seasonal variations of fatty acids in major milk processing steps and at the end of shelf life of commercially produced natural dairy products (UHT milk, strained yogurt, sour cream, curd cheese, and butter).

4. To apply the findings of the fatty acids dynamic for the development of a prototype computer program enabling procured raw milk screening according to the set normative fatty acid composition of the manufactured dairy produce.

1. LITERATURE REWIEV

1.1. Consumption of milk and dairy products

The dairy sector in Lithuania has a long tradition. Primary milk production is one of the major ones in the agricultural industry, and dairy processing is the most critical area of food processing. The sector is an essential source of employment for the population. The country processed 1.7 million tonnes of raw milk in 2018. Dairy processing companies exceed the needs of the Lithuanian domestic market; therefore, 50–60% of production is exported.

Among the Baltic States, Lithuania is the market leader in terms of the amount of milk procured and processed. Lithuania's share in the common Baltic milk production market was 47%, Latvia's – 30%, Estonia's – 23% in 2017 [22].

However, due to sector specificities and external factors, the dairy sector remains quite vulnerable, and dairy farms are among the most susceptible state-sponsored agricultural areas.

Consumption of milk and dairy products varies significantly across regions. Fig. 1.1.1 shows the use of fluid milk worldwide in 2018.

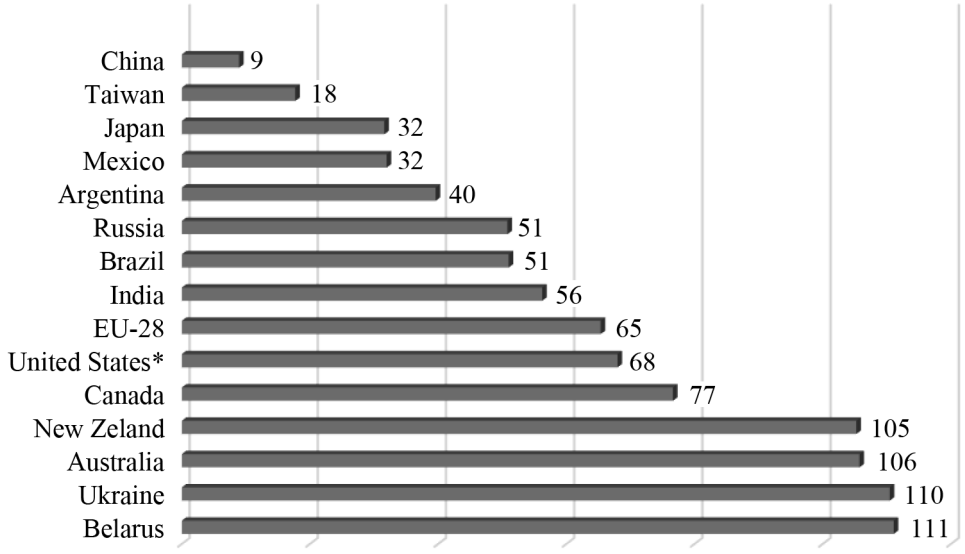


Fig. 1.1.1. *Consumption of fluid milk (kg/per capita) worldwide in 2018* [23].
*Include conventional, organic, and other various milk products.

Belarus and Ukraine are leading in milk consumption. Large quantities of milk are consumed in New Zealand and Australia, compared to other countries. The lowest use of milk is in Asia. The EU countries are in an intermediate position [23].

The Food and Agriculture Organization of the United Nations reported that the demand for milk and milk products is increasing worldwide, mainly due to rising incomes, population growth and changes in diets in developing countries; according to their report, milk production is expected to increase by 20% by 2025 worldwide. The main driver of growth is the growing population number and rising standard of living in developing countries, especially in East and Southeast Asia [24].

Milk consumption in Western societies has declined in recent decades. This trend can be partly explained by the adverse health effects attributed to milk and milk products. This criticism came in particular because of the high proportion of milk fat SFA assumed to contribute to heart diseases, weight gain, and obesity [25].

Meantime in Lithuania, dairy products are an integral part of the daily diet and accounted for 328 kg per capita in 2018 [22].

With the increasing use of food processing and the use of various food additives, more and more people are opting for natural products. This trend is also visible among dairy consumers. In recent years, an increase in the consumption of raw milk was observed. Assuming that raw milk gives higher health benefits, nutrition values, potential probiotic bacteria compared with heat-treated milk [26]. However, the consumption of raw milk imposes a significant health risk associated with the ingestion of foodborne pathogens and consequent zoonotic illnesses [27].

1.2. Nutritional and functional milk value

In human nutrition, milk consumption has long traditions, which began 12,000–15,000 years ago, when humans domesticated small ruminants and learned to raise and breed goats and sheep. Later, around 10,500–10,000 years ago, people domesticated taurine cattle [28]. In our days, bovine milk is most commonly consumed with 85% of total milk quantity produced worldwide [29]. Meantime, 11% of world milk production comes from buffalo, followed by 2.3% from goats, 1.4% from sheep, and 0.2% from camels [30].

Milk is the first food for mammals and supplies all the energy and nutrients needed to ensure proper growth and development in the postnatal period [26]. Milk consumption generally stops after the end of the weaning period, except in humans, as it is ingested even during adulthood [26]. Milk appeared to occupy a unique position among the many foods; therefore, milk contains

everything the young organism needs for growth and development [31, 32]. Dairy foods, in general, are commonly considered as balanced and nutritious foods, being frequently included as essential components of a healthy diet [26]. Dairy product popularity is due to the unique composition of the milk and its nutritional value. Milk contains all the necessary nutrients for the human body: proteins, fats, lactose, micro and macro elements, vitamins, and enzymes ensure normal human growth, development, and vital functions of the body [33].

Milk is often described as a colloidal suspension containing emulsified fat globules, a heterogeneous family of basic and minor proteins, carbohydrate lactose, minerals, and vitamins. In addition to essential nutrients, milk contains protective substances such as enzymes (lysozyme, catalase, lactoperoxidase, etc.), and growth factors [33].

Despite the nutritional value of milk, for a prolonged time consumption of dairy products were associated with many adverse health effects due to their saturated fatty acid (SFA) content, which may lead to increased low-density lipids (LDL) level, thus an increased risk of cardiovascular disease (CVD). It was widely accepted that the intake of C12:0, C14:0, and C16:0, which are detected in relevant quantities in dairy fat, would seem to be unhealthy in excessive amounts [25].

Nevertheless, other SFA found in milk neutralize their effect since they increase high-density lipids (HDL) level [34]. C18:0 is not considered to be a promoter of elevated cholesterol [35]. Indeed, increased levels of circulating C18:0 lipids are associated with reduced blood pressure, improved heart function, and reduced cancer risk [36]. It was found that even C12:0 can have positive antibacterial effects and might act as an anticaries and antiplaque agent [14]. Middle-chain fatty acids (MCFA) do not pose an obesity risk; they prevent ulcerative colitis, cancer, atherosclerosis, and hypertension; they have anti-inflammatory and antibacterial effects, and they boost natural immunity [42].

The matrix in which these SFA are contained may also influence health outcomes. Dairy matrix components, mainly calcium, peptides, phosphorus, and the milk fat globule membrane (MFGM), modify blood lipid responses to SFA intake [37].

Recent decade findings have indicated that the impact of SFA to CVD may be less pronounced than previously assumed. It has been shown that not all SFA are created equal and that the presence of specific fatty acids in circulation is associated with a lower incidence of several cardiometabolic diseases [38, 39].

The negative opinion on milk SFA is also debatable because of the high milk content components promoting health benefits, including C18:1n9c,

18:2c9t11 (CLA), omega-3 fatty acids, whey proteins, vitamins, minerals, and bioactive compounds and various milk proteins and their peptides which have anticancer activities [40, 41].

Short-chain fatty acids (SCFA), which exclusively synthesized in the cow mammary gland during de-novo synthesis, have beneficial properties for human health either. C4:0 can have an anticarcinogenic effect. C8:0 and C10:0 might play antiviral roles, and the C8:0 can even have functions related to the delay of tumor growth and act against gram-negative coliform [16]. In the human body, these fatty acids are used as sources of energy for the muscles, heart, liver, kidneys, blood platelets, and nervous system [42].

The most abundant trans fatty acid in bovine fats is 18:1t11 (VA) [43]. The effects of trans fatty acids on health are very complex. Many are potentially harmful, especially those of industrial origin, while VA seems to be beneficial in preventing cardiovascular diseases, cancer, and obesity [18]. VA is also a precursor of CLA, which has an anticarcinogenic, antiatherogenic, anti-diabetic, and immunomodulatory effect on human health [18, 44]. Only small amounts of natural trans fats are found in ruminant milk, and they are not associated with cardiovascular diseases [43].

Milk fat supplies the two essential polyunsaturated fatty acids (PUFA): 18:2n6 (LA) and 18:3n3 (ALA), which are predominant fatty acids of omega-6 and omega-3 groups, respectively. They are not synthesized in the human body and must be obtained with food [45]. In vitro and in vivo studies have demonstrated a wide range of health benefits of omega-6 and (in particular) omega-3 PUFA. Such as lowering the risk of cardiovascular disease, type 2 diabetes, hypertension, cancer, and specific neurological dysfunctions [42].

Bovine milk lipids characterized as a very high digestibility fat. The human body assimilates 97–99% of milk fat [42].

1.3. Chemical milk composition

Milk is defined as a colloidal suspension containing emulsified globules of fat, a heterogeneous family of major and minor proteins, the carbohydrate lactose, minerals, vitamins, enzymes, and many other minor components [46]. On average, bovine milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat, 0.8% minerals, and 0.1% vitamins [47, 48]. Several factors – breed [49], individual animal health status, lactation period [5], climatic conditions, feeding rations [10], milking system [50] can influence the composition of milk. Still, first and foremost, milk composition is determined by the animal species (Table 1.3.1).

Table 1.3.1. *The average composition of cow, goat and sheep milk* [51].

Milk components	Cow	Goat	Sheep
Fat (%)	3.6	3.8	7.9
Lactose (%)	4.7	4.1	4.9
Protein (%)	3.2	3.4	6.2
Energy (kcal/100 mL)	69	70	105
Calcium (mg/100 mL)	122	134	193
Phosphorus (mg/100 mL)	119	121	158
Vitamin A (IU)	126	185	146
Vitamin D (IU)	2.0	2.3	0.18

Bovine milk contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes, and other bioactive peptides. The lipids in milk are emulsified in globules coated with membranes. The proteins are in colloidal dispersions as micelles. The casein micelles occur as colloidal complexes of protein and salts, primarily calcium. Lactose and most minerals are in solution [32].

Basic milk constituents such as proteins and lipids are essential for dairy processors as these ingredients are mainly responsible for forming dairy products and influence the yield of the final product. It also affects the base price of raw milk, along with qualitative indicators [52].

1.3.1. Milk lipids

Lipids represent an essential component of milk, being able to influence the physical, organoleptic, and nutritional properties of dairy products, and playing an important role in human health promotion and disease prevention [53].

Triacylglycerols (TG) are the main component of bovine milk fat (~98% of the lipid fraction) [1, 14, 54]. Other milk lipids are diacylglycerols (~2%), cholesterol (less than 0.5%), phospholipids (~1%), and free fatty acids (FFA), accounting for less than 0.5% of total milk lipids [1].

The lipids in bovine milk are mainly present in globules as an oil-in-water emulsion [55]. These fat droplets are formed by the endoplasmic reticulum in the epithelial cells in the alveoli and coated with a surface material of proteins and polar lipids. When secreted, they are surrounded by the plasma membrane of the cell. Membrane-associated elements can comprise 2–6% of the globule mass [56].

The fat globules in cow milk are coated by a thin protective film with external layers comprised of proteins and mainly phospholipids in unprocessed milk (Fig. 1.3.1.1) [29, 57].

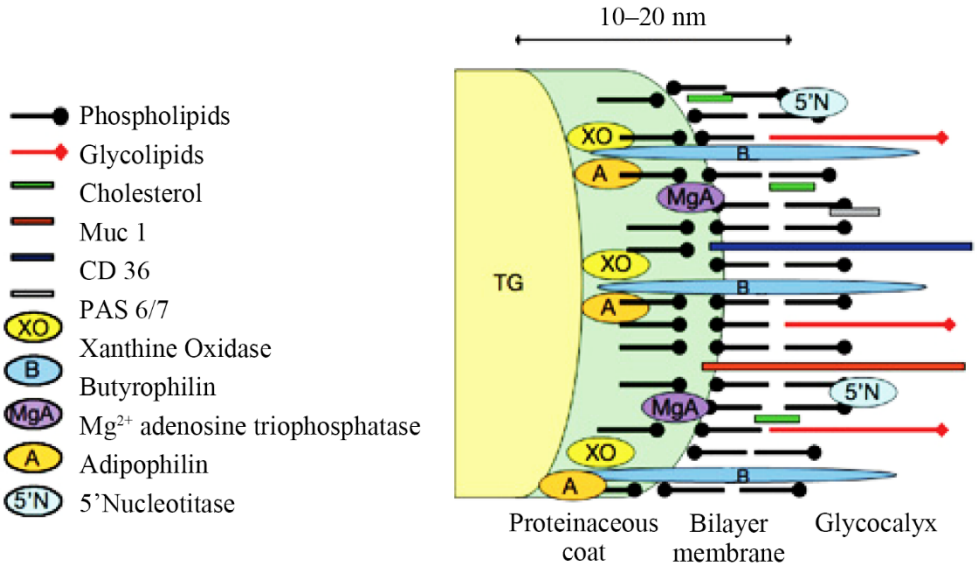


Fig. 1.3.1.1. *Milk fat globule membrane structure* [58].

These membranes are rich in phospholipids, sphingolipids, cholesterol, enzymes, and other minor components [58]. Phospholipids and sphingolipids comprise about 1% of total milk lipids. They may beneficially influence lipid metabolism, gut dysbiosis, inflammation, cardiovascular disease, gut health, and neurodevelopment [59].

This biological membrane ensures the protection and stability of the milk fat in the aqueous phase. During cream churning, this membrane ruptures and released from milk fat globule into the aqueous phase – buttermilk [60].

1.3.2. Milk fatty acids

Bovine milk fat is one of the most complex natural oils and fats. TG, as the main component of bovine milk fat, present a highly complicated structure due to the great number of fatty acids and their binding positions along the glycerol backbone [1, 14, 54]. Each TG molecule is built with a specific fatty acid combination [1].

Because of their high abundance in milk fat, TG has a major effect on the physical properties of milk fat, for instance, melting characteristics, solid fat content, rheology, and crystallization behavior [54].

Fatty acids are not randomly esterified at the three positions (stereospecific numbering, sn) of the triacylglycerol molecule. The SCFA (C4:0 and C6:0) are esterified almost entirely at sn-3. MCFA (C8:0–C14:0) as well as C16:0, are preferentially esterified at positions sn-1 and sn-2. C18:0 is selectively placed at position sn-1, whereas C18:1 shows a preference for positions sn-1 and sn-3 [61].

Gas-liquid chromatography analyses have identified 406 fatty acids in bovine milk lipids, most of which are present in amounts of <1% of total lipids. Only 12 to 14 fatty acids are present in concentrations higher than 1% of the milk fat (Table 1.3.2.1) [62].

Table 1.3.2.1. *The most abundant fatty acid composition of bovine, ovine and caprine milk (% of total fatty acids)* [6].

FA	Bovine	Ovine	Caprine
C4:0	2.87	2.57	2.03
C6:0	2.01	1.87	2.78
C8:0	1.39	1.87	2.92
C10:0	3.03	6.63	9.59
C12:0	3.64	3.99	4.52
C14:0	10.92	10.17	9.83
C16:0	28.7	25.1	24.64
C18:0	11.23	8.85	8.87
C18:1	22.36	20.18	18.65
C18:2	2.57	2.32	2.25
C18:3	0.5	0.92	0.77

Bovine milk fatty acids are obtained almost equally from two sources: feed and microbial activity in the rumen. During de novo synthesis in the mammary gland, the C4:0–C14:0 acids are synthesized, together with about half of the C16:0, and these fatty acids account for 45–60% of total fatty acids. The remaining C16:0 and the long-chain fatty acids (LCFA) originate from dietary lipids and lipolysis of adipose tissue TG. C15:0 and C17:0 are synthesized by bacterial flora in the rumen. MCFA, but mainly C18:0, may be desaturated in the mammary gland to form the corresponding mono-unsaturated acids [61].

On average, 70% of the fat fraction is composed of SFA and 30% by unsaturated fatty acids (UFA) [63]. The composition and content of lipids in milk fat vary widely among mammalian species (Table 1.3.2.2).

Table 1.3.2.2. *Main fatty acid composition of different animal species (minimum and maximum values, % of total fatty acids)* [29].

Milk	SFA	MUFA	PUFA	C18:2	C18:3	CLA
Mare	37–55	18–36	13–51	3.6–20.3	2.2–31.2	0.02–0.1
Donkey	46–68	15–35	14–30	6–15.2	4–16.3	trace
Buffalo	62–74	24–29	2.3–3.9	2.0	0.2–1.4	0.4–1
Cow	55–73	22–30	2.4–6.3	1.2–3.0	0.3–1.8	0.2–2.4
Goat	59–74	22–36	2.6–5.6	1.9–4.3	0.3–1.2	0.3–1.2
Ewe	57–75	23–39	2.5–7.3	1.6–3.6	0.5–2.3	0.6–1.1

Within SFA, the most important from a quantitative viewpoint are C16:0, C14:0, and C18:0, and they account for 30%, 11%, and 12% of the total fatty acids content, respectively. The SCFA (C4:0-C6:0) and MCFA (C8:0-C15:0), which accounts for 25% of total fatty acids is a unique attribute of bovine milk. C15:0 and C17:0, respectively, account for 1.05%, and 0.61% of milk fatty acids, are specific for ruminant fat. They cannot be synthesized in the human body [61, 62].

In the UFA fraction, C18:1 is present in concentrations within 24% to 35% of total fatty acids. PUFA constitute around 2.3% of total fatty acids, with LA and ALA accounting for 1.6% and 0.7%, respectively. Milk also includes trans fatty acids like VA and CLA, which accounts for 2.7% and 0.34–1.37% of total fatty acid content, respectively [61].

Milk fat is valued for its energy, supply of essential fatty acids, and for the dissolution and absorption of fat-soluble vitamins and essential nutrients [62].

1.3.3. Indices of lipid quality

To correctly assess the diet fat value, it is not enough to study the individual fatty acids and their main groups. Particularly lipid quality indices (LQI) that better reflect the dietary lipid value are calculated.

Mammals are incapable of synthesizing omega-6 and omega-3 fatty acids; consequently, their diets should be supplemented with these crucial fatty acids. Since LA and ALA represent the parental fatty acids of omega-6 and omega-3, respectively, the omega-6/omega-3 ratio closely tracks the LA/ALA ratio. The omega-6/omega-3 ratio of 3: 1 is required for healthy lipid control in the human body. Meantime, both rates in the diet of most people range from 15:1 [64] due to increased omega-6 intakes and decreased omega-3 intakes [65]. With the increase in the ratio, there is an increased incidence of chronic inflammatory diseases (CID) such as non-alcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease. By increasing the omega-3

fatty acids in the diet, reductions may be achieved in the incidence of CID [66]. However, foods based on omega-6/omega-3 ratios lower than 1:1 are not recommended because they inhibit the transformation of linoleic acid into longer-chain polyunsaturated fatty acids [67].

A growing tendency to replace animal fats, mainly milk fat, with vegetable fats is a matter of concern. Since the omega-6/omega-3 ratio of most popular vegetable oils is extremely higher than the recommended rates. E.g., sunflower oil, corn oil, and grape seed oil have omega-6/omega-3 ratios of 335:1, 141:1 and 173:1, respectively [64]. A high rate of omega-6/omega-3 was observed in the pumpkinseed (275.7:1) and sesame (136.2:1) oil [68].

Meantime, in the milk fat of Holstein-Friesian and Simmental cow breeds, the ratio of omega-6/omega-3 was found within the recommended levels, 1.69:1 and 1.72:1, respectively [69]. The ratio of omega-6/omega-3 differs among cow breeds (Braunvieh 4.40:1; Holstein 4.92:1; Pinzgau 2.32:1; Red Holstein 3.23:1) [70], but in general, bovine milk is consistent with recommendations.

In 1991, Ulbricht and Southgate suggested calculating the atherogenicity (AI) and thrombogenicity (TI) indices to measure the potential of the diet fat [71]. AI indicates the relationship between the main proatherogenic C12:0, C14:0, and C16:0 and PUFA and MUFA that are designated as anti-atherogenic fatty acids. Proatherogenic SFA is favoring the adhesion of lipids to cells of the immunological and circulatory system. Meantime, PUFA and MUFA inhibit the aggregation of plaque and diminish the levels of esterified fatty acid, cholesterol, and phospholipids, thus preventing the appearance of micro- and macro-coronary diseases.

TI shows the tendency to form clots in the blood vessels. This index defined the relationship between the prothrombogenic SFA and the anti-thrombogenic MUFA, omega-6 PUFA, and omega-3 PUFA [71, 72].

The smaller AI and TI index values show a higher amount of anti-atherogenic and anti-thrombogenic fatty acid present in fats, and the higher potential for preventing the onset of coronary heart disease [73].

The hypocholesterolemic/hypercholesterolemic ratio (h/H) is related to the functional activity of fatty acid in the metabolism of lipoproteins regarding plasma cholesterol transport and the risk of cardiovascular disease. This index permits an improved nutritional assessment of milk fat, considering to a ratio of the beneficial MUFA and PUFA and negative C14:0 and C16:0. High h/H indices specify the higher nutritive value of fat [74].

A PUFA/SFA ratio above 0.45 is recommended in the diet to prevent coronary heart disease and cancers [69, 75]. Bovine milk does not meet the PUFA/SFA ratio recommendations. It is significantly lower than recommended and varies between 0.026–0.08 among different cow breeds or

feeding ration [69, 76]. The herbaceous forages during the grazing period or cow diet modifications with individual plant seeds or oils can improve this ratio [77, 78].

UFA and C18:0 are included in the formula for the calculation of desirable fatty acids (DFA) [74, 79]. PUFA and MUFA showed biological significance by exerting antimicrobial, anti-carcinogenic and anti-inflammatory activities, as well as regulatory effects on blood serum lipid profile. Therefore, increaseing their concentration in milk would enrich milk's nutritional profile [80]. Dietary C18:0 does not increase atherosclerosis risk and reduces LDL [36]. Meantime C12:0, C14:0, and C16:0 are described as hypercholesterolemic fatty acids [14, 74] since they increased LDL blood concentration [34]. C14:0 is the most hypercholesterolemic among them and has a four times greater potential than C16:0 to raise the plasma cholesterol concentration [81].

Many factors are associated with the variations in the amount and fatty acid composition of bovine milk lipids. They may be of animal origin, which includes genetics (breed and selection), stage of lactation, mastitis, and ruminal fermentation, or they may be feed-related factors, which comprise fiber and energy intake, dietary fats, and seasonal and regional effects [48].

1.4. Milk processing

For a long time, it was not clear when dairy products were started to produce. But in 1970, archaeologist Peter Bogucki was excavating a Stone Age site in the fertile plains of central Poland when he came across an assortment of odd artifacts. The people who had lived there around 7,000 years ago were among central Europe's first farmers, and they had left behind fragments of pottery dotted with tiny holes. The mystery potsherds sat in storage until 2011 when they were pulled out, and fatty residues preserved in the clay were analyzed. Roffet-Salque, a geochemist at the University of Bristol, UK, found signatures of abundant milk fats – evidence that the early farmers had used the pottery as sieves to separate fatty milk solids from liquid whey. That makes the Polish relics the oldest known evidence of cheese-making in the world [82].

Raw milk is a perishable product, thus people have learned how to process raw milk into various dairy products. Fermentation is one of the oldest methods used to extend the shelf-life of milk. The exact origin of the manufacture of fermented milks is challenging to detect, but it is safe to assume that it could date to more than 10,000 years ago as the way of life of humans changed from food gathering to food-producing [83]. Over time humans learned to control fermentation processes from the first accidental events in fermentation. This learning of controlled fermentation of milk in

domestic practices gave rise to several dairy products influenced by habits of different ethnicities, and geographical environments [84]. In our days, fermented milks are manufactured throughout the world, and approximately 400 generic names are applied to traditional and industrialized products [83].

It is known that in ancient times humans used animal stomachs as vessels for storing water/milk; therefore, the rennet naturally present in the stomachs of calves would have turned any milk being carried in them into curds and whey. The processing of milk, particularly the production of cheese, not only allowed the preservation of milk products in a non-perishable and transport-table form, but it also made milk a more digestible (breakdown of lactose) commodity for early prehistoric farmers [82].

Louis Pasteur, in 1860 discovered and in 1870 demonstrated that heating liquids, especially wines, to relatively low temperatures, such as 60°C, improved the quality during storage. The first application of pasteurizing heat treatments to milk have been performed by Soxhlet, who pasteurized bottled milk fed to infants. Gerber and Wieske pasteurized milk in bottles at 65°C for 1 h as early as 1888. The first commercially-operated milk pasteurizer in the United States of America was installed in Bloomville, New York, in 1893 [85].

Milk is a universal raw material that, with relative ease, can be converted into a wide variety of products. In some cases, milk undergoes relatively limited processing, consisting of heat treatment to increase the product microbial shelf life and homogenization to improve the physical shelf life through retarding fat separation. Other well-known processes involve the acid-induced coagulation of milk to produce fermented milks, or the enzy-matic coagulation of milk to manufacture cheese. Besides, milk may be spray-dried or used as a base from which constituents, e.g., proteins, fats, or minor constituents, are isolated [46].

1.4.1. The effect of milk heat treatment on fatty acids profile

The primary aim of heat-treatment processes is to destroy pathogenic organisms in liquid foods to extend the shelf-life of the product for a limited period [86].

Milk pasteurization can be defined as “microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a signi-ficant health hazard.” Pasteurization conditions depend on the raw milk microbiological quality, on milk fat or sugar content, and also vary from country to country based on microorganism strain heat resistance [87].

There are two methods of pasteurization: Low-Temperature Long Time (LTLT) and High-Temperature Short Time (HTST). The LTLT method of pasteurization includes heating milk to 63°C and holding at such temperature for 30 min. The HTST method of pasteurization involves heating milk at 72–75°C for 15–20 sec [88]. In Lithuania, legislation requires that the milk must be heat treated at least 71.7°C for 15 sec during pasteurization and at least 135°C for 1 sec during ultra-high temperature (UHT) treatment [89]. At the same time, elsewhere, UHT processes vary from 130 to 150°C for 1 to 4 sec [88].

UHT treatment is a sterilization process. UHT process can be performed by "direct" or "indirect" heat transfer. During direct UHT treatment, super-heated steam is mixed with milk. In detail, steam may be injected into milk (steam injection), or milk may be sprayed into steam (steam-infusion). In the indirect system, the heat exchanger transfers heat across a partition between milk and steam or hot water [87].

Pasteurization kills most of the microorganisms in milk but does not render the milk sterile. Hence, pasteurized milk must be kept refrigerated throughout distribution and storage [88]. Spore-forming bacteria that are not affected by pasteurization remains a severe problem for dairy processors [90]. Consequently, spore-forming bacteria in conventionally pasteurized milk mainly originate from raw milk and are transmitted through the entire process [91]. UHT temperature treatment destroys both vegetative microorganisms and spores, leaving the product commercially sterile. This thermal process inactivates most of the harmful microorganisms and enzymes present so that UHT milk can be stored for several months under ambient temperature conditions. As a complement to pasteurized milk, UHT milk has gained acceptance and popularity due to its long shelf-life in ambient temperature [57].

Pasteurization is associated with minimal chemical, physical and organoleptic changes in the product. Meantime, nutritional composition and sensory properties changed in milk treated by UHT [88, 92]. Previous studies have found several sensory differences in milk treated by UHT methods, including cooked flavor and aroma, caramelized flavor, sweet, bitter, astringency, and color differences [93].

Pasteurization had no significant effect on fatty acid profile and content, although different pasteurization temperatures and applications were used in several studies [15, 94–99]. Santos et al. (2012) revealed minor changes after pasteurization (85°C/10 min) in milk fats: C14:0 and SFA increased, and 18:2c9t11 and 14:1 decreased, while all the rest fatty acid did not differ significantly [96].

Meantime, the data on fatty acid content of UHT treated milk is quite controversial.

Dias et al. (2020) found that bath pasteurization (BP) (63°C/30 min), HTST (72°C/15 sec), and UHT (135°C/3 sec) treatment did not statistically alter fatty acid concentrations comparing to raw bovine milk. Except for a 30% reduction in C10:0 after UHT compared to BP, no significant differences in total fatty acid concentrations were detected amongst heat treatments [100].

Pestana with co-workers stated that raw, pasteurized and UHT milk had very similar fatty acid profiles and found that pasteurization (75°C/15 sec) and UHT (140°C/3 sec) changed (decreased) only SCFA (C4:0, C6:0, and C8:0) in his study [92].

Khan et al. (2017) estimated a significant increase of SCFA and MCFA, and decrease of LCFA after pasteurization and boiling (1 min) of cow and buffalo milk [101]. Some authors referred to the significant decrease of SCFA, MCFA, and LCFA due to UHT treatment [17], but it's not clear what temperature modes were used in their study.

Data on the impact of UHT treatment on fatty acid profiles are varying, and changes in fatty acids appear to be closely related to temperature and application time.

1.4.2. The effect of milk homogenization on fatty acids profile

Homogenization is a widely used process in the food, pharmaceutical, and biotechnology industries. This process allows the mixing of two immiscible phases. The intense, disruptive forces of homogenization can break down fat globules and improve the stability of emulsions by reducing the creaming rate [102].

Homogenization, through the application of pressure, prevents fat separation from the milk. Homogenization of milk causes a reduction of fat globule size and a parallel increase in the milk fat surface area, which alters the original MFGM. Then milk proteins, especially casein, cover this newly generated surface of the fat globules. [103].

Homogenization temperature must be over 45°C because milk lipase and many microbial lipases are rendered inactive at this temperature. The effect of pressure on the size and distribution of fat globules starts at 50°C. Below this temperature, no changes have occurred due to the stability of the fat particle membrane [104]. The homogenization pressure depends on the fat content of the emulsion and commonly ranges from 10 to 25 MPa [105]. The higher pressure during homogenization of dairy cream-based emulsions results in smaller droplet size and narrower droplet size distribution [102].

Frahm et al. (2012) found more of C10:0 and less of C18:1 in milk after homogenization. The authors concluded that there were more differences in fatty acid content between different breeds of cows than as a result of processing [106]. Another study revealed that the total amount of fat extracted from the milk samples decreased as the homogenization pressure increased. In contrast, no significant differences were found in the fatty acid composition of raw milk treated by high-pressure homogenization up to 350 MPa [107]. Pirisi et al. (2007) stated that the fatty acid composition of cream was not significantly influenced by homogenization [95].

Tunick et al. (2016) found that milk fat homogenization significantly increased the FFA release during intestinal digestion (in vitro) compared to raw milk. Improved digestion may be attributed to the dislodging of the MFGM by homogenization, which increased the surface available to the lipase [108].

Thus, it seems that the homogenization of milk fat has a more significant impact on the size of the milk fat globule and their membrane than on the composition of the fatty acids.

1.4.3. The effect of milk fermentation on fatty acids profile

Milk fermentation is not only used for the preservation and increasing the shelf life of the product but also enhancing its tastes, forms, sensory properties, and improving the digestibility of milk. Gradually, consumers started to recognize the therapeutic and nutritional aspects of fermented foods, which increased consumption as well as the popularity of these foods. Validation of health benefits (i.e., anti-obesity, anti-diabetes, anti-cholesterol level, anti-cancer, immune modulation, etc.) of some of the fermented milks and its products have changed the preferences of consumers, thereby causing a concomitant rise in its production [109].

Fermented milks are manufactured throughout the world, and around 400 generic names are applied to traditional and industrialized products [83]. Taking into account the microorganisms that dominate in the product, Robinson (1990) suggested a classification of fermented milks as follows:

- lactic fermentation (mesophilic type (e.g., cultured butter-milk, filmjölk, tätmjölk, langofil); thermophilic type (e.g., yogurt, zabadi, dahi); probiotic type (e.g., acidophilus milk, Yakult, Onka, Vifit);
- yeast-lactic fermentations (e.g., kefir, koumiss, acidophilus yeast milk);
- mould-lactic fermentations (e.g., villi) [110].

Yogurt and strained yogurt are two examples of popular fermented milk products. Yogurt is produced by lactic acid fermentation of lactose in milk by lactic acid bacteria, such as *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*. The synergistic actions of these two bacteria contribute to the specific texture, composition, and sensory properties of yogurt. Fresh yogurt can be processed further into concentrated yogurt by partial removal of its whey using traditional cloth-bag or centrifugal separator methods [19].

Sour cream is a widely popular acidified dairy product. Sour cream is defined as the souring of pasteurized cream by LAB. Different types of sour creams exist that are determined based on fat content (e.g., full-fat, reduced-fat, low-fat) [111].

Milk lactose is particularly affected during fermentation. The lactose is a fermentable substrate, first being hydrolyzed by anaerobic microorganisms, allowing for anaerobic metabolism of the resultant simple sugars [112]. The lactic acid is the main compound produced, which gives the fermented dairy product the sharp and acidic taste. Other organic acids, such as acetic, butyric, pyruvic, and formic, can also be generated [113].

While fermentation impact on milk fatty acids is still unclear. 2015 study showed different individual fatty acids changing patterns in processing milk during yogurt production. E.g., there was found a consistent decline of C6:0 content from raw milk to strained yogurt. Meantime the content of C18:1 increased during milk fermentation and decreased after yogurt straining [19].

Camel milk fermentation with the thermophilic LAB (*L. delbrueckii* subsp. *Bulgaricus*, *Lactococcus lactis*, and *S. thermophilus*) increased the MCFA and LCFA (except C16:1, and C20:0) content [114]. Buffalo milk fermentation with *Lactobacillus acidophilus* and *L. lactis* increased the content of SCFA and MCFA (except C14:0) [115].

The increase of LCFA was observed when cream was fermented with probiotic mesophilic bacteria *Bifidobacterium lactis*. Whereas, cream fermentation with *L. acidophilus* revealed the rise in MCFA [116].

The authors of the study conducted in 2019 found that the manufacturing of yogurt using YoFlex Harmony® (Chr. Hansen, Denmark) culture did not influence the fatty acid composition. Volatile fatty acid slightly decreased, and the MCFA, LCFA, and UFA increased in yogurt compared to raw goat milk, but the differences were not significant [117]. When sheep milk was fermented with *S. thermophilus*, *L. delbrueckii* subsp. *Bulgaricus*, the fatty acid profile, did not undergo any changes [16]. Bovine milk fermentation using dried mixed starter culture (Danisco, Denmark) as well as yogurt straining (cloth sack method) did not reveal any changes in the fatty acid composition either [15]. In the 2002 study, when bovine milk was fermented

with *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* or probiotic *L. acidophilus* and *B. lactis*, the content of CLA, VA, and omega-3 fatty acids did not alter [118]. Bovine milk processing into yogurt using *Streptococcus salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* also did not impact significant changes in CLA content and fatty acid profile [119].

Fatty acid composition data when milk or cream from different animal species was fermented with different bacterial cultures are very heterogeneous and scattered. Data variety suggests that the substrate and bacterial culture of the starter are of particular importance to the fatty acid profile and content in fermented dairy products.

1.4.4. The effect of churning on butter fatty acids profile

Butter processing includes the cream separation from the milk to reach a 35-40% fat content in it. After cream pasteurization, it undergoes cooling and ripening. Churning is the next step, then the cream is separated into butter grains and buttermilk in a churning cylinder, after butter grain draining from buttermilk follows butter forming. The manufacturing process strongly affects the rheological behavior of the final products, as cooling rate, shear, and temperature during processing all affect fat crystallization, hence, network formation and microstructure [120].

The fatty acid composition influences the crystallization of milk fat and thus the level of solid fat content during cream ripening. By increasing the unsaturation degree of the fatty acid, the onset crystallization temperature decreases. Therefore, the same ripening temperature used for winter cream leads to lower levels of solid fat content and more extended ripening and churning times for summer cream [121].

Modifying the fatty acid composition of butter by decreasing the proportions of C12:0, C14:0, C16:0, and C18:0 and increasing the percentages of UFA and SCFA improves its spreadability [122]. Such changes can be achieved by processing technologies such as milk fat fractionation, cow nutrition, or cow selection. Cows that produced a more unsaturated and healthful milk fat maintained a higher LQI in their butter than did the cows with a low LQI in milk. Raw milk LQI reflection in the butter indicates that the fatty acids present in the product are directly related to the fatty acids present in the raw material [123].

2001 study showed similar trends of fatty acid content in the cream, butter, and buttermilk made from the milk of a control group cow and fed with fish oil additives [124]. 2007 study revealed that the butter-making process had no significant influence on the fatty acid and CLA content, either of organic cream processed into butter or of cream from integrated farming processed

into butter. Fatty acid profile of cream corresponded butter fatty acid profile [125].

1.4.5. The effect of dairy products storage/shelf life on fatty acids profile

Many factors such as quality of raw milk, steps in milk processing, level of recontamination after pasteurization, packaging material and technology, storage conditions, care during transportation, handling by the retail trade, determine the shelf life of dairy products [126–129]. Each category of dairy food has a unique shelf life. For example, UHT milk has several months of shelf life in ambient temperature [130]. Yogurt and similar fermented products remain fresh for 20–40 days under refrigerated storage conditions [131].

Some types of cheese, properly prepared and packaged retain their texture and specific sensory characteristics for several months while the application of edible coatings on cheese can extend their shelf life for several years in refrigerated storage conditions [128].

Regular pasteurization destroys vegetative microorganisms and native milk lipases. In contrast, the UHT process inactivates even bacterial spores and enzymes present, thus UHT milk (or cream) can be stored for several months under ambient temperature conditions [57].

Packaging strongly influences the dairy product's shelf life. For example, the UHT process, 135°C for few seconds, and aseptic packaging gives a shelf-life for several months to milk [130] while pasteurized liquid milk in regular package remains fresh for 12–14 days if kept below 4°C [132].

The study where raw milk was processed at various temperatures and packaged into six different packaging boards, showed that the flavor of milk packaged in standard deteriorated at a faster rate ($P \leq 0.05$) than milk packaged in barrier and foil boards over 15 weeks of storage [133]. In the 2019 study, during Mozarella storing for 90 days, the concentration of UFA decreased. The author state that transparent plastic pouches where cheese was wrapper increased the phot-oxidation of lipids [134]. Alves et al. (2007) also found out that cheese got oxidized after exposure to light [135]. Fletouris et al. (2015) indicated the importance of modified atmosphere packaging (MAP) in retail display storage of cheeses. The detected fatty acids changes (i.a. MUFA and PUFA decrease and SFA increase) in the light-exposed MAP samples were much lower than that exhibited by the aerobically packaged and light-exposed samples [136]. Lipid oxidation of milk is highly influenced by long-chain UFA, which is particularly susceptible to oxidation and can give rise to the development of off-flavor [137].

Karlsson, with coworkers (2019), discovered that storing temperature is also of great importance. UHT milk stored at 4°C and 20°C had the most extended shelf-life of 34-36 weeks, limited by sediment formation. While storage at 30°C and 37°C considerably decreased the shelf-life of UHT milk to 16–20 weeks, whereby changes in sediment formation, taste, and color were the limiting factors [130].

Commonly, three types of fat lipolysis may arise in milk: spontaneous, induced, and microbial. The activity of native milk lipases causes spontaneous lipolysis. Induced lipolysis is the result of mechanical damage to the fat globule membranes arising during milking, milk transport, storage, and processing. It is followed by the contact of free fat with milk lipases. The microbial lipolysis depends on the incidence of lipolytic bacteria. Principally psychrotrophic microorganisms are considered as hazardous, as they are capable of reproducing even at refrigerated storage temperatures [138].

During cold storage, psychrotrophic bacterial populations dominate the microflora, and their extracellular enzymes, mainly proteases and lipases, contribute to the spoilage of dairy products [139]. Among the psychrotrophic bacteria, the genus *Pseudomonas* (represented primarily by *P. fluorescens*) has been highlighted as the cause of numerous defects in dairy products [140].

Although these microorganisms have optimal and maximal growth temperatures above 15°C and 20°C, respectively, they can grow at low temperatures, such as 2–7°C. This means that over time psychrotrophic populations can develop in cold stored raw milk, and their presence in the raw milk microbiota can become a matter of concern [87]. The extracellular psychrotroph enzymes can resist pasteurization (72°C/15 s) and UHT (138°C/2 sec or 149°C/10 sec) [139, 141]. With the hydrolysis of milk fat through the activity of the bacterial lipases of psychrotrophic bacteria, FFA are released. These are the primary cause for the changes in product flavor that is described as rancid, unclean, soapy, or bitter. The lipolytic flavor defects are particularly pronounced in cream, butter, cheese, and sterilized (UHT) milk [142]. Meantime fermented milk due to the lower pH value (4.2–4.6) is not a appropriate environment for psychrotrophic bacteria and many other bacteria that damage dairy products [143]. The problem can arise when raw milk is stored at low temperatures for a more extended period before fermentation. In approximately 25% of cases, psychrotrophic bacteria are the leading causes of spoilage and reduced shelf-life of those products of cream and butter [142]. These days, due to consumer perceptions concerning health, many dairy products are enriched in omega-3 [144]. As the concentration of unsaturated double bonds increases in product fats, they become more susceptible to oxidation. Such dairy products become more vulnerable to rancidity [145]. Enriched with PUFA food products need to be processed, packed, and stored

under conditions that avoid such factors as a high level of oxygen, ultraviolet light, high temperature, and humidity [107, 146]. The most suitable foods for fortification with omega-3 are those that are frequently consumed and stored for only short periods at low temperature in airtight, light-excluding packages [107].

2. MATERIALS AND METHODS

2.1. Design of the study

The study has been done between 2015 and 2019 at the Lithuanian University of Health Sciences Veterinary Faculty Department of Food Safety and Quality (DFSQ) in collaboration with Lithuanian Central Milk Testing Laboratory, UAB 'Pieno Tyrimai' (LCMTL) and one of the largest dairy processing companies in Lithuania and its subdivisions located in Kaunas, Panevėžys and Mažeikiai districts.

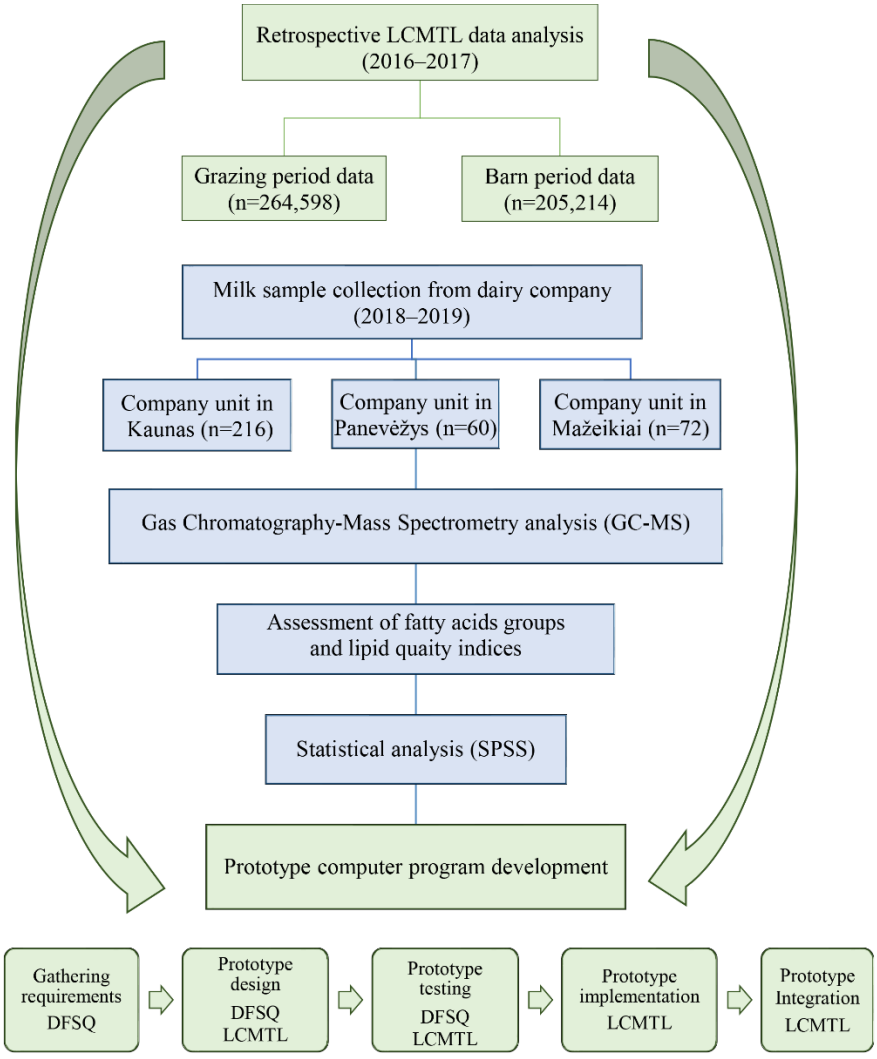


Fig. 2.1.1. *Chart of the research workflow.*

2.2. Data collection in Lithuanian central milk testing laboratory to evaluate main fatty acids and their groups in procured Lithuanian raw milk

In 2015, LCMTL started to analyze routinely main fatty acids in raw procured milk. According to fundamental knowledge and the dominant fatty acid composition in milk fats, SFA, UFA, MUFA, PUFA, C16:0, C18:0, and C18:1n9c as analytes have been examined in LCMTL.

To monitor the composition of main fatty acids of procured raw cow's milk intended for processing into dairy products in Lithuanian dairy plants, the retrospective analysis of data stored at the database of LCMTL was carried out.

The 264,598 samples from the grazing period (May to October) and 205,214 samples from the barn period (November to April) obtained in 2016–2017 were analyzed in this study. The SFA, UFA, MUFA, PUFA, C16:0, C18:0, and C18:1n9c were examined in these samples.

LCMTL provides the services of testing of composition and quality of procured cow's milk, which is required for settlement among parties of raw milk procurement agreements. Since 2001 according to LST EN ISO/IEC 17025 standard, there is an established and accredited Quality Management System in LCMTL. The laboratory is certified by the National Accreditation Bureau to carry out chemical, physical and microbiological tests of raw milk. The fatty acid, as well as fat, protein, lactose, urea content, and pH, are measured with the LactoScopeFTIR infrared meter.

2.3. Sample collection in the dairy processing company to evaluate the full fatty acid profile in raw processing milk

Dairy products that have a long tradition in production, are popular among consumers and are among the most frequently exported in Lithuania [147], were selected to evaluate the full fatty acid profile and content changes during raw milk processing. All dairy products chosen for the case study were natural and free from any additives that may influence the determination of the fatty acid composition. The selected products varied in fat content and represented differences in the technological approach.

UHT treated milk (fat content 2.5%), strained yogurt (fat content 3.9%), sour cream (fat content 25%), semi-fat curd (fat content 9%), and butter (fat content 82%) were chosen for the study.

Samples from the dairy company were collected during summer (June to August) and winter (January to March) period in 2018–2019.

For each technological step, the sample was taken [148] and analyzed six times in each season. A total of 288 samples were analyzed from a technological chain and storing. The sampling scheme and storing conditions are given in Table 2.1.2.1. Additionally, each time a raw milk sample (n=60) was taken from the bulk of the dairy plant.

All dairy products for this experiment were produced at one of the largest milk processing companies in Lithuania. Samples were collected at company units located in Kaunas, Panevežys, and Mažeikiai districts.

Dairy products were manufactured according to standard methods [149]. Their production flow charts are presented in Fig 2.1.2.1. Dairy products were stored in the original package: plastic polymeric containers with aluminum lids for yogurt and sour cream; a plastic polymeric bag for curd cheese; aluminum foil calibrated paper for butter; and aseptic multi-layer non-translucent cartons (Tetra Pack-type) for UHT milk under the conditions and time-frame given by the producer (commercial shelf life).

Table 2.1.2.1. *Sampling points at the main steps of technological process and at the end of shelf life.*

Product	**Sampling points**						**Storage environment**	**Evaluated technological process**
UHT milk	standardized milk	pasteurized milk	UHT milk	–	–	end of shelf-life	180 days in 20°C	pasteurization; homogenization; UHT treatment
Strained yogurt	standardized milk	pasteurized milk	milk with starter	yogurt before straining	strained yogurt	end of shelf-life	25 days in 5°C	pasteurization; homogenization; fermentation; straining
Sour cream	standardized cream	pasteurized cream	sour cream	–	–	end of shelf-life	25 days in 5°C	pasteurization; homogenization; fermentation
Curd cheese	standardized and pasteurized milk	curd cheese	whey	–	–	end of shelf-life	25 days in 5°C	pasteurization; fermentation; by-product
Butter	standardized cream	pasteurized cream	butter	buttermilk	–	end of shelf-life	90 days in 5°C	pasteurization; churning; by-product

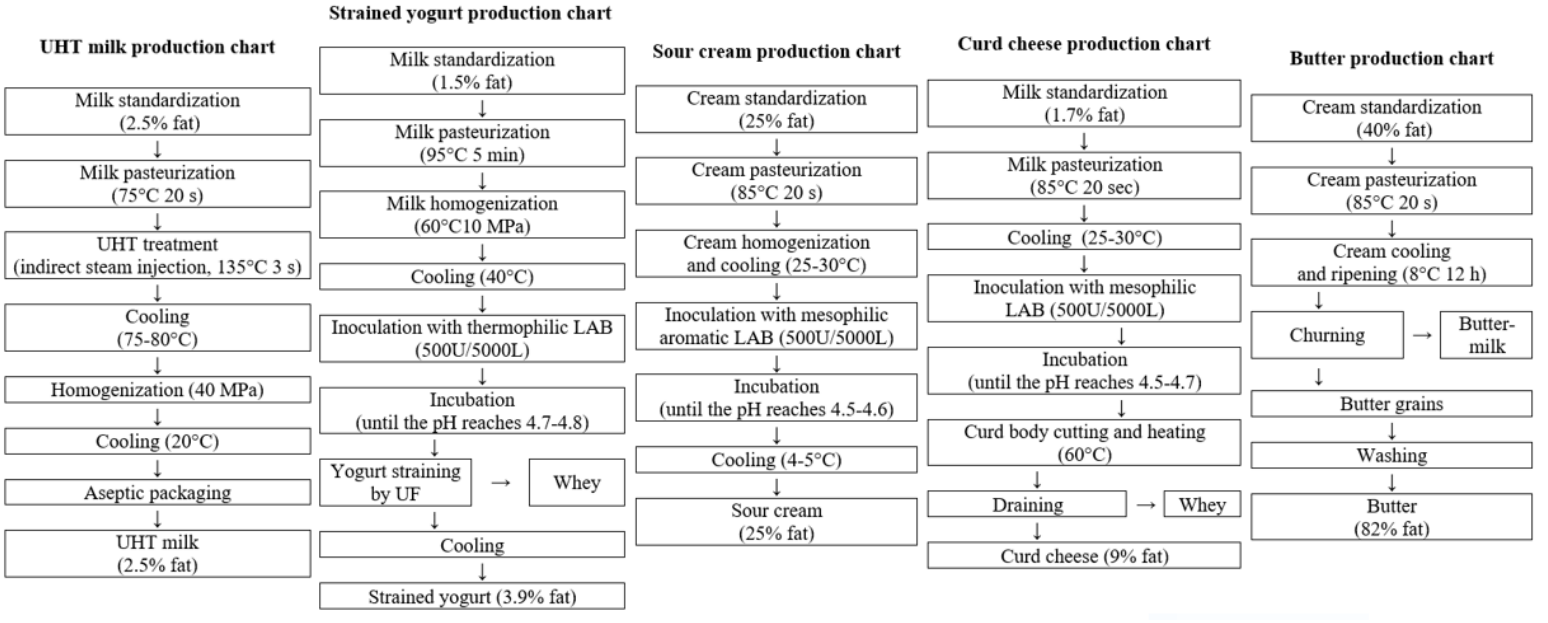


Fig. 2.1.2.1. *UHT milk, strained yogurt, sour cream, curd cheese, and butter production flow charts.*

2.4. Lipid extraction

Lipids from curds and strained yogurts were extracted using n-hexane. Strained yogurt was previously centrifuged to separate the whey residues. Then 10 g of sample was dispersed in 15 ml n-Hexane using a homogenizer (IKA T25 digital ULTRA TURAX) for 3 min, shaken mechanically with a shaker (Vortex) and then centrifuged (Heraeus Multifuge X1R Centrifuge, Thermo Scientific) at 5,000 rpm for 20 min at 20°C. The upper solvent was removed, and the extraction procedure was repeated. Two combined n-Hexane fractions with dissolved fats were combined and evaporated with a rotary evaporator (IKA, RV 10 basic) under vacuum [150]. After evaporation, fat was collected and directed for fatty acid methyl esters (FAME) preparation. The butter sample was melt in a warm water bath, mixed, weighed to 60 mg, and directed for FAME preparation.

The lipid separation from liquid samples was done by double centrifugation. Depending on the fat content of the sample, 20 ml of cream/soured cream, 40 ml of raw milk, 80 ml of standardized/UHT milk, and 320 ml of whey/buttermilk sample were poured into 50 ml conical tubes and centrifuged for 30 min at 12,000 rpm in 4ºC (Thermo Scientific, Heraeus Multifuge X1R Centrifuge). The settled fat layer at the top of the tube was collected and transferred to 1.5 ml tubes (Eppendorf) for further fat separation (20 min 13,000 rpm, at 20ºC) by microcentrifuge (Eppendorf Centrifuge 5418). The concentrated fat was collected and directed for FAME preparation [151].

2.5. Preparation of fatty acid methyl esters

The fatty acids were converted into FAME. 60 mg of concentrated fat was mixed with 2 ml of n-Hexane and 200 µl of anhydrous potassium hydroxide/ methanol (2 mol/L). The sample was shaken (Vortex) 1 min intensively, and after 10 min of standing, the top layer was collected and filtered (CHROMAFIL Xtra filter H-PTFE-20/25, 0.20 µm, 25 mm) into chromatography vial [152].

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analysis (GC-MS) was carried out using a Perkin Elmer Clarus 680 apparatus coupled to Perkin Elmer Mass Spectrometer detector. A fused silica SP-2560 capillary column was used (100 m × 0.25 mm i.d. × 0.20 µm film thickness). Conditions for chromatographic analysis were as following: the injector and detector temperatures were maintained at 230°C. Injection volume was 1 µl, a split ratio of 1:19.

The oven temperature was held at 100°C for 4 min, increased to 240°C (4°C/min) and held for 30 min. The total analysis time was 70 min. Carrier gas (He) flow rate was 1 ml/min.

Fatty acids peaks were identified using Supelco® 37 Component FAME Mix. Each fatty acid and their groups were expressed in percent (%) of total fatty acid content. All chemical reagents and standard Supelco® 37 Component FAME Mix used in this research were purchased from Sigma-Aldrich (Merck, KGaA, Darmstadt, Germany).

2.7. Evaluation of fatty acids and lipid quality indices

According to the saturation degree of the carbon chain, fatty acids were quantified into SFA, UFA, MUFA, and PUFA. Depending on the number of carbon atoms, fatty acids were grouped into three main groups: SCFA (C4-C6), MCFA (C8-C15), and LCFA (C16 and more) [116]. PUFA/SFA ratio was evaluated. The total content of hypocholesterolemic fatty acids (UFA and C18:0) was expressed as desirable fatty acids (DFA) [79].

The following lipid quality indices (LQI) were calculated: the ratio of LA/ALA, atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic and hypercholesterolemic index (h/H), and

AI and TI indices were estimated according to the following formulas [71]:

$$AI = \frac{(C12:0 + (4 \times C14:0) + C16:0)}{(PUFA + MUFA)}$$

$$TI = \frac{(C12:0 + C16:0 + C18:0)}{(0.5 \times MUFA) + (0.5 \times n6PUFA) + (3 \times n3PUFA) + (n3PUFA / n6PUFA)}$$

The h/H index was calculated as follows [69]:

$$h/H = \frac{(C18:1 + PUFA)}{(C14:0 + C16:0)}$$

2.8. Statistical analysis

All statistics were performed with the SPSS statistical package (IBM SPSS Statistics 20, SPSS Inc.).

Descriptive statistics were used to evaluate the homogeneity and scattering of data obtained from LCMTL. Identified outliers have been removed. The

influence of the month and season (grazing and barn period) on the main fatty acid composition (C16:0, C18:0, C18:1c9, SFA, UFA, MUFA, PUFA, and PUFA/SFA) was determined by One-way Analysis of Variance (ANOVA). Tukey HSD method was used in the statistical model to determine the between-group reliability criterion (*P*). The difference was considered statistically significant if $P \leq 0.05$. The fatty acid data obtained from LCMTL were expressed in grams per 100 ml. The conversion of these units into percents of total fatty acid content was possible only with a small loss of accuracy for some of the fatty acids [49].

To evaluate full fatty acid profile, as dependent variables individual fatty acids (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1c9, C18:1t9, C18:2n6, B18:3n3, C20:0, and C21:0), their groups (SFA, UFA, MUFA, PUFA, SCFA, MCFA, and LCFA), and LQI (AI, TI, PUFA/SFA, LA/ALA, and DFA) were analyzed in raw milk and various dairy product samples collected from milk processing plants.

Multivariate analysis of variance (MANOVA) was chosen to analyze the impact of season, processing, and storage factors and their interaction on the full fatty acid profile of dairy products. MANOVA was used to test whether or not the independent grouping variables (season, processing, and storage) simultaneously explain a statistically significant amount of variance in the dependent variables: individual fatty acids, their sums, and lipid quality indices. The difference was considered statistically significant if $P \leq 0.05$.

2.9. Prototype computer program development

As this study result, a computer program was developed in collaboration with LCMTL. This program allows the dairy processor to screen and select raw milk according to the chosen fatty acids parameter all over Lithuania.

Development of the program followed these steps: a gathering of requirements, designing prototypes, testing, implementation, and integration.

The results of our case study enabled us to gather the requirements for the development of this program.

After our requirements were presented, discussed, and the design of the program prototype was approved at the senior management level of LCMTL, the information technology specialists started to develop the prototype program.

The developed program was implemented on LCMTL servers. This prototype program was designed to work exclusively with databases of LCMTL. The prototype was launched for testing in November 2019.

After the trial period is over, the deviations and errors will be corrected, and the program will be integrated into the LCMTL database.

3. RESULTS AND DISCUSION

3.1. Retrospective analysis of seasonal variations of major fatty acids, estimated spectrometrically at LCMTL in procured Lithuanian cow's milk

The retrospective analysis was carried out using the database of LCMTL to monitor the composition of fatty acids of procured raw cow's milk intended for processing into dairy products in Lithuanian dairy plants.

Fatty acids have been extensively researched in milks and many factors (stage of lactation, pregnancy, the physiological and physical status of animal, milking frequency, cow breed, genotype, season, feeding ration) can influence the composition of fatty acids in milk [5, 11, 12]. All listed aspects can vary significantly among the countries, so the actual information regarding fatty acid composition in milk fat of Lithuanian cows is lacking.

Descriptive statistical analysis of 469,812 data of procured raw milk samples revealed such total means (% of total fatty acid content): 67.52±5.33 (range 47.82–87.46), 31.96±5.62 (range 12.84–52.74), 27.32±4.56 (range 11.47–43.91), 4.16±1.34 (range 0.02–9.27), 29.37±3.76 (range 15.13–44.0), 10.58±2.57 (range 1.98–19.64), and 20.10±3.84 (range 6.76–33.29) for SFA, UFA, MUFA, PUFA, C16:0, C18:0, and C18:1n9c, respectively.

Earlier studies pointed out that SFA in bovine milk accounts for 65%–75% and UFA for 25%–35%. The main individual fatty acids C16:0, C18:0, and C18: 1n9c account for 32.60%, 8.70%, and 18.00%, respectively, of the total fat content [1, 29, 63,153]. In general, the distribution of fatty acids in Lithuanian raw milk fat is consistent with overall trends.

The difference between grazing and barn periods for all fatty acids investigated were statistically significant. Bovine milk synthesized during the grazing period had a lower (P<0.05) amount of SFA and, conversely, a more considerable (P<0.05) amount of UFA, MUFA, and PUFA compared to winter milk (Fig. 3.1.1).

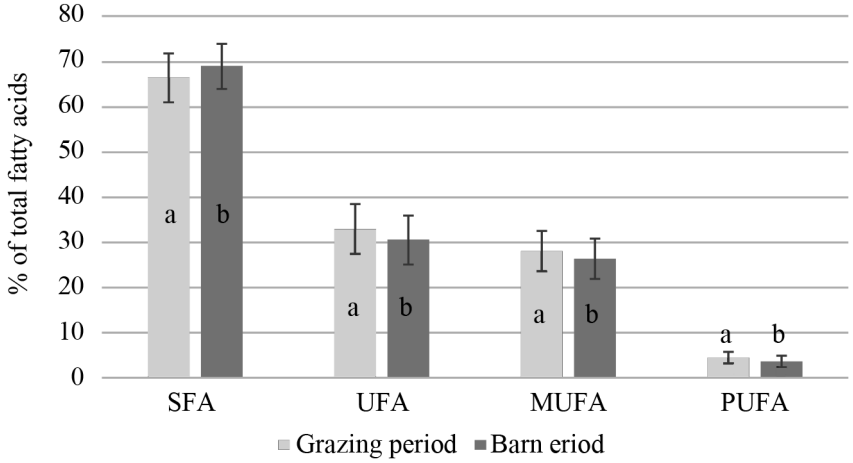


Fig. 3.1.1. *Composition of basic fatty acid groups in Lithuanian raw procured milk during different periods.*
Grazing period – May to October; Barn period – November to April;
SFA – saturated fatty acids, UFA – unsaturated fatty acids;
MUFA-monounsaturated fatty acids; PUFA –polyunsaturated fatty acids.

The content of C16:0 significantly increased during the barn period. Meantime the increase (P<0.05) of C18:0 and C18:1n9c was observed during the grazing period (Fig. 3.1.2). The distribution of main fatty acid groups and individual fatty acids in Lithuanian bovine milk fats during grazing and barn periods has a similar pattern as in other countries [77, 78, 154]. Seasonal changes in milk fat are closely related to changes in an animal's diet. Herbaceous grazing plants with a higher nutritional value and higher UFA concentration than silage feed and concentrates lead to an increase of UFA in milk fat [10–12].

Despite overall SFA dynamics in milk fats, an increase (P<0.05) in C18:0 concentration was observed during the grazing period in our study. The data on C18:0 is controversial: some researchers found a rise of this fatty acid over the grazing period [77, 154–156], while others observed a decrease [12, 157]. But overall, an increase of C18:0 in summer milk was more commonly detected.

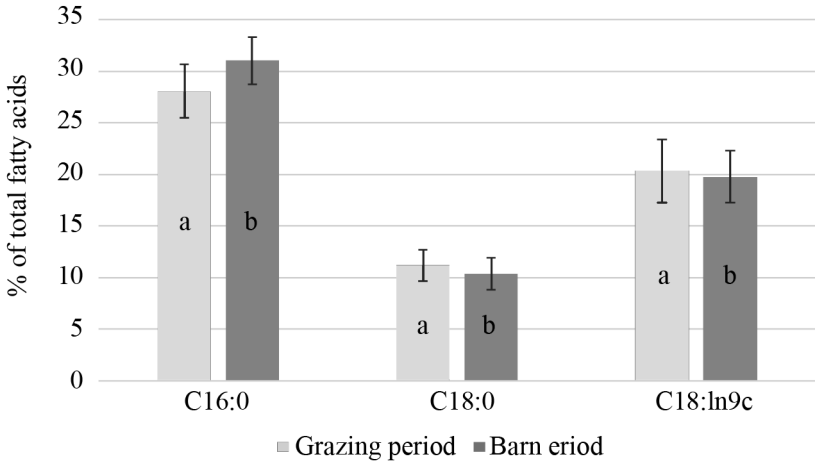


Fig. 3.1.2. *Content of most abundant individual fatty acid in Lithuanian raw procured milk during different feeding periods.*
Grazing period – May to October; Barn period – November to April.

For a long time, bovine milk was thought to contribute to CVD due to the adverse effects of SFA. Now it is stated that an increased concentration of LDL in blood is attributable to C12:0, C14:0, and C16:0, while other SFA found in milk neutralize their effect since they increase HDL level [34] or has no effect on LDL because of their poor absorption in the gut (e.g., C18:0) [35]. Some authors indicate that only C16:0 has shown adverse metabolic effects in vitro, while medium-chain (C6:0 to C12:0), odd chain (C15:0 to C17:0), and very-long-chain (C20:0 to C24:0) SFA expresses metabolic benefits [158]. C18:0 is not considered to be a trigger for elevated cholesterol and is classified as a desirable and health-promoting fatty acid [79].

The analysis revealed a statistically significant (P<0.05) influence of month factor on means of SFA, UFA, MUFA, PUFA, and some single fatty acid in milk fat (Table 3.1.1).

Table 3.1.1. *The effect of month factor on major fatty acid groups and some individual fatty acid content (% of total fatty acids).*

Month	ΣSFA	ΣUFA	ΣMUFA	ΣPUFA	C16:0	C18:0	C18:1n9c
January	69.63±4.69^{a}	31.38±5.30^{a}	27.15±4.33^{a}	3.70±1.26^{a}	31.49±3.19^{a}	10.20±2.47^{a}	20.28±3.38^{a}
February	67.53±4.20^{b}	29.33±4.83^{b}	25.36±3.94^{b}	3.49±1.23^{b}	31.00±3.07^{b}	9.33±2.41^{b}	19.74±3.24^{b}
March	67.28±4.49^{c}	28.95±5.15^{c}	24.96±4.20^{c}	3.60±1.24^{c}	30.47±3.19^{c}	10.19±2.73^{a}	19.40±3.54^{c}
April	66.75±4.85^{d}	31.32±5.96^{a}	27.10±4.936^{a}	3.84±1.29^{d}	30.00±3.32^{d}	10.81±2.79^{c}	20.46±3.88^{d}
May	66.60±5.58^{e}	31.90±6.15^{d}	27.45±5.05^{d}	4.03±1.42^{e}	29.14±3.72^{e}	10.89±2.69^{d}	20.92±3.80^{e}
June	67.47±6.07^{b}	31.85±5.83^{d}	27.39±4.81^{d}	4.01±1.26^{e}	28.82±3.62^{f}	11.33±2.36^{e}	20.88±3.54^{e}
August	64.11±3.95^{f}	34.83±4.77^{e}	29.36±3.96^{e}	5.00±1.10^{f}	25.97±2.62^{g}	11.96±2.31^{f}	19.63±4.87^{f}
September	65.74±4.75^{g}	33.75±4.94^{f}	28.43±4.04^{f}	4.81±1.17^{g}	27.45±3.39^{h}	11.18±2.49^{g}	20.13±4.06^{g}
October	69.98±4.69^{h}	32.39±5.13^{g}	27.32±4.17^{g}	4.53±1.27^{h}	30.24±3.08^{i}	10.41±2.45^{h}	20.23±3.28^{a}
November	69.87±4.32^{h}	31.61±5.24^{h}	27.16±4.27^{a}	4.08±1.25^{i}	31.57±3.11^{a}	10.38±2.33^{h}	19.67±3.30^{b}
December	72.58±4.16^{i}	29.93±5.11^{i}	25.67±4.10^{h}	3.60±1.20^{c}	31.92±3.20^{j}	10.87±2.33^{d}	18.93±3.33^{h}

Values are means±SD; means with different lowercase letters within the same column show significant (P<0.05) difference between months; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; C16:0: palmitic acid; C18:0: stearic acid; C18:1n9c: oleic acid.

For the SFA, we detected significant differences (P<0.05) among barn months (November, December, January, February, March, April). During the grazing period, the significant variations (P<0.05) in SFA content were estimated among May, June, August, September, and October. A similar pattern was found for UFA, MUFA, and PUFA. During winter months, the mean of UFA varied signifycantly (P<0.05) in the range of 28.95±5.15% to 31.61±5.24% of the total fatty acid content. While in the grazing period, the lowest (P<0.05) amount (31.85±5.83%) was detected in June, and the highest (P<0.05) amount (34.83±4.77%) of UFA was found in August. Variations in the content of individual fatty acids among different months in the barn or grazing period were detected as well. This dynamic was particularly notable for C16:0.

These results suggest that barn and grazing periods are not the only factor affecting fatty acid profile. To identify other factors affecting fatty acid profile, it is necessary to supplement the data array collected by LCMTL with information on the animal status (animal health indices, date of birth, inseminations, and calving to calculate reproduction parameters (lactation stage, parity, etc.) and its environment (feeding, keeping, milking systems, etc.).

3.2. The effect of season on full profile of raw milk fatty acids and lipid quality indices

The effect of season on full fatty acid profile assessed chromatographically in raw bulk milk collected during the 2018–2019 period is presented in Table 3.2.1.

In our study, summer milk had significantly higher (P<0.05) amount of UFA and MUFA and less (P<0.05) SFA than winter milk, which is in agreement with other studies [13, 78, 154].

Despite that many authors found a higher level of PUFA in summer milk [49, 78, 154], we did not confirm significant differences in PUFA content between winter and summer milk. Milk samples collected in wintertime had even a higher (P<0.05) content of 18:2n6c than summer milk. Meantime, the C18:3n3c content was higher in summer milk, but not significantly. The parallel results to ours were observed in the 2015 study [12].

Summer milk of the present study was more abundant (P<0.05) in individual C16:1, C18:0, C18:1n9c, C18:1n9t compared with those of winter milk. This is due to the higher consumption of fresh grass: grazing dairy cows produce milk with high levels of UFA [159].

Table 3.2.1. *The full fatty acid profile and content (% of total fatty acids) in raw cow milk during the summer and winter seasons.*

FA and their main groups	Range		Mean±SD		*P*
	Summer	Winter	Summer	Winter	
C4:0	0.45–2.78	1.66–2.50	1.97±0.48	2.04±0.23	ns
C6:0	1.39–4.46	0.20–1.89	1.76±0.71	1.50±0.40	ns
ΣSCFA	3.25–4.91	2.70–4.21	3.73±0.45	3.54±0.41	ns
C8:0	0.79–2.65	0.94–1.43	1.14±0.42	1.14±0.16	ns
C10:0	2.01–3.65	2.29–3.63	2.83±0.38	2.93±0.35	ns
C11:0	0.00–0.22	0.20–0.37	0.03±0.07	0.28±0.05	<0.05
C12:0	3.16–4.50	3.02–4.57	3.66±0.36	3.78±0.44	ns
C13:0	0.00–0.17	0.00–0.24	0.05±0.07	0.17±0.06	<0.05
C14:0	12.11–14.21	11.21–14.96	13.18±0.72	13.47±0.86	ns
C14:1	0.83–1.87	1.10–1.29	1.48±0.91	1.20±0.07	ns
C15:0	1.19–1.70	1.31–1.58	1.38±0.14	1.47±0.09	ns
ΣMCFA	20.39–28.72	20.30–28.01	23.76±2.00	24.43±1.79	ns
C16:0	30.61–38.46	32.23–40.55	34.44±2.22	37.99±2.54	<0.05
C16:1	1.83–2.40	1.54–2.59	2.19±0.16	1.97±0.32	<0.05
C17:0	0.61–1.18	0.00–1.22	0.92±0.14	0.83±0.27	ns
C17:1	nd	0.00–0.28	nd	0.04±0.04	ns
C18:0	9.44–12.19	7.99–11.35	10.60±0.90	9.12±1.06	<0.05
C18:1n9t	0.86–2.62	0.76–1.62	1.75±0.44	1.10±0.26	<0.05
C18:1n9c	17.17–23.40	15.26–23.65	19.64±1.67	18.07±2.03	<0.05
C18:2n6	1.09–1.76	1.38–1.89	1.41±0.17	1.61±0.19	<0.05
C18:3n3	0.42–0.82	0.38–0.88	0.64±0.10	0.57±0.16	ns
C20:0	0.00–0.27	0.00–0.30	0.06±0.10	0.18±0.09	<0.05
C21:0	0.02–4.27	0.35–0.93	0.86±0.91	0.56±0.15	ns
ΣLCFA	67.96–76.36	69.21–76.39	72.50±2.14	72.02±1.91	ns
ΣSFA	66.55–76.21	68.53–77.66	72.88±2.44	75.44±2.42	<0.05
ΣUFA	23.79–33.45	22.34–31.47	27.12±2.43	24.56±2.39	<0.05
ΣMUFA	21.89–31.41	19.73–28.78	25.07±2.36	22.38±2.28	<0.05
ΣPUFA	1.71–2.59	1.82–2.69	2.05±0.22	2.18±0.26	ns

Summer: June to August; Winter: January to March; ns: none of compared means differ significantly at $P<0.05$ level; nd: not detected; FA: fatty acids; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle chain fatty acids; LCFA: long-chain fatty acids.

Content of C11:0, C13:0, C16:0, C20:0, and SFA was found in higher (P<0.05) concentrations in winter milk of the present study. The increase in total SFA in winter milk is determined by many researchers [12, 13, 78, 154]. However, the results of individual SFA vary among different studies. Hanuš et al. (2016) did not confirm seasonal differences for C11:0, C13:0, and C20:0, but C16:0 differed significantly in summer and winter milk [13]. Meantime 2010 study revealed a significant increase of C11:0 and C16:0, but C13:0 and C20:0 remained the same in the milk of different seasons [154].

The effect of season on lipid quality indices (LQI) is presented in Table 3.2.2.

Table 3.2.2. *LQI in raw cow milk during the summer and winter seasons.*

LQI	Range		Mean±SD		*P* values
	summer	winter	summer	winter	
AI	2.50–4.12	2.55–4.46	3.39±0.43	3.94±0.51	<0.05
TI	2.36–3.77	2.55–4.07	3.15±0.34	3.66±0.42	<0.05
h/H	0.39–0.60	0.35–0.64	0.49±0.06	0.42±0.07	<0.05
LA/ALA	1.77–3.11	1.64–4.61	2.25±0.41	3.02±0.86	<0.05
DFA	33.41–43.5	30.53–42.82	37.7±2.87	33.67±3.17	<0.05
PUFA/SFA	0.02–0.04	0.02–0.04	0.03±0.00	0.03±0.00	ns

Summer: June to August; Winter: January to March; ns: none of compared means differ significantly at P<0.05 level; AI: atherogenicity index; TI: thrombogenicity index; h/H: hypocholesterolemic and hypercholesterolemic index; LA/ALA: ratio of linoleic and α-linolenic acids; DFA: desirable fatty acids; PUFA/SFA: ratio of polyunsaturated fatty acids and saturated fatty acids.

The seasonal impact is evident on milk fatty acid composition and LQI of raw milk. AI and TI values were lower (P<0.05) in summer raw milk. These two indices are good indicators of food fat quality and its effect on human health [6, 69]. The lower values of AI and TI indicate a higher content of anti-atherogenic fatty acids in milk fat [69].

Although the LA/ALA ratio was found lower (P<0.05) in summer (2.25±0.41:1) than in winter raw milk (3.02±0.86:1), both seasons milk met European Food Safety Authority (EFSA) recommendations [161] in this case study. Usually, both omega-6/omega-3 and LA/ALA ratios are high in most diets due to increased omega-6 intake [65, 160]. The optimal ratio should be considered as 2:1 to 3:1 to reduce the risk of many chronic diseases [161].

The PUFA/SFA ratio is a good nutritional fat indicator, and above 0.45 is recommended in the diet to prevent coronary heart disease and cancers [162]. A low PUFA/SFA ratio was observed in this study: 0.33 in both seasons. Fat

from ruminants typically shows PUFA/SFA values below the recommendation. That is due to a high content of predominant C12:0, C14:0, C16:0, and C18:0, resulting in a very high total content of SFA [69, 76].

Summer milk analyzed in this study was a healthier option, according to DFA and h/H ratio. The DFA value (% of total fatty acid content) was more favorable (P<0.05) in summer (37.71±2.87) than in winter (33.67±3.17) milk. This finding is supported by other researchers [77, 154].

The h/H ratio was significantly higher (P<0.05) in summer (0.49±0.06) than in winter (0.42±0.07) milk. Our findings are in agreement with the 2019 study, where the h/H ratio of cow milk was 0.44±0.03[163]. The h/H ratio is associated with the activity of fatty acids in the metabolism of lipoproteins for plasma cholesterol transport and to the risk of cardiovascular disease. Fats with a higher h/H ratio have a higher content of health-promoting (hypocholesterolemic) fatty acids in the fats and, as a result, are more desirable [79].

According to LQI data of this case study, raw summer milk is a healthier choice for the human diet.

3.3. The impact of season, processing, and storage on fatty acid profile of natural dairy products

The effect of season, processing, and storage and their interaction on fatty acid profiles and LQI of dairy products chosen for our study is presented in Table 3.3.1. The two factor (season, processing, and storage) interaction showed no significant effect on the fatty acid profile of UHT milk, yogurt, and sour cream. Only one fatty acid (C18:1n9c) expressed the same pattern of seasonal variations during the technological process of curd cheese. The significant impact of factors interaction on PUFA/SFA was observed during the production of butter.

Between single factors – season and processing and storage of dairy products, the season was more influential on the fatty acid profile of dairy products (except UHT milk) than technological processes. The fatty acids affected by the season varied greatly among the products.

Since the only minor impact of factors interaction was detected in the study, the particular effect of single factors – season, processing, and storage on specific dairy products (UHT milk, yogurt, sour cream, curd cheese, and butter) fatty acid profiles are addressed in subsequent chapters.

Table 3.3.1. *The efect of single factors (processing and storage, PS; season, S) and their interaction (PS*S) on fatty acid profiles and LQI of dairy products.*

FA, their groups, and LQI	Single factors and their interaction														
	UHT milk			Strained yogurt			Sour cream			Curd cheese			Butter		
	PS	S	PS*S	PS	S	PS*S	PS	S	PS*S	PS	S	PS*S	PS	S	P*S
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C4:0	0.197	0.322	0.280	0.883	0.994	0.842	0.426	0.989	0.653	0.375	0.073	0.852	0.107	0.052	0.116
C6:0	0.121	0.936	0.994	0.617	0.168	0.567	0.357	0.803	0.599	0.250	0.086	0.958	0.412	0.766	0.422
ΣSCFA	0.165	0.571	0.580	0.875	0.622	0.798	0.394	0.926	0.628	0.275	0.061	0.976	0.130	0.042	0.486
C8:0	0.019	0.264	0.649	0.698	0.347	0.655	0.441	0.219	0.297	0.107	0.513	0.291	0.540	0.168	0.117
C10:0	0.079	0.909	0.622	0.911	0.440	0.448	0.356	0.742	0.391	0.175	0.191	0.827	0.229	0.014	0.194
C11:0	0.216	0.949	0.962	0.856	0.941	0.959	0.352	0.001	0.599	0.032	0.001	0.383	0.627	0.002	0.800
C12:0	0.069	0.887	0.629	0.422	0.509	0.407	0.171	0.045	0.582	0.364	0.687	0.260	0.201	0.007	0.601
C13:0	0.630	0.946	0.296	0.915	0.140	0.996	0.151	0.007	0.100	0.008	0.000	0.602	0.614	0.000	0.223
C14:0	0.155	0.644	0.853	0.891	0.011	0.354	0.257	0.028	0.685	0.125	0.608	0.740	0.121	0.001	0.186
C14:1	0.561	0.577	0.932	0.699	0.000	0.853	0.057	0.004	0.223	0.245	0.525	0.295	0.117	0.041	0.170
C15:0	0.550	0.387	0.896	0.054	0.074	0.490	0.277	0.122	0.461	0.211	0.243	0.658	0.291	0.000	0.068
ΣMCFA	0.068	0.718	0.995	0.869	0.128	0.320	0.116	0.264	0.530	0.378	0.227	0.109	0.043	0.001	0.211
C16:0	0.875	0.732	0.762	0.069	0.000	0.655	0.584	0.796	0.856	0.669	0.003	0.348	0.003	0.003	0.136
C16:1	0.640	0.470	0.535	0.956	0.070	0.831	0.801	0.555	0.638	0.386	0.002	0.347	0.630	0.108	0.810
C17:0	0.265	0.377	0.241	0.370	0.663	0.276	0.191	0.034	0.898	0.836	0.710	0.266	0.322	0.497	0.292

Table 3.3.1 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C18:0	0.360	0.837	0.699	0.996	0.000	0.655	0.099	0.414	0.208	0.815	0.000	0.246	0.410	0.002	0.209
C18:1n9t	0.628	0.800	0.406	0.848	0.000	0.580	0.981	0.061	0.385	0.668	0.081	0.549	0.561	0.091	0.544
C18:1n9c	0.148	0.906	0.921	0.999	0.000	0.679	0.036	0.715	0.386	0.260	0.000	0.043	0.553	0.002	0.454
C18:2n6	0.385	0.449	0.398	0.075	0.010	0.405	0.590	0.139	0.453	0.238	0.868	0.454	0.376	0.051	0.148
C18:3n3	0.429	0.982	0.389	0.446	0.001	0.472	0.473	0.051	0.102	0.749	0.061	0.936	0.845	0.052	0.428
C20:0	0.498	0.255	0.398	0.167	0.886	0.680	0.598	0.055	0.598	0.769	0.348	0.828	0.950	0.684	0.615
C21:0	0.722	0.930	0.290	0.475	0.000	0.055	0.797	0.293	0.599	0.900	0.070	0.639	0.061	0.416	0.189
ΣLCFA	0.060	0.851	0.940	0.855	0.303	0.442	0.169	0.438	0.560	0.348	0.383	0.127	0.019	0.032	0.093
ΣSFA	0.131	0.944	0.825	0.685	0.000	0.371	0.066	0.715	0.289	0.317	0.000	0.150	0.552	0.004	0.495
ΣUFA	0.131	0.944	0.825	0.685	0.000	0.371	0.066	0.715	0.289	0.317	0.000	0.150	0.332	0.002	0.346
ΣMUFA	0.129	0.949	0.792	0.971	0.000	0.531	0.040	0.915	0.308	0.308	0.000	0.094	0.418	0.001	0.465
ΣPUFA	0.344	0.513	0.399	0.062	0.000	0.209	0.580	0.031	0.125	0.374	0.095	0.618	0.215	0.015	0.092
PUFA/SFA	0.289	0.543	0.450	0.077	0.185	0.205	0.494	0.048	0.151	0.354	0.031	0.715	0.118	0.118	0.049
LA/ALA	0.617	0.408	0.371	0.989	0.022	0.988	0.708	0.317	0.291	0.884	0.045	0.913	0.860	0.227	0.575
h/H	0.245	0.843	0.860	0.411	0.000	0.360	0.083	0.396	0.438	0.758	0.000	0.385	0.365	0.001	0.295
DFA	0.166	0.979	0.793	0.887	0.000	0.432	0.056	0.987	0.280	0.525	0.000	0.143	0.359	0.000	0.213
AI	0.135	0.865	0.893	0.701	0.000	0.256	0.091	0.131	0.622	0.233	0.000	0.082	0.444	0.002	0.380
TI	0.182	0.981	0.783	0.199	0.000	0.308	0.554	0.250	0.175	0.295	0.001	0.340	0.313	0.018	0.247

The impact of the factor is significant when $P<0.05$.

3.3.1. Seasonal variations of milk fatty acids in major processing steps and at the end of shelf life of UHT milk

UHT milk was chosen for this study to evaluate heat treatment impact on processing milk. First, the milk standardized according to fat content (2.5%) was preheated to a noncritical temperature. Later the temperature was raised as required the UHT process to 135°C and was kept for 3 sec. The detailed production flow chart of UHT milk is presented in Fig. 2.1.2.1 in the Materials and methods section.

Seasonal fatty acid variation was not confirmed in standardized milk directed for UHT milk production, in contrast to other dairy products of this study. This trend persisted throughout the entire process of UHT milk production. The absence of seasonal impact could be explained by the milk standardization procedure processing this particular product.

Since individual fatty acids and their main groups were not affected by season, the LQI remained unchanged in milk samples (Fig. 3.3.1.1).

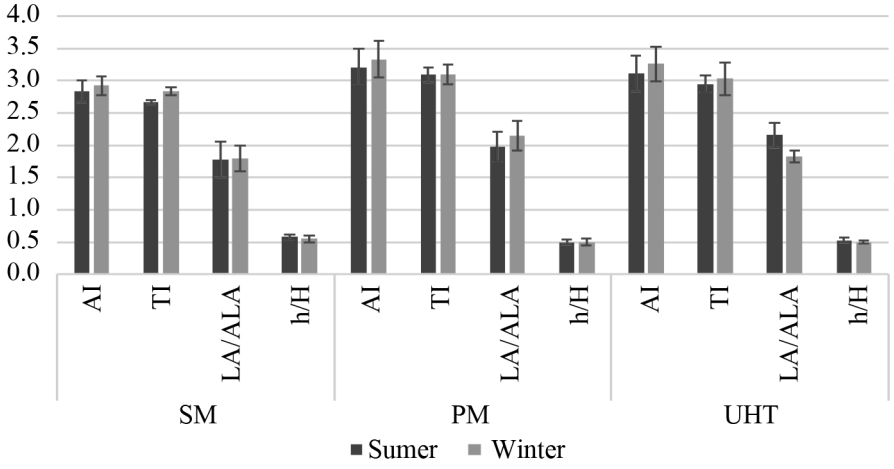


Fig. 3.3.1.1. *LQI in processing milk during UHT milk production in both seasons.*

SM – standardized milk; PM – pasteurized milk; UHT – UHT milk.

Pasteurization, UHT treatment, and homogenization effect on fatty acid profile and content was evaluated in processing milk.

Present study showed a slight variation in the content of certain fatty acids during milk processing. The most of SCFA and MCFA were prone to increase during milk processing, while most of LCFA slightly decreased after heat treatment; however, the changes were not confirmed as significant. In

general, the fatty acid profile remained the same; no individual fatty acid losses were detected during the production of UHT milk (Table 3.3.1.1). Thus, we conclude that the technological processes did not have a significant impact on the processing of milk under this particular UHT milk technology.

Many authors have found that pasteurization or homogenization has negligible effect on milk or cream fatty acids [15, 95–97, 99, 107].

Some authors suggest that even UHT has little or no impact on fatty acid content during milk processing. Dias et al. (2020) found that BP (63°C/30 min), HTST (72°C/15 sec) and, UHT (135°C/3 sec) treatments did not statistically alter fatty acid concentrations comparing to raw bovine milk. Except for a reduction in C10:0 after UHT compared to BP, no significant differences in total fatty acid concentrations were detected amongst the heat treatments [100]. Pestana et al. (2015) found that raw, pasteurized (75°C/15 sec) and UHT (140°C/3 sec) treated milk had very similar fatty acid profiles, only for C4:0, C6:0, C8:0, and C20:0 was found a significant decrease [92]. The higher UHT temperature used in the Pestana study compared to ours (140°C versus 135°C) could have revealed the changes in C4:0, C6:0, C8:0, and C20:0 content.

The 2018 study, where milk was treated with 130°C/3 sec or 145°C/2 sec, showed the decrease only in C4:0, C6:0, and C8:0 content then compared to those of raw milk [164].

Contrary to our results, several studies revealed more intense changes in fatty acid content in milk during UHT treatment.

Xu et al. (2020) found that UHT (135°C/15 sec) milk contained significantly fewer UFA including C14:1, C15:1, C16:1, C17:1, C18:1n9c, C18:2n6c, C18:3n3, C18:3n6, C20:1, C20:2, C20:3n6, C20:3n3, C20:4n6, and C20:5n3 than raw or pasteurized milk [98]. We did not observe similar changes, but the UHT temperature retention in our study was significantly shorter compared to the latter study (15 sec versus 3 sec).

Khan et al. (2017) pointed out that pasteurization (65°C/30 min) and boiling (1 min) increased the concentration of SCFA in both cow and buffalo milk, and this increase is due to the conversion of LCFA into SCFA and MCFA during heat treatment [101]. Ajmal et al. (2018) reported about the significant decrease of SCFA, MCFA, and LCFA due to UHT treatment, but information on temperature regimes used in their study is lacking [17].

In this study, the effect of storage on fatty acid content in UHT milk was estimated at the end of commercial shelf life (on the 180th day).

Table 3.3.1.1. *The profile and content (% of total fatty acids) of individual fatty acids and their groups during UHT milk production and storage in different seasons.*

FA and their main groups	Summer				Winter			
	standardized milk	pasteurized milk	UHT milk	end of shelf life	standardized milk	pasteurized milk	UHT milk	end of shelf life
1	2	3	4	5	6	7	8	9
C4:0	1.82±0.15	1.77±0.19	2.36±0.37	1.96±0.19	1.72±0.09	1.69±0.08	1.86±0.12	2.09±0.05
C6:0	1.29±0.02	1.34±0.18	1.54±0.13	1.71±0.20	1.31±0.11	1.34±0.18	1.54±0.02	1.74±0.17
ΣSCFA	3.11±0.15	3.11±0.37	3.90±0.51	3.66±0.39	3.03±0.21	3.02±0.25	3.41±0.10	3.84±0.21
C8:0	0.98±0.04	0.86±0.10	0.92±0.01	1.18±0.20	0.94±0.09	0.86±0.11[a]	1.02±0.06	1.38±0.01[b]
C10:0	2.43±0.15	2.62±0.08	2.82±0.20	3.25±0.31	2.35±0.14	2.79±0.12	2.59±0.05	3.22±0.34
C11:0	0.02±0.01	0.04±0.00	0.05±0.01	0.10±0.06	0.06±0.02	0.03±0.00	0.04±0.01	0.11±0.10
C12:0	3.06±0.06	3.31±0.03	3.65±0.44	4.03±0.47	3.19±0.09[a]	3.45±0.11	3.36±0.08	4.28±0.22[b]
C13:0	0.05±0.01	0.03±0.00	0.04±0.01	0.08±0.07	0.04±0.01	0.08±0.05	0.05±0.01	0.04±0.00
C14:0	10.97±0.58	12.47±0.80	12.09±0.90	13.75±0.42	11.41±0.09	12.52±0.80	12.79±0.51	13.83±0.54
C14:1	1.16±0.04	1.03±0.14	1.03±0.06	1.11±0.07	1.19±0.05	1.03±0.14	1.10±0.06	1.19±0.15
C15:0	1.70±0.18	1.44±0.09	1.53±0.11	1.48±0.05	1.53±0.17	1.40±0.03	1.43±0.08	1.42±0.07
ΣMCFA	20.38±0.56	21.79±1.02	22.13±1.72	24.97±1.42	20.72±0.92	22.17±1.410	22.38±0.47	25.48±0.92
C16:0	33.88±0.36	34.49±0.90	34.10±0.03	34.62±0.08	33.74±0.16	34.33±0.32	34.51±1.34	34.10±0.33
C16:1	2.59±0.02	2.48±0.14	2.10±0.39	2.10±0.27	2.39±0.07	2.35±0.02	2.59±0.03	2.23±0.40
C17:0	1.06±0.01	1.12±0.04	0.85±0.01	0.99±0.07	1.05±0.05	1.00±0.08	1.01±0.15	1.15±0.09
C18:0	10.97±0.57	11.54±0.77	10.84±0.12	10.02±0.39	11.53±0.59	10.97±1.34	11.31±0.54	10.48±0.08
C18:1n9t	2.36±0.40	2.14±0.31	2.28±0.09	1.73±0.15	1.96±0.40	2.03±0.41	1.92±0.09	2.04±0.21

Table 3.3.1.1 continued

1	2	3	4	5	6	7	8	9
C18:1n9c	20.54±0.92	19.36±1.05	19.59±1.03	17.18±0.21	20.00±0.92	18.79±1.62	19.59±1.28	17.43±0.25
C18:2n6	1.75±0.07	1.58±0.16	1.96±0.42	1.42±0.20	1.81±0.07	1.68±0.06	1.47±0.05	1.39±0.17
C18:3n3	1.26±0.21^{a}	0.80±0.09	0.87±0.02	0.70±0.03^{b}	1.07±0.15^{a}	0.88±0.12	0.80±0.13	0.80±0.00^{b}
C20:0	0.11±0.06	0.14±0.07	0.13±0.07	0.10±0.06	0.16±0.06	0.15±0.09	0.11±0.02	0.12±0.02
C21:0	1.30±0.27	0.99±0.20	1.24±0.19	2.48±1.79	1.23±0.22	1.73±0.74	0.93±0.13	0.84±0.15
ΣLCFA	74.52±0.42	75.07±1.39	73.96±1.22	71.36±1.80	73.63±1.12	73.83±1.65	74.25±0.56	70.68±1.13
ΣSFA	70.35±1.03^{a}	72.62±1.23	72.17±1.11	75.76±0.46^{b}	71.57±1.45	73.35±1.95	72.53±1.32	74.91±1.30
ΣUFA	29.65±1.01^{a}	27.38±1.22	27.83±1.10	24.24±0.44^{b}	28.43±1.02	26.67±1.92	27.47±1.30	25.09±1.29
ΣMUFA	26.64±1.30	25.00±1.07	25.00±0.68	22.12±0.23	25.55±1.24	24.21±1.86	25.20±1.27	22.90±1.00
ΣPUFA	3.01±0.27^{a}	2.38±0.15	2.83±0.40	2.12±0.21^{b}	2.88±0.0.18^{a}	2.46±0.09	2.27±0.05	2.19±0.30^{b}
PUFA/SFA	0.04±0.00^{a}	0.03±0.01	0.03±0.01	0.03±0.00^{b}	0.04±0.00^{a}	0.03±0.01	0.03±0.01	0.03±0.00^{b}
DFA	40.62±0.46	38.92±1.99	38.67±0.44	34.26±0.07	39.86±0.52	37.64±1.29	38.79±1.86	35.57±1.38

Values are mean±SD of six replicates; means with different lowercase letters within the same row show significant (P<0.05) difference between different processing steps; means with different uppercase within the same row show significant (P<0.05) difference between analogous samples in different seasons; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle-chain fatty acids; LCFA: long-chain fatty acids, PUFA/SFA: ratio of polyunsaturated and saturated fatty acids; DFA – desirable fatty acids.

We revealed a decrease (P<0.05) in C18:3n3c, UFA, and PUFA, and increase (P<0.05) in SFA at the end of shelf life in summer UHT milk comparing to standardized milk. In winter UHT milk, the C8:0 and C12:0 increased (P<0.05) while C18:3n3c and PUFA decreased (P<0.05) at the end of shelf-life comparing to standardized or pasteurized milk. However, no significant differences were found between freshly produced UHT milk and at the end of its storage. This may indicate a low hydrolysis level of milk fat. Such a result could be related to the high primary quality of raw milk, sterilization effect of UHT treatment, and aseptic light-proof packaging.

Usually, the increase of SFA is related to the hydrolysis (decrease) of UFA during storage. Lipid oxidative stability depends on several intrinsic and extrinsic factors (environment conditions, processing techniques, light, and oxygen exposure, the use of antioxidants, the presence of pro-oxidants and, the storage conditions). First, fat oxidation is influenced by the unsaturation of their fatty acids composition [165]. As the concentration of highly unsaturated double bonds increases, the fats become more susceptible to oxidation. UFA, especially PUFA, is less stable than SFA. Unsaturation of fat makes them more vulnerable to rancidity [145].

In the present study, UHT milk was packaged aseptically in a nontransparent (light-barrier) package. Hence, the degradation of UFA could have been conditioned to the heat-resistant microbial lipases.

Psychrotrophs (mostly *Pseudomonas* species) yield extremely heat-resistant lipases during the storage of raw milk even at refrigeration temperatures [140, 166]. Microbial lipases may keep their activity even after UHT treatment in milk during storage. Commonly, milk lipoprotein lipases are nonspecific. Meantime microbial lipases derived from different species or strains differ in specificity for liberating fatty acids from milk fat [166]. Thus, we speculate that microbial lipases were more specific for UFA and PUFA in our study.

Rodríguez-Alcalá et al. (2019) study revealed that during storage at room temperature for 12 weeks, the fatty acid profile (SFA, MUFA, and PUFA) of omega-3 enriched UHT milk samples was stable [167]. According to the study authors, the absence of variation in the fatty acid profile was related to an environment of reduced potential redox and the low content oxygen: UHT milk was stored sealed and in the dark [167]. The storage conditions of this study were very similar to ours. Only the time of storage was shorter in their research because shelf life for PUFA enriched dairy products is shorter than for regular ones.

In the 2018 study, 30 days of storage in the aseptic package and ambient temperature did not reveal a significant effect on the fatty acid profile of UHT milk. Significant changes were recorded in the fatty acid profile when milk

samples were stored for 60 and 90 days; SCFA, MCFA, and LCFA continually decreased during storing [17]; however, it is not clear what UHT temperature and the exposure time were used in this study.

Meantime, Martini et al. (2018) found a poorly expressed fat degradation and oxidation processes even in pasteurized milk during prolonged cold storage. The author analyzed the impact of 21 days of storage at 3°C on the fatty acid composition of pasteurized (65°C/30 min) donkey milk and did not reveal fatty acid changes after storage. Only an extended 90 days storage at freezing −20°C temperature significantly decreased C18:2 and increased C6:0, C14:0, C14:1, C21:0, and 20:3 [97].

3.3.2. Seasonal variations of milk fatty acids in major processing steps and at the end of shelf life of strained yogurt

Strained yogurt is popular among fermented milk products. Yogurt is produced by fermentation of milk lactose by synergistic LAB, which contributes to the specific texture, composition, and sensory properties of yogurt [19]. In this study, yogurt was made exclusively from milk and thermophilic starter containing *S. thermophiles* and *L. delbrueckii* subsp. *Bulgaricus*. It did not contain any flavor additives, thickeners, or added sugar. Due to the straining process, it had a threefold higher amount of protein (9%) compared to regular yogurt. Strained yogurt is much thicker and creamier than yogurts that have not been strained; therefore, it is highly valued by consumers.

The detailed production flow chart of strained yogurt is presented in Fig. 2.1.2.1 in the Materials and methods section. The sampling points, profile and content of individual fatty acids and their major groups are presented in Table 3.3.2.1.

Seasonal differences in fatty acid content were confirmed between analogous summer and winter samples during strained yogurt production. Significantly higher levels of C8:1n9t, C18:0, UFA, MUFA and, DFA and lower (P<0.05) levels of C16:0, C20:0, and SFA were detected in most summer yogurt samples. Besides, as seen in Fig. 3.3.2.1, summer strained yogurt was a healthier option due to higher (P<0.05) h/H and lower (P<0.05) AI and TI indices. Only the LA/ALA ratio did not differ significantly in summer and winter samples.

Pasteurization of standardized milk did not significantly affect fatty acid content in milk fats during strained yogurt production.

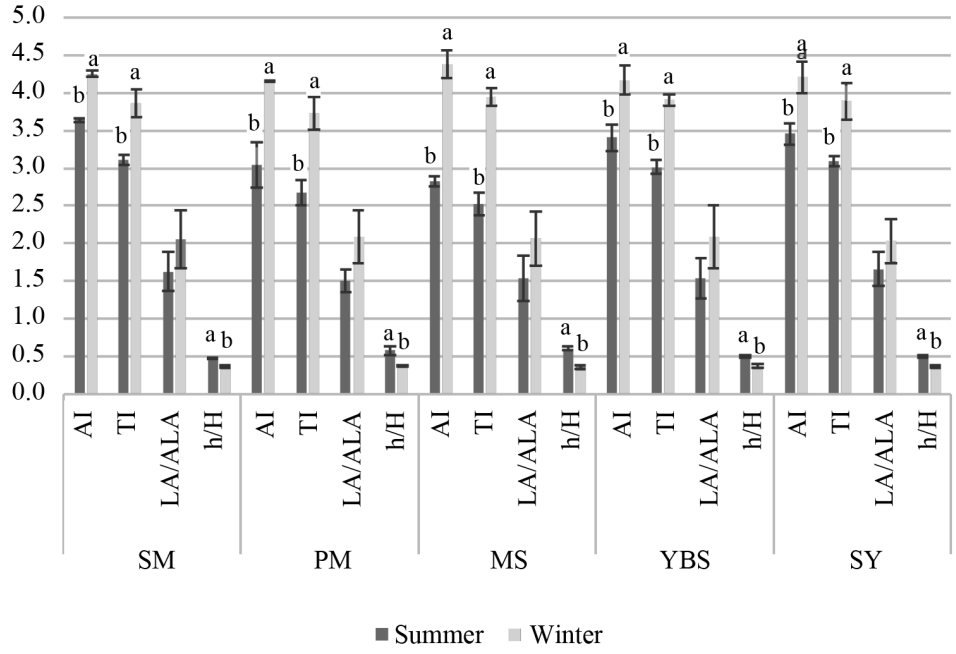


Fig. 3.3.2.1. *LQI in processing milk during strained yogurt production.*
SM – standardized milk; PM – pasteurized milk: MS – milk with starter;
YBS – yogurt before straining; SY – strained yogurt; different lowercase show significant between analogous samples in different seasons.

Our findings are in agreement with other studies where the difference in fatty acid content between raw and pasteurized at 95°C/5 min, 95°C/15 min, 85–90°C/2 min [15] or 75°C/15 sec milk was not confirmed [99].

In this study, the higher quantities (P<0.05) of C15:0, C18:2n6c, C21:0, PUFA, and the ratio of PUFA/SFA were observed in summer milk inoculated with starter when compared to the fresh-made yogurt product and the yogurt at the end of the shelf life. However, standardized and pasteurized milk and fresh-made strained yogurt did not vary significantly regarding fatty acid composition. Only a few researchers studied the effect of pasteurization [96] or straining processes [19] on fatty acid profiles during yogurt processing. In most of the studies investigating the impact of processing on fatty acid profile, only raw materials and the end products were taken into account, bypassing the technological steps in between [117, 154]. As a result, it is not always possible to compare our data gathered at intermediate processing steps with similar studies of other researchers. Therefore, we can assume that the increase of C15:0, C18:2n6c, C21:0, PUFA, and PUFA/SFA was due to the milk inoculation with LAB cultures present in the starter. No such change has been observed in winter.

Table 3.3.2.1. *The profile and content (% of total fatty acids) of individual fatty acids and their groups during strained yogurt production and storage in different seasons.*

FA and their main groups	Summer						Winter					
	standardized milk	pasteurized milk	milk with starter	yogurt before straining	yogurt	yogurt at the end of shelf life	standardized milk	pasteurized milk	milk with starter	yogurt before straining	yogurt	yogurt at the end of shelf life
1	2	3	4	5	6	7	8	9	10	11	12	13
C4:0	2.21±0.08	1.85±0.08	2.26±0.30	2.09±0.14	2.12±0.16	2.46±0.20	1.85±0.19	2.07±0.01	2.19±0.29	1.80±0.11	1.86±0.21	1.97±0.02
C6:0	1.79±0.11	1.46±0.05	1.76±0.23	1.67±0.08	1.75±0.18	1.92±0.14	1.36±0.11	1.64±0.06	1.62±0.20	1.36±0.02	1.54±0.14	1.60±0.03
ΣSCFA	4.00±0.13	3.31±0.14	4.01±0.52	3.76±0.20	3.87±0.34	4.38±0.34	3.21±0.30	3.71±0.04	3.82±0.06	3.16±0.09	3.40±0.35	3.57±0.01
C8:0	1.19±0.02	1.00±0.02	1.14±0.22	1.11±0.06	1.21±0.13	1.30±0.08	1.07±0.08	1.09±0.08	1.18±0.22	0.94±0.05	1.03±0.10	1.05±0.03
C10:0	3.18±0.23	2.65±0.17	2.72±0.20	2.95±0.27	3.24±0.39	3.36±0.18	2.60±0.22	2.83±0.29	3.04±0.21	2.56±0.02	2.68±0.29	2.88±0.24
C11:0	0.32±0.05	0.27±0.04	0.24±0.12	0.19±0.10	0.27±0.03	0.28±0.02	0.26±0.01	0.31±0.04	0.28±0.11	0.22±0.03	0.28±0.02	0.22±0.01
C12:0	3.96±0.12	3.31±0.21	3.93±0.20	3.72±0.19	4.00±0.39	4.05±0.17	3.49±0.17	3.74±0.41	4.10±0.23	3.42±0.01	3.63±0.43	3.29±0.28
C13:0	0.24±0.03	0.14±0.01	0.16±0.08	0.10±0.05	0.13±0.01	0.12±0.01	0.16±0.01	0.19±0.00	0.20±0.09	0.18±0.00	0.17±0.03	0.17±0.01
C14:0	13.57±0.23	12.15±0.45	11.68±0.47^{A}	13.00±0.44	13.21±0.56	13.19±0.57	13.52±0.15	13.62±1.01	14.00±0.26^{B}	13.24±0.41	13.46±0.86	13.83±0.08
C14:1	0.98±0.06	0.92±0.05^{A}	1.00±0.02^{A}	0.97±0.02	0.95±0.06	0.95±0.07	0.70±0.59	1.19±0.04^{B}	1.22±0.03^{B}	1.12±0.13	1.14±0.00	1.14±0.05
C15:0	1.48±0.01	1.36±0.05	1.53±0.07^{a}	1.40±0.02	1.25±0.06^{b}	1.25±0.05^{b}	1.49±0.15	1.48±0.11	1.57±0.06	1.45±0.10	1.45±0.04	1.43±0.05
ΣMCFA	24.91±0.56	21.80±0.88	22.39±0.33	23.43±0.90	24.26±1.58	24.50±1.14	23.29±1.38	24.46±1.67	25.70±0.78	23.12±0.59	23.84±1.62	24.01±0.50
C16:0	34.17±0.77^{A}	34.30±0.68^{A}	31.29±0.64^{A}	34.44±0.62^{A}	33.91±1.32^{A}	33.38±0.49^{A}	40.67±0.55^{B}	40.57±0.23^{B}	39.62±1.36^{B}	41.04±0.35^{B}	40.24±0.26^{B}	40.21±0.62^{B}
C16:1	1.71±0.23	1.79±0.29	2.00±0.33	1.85±0.31	1.60±0.17	1.32±0.05^{A}	2.17±0.25	2.18±0.22	2.24±0.23	2.16±0.19	2.32±0.08	2.42±0.13^{B}
C17:0	0.87±0.07	0.95±0.05	1.02±0.14	0.86±0.08	0.77±0.06	0.67±0.02	0.88±0.02	0.87±0.08	0.78±0.9	0.97±0.04	0.95±0.06	1.32±1.23
C18:0	10.73±0.42^{A}	11.54±0.07^{A}	11.44±0.68^{A}	10.92±0.51^{A}	10.97±0.28^{A}	10.80±0.61	8.84±0.30^{B}	8.28±0.42^{B}	8.19±0.49^{B}	8.78±0.38^{B}	8.72±0.73^{B}	8.86±1.16
C18:1n9t	1.78±0.16^{A}	2.00±0.15^{A}	2.38±0.30^{A}	1.95±0.29	1.87±0.19^{A}	1.81±0.23^{A}	0.85±0.00^{B}	0.88±0.01^{B}	0.78±0.02^{B}	0.84±0.04	0.92±0.06^{B}	0.87±0.07^{B}

Table 3.3.2.1 continued

1	2	3	4	5	6	7	8	9	10	11	12	13
C18:1n9c	18.49±0.17	20.71±0.60^{A}	20.26±0.31^{A}	19.51±0.60^{A}	19.76±0.88	20.21±1.91	17.40±1.00	16.42±0.59^{B}	16.70±0.60^{B}	17.47±0.77^{B}	17.06±0.68	16.41±0.91
C18:2n6	1.48±0.05	1.49±0.04	1.96±0.26^{a}	1.36±0.06	1.32±0.03^{b}	1.24±0.07^{b}	1.31±0.19	1.25±0.09	1.37±0.15	1.22±0.23	1.29±0.02	1.30±0.18
C18:3n3	0.96±0.14	0.96±0.11	1.36±0.26	0.93±0.12	0.83±0.11	0.82±0.01	0.66±0.15	0.66±0.16	0.55±0.12	0.59±0.14	0.60±0.17	0.55±0.13
C20:0	0.06±0.05^{A}	0.11±0.10^{A}	0.12±0.07^{A}	0.08±0.08^{A}	0.06±0.06^{A}	0.10±0.08^{A}	0.25±0.03^{B}	0.23±0.00^{B}	0.20±0.00^{B}	0.22±0.03^{B}	0.24±0.01^{B}	0.25±0.02^{B}
C21:0	0.85±0.06^{A}	1.04±0.05^{A}	1.77±0.49^{aA}	0.91±0.02^{A}	0.73±0.06^{bA}	0.76±0.08^{bA}	0.46±0.04^{B}	0.48±0.10^{B}	0.17±0.26^{B}	0.44±0.03^{B}	0.42±0.08^{B}	0.23±0.22^{B}
ΣLCFA	71.09±0.66	74.89±0.96	73.60±0.73	72.81±1.09	71.87±1.91	71.12±1.49	73.50±1.68	71.83±1.71	70.49±0.89	73.73±0.68	72.76±1.98	72.42±0.51
ΣSFA	74.61±0.13^{A}	72.12±0.35^{A}	71.04±0.67^{A}	73.43±0.79^{A}	73.68±0.61^{A}	73.64±1.05^{A}	76.90±0.19^{B}	77.43±0.93^{B}	77.14±0.25^{B}	76.61±0.58^{B}	76.68±0.84^{B}	77.31±0.45^{B}
ΣUFA	25.39±0.12^{A}	27.88±0.34^{A}	28.96±0.66^{A}	26.57±0.77^{A}	26.32±0.60^{A}	26.36±1.04^{A}	23.10±.19^{B}	22.57±0.92^{B}	22.86±0.14^{B}	23.39±0.56^{B}	23.32±0.83^{B}	22.69±0.44^{B}
ΣMUFA	22.95±0.28^{A}	25.43±0.26^{A}	25.64±0.64^{A}	24.27±0.75^{A}	24.17±0.51^{A}	24.29±0.99^{A}	21.13±0.15^{B}	20.66±0.86^{B}	20.94±0.21^{B}	21.59±0.49^{B}	21.43±0.70^{B}	20.83±0.40^{B}
ΣPUFA	2.44±0.16	2.45±0.15	3.32±0.47^{a}	2.29±0.10	2.15±0.14^{b}	2.06±0.06^{b}	1.97±0.03	1.91±0.07	1.92±0.05	1.81±0.09	1.89±0.15	1.86±0.05
PUFA/SFA	0.03±0.00	0.04±0.00	0.05±0.01^{a}	0.03±0.00^{b}	0.03±0.00^{b}	0.03±0.00	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
DFA	36.12±0.48	39.42±2.19	40.40±0.89	37.49±1.29	37.35±0.88	37.16±1.64	31.94±0.48	31.97±0.02	31.25±0.17	32.40±0.97	32.05±1.58	31.80±1.51

Values are mean±SD of six replicates; means with different lowercase letters within the same row show significant (P<0.05) difference between different processing steps; means with different uppercase within the same row show significant (P<0.05) difference between analogous samples in different seasons; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle chain fatty acids; LCFA: long-chain fatty acids, PUFA/SFA: ratio of polyunsaturated and saturated fatty acids; DFA – desirable fatty acids.

In this research, milk fermentation with thermophilic starter (containing *S. thermophilus, L. delbrueckii* subsp. *Bulgaricus*) did not affect significantly fatty acid profile and content during yogurt manufacturing.

A study conducted in 2019 states that the manufacturing of yogurt using YoFlex Harmony® (Chr. Hansen, Denmark) starter had no major influence on the fatty acid composition. Although the volatile fatty acids decreased and the MCFA, LCFA, and UFA increased in yogurt compared to raw goat milk, though the differences in the fatty acid composition between milk and yogurt were not significant [117]. Sheep milk fermentation with the analogous starter, as in our study, did not reveal any changes in fatty acid profile [16]. Bovine milk fermentation using dried mixed starter culture (Danisco, Denmark) as well as yogurt straining (cloth sack method) did not reveal changes in the fatty acid composition [15].

On the contrary, a 2015 study under similar conditions to ours, showed that individual fatty acids underwent changes and exhibited different patterns during yogurt processing. E.g., there was seen a consistent decline in C6:0 from 1.95% (of total fatty acid content) in raw milk to 1.88% in strained yogurt. Meantime C18:1 content (% of total fatty acid) increased from 19.46% in milk to 21.06% in yogurt and then decreased again in strained yogurt to 18.73% [19].

In the present study, storage of the strained yogurt for 25 days at 5°C in the original package did not cause any changes in the fatty acid profile and content when compared to freshly made strained yogurt. Our findings are partially supported by another study where 30 days of storage did not alter CLA, VA, or omega-3 fatty acids in yogurt [118].

Results similar to ours were obtained in a study with kefir. Although kefir was produced with a different starter culture (LAB, acetic acid bacteria, and yeasts) than yogurt, the analysis showed that fatty acid profile remained unchanged during both manufacturing and storage for 21 days compared to the fatty acid profile of raw goat milk [168].

Meantime, Serafeimidou et al. (2013) found out that 14-day storage at 5°C had an impact on bovine milk yogurt: SFA increased and UFA decreased significantly after storage. While no significant change was observed in the fatty acid content of sheep milk yogurt during storage in the same study [169]. 2020 study showed significant decreases of CLA, MUFA, PUFA in yogurts made from cow milk on the 21 days of storage [170].

A variety of data on changes in fatty acids during milk fermentation and storage [69, 114–116, 170] suggest that the substrate and bacterial culture of the starter are of particular importance to the fatty acid profile and composition in fermented dairy products.

3.3.3. Seasonal variations of milk fatty acids in major processing steps and at the end of shelf life of sour cream

Sour cream, also known as cultured cream, is produced by fermentation of pasteurized cream that contains 18–25% fat [171].

Raw cream was standardized, homogenized, pasteurized, and inoculated with a mesophilic aromatic starter (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc* spp.) to produce sour cream used in our study. The fermentation lasted until the acidity of the cream reached pH 4.5–4.6. The detailed sour cream production flow chart is presented in Fig. 2.1.2.1 in the Materials and methods section.

Some seasonal variations in fatty acid content were observed during sour cream production. All winter samples had a higher (P<0.05) content of C11:0, C13:0 (except for sour cream), and C20:0 than analogous summer samples. Summer standardized cream had a higher (P<0.05) content of C6:0, C10:0, and lower (P<0.05) content of C18:3n3c than winter standardized cream. Pasteurized summer cream was found in a higher (P<0.05) content of C12:0, and lower (P<0.05) content of C14:1 and C15:0 when compared to analogous winter samples. The C17:0 content was significantly higher in winter sour cream at the end of storing.

However, the seasonal variations of the individual fatty acids did not affect their main groups and LQI significantly; thus, the latter remained similar in samples during cream processing in both seasons (Fig. 3.3.3.1).

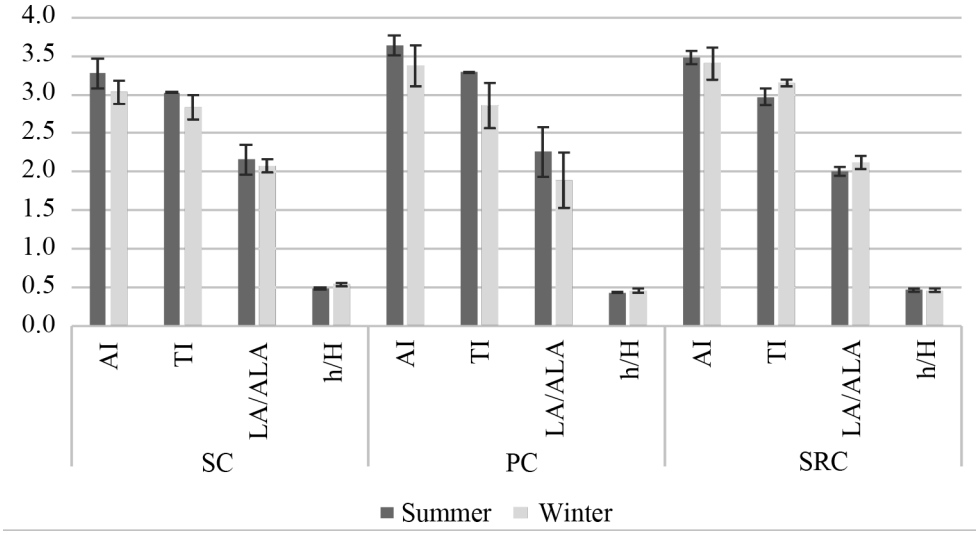


Fig. 3.3.3.1. *LQI in processing milk during sour cream production.*
SC – standardized cream; PC – pasteurized cream, SRC – sour cream.

Technological operations such as cream homogenization, pasteurization, and fermentation did not affect fatty acid profile and content significantly neither in winter nor summer samples. The individual fatty acids and their groups in samples during sour cream processing are presented in Table 3.3.3.1.

Our results are supported by other findings where no pasteurization impact on milk [99], no homogenization impact on cream [95], or milk [172] fatty acid profiles were found.

Whereas, previous studies with milk or cream fermentation provided controversial data. Sheep milk fermentation with *L. delbrueckii* subsp. *Bulgaricus* and *S. thermophilus* did not reveal any change in the fatty acid profile [16]. Meantime, camel milk fermentation with the same thermophilic LAB increased the MCFA and LCFA (except C16:1, C20:0) content [114]. Bovine milk fermentation using dried mixed starter culture (Danisco, Denmark) did not change fatty acid composition [15]. An increase of SCFA and MCFA (except C14:0) in buffalo milk fermented with *L. acidophilus* and *L. lactis* has been reported in the 2007 study [115]. The increase of LCFA was observed when cream was fermented with probiotic mesophilic bacteria *B. lactis*, whereas the increase of MCFA was observed in cream fermented with *L. acidophilus*, in 2013 study [116].

In general, the storing for 25 days at 5°C in the original package did not cause significant changes in the fatty acid profile and content of sour cream in our study. Only C15:0 found in higher (P<0.05) content at the end of shelf life in summer sour cream.

Similar results to ours were obtained in 2015 study; the fatty acid profile remained unchanged in kefir during 21-day storage [168]. No storage effect also was observed on yogurt and labaneh fatty acid profile under various storage conditions in a few studies as well [15, 118, 119].

The native milk lipoprotein lipases are very sensitive to high temperature and do not survive pasteurization. During milk fermentation and storage, commonly, the bacterial lipase causes to release FFA from the TG and can cause changes in fatty acid profile [96]. Nevertheless, not all LAB strains have lipolytic activity. The 2012 study revealed only two lipolytic strains from 76 LAB isolates: *L. delbrueckii* subsp. *delbrueckii* and *L. delbrueckii* subsp. *Bulgaricus* [173]. It seems that bacteria used in our study for cream fermentation did not appear to have lipolytic properties.

Table 3.3.3.1. *The profile and content (% of total fatty acids) of individual fatty acids and their groups during sour cream production and storage in different seasons.*

FA and their main groups	Summer				Winter			
	standardized cream	pasteurized cream	sour cream	end of shelf-life	standardized cream	pasteurized cream	sour cream	end of shelf-life
1	2	3	4	5	6	7	8	9
C4:0	2.12±0.02	2.27±0.04	2.34±0.28	2.09±0.05	1.65±0.21	2.29±0.44	2.82±1.08	1.72±0.20
C6:0	1.70±0.03^{A}	1.76±0.11	1.87±0.22	1.68±0.09	1.27±0.08^{B}	1.71±0.20	2.15±0.76	1.41±0.16
ΣSCFA	3.82±0.01	4.03±0.06	4.21±0.50	3.77±0.14	2.91±0.29	4.00±0.64	4.97±1.83	3.13±0.36
C8:0	1.03±0.01	0.92±0.08	1.01±0.06	1.24±0.13	0.87±0.07	1.15±0.12	1.41±0.43	0.91±0.06
C10:0	2.79±0.05^{A}	3.14±0.19	2.75±0.19	2.85±0.19	3.25±0.01^{A}	2.88±0.05	3.24±0.85	2.60±0.24
C11:0	0.05±0.01^{A}	0.03±0.00^{A}	0.06±0.02^{A}	0.07±0.01^{A}	0.28±0.01^{B}	0.34±0.03^{B}	0.34±0.07^{B}	0.30±0.02^{B}
C12:0	3.75±0.23	4.08±0.02^{A}	3.99±0.10	3.60±0.17	2.86±0.09	3.58±0.11^{B}	3.68±0.61	3.29±0.32
C13:0	0.04±0.01^{A}	0.05±0.00^{A}	0.09±0.01	0.06±0.01^{A}	0.20±0.09^{B}	0.56±0.21^{B}	0.16±0.12	0.70±0.35^{B}
C14:0	13.03±1.06	13.58±0.87	14.14±0.30	12.61±1.22	11.23±0.18	12.75±0.72	12.26±0.39	12.65±0.87
C14:1	0.97±0.07	1.04±0.03^{A}	1.04±0.01	1.24±0.13	1.11±0.02	1.34±0.01^{B}	1.16±0.09	0.73±0.57
C15:0	1.28±0.09^{a}	1.34±0.00^{aA}	1.35±0.08^{a}	1.79±0.05^{b}	1.36±0.00	1.52±0.03^{B}	1.36±0.09	1.53±0.17
ΣMCFA	22.94±1.51	24.18±0.53	24.43±0.44	24.55±1.59	21.14±1.04	24.12±0.21	23.61±2.35	22.73±1.90
C16:0	35.09±1.01	35.68±1.28	33.65±0.76	32.60±0.93	35.81±1.38	35.25±1.64	34.68±2.43	37.85±0.83
C16:1	2.11±0.11	2.12±0.20	2.16±0.10	2.18±0.05	2.12±0.31	2.13±0.15	1.86±0.12	1.91±0.03
C17:0	0.68±0.16	0.88±0.02	0.73±0.07	0.58±0.19^{A}	0.93±0.06	1.05±0.06	0.90±0.09	0.94±0.07^{B}
C18:0	9.72±0.01	9.46±0.24	9.66±0.40	9.74±0.35	9.35±0.12	8.43±0.61	9.16±0.84	9.40±0.58
C18:1n9t	1.81±0.16	1.55±0.23	1.86±0.26	1.42±0.42	1.32±0.02	1.51±0.36	1.20±0.02	1.25±0.18

Table 3.3.3.1 continued

1	2	3	4	5	6	7	8	9
C18:1n9c	20.88±0.55	19.27±0.10	19.92±0.55	20.29±0.89	22.42±1.63	19.00±0.11	19.36±1.66	19.35±1.04
C18:2n6	1.45±0.16	1.37±0.14	1.48±0.07	2.58±1.32	1.73±0.14	1.61±0.05	1.46±0.12	1.57±0.05
C18:3n3	$0.67±0.01^{A}$	0.61±0.09	0.74±0.01	0.65±0.06	$0.83±0.03^{B}$	1.06±0.21	0.69±0.09	0.68±0.10
C20:0	$0.11±0.02^{A}$	$0.12±0.04^{A}$	$0.09±0.01^{A}$	$0.14±0.01^{A}$	$0.35±0.08^{B}$	$0.54±0.18^{B}$	$1.03±0.78^{B}$	$0.34±0.07^{B}$
C21:0	0.71±0.02	0.75±0.03	1.05±0.43	2.59±1.84	1.07±0.35	1.30±0.52	1.07±0.13	0.86±0.21
ΣLCFA	73.24±1.50	71.80±0.05	71.40±0.06	71.75±1.73	75.94±0.62	71.88±0.43	71.42±4.18	74.14±2.26
ΣSFA	72.11±0.61	74.03±0.05	72.80±0.78	71.63±1.77	70.47±0.82	73.36±0.78	74.27±1.63	74.51±0.68
ΣUFA	27.89±0.60	25.97±0.04	27.20±0.76	28.37±1.75	29.53±0.80	26.64±0.77	25.73±1.60	25.49±0.68
ΣMUFA	25.77±0.43	23.99±0.15	24.98±0.69	25.13±0.39	26.97±0.95	23.98±0.62	23.58±1.42	23.25±0.62
ΣPUFA	2.12±0.17	1.99±0.10	2.22±0.08	3.24±1.39	2.57±0.17	2.67±0.16	2.16±0.21	2.25±0.05
PUFA/SFA	0.04±0.01	0.04±0.01	0.03±0.00	0.05±0.02	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
DFA	37.62±0.59	35.43±0.29	36.86±1.18	38.11±2.13	39.89±2.70	35.07±0.46	34.89±2.48	34.89±1.26

Values are mean±SD of six replicates; means with different lowercase letters within the same row show significant (P<0.05) difference between different processing steps; means with different uppercase within the same row show significant (P<0.05) difference between analogous samples in different seasons; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle chain fatty acids; LCFA: long-chain fatty acids, PUFA/SFA: ratio of polyunsaturated and saturated fatty acids; DFA – desirable fatty acids.

3.3.4. Seasonal variations of milk fatty acids in major processing steps and at the end of shelf life of curd cheese

The raw milk was standardized, pasteurized, cooled, and inoculated with a mesophilic starter culture (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc* spp.) to produce curd cheese analysed in this study. When ph reached 4.5–4.7, the curd body was heated to 60°C and sliced to separate the whey. The detailed production diagram of curd cheese is presented in Fig. 2.1.2.1 in the Materials and methods section.

The season impact, in contrast to the technological process, was significant for curd cheese, whey, and stored samples. Summer curd cheese had a significantly lower content of C14:1, C16:0 and, SFA and a higher (P<0.05) content of 18:0, C18:1n9c, UFA, MUFA and, DFA than winter cheese. At the end of storing, summer curd cheese had a significantly higher content of C4:0, C6:0, C8:0, C18:0, C18:1n9c, and C18:n3c, lower (P<0.05) content of C16:0 and C17:0 than winter curd cheese at the end of storing. Major fatty acid groups showed a similar pattern in all summer samples: the content of UFA and MUFA was higher, and the content of SFA was lower than in winter samples.

Our findings are in agreement with other studies that analyzed the seasonal impact on the milk fat profile [5, 13, 77].

Fig. 3.3.4.1 shows that summer curd cheese was more beneficial to human nutrition since (P<0.05) lower AI, TI, and a higher (P<0.05) h/H value was detected in it.

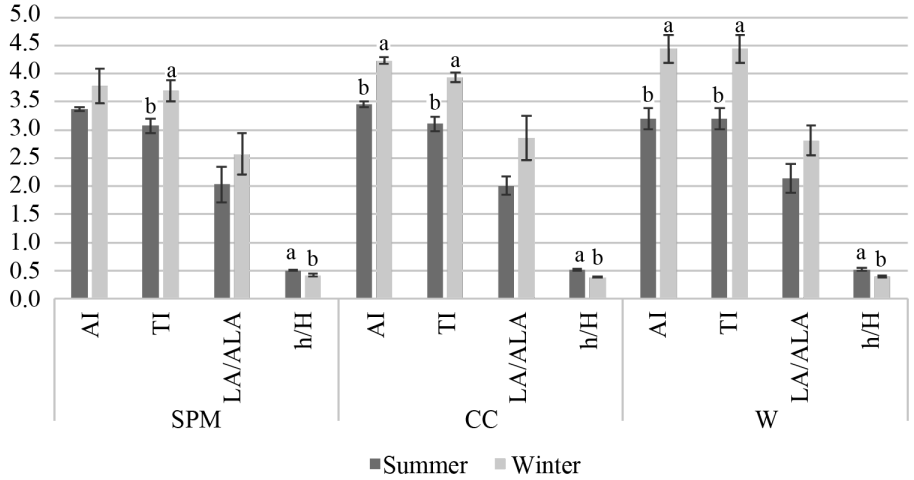


Fig. 3.3.4.1. *LQI in processing milk during curd cheese production.*
SPM – standardized and pasteurized milk; CC – curd cheese, W – whey; different lowercase show significant diference between analogous samples in different seasons.

In the present study, pasteurization, fermentation, and syneresis did not affect the fatty acid profile significantly in milk directed to process curd cheese during both summer and winter seasons.

Many authors have found that pasteurization did not affect fatty acid profiles [15, 95–97, 99,107]. We did not reveal a significant effect of heat treatment on other products analyzed in this study. Changes in fatty acid composition during milk fermentation have already been described in detail in previous (3.3.2 and 3.3.3) sections of this thesis.

Similar to our fatty acid dynamic between product (fresh cheese) and by-product (whey) was detected in the 2017 study [18]. In a 2005 study, where sheep's milk was processed into fresh cheese, no changes in dairy fats were detected. The fatty acid content of the final product was primarily dependent on the fatty acid content of raw milk [174]. This statement is following our findings. Although 0.60±0.04% of fat was removed with whey during curd cheese processing, the composition and content of many fatty acids in it remained similar as in curd cheese and standardized and pasteurized milk. Herzallah et al. (2005) did not find a difference in fatty acids between yogurt and yogurt after straining either [15]. An exception was found only for two fatty acids during summer curd cheese production in this study. Only traces (below detectable level) of C11:0 and C13:0 were detected in milk and later in whey. The appearance of these fatty acids in fresh-made curd cheese was due to the curd concentration specificity. In winter curd cheese, a similar pattern was not observed due to the high levels of above mentioned fatty acids in winter milk. The seasonal differences in C11:0 and C13:0 content might be feed related.

Storing, as can be seen in Table 3.3.4.1, had a significant impact on some fatty acid content in summer curd cheese.

Table 3.3.4.1. *The profile and content (% of total fatty acids) of individual fatty acids and their groups during curd cheese production and storage in different seasons.*

FA and their main groups	Summer				Winter			
	standardized and pasteurized milk	curd cheese	whey	end of shelf-life	standardized and pasteurized milk	curd cheese	whey	end of shelf-life
1	2	3	4	5	6	7	8	9
C4:0	1.95±0.03	2.08±.05	1.93±0.23	2.36±0.07^{A}	1.59±0.30	1.90±0.06	1.67±0.12	2.02±0.00^{B}
C6:0	1.55±0.07^{a}	1.75±0.09	1.53±0.06^{a}	1.89±0.04^{bA}	1.40±0.27	1.53±0.05	1.35±0.16	1.65±0.02^{B}
ΣSCFA	3.50±0.08	3.83±0.14	3.46±0.28	4.25±0.11	2.98±0.57	3.43±0.10	3.02±0.28	3.67±0.02
C8:0	0.99±0.06	1.26±0.08	0.93±0.11^{a}	1.29±0.03^{bA}	1.03±0.17	1.04±0.03	0.96±0.10	1.14±0.03^{B}
C10:0	2.94±0.23^{a}	3.32±0.27	2.66±0.10^{a}	3.19±0.14^{b}	2.62±0.46	2.90±0.07	2.54±0.23	2.89±0.15
C11:0	nd^{aA}	0.18±0.09	nd^{aA}	0.27±0.00^{b}	0.27±0.01^{B}	0.30±0.05	0.23±0.02^{B}	0.26±0.03
C12:0	3.53±0.25	3.99±0.29	3.54±0.20	4.08±0.12	3.38±0.51	3.74±0.05	3.51±0.06	4.08±0.03
C13:0	nd^{aA}	0.12±0.06	nd^{aA}	0.16±0.02^{b}	0.14±0.06^{B}	0.27±0.07	0.18±^{B}0.00	0.20±0.00
C14:0	13.28±0.52	13.91±0.36	12.51±0.29	13.53±0.30	12.63±0.92	13.68±0.01	12.70±0.87	14.26±0.00
C14:1	0.96±0.07	0.93±0.05^{A}	0.92±0.02	1.00±0.08	1.12±0.05	1.23±0.03^{B}	0.71±0.49	1.22±0.02
C15:0	1.45±0.12	1.41±0.04	1.29±0.01	1.35±0.07	1.45±0.02	1.55±0.02	1.39±0.13	1.50±0.01
ΣMCFA	23.15±0.98	25.26±1.14	21.85±0.63	24.88±0.63	22.63±2.15	24.71±0.17	28.23±4.18	25.55±0.17
C16:0	34.59±1.36	32.04±1.54^{A}	34.54±0.98	33.77±0.90^{A}	39.52±0.85	39.54±0.63^{B}	37.47±2.96	40.62±0.33^{B}
C16:1	2.27±0.03^{a}	2.12±0.03^{a}	2.19±0.08^{aA}	1.61±0.19^{b}	1.94±0.19	1.96±0.11	1.75±.13^{B}	2.13±0.30
C17:0	0.86±0.05	0.87±0.02	0.95±0.03	0.71±0.01^{A}	0.92±0.05	0.92±0.05	0.88±0.03	0.82±0.02^{B}
C18:0	10.86±0.22	11.76±0.40^{A}	11.50±0.77	10.49±0.34^{A}	9.40±0.58	8.76±0.29^{B}	8.36±0.11	8.06±0.27^{B}

Table 3.3.4.1. continued

1	2	3	4	5	6	7	8	9
C18:1n9t	1.74±0.26	1.23±0.56	1.96±0.31	1.76±0.21	1.03±0.08	1.11±0.07	1.04±0.02	1.01±0.11
C18:1n9c	19.81±0.41	20.23±0.20^{A}	20.42±0.52^{A}	19.76±0.36^{A}	18.79±0.88	16.94±0.33^{B}	16.58±0.57^{B}	15.88±0.34^{B}
C18:2n6	1.50±0.07^{a}	1.32±0.04	1.47±0.02^{a}	1.15±0.09^{b}	1.40±0.12	1.39±0.05	1.47±0.10	1.23±0.06
C18:n3	0.78±0.13	0.66±0.04	0.71±0.08	0.85±0.11^{A}	0.56±0.13	0.52±0.13	0.53±0.09	0.44±0.03^{B}
C20:0	0.15±0.10	0.14±0.07	0.13±0.07	0.10±0.06	0.26±0.06	0.15±0.09	0.21±0.02	0.21±0.02
C21:0	0.79±0.16	0.67±0.09	0.81±0.16	0.66±0.02	0.57±0.06	0.57±0.10	0.45±0.05	0.38±0.01
ΣLCFA	73.35±1.04	70.90±1.24	74.69±0.90	70.87±1.57	74.39±2.72	71.87±1.07	68.75±3.89	70.78±1.15
ΣSFA	72.93±0.06	73.50±0.56^{A}	72.33±0.93^{A}	73.87±0.56^{A}	75.16±1.19	76.85±0.18	77.91±1.34^{B}	78.08±0.17^{B}
ΣUFA	27.07±0.05	26.50±0.54^{A}	27.67±0.90^{A}	26.13±0.53^{A}	24.84±1.17	23.15±.17^{B}	22.09±1.32^{B}	21.92±0.15^{B}
ΣMUFA	24.79±0.13	24.51±0.56^{A}	25.50±0.84^{A}	24.13±0.69^{A}	22.88±0.94	21.24±0.26^{B}	20.08±1.16^{B}	20.24±0.13^{B}
ΣPUFA	2.28±0.12	1.99±0.00	2.18±0.08	2.00±0.17	1.96±0.25	1.91±0.08^{B}	2.01±0.18	1.67±0.03
PUFA/SFA	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.02±0.00
DFA	37.93±0.20	38.26±0.29^{A}	39.18±1.66^{A}	36.62±0.46^{A}	34.24±1.77	31.91±0.47^{B}	30.45±1.45^{B}	29.97±0.44^{B}

Values are mean±SD of six replicates; means with different lowercase letters within the same row show significant (P<0.05) difference between different processing steps; means with different uppercase within the same row show significant (P<0.05) difference between analogous samples in different seasons; nd: not detected; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle chain fatty acids; LCFA: long-chain fatty acids, PUFA/SFA: ratio of polyunsaturated and saturated fatty acids; DFA – desirable fatty acids.

A decrease of C18:2n6 and an increase of C6:0, C8:0, C10:0 in stored summer curd cheese was significant (P<0.05) when compared to whey or standardized and pasteurized milk. However, comparing fresh-made curd cheese with the same product at the end of storing only a decrease (P<0.05) of C16:1 was found. The degradation of fat in stored curd cheese did not appear to be intense.

Silva-Kazama et al. (2010) reported the similar findings in the butter storage study. They claimed that the relative percentage of SFA and MCFA increased due to the oxidation (decrease) of UFA [175]. Gulzar et al. (2019) confirmed similar findings when Mozarrella was stored for 90 days and state that the concentration of UFA decreased, and SFA increased on a percentage basis [134].

3.3.5. Seasonal variations of milk fatty acids in major processing steps and at the end of shelf life of butter

Natural unsalted 82% fat butter was chosen for the study. Standardized cream was pasteurized, cooled, and ripened at low temperature for butter production. After ripening, the cream was churned until the butter grains formed, and the buttermilk separated. The detailed production flow chart is given in Fig. 2.1.2.1 in the Materials and methods section.

The major seasonal impact was determined in the end product – butter, while it was less pronounced in standardized and pasteurized cream (Table 3.3.5.1.). Summer butter had significantly (P<0.05) lees content of SCFA, C10:0, C11:0, C12:0, C14:1, C15:0, MCFA, and SFA, and a higher content of C18:0, C18:1n9c, LCFA, UFA, and MUFA than winter butter. The seasonal butter differences found in our study, corresponding to the results found by many other researchers. Both raw and various dairy products produced during the summer period usually have higher levels of health-promoting fatty acid composition (MUFA and PUFA), and they are more beneficial to human health [13, 154, 156]. Frequently, this phenomenon can also be related to the faster deterioration of dairy products [176].

Since summer butter had more UFA, MUFA, and DFA, the AI, and h/H values were rated as more beneficial (P<0.05) fo human health (Fig. 3.3.5.1).

This study assessed the effect of cream pasteurization, churning, and byproduct (buttermilk) separation on fatty acid profile and content during butter processing.

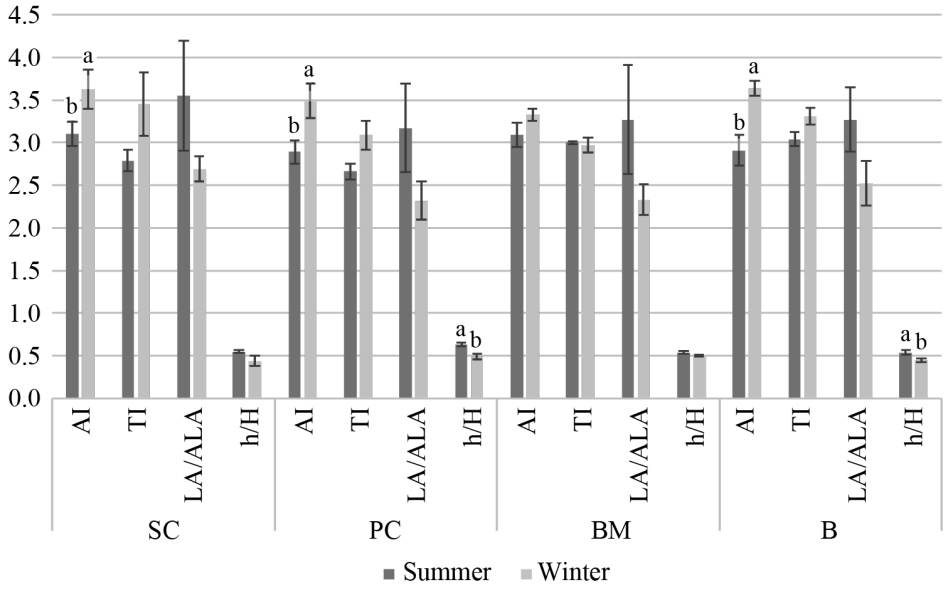


Fig. 3.3.5.1. *LQI in processing milk during butter production.*
SC – standardized cream; PC – pasteurized cream; BM-buttermilk; B – butter; different lowercase show significant diference between analogous samples in different seasons.

Fatty acid distribution in processing cream during summer and winter is presented in Table 3.3.5.1. In summer processing cream, some minor differences between major fatty acids groups were seen; the total content of SCFA and MCFA was prone to a slight decrease, whereas LCFA to a slight increase. However, neither changes in the major fatty acid group nor the differences between individual fatty acids in the processing of summer cream have been confirmed as significant.

In winter processing cream, only a higher (P<0.05) content of SCFA was found in standardized and pasteurized cream compared to that in butter and buttermilk. Meantime the individual C4:0 and C6:0 did not show significant changes between raw and the end product.

Hence, cream pasteurization and churning did not have a significant effect on individual fatty acid profile and content. The average 0.5±0.02% of total fat was removed with buttermilk, but the fatty acid profile and content in it remained similar as in butter and standardized cream.

Similar trends of fatty acid content were observed in cream, butter, and buttermilk made from the milk of a control group cow and fed with fish oil additives in a 2001 study [124]. 2007 study revealed that the butter-making process had no significant influence on the fatty acid and CLA content when organic cream or the cream from integrated farming was processed into butter. Fatty acid profile of cream corresponded butter fatty acid profile [125].

Table 3.3.5.1. *The profile and content (% of total fatty acids) of individual fatty acids and their groups during butter production and storage in different seasons.*

FA and their main groups	Summer					Winter				
	standardized cream	pasteurized cream	butter milk	butter	end of shelf-life	standardized cream	pasteurized cream	butter milk	butter	end of shelf-life
1	2	3	4	5	6	7	8	9	10	11
C4:0	1.66±0.21	1.72±0.09	1.78±0.02	1.60±0.03	2.02±0.01^{A}	2.25±0.24	2.10±0.08	1.67±0.08	1.76±0.04	1.90±0.00^{B}
C6:0	1.86±0.46	1.44±0.13	1.23±0.28	1.28±0.07	1.39±0.23	1.44±0.23	1.68±0.07	1.37±0.12	1.50±0.02	1.52±0.01
ΣSCFA	3.52±0.25	3.16±0.22	3.01±0.27	2.88±0.04^{A}	3.41±0.24	3.69±0.09^{a}	3.78±0.14^{a}	3.04±0.16^{b}	3.27±0.05^{bB}	3.42±0.2
C8:0	1.32±0.33	1.06±0.17	1.09±0.06	0.69±0.34	1.21±0.11	1.26±0.16	1.19±0.06	1.01±0.02	1.03±0.11	0.90±0.09
C10:0	2.79±0.32	2.35±0.10	2.50±0.34	1.91±0.14^{A}	2.75±0.15	2.79±0.15	2.90±0.11	2.75±0.03	2.80±0.07^{B}	2.78±0.06
C11:0	0.06±0.00^{A}	0.08±0.02^{A}	0.04±0.00	0.03±0.03^{A}	0.06±0.02^{A}	0.31±0.06^{B}	0.28±0.01^{B}	0.17±0.17	0.27±0.01^{B}	0.23±0.00^{B}
C12:0	3.56±0.19	2.51±0.20^{A}	2.96±0.63	2.94±0.12^{A}	3.69±0.27	4.09±0.48	3.80±0.05^{B}	3.53±0.16	3.66±0.03^{B}	3.67±0.13
C13:0	0.09±0.00^{A}	0.02±0.00^{A}	0.03±0.02^{A}	0.05±0.05	0.29±0.16	0.17±0.01^{B}	0.19±0.02^{B}	0.27±0.06^{B}	0.19±0.04	0.18±0.05
C14:0	12.64±0.10	11.34±0.50	12.75±0.18	11.64±0.50	12.24±0.45	14.23±0.74	13.60±0.33	13.17±0.07	13.44±0.08	13.91±0.30
C14:1	1.11±0.25	1.29±0.10	1.07±0.01^{A}	0.85±0.03^{A}	1.48±0.15	1.24±0.04	1.30±0.04	1.33±0.03^{B}	1.26±0.06^{B}	1.15±0.03
C15:0	1.28±0.08^{a}	1.28±0.01^{aA}	1.38±0.08^{a}	1.17±0.12^{aA}	1.76±0.07^{bA}	1.56±0.00^{a}	1.51±0.02^{B}	1.47±0.13	1.52±0.02^{B}	1.49±0.01^{bB}
ΣMCFA	22.86±1.28	19.93±1.04^{A}	21.82±1.03	19.28±0.60^{aA}	23.47±1.55^{b}	25.66±1.35	24.76±0.45^{B}	23.69±0.23	24.16±0.20^{B}	24.31±0.04
C16:0	32.54±1.43^{A}	33.50±0.16	34.84±0.19	35.75±0.93	33.92±0.26^{A}	34.91±0.40^{B}	34.81±0.84	35.56±0.44	36.70±0.61	37.03±0.68^{B}
C16:1	2.21±0.08	2.19±0.22	2.26±0.18	2.15±0.15	2.17±0.14	1.74±0.18	1.87±0.06	2.04±0.06	1.87±0.03	2.32±0.19
C17:0	0.91±0.10	1.14±0.19	1.03±0.04	0.85±0.02	0.96±0.31	0.96±0.04	0.89±0.04	1.00±0.03	0.90±0.03	0.89±0.05
C18:0	11.09±0.69	11.54±0.50^{A}	10.46±0.44	11.96±0.62^{A}	9.76±0.32	10.48±0.71	9.57±0.18^{B}	9.59±0.21	9.50±0.28^{B}	9.39±0.13

Table 3.3.5.1 continued

1	2	3	4	5	6	7	8	9	10	11
C18:1n9t	1.48±0.04	1.77±0.25^{a}	1.90±0.50^{a}	1.43±0.02	1.22±0.11^{b}	1.50±0.12	1.39±0.06	1.33±0.04	1.25±0.06	1.25±0.12
C18:1n9c	22.55±0.85	23.24±1.17	21.29±0.70	22.68±0.58^{A}	22.00±1.13	17.72±2.46	19.68±0.80	20.01±0.16	19.07±0.45^{B}	18.46±0.53
C18:2n6	1.56±0.09	2.10±0.35	1.72±0.07	1.62±0.08	1.54±0.18	1.84±0.06	1.70±0.03	2.01±0.17^{a}	1.74±0.05	1.52±0.03^{b}
C18:3n3	0.60±0.04	0.66±0.00	0.55±0.21	0.63±0.01	0.73±0.17	0.68±0.0.3	0.74±0.06	0.86±0.04	0.69±0.05	0.63±0.04
C20:0	0.11±0.10	0.21±0.01	0.24±0.01	0.17±0.17	0.20±0.02	0.23±0.01	0.22±0.01	0.15±0.08	0.23±0.03	0.21±0.02
C21:0	0.58±0.03	0.56±0.05	0.88±0.02^{A}	0.59±0.02	0.63±0.20	0.58±0.01	0.59±0.02	0.72±0.01^{B}	0.61±0.11	0.57±0.01
ΣLCFA	73.63±1.52	76.91±0.30^{aA}	75.17±1.29	77.83±0.64^{aA}	73.13±0.35^{b}	70.65±1.91	71.46±0.45^{B}	73.27±0.24	72.57±0.36^{B}	72.27±1.02
ΣSFA	70.49±0.70	68.75±1.66	71.21±0.89	70.63±0.75^{A}	70.87±0.73^{A}	75.27±2.39	73.32±0.92	72.42±0.50	74.12±0.43^{B}	74.67±0.44^{B}
ΣUFA	29.51±0.69	31.25±0.91^{A}	28.79±0.88	29.37±0.74^{A}	29.13±0.72^{A}	24.73±2.28	26.68±0.90^{B}	27.58±0.50	25.88±0.42^{B}	25.33±0.42^{B}
ΣMUFA	27.35±0.56	28.49±1.04	26.51±1.03	27.11±0.68^{A}	26.86±0.72	22.21±2.48	24.23±0.88	24.71±0.29	23.45±0.40^{B}	23.18±0.42
ΣPUFA	2.16±0.14	2.76±0.13	2.27±0.14	2.26±0.07	2.27±0.00	2.52±0.09	2.45±0.03	2.87±0.21^{a}	2.43±0.00	2.15±0.00^{b}
PUFA/SFA	0.03±0.00^{A}	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.04±0.00^{B}	0.03±0.00	0.04±0.00	0.03±0.00	0.03±0.00
DFA	40.60±1.38	42.79±0.41^{A}	39.25±1.33	41.33±1.37^{A}	38.90±1.05^{A}	35.21±1.68	36.25±1.69^{B}	37.17±0.71	35.38±0.70^{B}	34.72±0.57^{B}

Values are mean±SD of six replicates; means with different lowercase letters within the same row show significant (P<0.05) difference between different processing steps; means with different uppercase within the same row show significant (P<0.05) difference between analogous samples in different seasons; SFA: saturated fatty acids, UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: middle chain fatty acids; LCFA: long-chain fatty acids, PUFA/SFA: ratio of polyunsaturated and saturated fatty acids; DFA – desirable fatty acids.

No effect of storage on the fatty acid profile was observed in winter butter. Meantime an increase ($P<0.05$) of C15:0 and MCFA and a decrease ($P<0.05$) of LCFA were detected in summer butter at the end of shelf life compared with freshly made butter.

Parallel findings to our were observed in the 2010 study; MUFA and LCFA decreased, while relative percentages of SFA and MCFA increased with more extended (45 versus 15 days) storage period in butter [175]. Usually, summer milk contains a higher level of UFA and is more prone to be oxidized than milk produced in other months of the year [156]. Buttermilk made from the more unsaturated milk also was less oxidatively stable than buttermilk from the more saturated milk during storage at 4°C for 11 days [177]. Silva-Kazama et al. (2010) stated that butter enriched in PUFA had a shorter shelf life [175].

Commonly, neutral milk fats TG are quite stable as they are surrounded and protected by fat globule membranes. Butter-making technology involves special physical cream treatment (churning process) to break down the membranes of milk fat globules and release the TG to form the grains of butter. This is followed by the interaction of fat with lipases (milk-derived or bacterial) that release FFA from triglycerides [178]. Thus, butter could be identified as a sensitive lipase product.

Since cream was pasteurized, milk's indigenous enzymes LPL were inactivated (they are particularly sensitive to higher temperatures). Meantime, enzymes of psychrotrophic bacteria can survive even UHT treatment and can be related to flavors defects in cream, butter, cheese, and UHT milk [142].

In contrast, 2020 study with goat milk butter, did not reveal any significant changes in individual SFA, MUFA, and PUFA percentage weight neither between different processing treatment (salted or unsalted) nor refrigeration time (0, 1, 3, 6 months at 5°C). There were no significant interactions found between processing treatment and storage time, either [179]. Another 2017 study, where the butter made from cow milk was stored at 4°C or 12°C, showed a significant decrease of few PUFA only after 9 months of storage [180].

3.3.6. Development of computer program for raw milk screening according to the desired fatty acid composition

The implementation of strategies to improve milk process optimization is of great importance within the dairy industry. To reduce any time and resource wastage, unnecessary costs, and errors while attaining the process objective of creating a quality product suitable as for internal so for external

markets, a new screening tool for raw recourses is needed for the dairy business.

As an answer to the request of Lithuanian dairy producers facing challenges of conformity of fatty acids composition of exported dairy products to the standards of emerging markets, this case study was performed. One of the tangible outcomes of this study was the prototype-programming tool (software application) created for the screening of the composition of raw procured milk and launched on the LCMTL database server, which works exclusively with laboratory data stored at LCMTL. The program enables milk producers to screen procured raw milk fatty acid composition data applying various filters such as period, region, overall supplier Lithuania, and choose the milk (supplier) according to the selected composition parameters to produce a dairy product of required (standard) quality. To date, this prototype screens milk for SFA, UFA, MUFA, PUFA, C16:0, C18:0, C18:1n9c, and SFA/UFA ratio.

The prototype program allows to narrow data search according to the desired period, region of Lithuania or concrete fatty acid data value (Figs. 3.3.6.1 and 3.3.6.2).

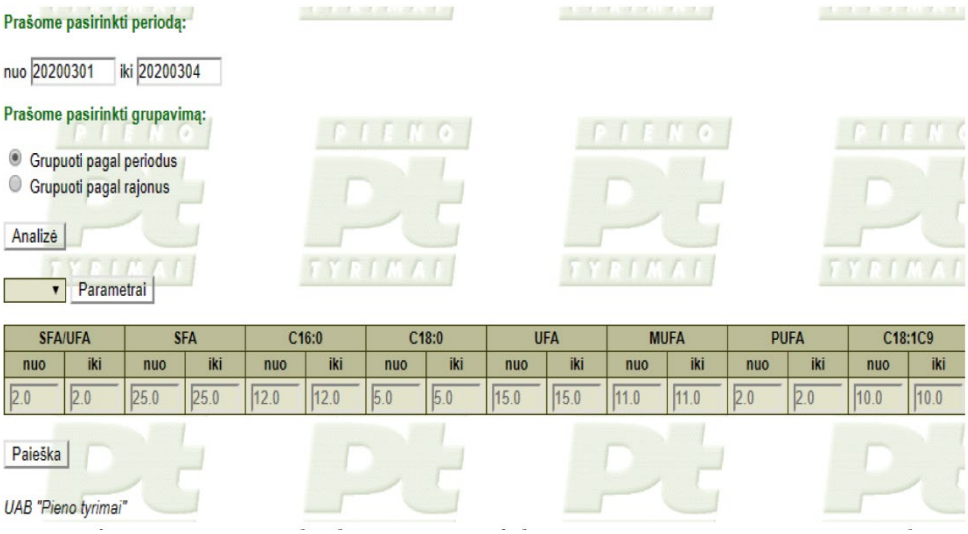


Fig. 3.3.6.1. *The home page of the prototype-programming tool.*

SFA/UFA		SFA		C16:0		C1
nuo	iki	nuo	iki	nuo	iki	nuo
2.0	2.7	25.00	28.00	8.00	12.00	5.0

Paieška

Periodas: nuo: 2020.03.01 iki: 2020.03.04

Rajonas	Punktas	Gamintojas	Mėginių	Tyrimų	SFA/UFA	SFA	C16:0	C1
Biržų r.	10878.Punktas	10878	1	1	2,14	26,92	11,31	5
Biržų r.	20266.Skrebiškiai	13	1	1	2	26,43	11,97	5
Pakruojo r.	10057.Guostagalio ŽŪB	41661	4	4	2,38	27,91	11,31	6
Šiaulių r.	31072.Punktas	31072	1	1	2,52	28	11,58	6

UAB "Pieno tyrimai"

Fig. 3.3.6.2. *Search for raw milk according to desired values of fatty acids and demonstration of the obtained data.*

The tool enables the filtering of data: by period, region, milk collecting center, etc. until the data of particular milk, supplier appears on the screen (Fig. 3.3.6.3).

Periodas: nuo: 2020.01.01 iki: 2020.03.04

Mėnuo	Mėginių	Tyrimų	SFA/UFA	SFA	C16:0	C18:0	UFA	MUFA	PUFA	C18:1C9
2020.01	375	375	2,6	30,25	14,07	4,58	11,94	10,52	1,28	7,82
2020.02	453	453	2,6	29,44	13,71	4,56	11,54	10,22	1,14	7,63
2020.03	64	64	2,68	30,02	13,46	5,78	11,41	10,02	1,17	7,11

Rajonas	Tyrimų	SFA/UFA	SFA	C16:0	C18:0	UFA	MUFA	PUFA	C18:1C9
Biržų r.	79	2,77	31,05	14,72	4,56	11,63	10,29	1,28	7,74
Joniškio r.	43	2,58	30,9	14,58	4,31	12,1	10,63	1,28	7,58
Pakruojo r.	65	2,28	28,81	12,69	4,46	12,84	11,08	1,66	8,43
Pasvalio r.	94	2,61	30,97	14,46	5,04	12,23	10,89	1,15	8,23
Šiaulių r.	94	2,67	29,58	13,85	4,34	11,22	9,91	1,14	7,16

Punktas	Tyrimų	SFA/UFA	SFA	C16:0	C18:0	UFA	MUFA	PUFA	C18:1C9
10358.Punktas	3	2,31	28,15	12,14	5,4	12,35	10,6	1,62	7,83
10390.Punktas	3	2,69	29,53	14,41	3,95	11	9,9	,85	7,11
10581.Punktas	3	2,42	30,24	13,76	4,82	12,55	10,99	1,39	7,85
10878.Punktas	1	1,94	25,78	11,11	4,75	13,27	11,26	1,9	8,4
10966.Punktas	3	1,98	26,27	12,16	4,96	13,31	11,72	1,36	8,8
20182.Ilgalaukiai	24	3,09	29,94	13,67	4,59	10,33	9,27	,96	7,58
20194.Smilgeliai	9	2,47	28,85	14,31	4,09	11,84	9,78	2,94	7,45
20196.Pasvaliečiai	12	2,95	35,77	17,05	5,57	12,27	10,97	1,01	7,55
20266.Skrebiškiai	8	2,63	32,34	16,63	3,31	12,41	11,25	,94	7,91
23917.Anciškiai	6	3,13	34,17	16,13	4,51	11,13	10,02	,99	8,07
23944.Suostas	4	2,19	28,2	13,6	4,06	13,11	11,63	1,29	8,12
31935.Punktas	3	2,71	33,54	16,31	4,7	12,41	11,2	,96	8,13

UAB "Pieno tyrimai"

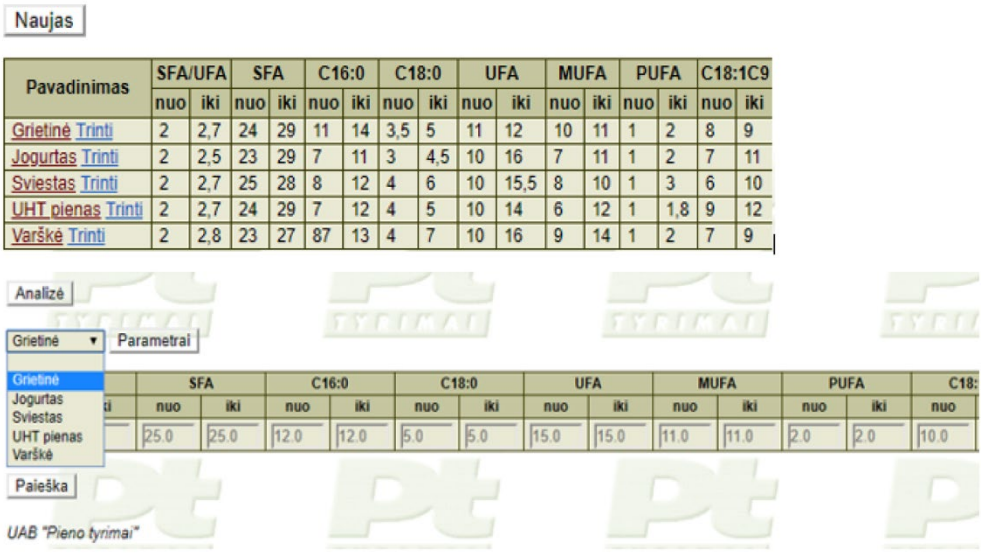
Fig. 3.3.6.3. *Raw milk data grouping by period, region and supplier.*

Special algorithms addressing certain composition parameters of a particular dairy product can be set in this program. These algorithms enable the correction of changes in fatty acids profile during milk processing. As follows, milk producers will be able to select milk according to the required (minimum mandatory) specifications on the regard of the fatty acid composition of dairy products in question.

Since no significant impact of the technological process on the most important individual fatty acid and their groups from a quantitative viewpoint tested routinely at LCMTL in procured milk has been identified in our study, adjusting (correcting) algorithms were not entered into the search engine at this stage.

Major seasonal variations in the fatty acid profile of procured raw milk were detected in our study. The same variations were subsequently tracked down in every examined dairy end product. According to the growing body of scientific literature, the fatty acid composition of raw milk shows rapid and significant variation in response to changes in the cow diet. By filtering raw milk according to a particular set and composition of fatty acids, dairy producers can expect the same fatty acid composition in the end product.

Certain fatty acid parameters for particular dairy end products can be set, saved, and used by the dairy processor for the new milk screening (Fig. 3.3.6.4).



Pavadinimas	SFA/UFA		SFA		C16:0		C18:0		UFA		MUFA		PUFA		C18:1C9	
	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki
Grietinė Trinti	2	2,7	24	29	11	14	3,5	5	11	12	10	11	1	2	8	9
Jogurtas Trinti	2	2,5	23	29	7	11	3	4,5	10	16	7	11	1	2	7	11
Sviestas Trinti	2	2,7	25	28	8	12	4	6	10	15,5	8	10	1	3	6	10
UHT pienas Trinti	2	2,7	24	29	7	12	4	5	10	14	6	12	1	1,8	9	12
Varškė Trinti	2	2,8	23	27	87	13	4	7	10	16	9	14	1	2	7	9

	SFA		C16:0		C18:0		UFA		MUFA		PUFA		C18:
	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo	iki	nuo
	25.0	25.0	12.0	12.0	5.0	5.0	15.0	15.0	11.0	11.0	2.0	2.0	10.0

Fig. 3.3.6.4. *Raw procured milk screening by a set fatty acid parameters for particular dairy products.*

CONCLUSIONS

1. The 2016–2017 year retrospective analysis of procured Lithuanian cow's raw milk samples showed that distribution of SFA, UFA, MUFA, PUFA, C16:0, C18:0, and C18:1c9 corresponds to the regularities of fatty acids described in the literature. Statistically significant differences in fatty acids content were detected not only in the milk samples collected during the grazing and barn periods but also in the different months of these periods.
2. The seasonal analysis of the full fatty acid profile of bulk tank raw milk collected during 2018-2019 revealed that summer raw milk fat contained a higher C16:1, C18:0, C18:1n9c, C18:1n9t, UFA, and MUFA counts, and a lower C11:0, C13:0, C16:0, 18:2n6c, C20:0, and SFA counts than winter milk fat. Significant seasonal changes of individual fatty acids also affected AI, TI, h/H, DFA, and LA/ALA ratio; more favorable values for human health have been found in summer milk fat.
3. The season was the major factor affecting the fatty acid profile of natural dairy products (except for UHT milk). Seasonal variations in the fatty acid profile detected in raw and standardized milk were subsequently tracked down in the dairy end products.

   The processing, as well as the interaction of season and processing, had minor or no significant impact on fatty acid profiles of dairy products. All fatty acids identified in the raw materials (except for the summer curd cheese) were detected in the final dairy products without significant changes in their concentration.

   The significant storage impact on fatty acid composition was observed in summer sour cream, curd cheese, and butter. The pattern of fatty acid dynamics during storage was similar in all affected summer products: some of SFA or MCFA increased, and some of UFA or LCFA – decreased.
4. As an outcome of the study on fatty acid dynamics, a prototype computer program was created for the screening of fatty acids in raw procured milk and was launched on the Lithuanian central milk laboratory (LCMTL) database server.

   4.1. After identifying significant seasonal changes in procured raw milk fatty acids profile, which is reflected in the end product, the program was set to screen the procured raw milk according to the normative fatty acid composition of the chosen dairy product.

   4.2. No significant impact of the technological process on individual fatty acids and major fatty acids groups that are tested routinely at LCMTL in procured milk has been identified in our study; thus, no adjusting algorithms were used in the program code at the testing stage.

RECOMMENDATIONS

Whereas no loss of fatty acids has been identified in this study due to milk processing, to ensure the appropriate composition of certain fatty acids in the end product the selection of raw milk for the production of dairy products intended for the export should be thoroughly performed.

We recommend dairy processors to test the new application for fatty acids screening in the procured raw milk. It was designed for screening fatty acids in raw milk by applying various filters such as period, region, supplier, etc. and choose the milk according to the selected fatty acid composition parameters to produce the dairy product of required (standard) quality.

The use of this program will enable Lithuanian milk processors to increase compliance of exported products to the quality standards applied in the target destinations and, as a result, to become more competitive in the international markets.

SANTRAUKA

1. Problemos aktualumas ir svarba

Pieno riebalus sudaro apie 70 proc. sočiųjų, 25 proc. mononesočiųjų ir 5 proc. polinesočiųjų riebalų rūgščių [1]. Jų sudėtį ir santykį galima koreguoti naudojant specialius pašarų priedus melžiamoms karvėms šerti. Taip optimizuojant žaliavinio pieno riebalų sudėtį, gali būti keičiamos pieno produktų savybės [2]. Šių procesų padariniai yra dvejopi: 1) toks pienas leidžia pagaminti pieno produktus, kurie patenkina vartotojų lūkesčius (pavyzdžiui, didesnis sviesto tepumas) [3]; 2) populiarėjant karvių pašarų priedams, pieno produktų riebalų rūgščių sudėtis gali neatitikti normų ir rekomendacijų, aprašytų daugelyje literatūros šaltinių ir teisės aktų [4–6].

Be to, produktų falsifikacija yra rimta problema. Falsifikuojama daugelis maisto produktų [7], tačiau pienas ir pieno produktai yra viena dažniausiai falsifikuojamų maisto produktų kategorijų. Pieno riebalams falsifikuoti dažniausiai vartojami augaliniai aliejai (sojų, saulėgrąžų, žemės riešutų, kokosų, palmių, žemės riešutų) bei gyvuliniai riebalai (karvės lajus ir kiaulienos taukai) [8]. Akivaizdu, kad pieno riebalų klastojimas gali pakeisti riebalų rūgščių sudėtį pieno produktuose bei nulemti normų/rekomendacijų neatitikimą. Kai kurios šalys dėl prastos pieno produktų kokybės, vartotojus klaidinančios informacijos ir galimo pieno produktų klastojimo nustatė riebalų rūgščių kiekio norminius reikalavimus ir šalies viduje gaminamiems, ir importuojamiems pieno produktams [9].

Pieno produktų asortimentas Lietuvoje nuolat plečiamas, vidaus rinka yra užpildyta. Gamintojai nuolat ieško naujų užsienio rinkų savo produkcijai realizuoti. Viena iš priežasčių, kodėl lietuviška produkcija nepatenka į kai kurių šalių rinkas arba dėl ko pieno produktų siuntos grąžinamos gamintojui, yra ta, kad pieno produktuose esančių riebalų rūgščių kiekis neatitinka užsienio šalių atnaujintų maisto produktus reglamentuojančių teisės aktų reikalavimų.

Lietuviški pieno produktai gali neatitikti užsienio šalių standartų dėl daugelio išorinių bei vidinių/biologinių veiksnių, lemiančių pieno sudėtį. Tai gali būti klimatinės-geografinės sąlygos, sezonas, karvių raciono sudėtis, karvių veislė, jų sveikatos būklė, laktacijos periodas, pieno ūkio valdymas, melžimas, ir kt. [5, 10–14, 181]. Įvairiose šalyse visi šie veiksniai gali skirtis, todėl analogiškas pieno produktas, pagamintas skirtingose šalyse, gali būti nevienodos sudėties. Analizuojant galimas skirtumų priežastis, labai svarbu įvertinti gamybos proceso poveikį. Kol kas neaišku, ar perdirbant pieną visos žaliavinio pieno riebalų rūgštys patenka į galutinį produktą, ar/kaip keičiasi

jų sudėtis, ar tam tikrų riebalų rūgščių netenkama vykstant technologiniam procesui.

2. Mokslinis naujumas

Komercinėmis sąlygomis atliekama labai mažai tyrimų dėl technologinio proceso poveikio pieno produktų riebalų rūgščių sudėčiai nustatyti. Dažniausiai tyrėjai analizuoja laboratorijose pagamintus pieno produktus [15–17], arba riebalų rūgščių sudėties pokyčiai vertinami tradiciniuose tik tam tikrose šalyse vartojamuose pieno produktuose [18–21].

Iki šiol Lietuvoje nebuvo atlikta jokių tyrimų siekiant nustatyti riebalų rūgščių profilį pieno technologinėje grandinėje, apimančioje daugelį technologinio proceso etapų; be to, nebuvo analizuotos žaliavos, galutinio bei šalutinio produkto riebalų rūgščių kompozicijos ir santykiai atsižvelgiant į gamybines sąlygas. Dėl to šis tyrimas yra itin aktualus, naujas ir savalaikis.

Atsižvelgiant į atliktų tyrimų rezultatus, buvo sukurtas naujos kompiuterinės programos prototipas, pritaikytas specifiniams pieno perdirbėjų poreikiams. Ši programa leidžia filtruoti Lietuvos centrinėje pieno tyrimų laboratorijoje UAB „Pieno tyrimai“ ištirto žaliavinio pieno sudėties ir kokybės duomenis pagal tam tikrą laikotarpį, regioną ar pasirinktus žalio pieno gamintojus. Tokiu būdu galima prognozuoti pageidaujamos kokybės pieno produktus bei jų gamybai atrinkti atitinkamos riebalų rūgščių sudėties žaliavinį pieną, gaminamą bei parduodamą Lietuvoje. Šis įrankis gali padėti pieno perdirbėjams gaminti standartinės sudėties produkciją eksportui bei tapti konkurencingesniems ir vidaus, ir užsienio rinkose.

3. Darbo tikslas ir uždaviniai

Šio darbo tikslas – įvertinti sezono, perdirbimo ir laikymo įtaką pieno riebalų rūgščių sudėčiai superkamo žalio pieno riebalų rūgščių atrankos kompiuterinės programos prototipui sukurti.

Darbo uždaviniai:

1. Atlikti retrospektyvią pagrindinių riebalų rūgščių rutiniškai tiriamų Lietuvos akredituotoje centrinėje pieno tyrimų laboratorijoje kiekio analizę 2016–2017 m. supirktame Lietuvos karvių piene.
2. Įvertinti ir išanalizuoti sezono poveikį visai pieno riebalų rūgščių sudėčiai bei pieno riebalų kokybės rodikliams žalio pieno mėginiuose, surinktuose iš perdirbimo įmonių talpų 2018–2019 m.
3. Įvertinti ir išanalizuoti riebalų rūgščių sudėties sezoninius pokyčius, technologinių procesų ir laikymo įtaką riebalų rūgščių sudėčiai natūraliuose

pieno produktuose – UAT piene, koncentruotame jogurte, grietinėje, varškėje ir svieste.

4. Atsižvelgiant į gautus tyrimo rezultatus, sukurti kompiuterinę pieno riebalų rūgščių vertinimo ir atrinkimo programą tiksliniam žaliavinio pieno pagal pasirinktus standartinius gaminamo pieno produkto riebalų rūgščių sudėties reikalavimus.

4. Mėginių atrinkimas ir tyrimo metodika

Tyrimas atliktas 2015–2019 m. Lietuvos sveikatos mokslų universiteto Veterinarijos akademijos Veterinarijos fakulteto Maisto saugos ir kokybės katedroje (MSKK) bendradarbiaujant su akredituota Lietuvos centrine pieno tyrimų laboratorija UAB „Pieno tyrimai“ (LCPTL) ir viena didžiausių pieno perdirbimo įmonių Lietuvoje.

Perdirbimui skirto lietuviško žalio pieno pagrindinių rutiniškai tiriamų LCPTL riebalų rūgščių sudėties retrospektyvi analizė buvo atlikta naudojant dvejų 2016–2017 m. LCPTL duomenis. Viso išanalizuota 264 598 pieno mėginiai surinkti ganykliniu laikotarpiu (gegužės–spalio mėn.) ir 205 214 pieno mėginių surinktų tvartiniu laikotarpiu (lapkričio–balandžio mėn.). Juose įvertinti sočiųjų riebalų rūgščių (SRR), nesočiųjų riebalų rūgščių (NRR), mononesočiųjų riebalų rūgščių (MNRR), polinesočiųjų riebalų rūgščių (PNRR) bei C16:0, C18:0 ir C18:1n9c kiekiai.

Technologinio proceso įtakos pilnai riebalų rūgščių sudėčiai perdirbant žaliavinį pieną įvertinimui buvo atrinktas UAT (ultra aukšta temperatūra apdorotas) pienas (2.5 proc. rieb.), koncentruotas jogurtas (graikiško tipo, 3.9 proc. rieb.), grietinė (25 proc. rieb.), pusriebė varškė (9 proc. rieb.) ir sviestas (82 proc. rieb.). Visi pieno produktai buvo gaminami Lietuvos vienoje didžiausių pieno perdirbimo įmonių ir surinkti įvairiuose įmonės padaliniuose Kauno, Panevėžio ir Mažeikių rajonuose. Mėginiai buvo renkami 2018–2019 m. vasarą (birželio–rugpjūčio mėn.) ir žiemą (sausio–kovo mėn.). Mėginiai iš technologinių etapų buvo renkami ir tiriami po šešis kartus kiekviename sezone. Viso ištirta 288 mėginiai. Papildomai surinkti žalio pieno mėginiai (n = 60) iš pieno perdirbimo įmonės talpų.

Riebalai iš varškės ir jogurto mėginių buvo išskirti 10 g mėginio sumaišius su 15 ml n-heksano panaudojant homogenizatorių (IKA T25 digital ULTRA TURAX) 3 min. Mišinys po mechaninio sumaišymo 20 min. buvo centrifuguojamas (Heraeus Multifuge X1R Centrifuge, Thermo Scientific) esant 5 000 aps./min. Viršutinis sluoksnis su jame ištirpusiais riebalais buvo surinktas, o mėginio nuosėdos ekstrahuotos pakartotinai. Dvi sujungtos ekstrahento frakcijos buvo išgarintos vakuume naudojant rotacinį garintuvą (IKA, RV 10 basic) [150].

Riebalų ekstrakcija iš skystų mėginių buvo atlikta dvifaze centrifugacija. Atsižvelgiant į riebalų kiekį mėginyje, 20 ml grietinėlės/grietinės, 40 ml žalio pieno, 80 ml standartizuoto/UHT pieno ir 320 ml išrūgų/pasukų pieno mėginiai buvo supilti į 50 ml kūginius mėgintuvėlius ir centrifuguoti 30 min. esant 12 000 aps/min/4 °C (Heraeus Multifuge X1R Centrifuge, Thermo Scientific). Nusistojęs riebalų sluoksnis kūginio mėgintuvėlio viršuje buvo surinktas ir perkeltas į 1,5 ml mėgintuvėlius (Eppendorf) tolimesniam riebalų atskyrimui naudojant mikrocentrifugą (Eppendorf Centrifuge 5418) esant 13 000 aps/min, 20 °C, 20 min [151]. Sviesto mėginys buvo išlydomas šilto vandens vonelėje, išmaišomas ir atsveriama 60 mg.

Sukoncentruoti riebalai prieš chromatografinę analizę buvo metilinami: 60 mg riebalų sumaišoma su 2 ml heksano ir 200 µl KOH metanolyje (2 mol/l). Po 1 min intensyvaus mechaninio maišymo (Vortex) ir 10 min stovėjimo viršutiniame sluoksnyje susikaupusi riebalų rūgščių metilo esterių heksane frakcija buvo filtruojama į tamsaus stiklo chromatografinius indelius [152].

Riebalų rūgščių metilo esteriai buvo nustatyti dujų chromatografu (Clarus 680, Perkin Elmer), sujungtu su masių spektrometro (MS) detektoriumi ir kapiliarine kolonėle SP-2560, 100 m × 0,25 mm id × 0,20 µm (Supelco). Riebalų rūgščių metilo esterių identifikacija atlikta pagal riebalų rūgščių standartą Supelco® 37 Component FAME Mix.

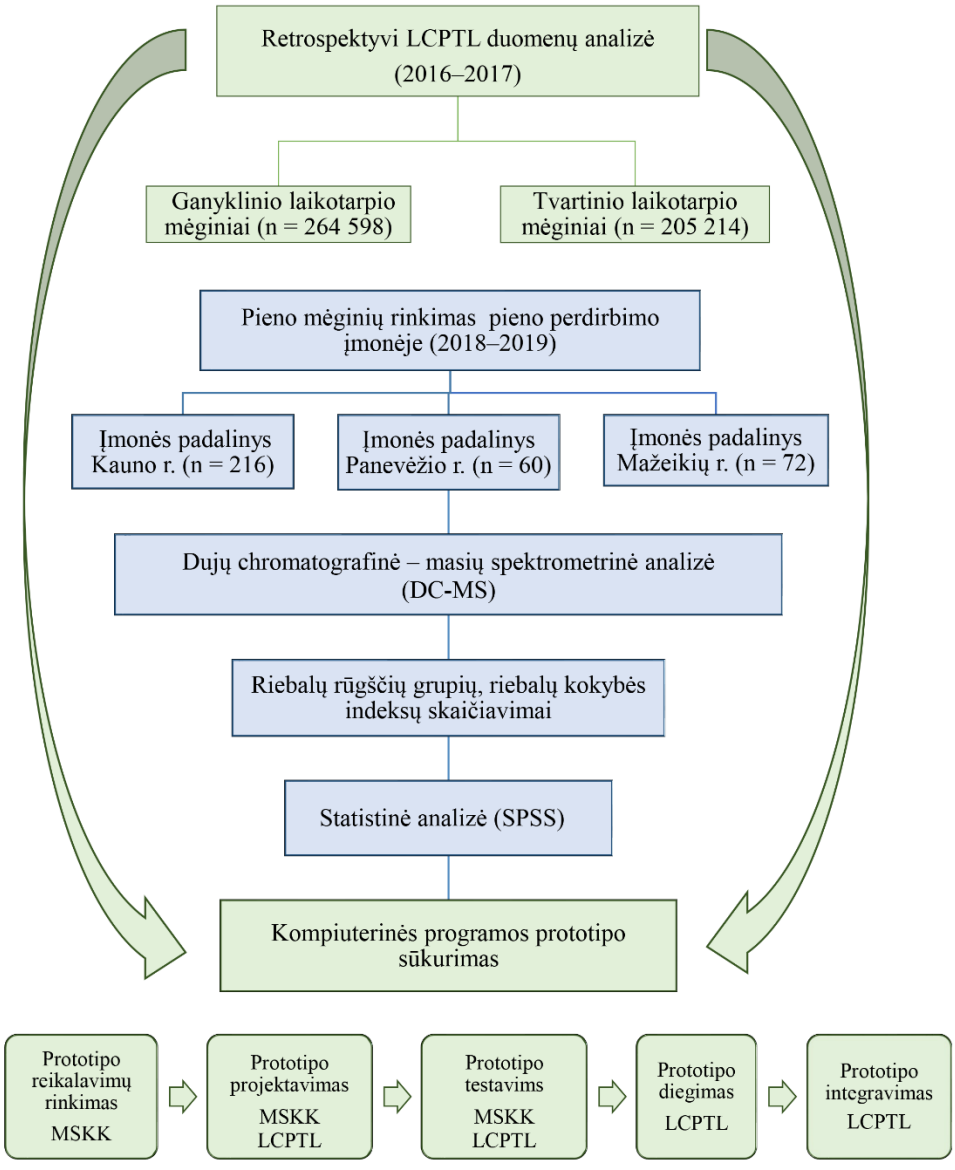
Kiekviena riebalų rūgštis ar jų grupė išreiškiama procentais nuo bendro riebalų rūgščių kiekio pieno riebaluose.

Atsižvelgiant į anglies atomų grandinės ilgį bei dvigubų ryšių skaičių, riebalų rūgštys buvo suskirstytos į trumpos grandinės (TGRR, C4-C6), vidutinės grandinės (VGRR, C8-C15), ilgosios grandinės riebalų rūgštis (IGRR, C16 ir daugiau) [116] bei SRR, NRR, MNRR ir PNRR. Buvo apskaičiuotas PNRR/SRR santykis bei pageidaujamų hipocholesteroleminių riebalų rūgščių kiekis (HRR) [79]. Be to, apskaičiuoti riebalų kokybės rodikliai: 18:2n6c/C18:3n3c (LA/ALA) santykis, bei hipocholesterolemijos ir hipercholesterolemijos (h/H) [69], aterogeniškumo (AI) ir trombogeniškumo (TI) indeksai [71].

Statistinė duomenų analizė buvo atlikta SPSS statistiniu paketu (IBM SPSS Statistics 20, SPSS Inc.). Pavienių priklausomų kintamųjų (riebalų rūgščių, jų grupių, indeksų) bei atskirų veiksnių (sezono, technologinių procesų, laikymo) poveikis buvo įvertintas aprašomosios statistikos ir ANOVA metodais. Tarpgrupinis sąveikos reikšmingumas buvo nustatytas Tukey HSD testu. Skirtumas buvo laikomas statistiškai reikšmingu, jei $p < 0,05$. Sezono, technologinio proceso ir laikymo veiksnių įtaka bei jų tarpusavio sąveika įvertinta ir daugiafaktorinės dispersijos metodu MANOVA. Skirtumas buvo laikomas statistiškai reikšmingu, jei $p < 0,05$.

Bendradarbiaujant su LCPTL informacinių technologijų skyriumi, buvo sukurtas pieno riebalų rūgščių vertinimo kompiuterinės programos prototipas. Ši priemonė leidžia pieno perdirbėjams pasirinkti superkamo žaliavinio pieno riebalų rūgščių duomenis pagal tam tikrą laikotarpį, regioną ar pasirinktus žalio pieno gamintojus ir pagal gatavos produkcijos standartų reikalavimus.

Atliktų tyrimų eigos schema pateikta 4.1 pav.



4.1 pav. *Tyrimų eigos diagrama.*

5. Rezultatai

Retrospektyvi žalio pieno mėginių, 2016–2017 m. ištirtų LCPTL, analizė parodė pagrindinių pieno riebalų rūgščių bei jų grupių reikšmingus sezoninius skirtumus. Per ganiavos laikotarpį surinktuose pieno mėginių riebaluose reikšmingai daugiau ($p < 0,05$) nustatyta NRR (33,06 ± 5,52), MNRR (28,09 ± 4,50), PNRR (4,49 ± 1,31), C18:0 (11,23 ± 2,51) ir C18:1n9c (20,35±4,05). Per tvartinį laikotarpį surinktuose pieno mėginių riebaluose reikšmingai daugiau aptikta SRR (68,97±4,95) ir C16:0 (31,04 ± 3,27). Nors pieno riebalų sudėtis pasižymi statistiškai reikšmingu sezoniškumu per ganiavos ir tvartinį laikotarpius, tačiau mėnesio įtaka riebalų rūgštims taip pat buvo reikšminga. Pavyzdžiui, SRR ir NRR kiekiai per lapkričio, gruodžio, sausio, vasario bei kovo mėnesius skyrėsi statistiškai patikimai ($p < 0,05$). Panašios tendencijos nustatytos MNRR, PNRR, C16:0, C18:0 bei C18:1n9c ir per tvartinio, ir per ganiavos laikotarpio mėnesius. Tokie rezultatai rodo, kad tvartinis ir ganiavos laikotarpiai nėra vienintelis veiksnys, turintis įtakos riebalų rūgščių profiliui piene. Norint nustatyti kitus įtaką darančius veiksnius, LCPTL duomenų išteklius reikėtų papildyti šia informacija: karvės sveikatos rodikliai, apvaisinimo ir veršiavimosi datos, laktacijos stadija, šėrimas, laikymas, melžimo sistemos, bandos dydis ir kt.

2018–2019 m. surinktuose pieno perdirbimo įmonėje žalio pieno mėginiuose sezoninis poveikis įvertintas pilnam riebalų rūgščių profiliui bei riebalų kokybę nusakantiems rodikliams. Nustatyta, kad vasaros pieno mėginiuose buvo daugiau ($p < 0,05$) C16:1, C18:0, C18:1n9c, C18:1n9t, NRR ir MNRR lyginant su žiemos mėginiais. Kadangi PNRR kiekis išliko nepakitęs abiejų sezonų mėginiuose, nepakito ir PNRR/SRR santykis. Nustatyta, kad vasaros mėginiuose LA/ALA santykis buvo 2,25±0,41, HRR – 37,7 ± 2,87, h/H – 0,49 ± 0,06, AI – 3,39 ± 0,43, TI – 3,15 ± 0,34, o žiemą surinktuose mėginiuose atitinkamai – 3,02 ± 0,86, 33,67 ± 3,17, 0,42 ± 0,07, 3,94 ± 0,51 ir 3,66 ± 0,42. Šie sezoniniai skirtumai buvo statistiškai reikšmingi ir įrodo, kad vasaros pienas turi daugiau hipocholesteroleminių riebalų rūgščių, todėl yra palankesnis žmogus sveikatai.

Analizuojat sezono, technologinio proceso ir laikymo įtaką perdirbamam/perdirbtam pienui, veiksnių tarpusavio sąveika iš esmės buvo statistiškai nereikšminga. Vertinant pavienių veiksnių įtaką riebalų rūgščių sudėčiai, paaiškėjo, kad sezonas bei produktų laikymas turėjo didesnį poveikį pieno produktų riebalų rūgščių profiliui nei technologiniai procesai. Sezoniniai riebalų rūgščių ir riebalų kokybės rodiklių skirtumai tarp įvairių technologinio proceso etapų – pradedant normalizuoto pieno ar grietinėlės etapu ir baigiant galutiniu produktu: jogurtu, grietine, varške bei sviestu (išskyrus UAT pieną) – daugeliu atveju buvo reikšmingi.

UAT pieno gamybos metu buvo įvertinta pasterizacijos, ultraaukštos temperatūros bei homogenizacijos įtaka pieno riebalų rūgštims. Nors ir buvo nežymių svyravimų tarp TGRR, VGRR ir IGRR gamybos procese, tačiau jie nebuvo statistiškai reikšmingi. Riebalų kokybės rodikliai taip pat išliko nepakitę technologinio proceso metu.

Analizuojant koncentruoto jogurto gamybą, buvo įvertinta pieno mišinio pasterizacija, fermentacija panaudojant termofilinį raugą (*S. thermophilus, L. delbrueckii* subsp. *Bulgaricus)* ir jogurto ultrafiltracija. Nustatyta, kad pieno mišinio terminis apdorojimas, fermentacija bei koncentravimas reikšmingos įtakos riebalų rūgščių profiliui neturėjo. Didesnis ($p < 0,05$) C15:0, C18:2n6c, C21:0 ir PNRR kiekis bei PNRR/SRR santykis buvo nustatytas užraugtame vasaros pieno mišinyje lyginant su šviežiai pagamintu jogurtu ir jogurtu laikymo pabaigoje. Tačiau reikšmingų skirtumų tarp pradinės žaliavos ir šviežiai pagaminto jogurto riebalų rūgščių profilio nenustatyta.

Panašūs duomenys gauti analizuojant ir varškės gamybą. Nustatyta, kad nei pieno mišinio pasterizacija, nei rauginimas mezofiliniu raugu (*L. lactis* subsp. *cremoris, L. lactis* subsp. *lactis, L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc spp*.), nei sinerezė reikšmingos įtakos riebalų rūgščių profiliui neturėjo. Nors su išrūgomis vidutiniškai pasišalino $0,60 \pm 0,34$ proc. riebalų, tačiau daugumos riebalų rūgščių kiekiai išliko panašūs ir išrūgose, ir varškėje. Išimtis nustatyta tik kelioms riebalų rūgštims vasaros varškės gamybos metu. Dėl itin mažos C11:0 ir C13:0 koncentracijos vasaros žaliavoje šių riebalų rūgščių pieno mišinyje, o vėliau ir išrūgose nenustatyta. Tačiau šviežiai pagamintoje vasaros varškėje jos buvo aptiktos.

Grietinėlės pasterizacija bei rauginimas mezofiliniu raugu *(L. lactis* subsp. *cremoris, L. lactis* subsp. *lactis, L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc spp.*) gaminant grietinę, reikšmingų riebalų rūgščių pokyčių nenulėmė. Manome, kad šiame tyrime pieno mišinių ar grietinėlės rauginimui naudotos raugų bakterijos lipolitinėmis savybėmis nepasižymėjo.

Gaminant sviestą, buvo įvertinta pasterizacijos bei grietinėlės mušimo įtaka riebalų rūgščių sudėčiai. Reikšmingai didesnis ($p < 0,05$) TGRR kiekis nustatytas žiemos metu standartizuotoje ir pasterizuotoje grietinėlėje lyginant su sviestu bei pasukomis. Tačiau pavienių TGRR (C4:0 ir C6:0) kiekiai reikšmingai nesiskyrė per visą sviesto gamybos procesą.

Kadangi technologiniai procesai riebalų rūgščių sudėtį bei koncentracijas paveikė minimaliai arba neturėjo jokios įtakos, todėl riebalų kokybės indeksai perdirbamame piene ar grietinėlėje išliko tapatūs per visą technologinį procesą.

Laikymas gamintojo nurodytomis sąlygomis lėmė riebalų rūgščių kiekio pokyčius vasaros periodu pagamintuose produktuose. Svieste nustatytas C15:0 ir VGRR padidėjimas ($p < 0,05$) bei IGRR sumažėjimas ($p < 0,05$),

grietinėje – C15:0 padidėjimas ($p < 0,05$), o varškėje – C16:1 sumažėjimas ($p < 0,05$). Nustatytas panašus riebalų rūgščių dinamikos modelis riebaluose visų produktų laikymo metu: SRR ar VGRR didėjimas ir NRR ar IGRR mažėjimas.

Atsižvelgiant į atlikto tyrimo rezultatus ir bendradarbiaujant su Lietuvos centrinės pieno tyrimų laboratorijos (LCPTL) informacinių technologijų skyriumi, buvo sukurtas pieno riebalų rūgščių vertinimo ir atrankos kompiuterinės programos prototipas. Ši programa leidžia pieno perdirbėjams analizuoti superkamo žaliavinio pieno sudėties bei kokybės duomenis pagal tam tikrą laikotarpį, regioną ar pasirinktus žalio pieno gamintojus ir atrinkti pageidaujamos riebalų rūgščių sudėties žaliavinį pieną visoje Lietuvoje.

6. Išvados

1. 2016–2017 m. Lietuvoje supirkto žaliavinio pieno duomenų retrospektyvinė analizė, parodė kad C16:0, C18:0, C18:1n9c, SRR, NRR, MNRR ir PNRR atitinka literatūroje aprašytus riebalų rūgščių pieno riebaluose dėsningumus. Statistiškai reikšmingi riebalų rūgščių skirtumai buvo nustatyti ne tik per ganiavos ir tvartinį laikotarpius surinktuose pieno mėginiuose, bet ir per skirtingus tų laikotarpių mėnesius.
2. 2018–2019 m. pieno perdirbimo įmonėse surinktų žalio pieno mėginių riebalų analizė patvirtino sezono įtaką riebalų rūgščių sudėčiai. Vasaros pieno riebaluose nustatytas reikšmingai didesnis C16:1, C18:0, C18:1n9c, C18:1n9t, NRR bei MNRR kiekis bei mažesnis C11:0, C13:0, C16:0, 18:2n6c, C20:0 ir SRR kiekis nei žiemos pieno riebaluose. PNRR bei PNRR/SRR rodikliams sezono įtaka nenustatyta. Statistiškai reikšmingi sezoniniai tam tikrų riebalų rūgščių pokyčiai paveikė ir riebalų kokybės indeksus. AI, TI, h/H, HRR ir LA/ALA palankesnės žmonių sveikatai reikšmės nustatytos vasaros pieno riebaluose.
3. Sezonas buvo pagrindinis veiksnys, turintis įtakos natūralių pieno produktų (išskyrus UAT pieną) riebalų rūgščių sudėčiai. Žaliaviniame ir standartizuotame piene nustatyti statistiškai reikšmingi sezoniniai riebalų rūgščių pokyčiai buvo nustatyti ir iš jo pagamintuose pieno produktuose.
   Technologinės operacijos, bei sezono ir technologinių operacijų sąveika daugumos tirtų produktų riebalų rūgštims reikšmingos įtakos neturėjo. Visos žaliavoje (išskyrus vasaros varškės žaliavą) nustatytos riebalų rūgštys po technologinio perdirbimo be reikšmingų koncentracijos pasikeitimų buvo aptiktos ir galutiniuose pieno produktuose.
   Reikšminga laikymo įtaka nustatyta vasaros periodu pagamintos grietinės, varškės bei sviesto riebalų rūgščių sudėčiai. Riebalų rūgščių dinamika

šiuose produktuose laikymo metu buvo panaši: kai kurių SRR ir VGRR kiekiai didėjo, o kai kurių NRR ir IGRR – mažėjo.

4. Atsižvelgiant į riebalų rūgščių dinamikos tyrimų rezultatus, buvo sukurtas pieno riebalų rūgščių vertinimo žaliaviniame piene kompiuterinės programos prototipas. Programa instaliuota ir testuojama UAB „Pieno tyrimai“ laboratorijos serveryje.

   4.1. Nustačius reikšmingus sezoninius pokyčių žaliaviniame piene, atsispindinčius ir gatavoje produkcijoje, programoje buvo numatyta žaliavinio pieno atranka pagal normatyvinę gaminamo pieno produkto riebalų rūgščių sudėtį.

   4.2. Tyrimo metu reikšminga technologinio proceso įtaka pavienėms riebalų rūgštims ir pagrindinėms riebalų rūgščių grupėms rutiniškai tiriamoms UAB „Pieno tyrimai“ laboratorijoje žaliaviniame piene nebuvo nustatyta, todėl programos testavimo etape jokie koreguojančių koeficientų naudojimas nenumatytas.

7. Rekomendacijos

Atlikus tyrimą, nenustatyta reikšmingų riebalų rūgščių pokyčių technologinio proceso metu. Riebalų rūgščių sudėtis galutiniame pieno produkte tiesiogiai priklausė nuo žaliavoje esančių riebalų rūgščių.

Siekiant pagaminti pageidaujamos (standartinės) riebalų rūgščių sudėties pieno produktus, žaliavinio pieno atranka pagal riebalų rūgštis tampa esminiu veiksniu ir turi būti atliekama itin kruopščiai.

Šiuo tikslu pieno perdirbėjams rekomenduojame naudotis testuojama pieno riebalų rūgščių vertinimo ir atrankos žaliaviniame piene kompiuterine programa, kurioje yra UAB „Pieno tyrimai“ laboratorijos duomenų ištekliai. Šis programinis įrankis leidžia analizuoti parduodamo Lietuvoje žaliavinio pieno riebalų rūgščių sudėties parametrus UAB „Pieno tyrimai“ laboratorijos duomenų masyve filtruojant juos pagal laikotarpius, regionus ar tiekėjus ir sudaro galimybę pieno perdirbėjams surasti ir atsirinkti pageidaujamos sudėties žaliavą norimos sudėties eksportinei produkcijai gaminti. Manome, kad šio programinio įrankio naudojimas leis Lietuvos pieno perdirbėjams gaminti eksportinę produkciją, atitinkančią priimančios šalies kokybės standartų reikalavimus ir tapti konkurencingais tarptautinėse rinkose.

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LIST OF PUBLICATION

Publications on the Ph.D. thesis:

1. **Laučienė, Lina**; Andrulevičiūtė, Vaida; Sinkevičienė, Ingrida; Kašauskas, Artūras; Urbšienė, Laima; Šernienė, Loreta. Impact of technology and storage on fatty acids profile in dairy products : original scientific paper // Mljekarstvo : journal for dairy production and processing improvement. Zagreb : Croatian Dairy Union. 2019, vol. 69, no. 4, p. 229-238. [IF: 0,806, Q3 (2018, InCites JCR SCIE)]
2. **Laučienė, Lina**; Andrulevičiūtė, Vaida; Sinkevičienė, Ingrida; Sederevičius, Antanas; Musayeva, Kristina; Šernienė, Loreta. Analysis of fatty acid composition and healthy lipids indices in raw and processed milk // Journal of food and nutrition research. Newark, De: Science and Education Publishing. 2019, vol. 7, no. 5, p. 386-390.

Abstracts on the Ph.D. thesis:

1. **Laučienė, Lina**; Andrulevičiūtė, Vaida; Sinkevičienė, Ingrida; Malakauskas, Mindaugas; Šernienė, Loreta. Changes in chromatographically assessed fatty acid profile during technology of dairy products // World Academy of Science, Engineering and Technology. International Science Index, Agricultural and Biosystems Engineering: ICAFB 2019: International Conference on Agriculture, Food and Biotechnology: Rome, Italy, January 17-18, 2019: conference proceedings. [S. l.]: World Academy of Science, Engineering and Technology. 2019, vol. 13, no. 1, p. 142-142 [1873-1878]. [0,200]
2. **Lina Laučienė**, Rūta Danilevičienė, Loreta Šernienė. Influence of technological process on fatty acids in commercial strained yoghurt. Accepted for //FoodBalt2020:14th Baltic Conference on Food Science: abstract book: August 24-26, 2020, Tallinn, Estonia//Center of Food and Fermentation Technologies. The conference was delayed due to the COVID-19 pandemic.
3. **Lina Laučienė**, Rūta Danilevičienė, Loreta Šernienė. Fat composition of probiotic yogurt during production and storage in different seasons. Accepted for //FoodBalt2020:14th Baltic Conference on Food Science: abstract book: August 24-26, 2020, Tallinn, Estonia//Center of Food and Fermentation Technologies. The conference was delayed due to the COVID-19 pandemic.

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Impact of technology and storage on fatty acids profile in dairy products

Lina Laučienė[1], Vaida Andrulevičiūtė[2], Ingrida Sinkevičienė[2], Artūras Kašauskas[2], Laima Urbšienė[3], Loreta Šernienė[1]*

[1]Lithuanian University of Health Science, Department of Food Safety and Quality, Tilžės st. 18, Kaunas, Lithuania
[2]Lithuanian University of Health Science, Department of Biochemistry, Tilžės st. 18, Kaunas, Lithuania
[3]Accredited central milk testing laboratory, Radvilų Dvaro st. 31, Kaunas, Lithuania
*Corresponding author: lina.lauciene@lsmuni.lt

Abstract

In the present study fatty acid (FA) composition in four main groups of dairy products was determined to investigate their development during processing and storage. Fresh cheese, sour cream, butter, and ultra-high temperature (UHT) milk representing differences in technological approach were chosen for the study. Fatty acids methyl esters (FAME) were quantified using a gas chromatograph (GC) equipped with a mass spectrometer (MS) and a capillary column SP-2560. The concentrations and profile of FA in final products were primarily dependent on the FA content of raw milk for UHT milk and fresh cheese production or in the raw cream for sour cream and butter. The shelf life had a significant impact ($P<0.05$) only in UHT milk and butter, whereby unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA) decreased significantly in UHT milk, while PUFA decreased signifcantly in butter.

Key words: **milk, fatty acids, dairy products, processing, storage**

Introduction

The range of dairy products is constantly expanding and the internal markets are overloaded, so manufacturers are looking for new markets for their products. As a result, the problem of product compliance with the increasing quality requirements for imported products is highlighted. This is especially true for the composition/ratio of milk fat in dairy products. Some non-EU countries, which are an important export market for EU dairy producers, formally regulate the limits of individual FA's in various imported dairy produce with non-compliance resulting in returned shipments. Non-compliance of dairy products in the FA profile with the standards of the export country may be influenced by quite a number of already known intrinsic factors such as stage of lactation, pregnancy (Samková et al., 2012), breed or genotype (Hanuš et al., 2016), or extrinsic factors like nutrition, season (Ozcan et al., 2015), dairy production system (Morales et al., 2015), feeding ration (Ferlay et al., 2006). All of previously listed factors can vary greatly among the countries, therefore, the same product produced in a different country can vary in composition. Furthermore, the impact of dairy processing technological stages such as heat treatment, homogenization, fermentation, churning, and storage on FA profile should be also considered. The research done in this area is either scarce or scattered. The influence

of high temperatures on milk has been studied, but the results vary widely among the studies. Some of them present an increase of SCFA, MCFA, and decrease of LCFA after pasteurization and boiling (Khan et al., 2017), others point at a decrease of SCFA (Pestana et al., 2015) or of all FA concentration during the UHT treatment (Ajmal et al., 2018). The results from previous studies on the milk and cream fermentation also vary (Gerchev and Mihaylova, 2012; Gassem et al., 2016; Jia et al., 2016) and were influenced by the chosen bacterial culture and the origin of the raw material.

The dynamics of dairy FA during the technological process are still unclear due to intermediate stages of each technological process being bypassed in all previous studies. No studies were done so far to estimate the FA profile in samples taken directly from the dairy production line. In none of them dairy by-products such as whey and buttermilk were analysed along with the final products (cheese and butter, respectively) as well as the end of shelf life impact of FA profile. For dairy processors exporting dairy products to countries where FA levels are regulated, to track any loss of FA or change in their quantity and ratio during the production is particularly relevant. Therefore, the aim of this study was to analyse the extent of FA transfer from milk fat to the fat of dairy product through the main processing stages and examine the effect of storage on FA content in selected dairy products – UHT milk, sour cream, fresh cheese, and butter.

Materials and methods

Samples and reagents

To analyse the impact of technological process on the FA profile several dairy products such as ultra-high temperature (UHT) milk (fat content 2.5 g 100 g^{-1}), fresh cheese (fat content 9 g 100 g^{-1}), sour cream (fat content 25 g 100 g^{-1}) and butter (fat content 82 g 100 g^{-1}), representing differences in technological approach were chosen for the study.

All dairy products for this experiment were produced and collected at one of the largest dairy processing companies in Lithuania during the summer (June - August) in 2008. Each sample was taken and analyzed in triplicate. The sampling scheme is given in Table 1.

The products were manufactured according to standard methods (Walstra, 1999). Production flow charts are presented in Figure 1.

TABLE 1. Sampling points at the main stages of the technological process and at the end of shelf life

Product	Sampling points					Conditions of storage	Evaluated effect of processing
UHT milk	raw milk	separated cream	standardized and pasteurized milk	UHT milk	end of shelf-life	180 days in ambient temperature	separation; pasteurization; UHT treatment
Sour cream	raw cream	standardized and pasteurized cream	sour cream	-	end of shelf-life	25 days in 5 °C	pasteurization; fermentation
Butter	raw cream	standardized and pasteurized cream	butter	buttermilk	end of shelf-life	90 days in 5 °C	pasteurization; churning; by-product
Fresh cheese	raw milk	standardized and pasteurized milk	fresh cheese	whey	end of shelf-life	25 days in 5 °C	pasteurization; fermentation; by-product

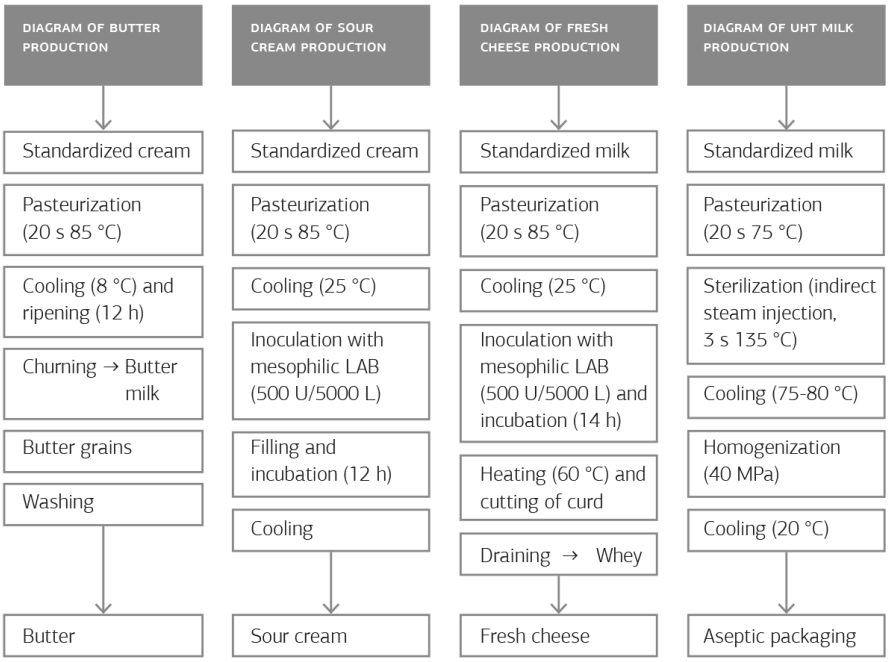

FIGURE 1. Production flow charts.

All chemical reagents and FAME standards for GC analysis were purchased from Sigma-Aldrich (Merck, KGaA, Darmstadt, Germany).

Lipid extraction

The lipid separation from liquid samples was done by double centrifugation. Depending on the fat content of the sample, 20 mL of cream/soured cream, 40 mL of raw milk, 80 mL of standardized/UHT milk, and 320 mL of whey/buttermilk sample were poured into 50 mL conical tubes and centrifuged for 30 min at 12.000 rpm at 4 °C (Thermo Scientific, Heraeus Multifuge X1R Centrifuge). The settled fat layer at the top of the tube was collected and transferred into 1.5 mL tubes (Eppendorf) for further fat separation (20 min 13.000 rpm, 20 min, 20 °C) by microcentrifuge (Eppendorf Centrifuge 5418). The concentrated fat was collected and directed for FAME preparation (Feng et al., 2004).

The lipids from curd were extracted using hexane: 10 g of sample was dispersed in 15 mL hexane using a homogenizer (IKA T25 digital ULTRA TURAX) for 3 min, shaken mechanically and then centrifuged at 5000 rpm for 20 min. The upper solvent was removed and the sediment extracted again twice. The solvents with dissolved fats were combined and evaporated with a rotary evaporator (IKA, RV 10 basic) under vacuum (GOST 32915 2014). After evaporation fat was collected and directed for FAME preparation.

Preparation of fatty acid methyl esters

The FA were converted into fatty acid methyl esters (FAME). 60 mg of concentrated fat was mixed with 4 mL of hexane and 200 µL of 2 mol L^{-1} KOH in methanol, then intensively vortexed for 1 min and after 10 min of resting, the top layer was collected and filtered into chromatography vial (Ficarra et al., 2010).

Gas chromatography (GC) analysis

FAME were quantified using a GC Clarus 680 (Perkin Elmer) equipped with a mass spectrome-

ter (MS) and a capillary column SP-2560, 100 m x 0.25 mm id x 0.20 µm. Conditions for chromatographic analysis were as following: the injector and detector temperatures were maintained at 230 °C. Injection volume was 1 µL, a split ratio of 1:19. Oven temperature was held at 100 °C for 4 min, increased to 240 °C (4 °C min^{-1}) and held for 30 min (total analysis time 70 min). Carrier gas (He) flow rate was 1 mL min^{-1}. FA peaks were identified using Supelco® 37 Component FAME Mix. Each FA were expressed in g 100 g^{-1} of total FAME content. FA was divided into four main groups depending on the number of carbon atoms: short-chain fatty acids (SCFA; C4-C6), medium-chain fatty acids (MCFA; C8-C15) and long-chain fatty acids (LCFA; C16 and more; Yilmaz-Ersan, 2013); and in four main groups depending on the presence and the number of double or triple bonds: saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Statistical analysis

Statistical analysis was performed by SPSS statistical package (Chicago, SPSS Inc., SPSS 17). The data were analyzed using Descriptive Statistics (Explore) and Analysis of Variance (ANOVA) methods. The significance of interactions among the groups assessed was determined by the Tukey HSD test. The differences were considered significant at $P<0.05$.

Results and discussion

Fresh cheese

To make fresh cheese raw milk was standardized, pasteurized, cooled and inoculated with mesophilic LAB (*Lactococcus lactis* subsp. *cremoris*, subsp. *lactis*, subsp. *lactis biovar diacetylactis* and *Leuconostoc* subsp.). Curd body was heated to 60 °C and sliced/mixed to separate the whey after 14 h fermentation. Processing of raw milk into fresh cheese and storage at 5 °C for 25 days, did not have a significant effect on the content of FA (Fig. 1).

The FA profile in whey remained similar to that of raw milk and fresh cheese. Nudda et al. (2005) did not find significant differences in FA profile between raw sheep milk and fresh cheese/ricotta fats either. The author state that concentrations of FA in fresh cheeses fat were primarily dependent on the FA content of the raw milk that is following findings of this study. High cooking temperature (up to 60 °C) traditionally applied in Lithuanian fresh cheese (quark) production does not leave many chances for mesophilic starter strains to survive. Prandini et al. (2009) stated the same findings - neither the LAB added to the milk, nor processing technology and ripening did influence the SFA, MUFA, PUFA and CLA content in dairy products during the production of Grana Padano cheese. Surprisingly, in the production of ripened cheese with lipolytic starter strains and molds technologically cultivated, lower pH, lower water activity and presence of the other FA are discussed as possible factors inhibiting the lipase activity (Bisig et al., 2007).

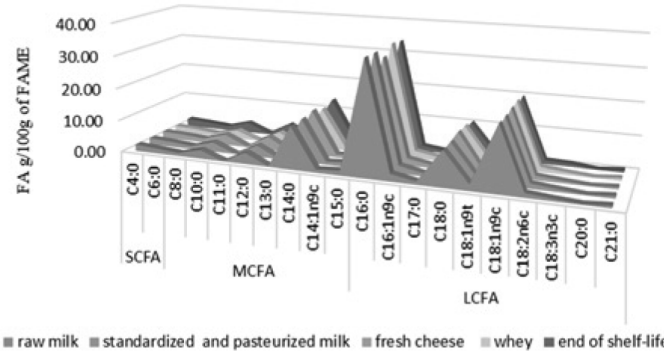

FIGURE 2. The profile and distribution of FA of different fresh cheese production stages

Sour cream

Raw cream was standardized, homogenized, pasteurized and inoculated with a mesophilic starter culture (*Lactobacillus lactis* subsp. *cremoris*, subsp. *lactis*, subsp. *lactis biovar diacetylactis* and *Leuconostoc* subsp.) during sour cream production. The fermentation lasted until the acidity of the cream reached pH 4.5-4.6. The profile of individual FA did not change during sour cream processing and storage at 5 °C for 25 days (Fig.2).

This comes in agreement with other findings - no pasteurization effect on milk fat profile (Pestana et al., 2015; Santos, 2012), no homogenization effect on cream (Pirisi et al., 2007) or milk (Michalski and Januel, 2006) FA profiles were found.

Meanwhile, data from previous studies on milk and cream fermentation are rather controversial. Usually, changes in FA content during milk fermentation and storage are related to the bacterial enzyme lipase that catalyzes the triglycerols to release free fatty acids (FFA) and glycerol (Santos, 2012). However, not all LAB strains have lipolytic activity (Dinçer and Kıvanç, 2018). Bettache and Fatma (2012) found that only two from 76 LAB isolates (from 4 genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Enterococcus*) were lipolytic: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Since FA profile of sour cream remained without significant changes during (*Lactobacillus bulgaricus*, *Lactobacillus lactis* and *Streptococcus thermophilus*) reported an increased contents of MCFA and LCFA (except C16:1, C20:0) in fermented camel milk (Gassem et al., 2016). Buffalo milk fermentation with *Lactobacillus acidophilus* and *Lactobacillus lactis* showed an increase in SCFA and MCFA (except C14:0) (Yadav et al., 2007). Sheep milk fermentation with thermophilic LAB (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) did not reveal a change in the FA profile (Gerchev and Mihaylova, 2019). Yilmaz-Ersan (2013) observed that cream fermentation with probiotic mesophilic bacteria *Bifidobacterium lactis* was associated with increases of LCFA and cream fermentation with *Lactobacillus acidophilus* showed an increase of MCFA. Apparently the storage time in combination with thermophilic LAB could increase the content of SFA in yogurt, however showing no impact on sheep yogurt SFA content (Serafeimidou et al., 2013).

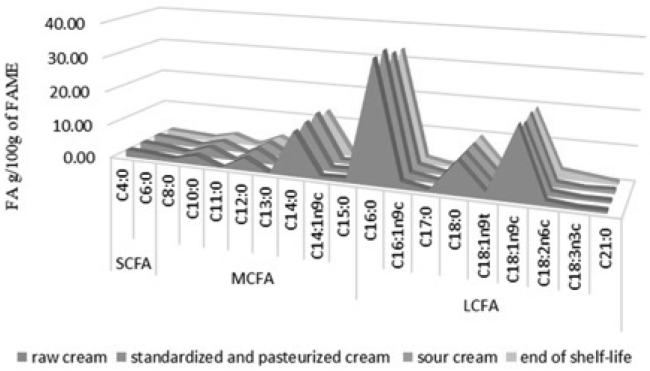

FIGURE 3. The profile and distribution of FA of different sour cream production stages

The reported results are pointing at the importance of LAB strains used for fermentation, and probiotic strains, in particular, contributing to significant changes in FA distribution.

UHT milk

The UHT milk was chosen for this study to assess the high-temperature effect on FA profile. At UHT treatment stage, the standardized milk is first preheated to a noncritical temperature (70-80 °C), and then quickly heated to the temperature required by the process. In heat treatment processes, various time/temperature combinations can be applied, depending on the product properties and

shelf-life requirements and this why time/temperature varies among factories and countries. Typically, temperature-time conditions for UHT treatment of milk are 130-150 °C for 1-3 sec (Manners and Craven, 2003). In our case, standardized and pasteurized milk was indirectly heated by steam for 3 sec at 135 °C.

The percentage of individual FA and their groups at the main technological stages of UHT milk processing are shown in Table 2.

TABLE 2. The profile and distribution of individual FA and their groups at the main technological stages during UHT milk processing.

FA (g 100g^{-1} of FAME)	Raw milk		Separated cream		Standardized and pasteurized milk		UHT milk		End of shelf life	
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
C4:0	1.52	0.15	1.89	0.15	1.80	0.19	2.41	0.37	1.95	0.19
C6:0	1.29	0.02	1.57	0.01	1.34	0.18	1.54	0.13	1.71	0.20
∑SCFA	2.81	0.13	3.47	0.16	3.14	0.37	3.95	0.51	3.66	0.39
C8:0	0.98	0.04	1.24	0.15	0.86	0.10	0.92	0.01	1.18	0.20
C10:0	2.77	0.09	2.76	0.13	2.62	0.08	2.82	0.20	3.25	0.31
C11:0	nd	-	nd	-	nd	-	nd	-	0.11	0.05
C12:0	3.06	0.06	3.76	0.31	3.31	0.03	3.65	0.44	4.03	0.47
C13:0	nd	-	nd	-	nd	-	nd	-	0.08	0.03
C14:0	10.95	0.58	12.89	0.61	12.52	0.80	12.09	0.90	13.75	0.42
C14:1n9c	1.16	0.04	1.19	0.15	1.03	0.14	1.03	0.06	1.11	0.07
C15:0	1.75	0.18	1.39	0.00	1.47	0.09	1.57	0.11	1.48	0.05
∑MCFA	20.67	0.56	23.22	1.34	21.79	1.02	22.08	1.72	24.98	1.42
C16:0	32.99	0.36	33.63	0.35	35.06	0.90	34.23	0.03	34.72	0.08
C16:1n9c	1.59	0.32	2.60	0.03	2.48	0.14	2.10	0.39	2.10	0.27
C17:0	1.06	0.01	1.05	0.19	1.12	0.04	0.85	0.01	0.99	0.07
C18:0	10.97	0.57	11.20	0.65	11.54	0.77	10.84	0.12	10.02	0.39
C18:1n9t	2.36	0.40	2.13	0.12	2.14	0.31	2.28	0.09	1.73	0.11
C18:1n9c	21.54	0.92	19.27	1.60	19.36	1.05	19.59	1.03	17.18	0.01
C18:2n6c	1.96	0.17	1.54	0.02	1.58	0.16	1.96	0.42	1.42	0.20
C18:3n3c	1.75^{a}	0.31	0.87^{a}	0.07	0.80^{a}	0.01	0.87^{a}	0.02	0.70^{b}	0.03
C21:0	2.30	0.27	1.02	0.04	0.99	0.20	1.24	0.19	2.48	1.79
∑LCFA	76.52	0.42	73.31	1.50	75.07	1.39	73.96	1.22	71.36	1.80
∑SFA	69.65^{a}	1.03	72.41^{a}	1.19	72.62^{a}	1.23	72.17^{a}	1.11	75.76^{b}	0.44
∑UFA	30.35^{a}	1.03	27.59^{a}	1.20	27.38^{a}	1.23	27.83^{a}	1.11	24.24^{b}	0.46
∑MUFA	26.64	1.30	25.19	1.29	25.00	1.07	25.00	0.68	22.12	1.23
∑PUFA	3.71^{a}	0.37	2.41^{a}	0.19	2.38^{a}	0.15	2.83^{a}	0.43	2.12^{b}	0.13

Means denoted in rows by different letters indicate statistically significant differences (P<0.05); SCFA - short chain fatty acids; MCFA - medium chain fatty acids; LCFA - long chain fatty acids; SFA - saturated fatty acids, UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Data of this study showed a slight change in the amount of certain FA but no individual FA losses were detected during the production of UHT milk. The amounts of SCFA and MCFA (except C8:0, C14:1n9c, C15:0) showed a tendency to increase, while LCFA (except C16:0, C16:1n9c, C18:2n6c) slightly decreased after the high-temperature treatment. However, the significance of these changes has not been statistically confirmed. Meanwhile, Khan et al. (2017) estimated a significant increase of SCFA and MCFA, and decrease of LCFA after pasteurization and boiling (1 min) in cow and buffalo milk. Unlike us, some authors referred to the significant decrease of SCFA, MCFA, and LCFA (Ajmal et al., 2018) due to UHT treatment but it's not clear what temperature modes were used in this study. Pestana et al. (2015) stated that raw, pasteurized (75 °C for 15 sec) and UHT (140 °C for 3 sec) milk had very similar fatty acid profiles. A significant decrease was found only for C4:0, C6:0, C8:0 and C:20 which indicated pasteurization and sterilization of milk had a little effect on FA profile (Pestana et al., 2015). Our results revealed no significant effect of temperature treatment on FA profile of UHT milk or other dairy product (fresh cheese, sour cream) analysed in this study. Concentrations of FA in UHT milk fat were primarily dependent on FA content of raw milk.

The storage period in ambient temperature for 180 days had a significant impact on FA content in UHT milk: UFA, PUFA and C18:3n3c significantly decreased in UHT milk fat while SFA showed the opposite tendency ($P<0.05$) at the end shelf-life. Ajmal et al. (2018) revealed a negative effect of 90 days storage on UHT milk FA profile: SFA and UFA decreased at the end of storage. According to the authors, heat, moisture, metal ions and bacterial lipases that survive the orthodox UHT treatment cleave the bonds between the fatty acids and glycerol, leading to the formation of free fatty acids in milk.

Butter

The European-style unsalted 82 % fat butter was chosen for our study. Processing of raw sweet (uncultured) cream into butter did not have a significant effect on the content of FA.

Despite the fat loss (0.5 %) with buttermilk during butter processing, the FA ratio in buttermilk remained similar to that of raw cream and butter. However, the storage at 5°C for 90 days significantly decreased PUFA and C18:2n6c content (Table 3).

TABLE 3. The profile and distribution of individual FA and their groups at the main technological stages during butter processing.

FA (g/100g of FAME)	raw cream		standardized and pasteurized cream		butter milk		butter		end of shelf-life	
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
C4:0	1.95	0.01	3.21	1.24	1.72	0.05	1.68	0.05	1.71	0.44
C6:0	0.96	0.63	1.31	0.26	1.30	0.12	1.39	0.07	1.94	0.70
∑SCFA	2.91	0.74	4.52	1.00	3.02	0.11	3.08	0.11	3.64	0.47
C8:0	1.21	0.11	0.83	0.27	0.80	0.21	0.86	0.12	1.54	0.38
C10:0	2.75	0.15	2.38	0.35	2.38	0.40	2.35	0.26	3.04	0.24
C11:0	0.36	0.07	0.16	0.07	0.10	0.08	0.15	0.07	0.17	0.09
C12:0	3.69	0.27	2.65	0.82	3.24	0.31	3.30	0.21	3.82	0.26
C13:0	0.65	0.36	0.10	0.05	0.15	0.07	0.12	0.05	0.11	0.04
C14:0	13.19	0.70	12.47	0.69	13.21	0.14	12.54	0.56	13.43	0.55
C14:1n9c	1.48	0.15	1.09	0.20	1.20	0.08	1.05	0.12	1.18	0.11
C15:0	1.71	0.32	1.39	0.07	1.43	0.04	1.35	0.10	1.42	0.09
∑MCFA	25.67	0.61	21.08	2.14	22.51	0.93	21.72	1.43	24.73	1.32
C16:0	32.80	0.26	34.30	0.81	35.20	0.27	36.23	0.53	33.22	1.06

FA (g/100g of FAME)	raw cream		standardized and pasteurized cream		butter milk		butter		end of shelf-life	
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
C16:1n9c	1.97	0.14	1.91	0.19	2.15	0.10	2.01	0.10	1.98	0.16
C17:0	0.46	0.39	1.02	0.11	1.01	0.09	0.88	0.02	0.93	0.05
C18:0	9.76	0.32	10.73	0.71	10.02	0.32	10.73	0.76	10.78	0.44
C18:1n9t	0.66	0.31	1.58	0.15	1.62	0.26	1.34	0.06	1.49	0.05
C18:1n9c	21.75	1.13	21.76	1.33	20.65	0.47	20.87	1.08	20.13	1.75
C18:2n6c	2.54[a]	0.28	1.90[a]	0.18	1.87[a]	0.21	1.68[a]	0.23	1.58[b]	0.09
C18:3n3c	0.73	0.18	0.59	0.13	0.71	0.13	0.66	0.03	0.75	0.05
C20:0	0.11	0.01	0.16	0.05	0.13	0.06	0.20	0.07	0.17	0.06
C21:0	0.63	0.20	0.45	0.15	1.11	0.34	0.60	0.04	0.58	0.01
∑LCFA	71.42	1.36	74.40	2.01	74.47	1.01	75.20	1.55	71.63	1.30
∑SFA	70.86	0.73	71.17	1.47	71.82	0.54	72.38	1.07	72.88	1.71
∑UFA	29.14	0.71	28.83	1.45	28.18	0.64	27.62	1.06	27.12	1.68
∑MUFA	25.87	0.65	26.34	1.46	25.61	0.68	25.28	1.11	24.78	1.81
∑PUFA	3.27[a]	0.38	2.49[a]	0.26	2.57[a]	0.20	2.35[a]	0.36	2.34[b]	0.02

Means denoted in rows by different letters indicate statistically significant differences (P<0.05); SCFA - short chain fatty acids; MCFA - medium chain fatty acids; LCFA - long chain fatty acids; SFA - saturated fatty acids, UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Findings presented by Bisig et al. (2007) are similar to the data obtained in this study: butter-making process had no significant influence on the conjugated linoleic acid (CLA) and FA content of cream processed into butter. Similarly to our findings, Silva-Kazama et al. (2010) confirmed the effect of butter storage and pointed out that the relative percentage of SFA and MCFA increased due to the oxidation (decrease) of UFA. Usually, the lipid oxidation involves UFA, especially PUFA, because the hydrogen atoms on the methylene groups in UFA are much easier to disassociate than in SFA (O'Connor and O'Brien, 2006). Lipolysis is limited since the membrane protects milk fat. Churning was the main stage in the butter-making process that destroyed fat globules membranes. Antioxidants naturally present in raw buttercream (enzymes, vitamin C and lactoferrin) are either destroyed by pasteurization or separated since antioxidative caseins are removed with buttermilk (Lindmark-Månsson and Akesson, 2000). According to this, butter seems to be the most favorable environment for lipolysis, but in our study, a significant decrease was observed only for PUFA.

In this study, the storage had an impact only on butter and UHT milk fats. The main differences between products in this study were the storage period (relatively short 25 day period for sour cream and fresh cheese and, 90 and 180 day period for butter and UHT milk, respectively) and the technological aspect (fermentation for sour cream and fresh cheese and ultra-high temperature and churning for UHT milk and butter, respectively). Lipolysis in raw milk is largely due to the indigenous enzyme lipoprotein lipase (LPL). Since all products have passed the pasteurization, the LPL, which is sensitive to higher temperatures, was inactivated. Some spores of gram-positive psychrotrophic bacteria and especially enzymes of psychrotrophic bacteria can survive raw milk pasteurization and UHT treatment and can be related to flavors defects pronounced in cream, butter, cheese and UHT milk (Samaržija et al., 2012). The optimal growth temperature for these cold-tolerant strains is 15 - 20°C, but they can also grow and multiply at low temperatures through as well (Moyer and Morita, 2007). We can speculate that FA changes in UHT milk and butter during storage were influenced by psychrotrophic bacterial lipases. Meanwhile, these microorganisms and their lipases could not function in the fermented products due to the acidic environment.

Conclusions

This study showed that various technological treatments such as pasteurization at various temperatures, fermentation, and the churning process had no significant influence on FA composition and percentage at various stages of UHT milk, sour cream, fresh cheese, and butter production. No loss of individual FA was observed in any of the final products during the technological process. The observed variability in FA content of processed dairy products has been attributed to the variability in the FA content of raw milk.

Having that in mind, the raw milk has to be carefully selected by the producer to ensure the compliance of FA content to standard requirements before and during launching them to the export markets. As an outcome of this study, a prototype software was created and installed at the Lithuanian accredited central milk-testing laboratory to equip dairy producers with the raw milk screening tool according to the standard FA composition of their choice.

The shelf-life period had an impact only on UHT milk and butter fats. Antioxidative additives and proper shelf life duration/ conditions combination might help to protect fats of these products from oxidation thus resulting in no or lesser FA profile changes.

Utjecaj tehnološkog postupka i skladištenja na profil masnih kiselina u mliječnim proizvodima

Sažetak

U ovom je radu u četiri glavne vrste mliječnih proizvoda utvrđivan sastav masnih kiselina (FA), kao i njihov udio tijekom prerade i čuvanja. Kako bi se utvrdio utjecaj tehnološkog postupka, za istraživanje su odabrani svježi sir, kiselo vrhnje, maslac i trajno mlijeka obrađeno režimom UHT toplinske obrade. Metilni esteri masnih kiselina (FAME) određeni su pomoću plinskog kromatografa (GC) opremljenog masenim detektorom (MS) i kapilarnom kolonom SP-2560. Koncentracije i profil FA u krajnjim proizvodima su prije svega ovisili o koncentraciji FA u sirovom mlijeku prije UHT obrade mlijeka ili proizvodnje svježeg sira, odnosno o udjelima FA u svježem siru i sirovom vrhnju. Rok trajanja imao je značajan utjecaj ($P<0,05$) samo u UHT obrađenom mlijeku i u maslacu gdje je utvrđen pad koncentracije nezasićenih (UFA - samo mlijeko) i višestrukonezasićenih (PUFA - mlijeko i maslac) masnih kiselina.

Ključne riječi: **mlijeko, masne kiseline, mliječni proizvodi, prerada, skladištenje**

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Analysis of Fatty Acid Composition and Healthy Lipids Indices in Raw and Processed Milk

Lina Laučienė[1,*], Vaida Andrulevičiūtė[2], Ingrida Sinkevičienė[2], Antanas Sederevičius[3], Kristina Musayeva[3], Loreta Šernienė[1]

[1]Department of Food Safety and Quality, Veterinary Faculty, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Department of Biochemistry, Veterinary Faculty, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Department of Anatomy and Physiology, Veterinary Faculty, Lithuanian University of Health Sciences, Kaunas, Lithuania
*Corresponding author: lina.lauciene@lsmuni.lt

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Abstract The aim of the present study was to examine the fatty acid composition and healthy lipids indices in raw milk and dairy products, covering the main stages of production. The fresh cheese, butter, sour cream, and ultra-high temperature treated milk, representing differences in technological approach were chosen for the study. Fatty acids methyl esters were quantified using a gas chromatograph equipped with flame ionization detector and a capillary column SP-2560. No significant influence of the technological process has been identified. The concentrations and profile of fatty acids and healthy lipids indices in final product fat were primarily dependent on the content of the raw milk or cream. The significant difference ($P<0.05$) in main groups of fatty acids and healthy lipids indices were determined according to the season (except for sour cream). Summer raw milk and cream was a healthier option for the production of fresh cheese, ultra-high temperature milk, and butter.

Keywords: *bovine milk, fatty acids, health lipid indices, milk processing*

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1. Introduction

Milk is the most valuable among all plant and animal products. This is due to the unique composition of the milk and its nutritional value, which makes it possible for milk to replace any of the foods. Milk contains all the necessary nutrients for the human body: proteins, fats, lactose, micro and macro elements, vitamins and enzymes ensure normal human growth, development and vital functions of the body [1]. Milk fat is one of the most complex natural fats that consist of approximately 400-500 fatty acids (FA) [2]. The human body absorbs about 97 percent of the milk fat and supplies the essential polyunsaturated fatty acids (PUFA). Two main and essential PUFA are linoleic acid (18:2n6; LA) and α-linolenic acid (18:3n3; ALA) which represent the n6 and n3 of PUFA group respectively, are not synthesized in the human body and should be obtained with food [3]. C18:1c9 (oleic acid) which is the most abundant fatty acid of monounsaturated fatty acids (MUFA) and the isomers of conjugated linoleic acid, related to the cholesterol reduction and to the anticarcinogenic effects respectively [4].

Despite benefits, for a long time bovine milk was thought to contribute to cardiovascular disease (CVD) due to negative effects of saturated fatty acids (SFA) which account for 65-75% of all milk fat. However, recer deacade findings have indicated that the impact of SFA t CVD may be less pronounced than previously assumed. has been shown that not all SFA are created equal an that the presence of specific fatty acids in circulatio are associated with a lower incidence of severa cardiometabolic diseases [5]. Now, it is stated th increased low-density lipid (LDL) blood concentration attributable to C12:0 (lauric), C14:0 (myristic), and C16: (palmitic) acids, while other SFA found in milk neutralis their effect since they increase high-density lipids (HDI level [6] or has no effect on LDL because poorly absorbed in the gut (eg. C18:0, stearic acid) [7 Even trans FA from ruminant sources may hav cardioprotective effects [1].

The FA profile of dairy fat is important for th nutritional quality of dairy products. To acsses the di nutrition value and consumer health the ratio PUFA/SFA and LA/ALA, desirable fatty acids (DFA hypocholesterolemic/hypercholesterolemic index (h/H atherogenicity index (AI) and thrombogenicity index (T are valued widly. In 1991, according to the different effe and link to coronary heart disease of the various FA, was suggested to calculate the AI and TI that can mor reliably measure potential of the diet than a ratio PUFA/SFA [8]. The h/H takes into account the know effects of certain FA (C18:1, PUFA, C14:0, C16:0) o cholesterol metabolism [9]. DFA are expressed as the sur

of unsaturated fatty acids (UFA) plus C18:0 (stearic acid) [10].

A PUFA/SFA ratio above 0.45 is recommended in the diet to prevent coronary heart disease and cancers [11]. According reglamentation from European Union, the levels of LA and ALA should cover a ratio between 5-6. Nevertheless, the optimal ratio should be 2:1 to 3:1 whereas lower ratio of omega-6/omega-3 FA is more desirable in reducing the risk of many of the chronic diseases [12]. The lower values of AI and TI, that indicate high quantities of anti-atherogenic FA in fat, are recommended for a healthy diet [9].

A number of already known factors such as feeding ration, season, stage of lactation, pregnancy, breed or genotype, parity and stage of lactation, dairy production system factors can greatly influence the profile of FA in milk [13,14,15,16]. There were described health lipid indices (HLI) in raw milk depending on the cow breed [9,17], feeding ratio [15], the species of animal [16,17]. But there is still lack of information on HLI in different stages of dairy technological process. It is not clear whether all of the FA are transferred from the raw material to the final product, whether and in what way their ratio changes, are there any loss of certain FA and changes in HLI during production, simultaneously. Therefore, the purpose of this study was to analyze the extent of FA transfer from raw milk fat to dairy product and evaluate the nutritional value of dairy fats according to PU FA/SFA, LA/ALA, h/H, DFA, AI and TI during raw milk processing.

2. Materials and Methods

2.1. Samples and Reagents

Several dairy products such as fresh cheese, butter, sour cream and ultra-high temperature treated (UHT) milk, representing differences in technological approach were chosen for the study. Each product was examined two times. The samples from the main technological stages (Table 1) were collected at one branch of Lithuanian dairy factory during June-July period and January-February period in 2018. The products were manufactured following standard methods [18]. All chemicals and FAME standards for Gas Chromatographic (GC) Analysis were purchased from Merck (KGaA, Darmstadt, Germany).

Table 1. Sampling scheme

Product	Sampling points*			
Fresh cheese	raw milk	standardized milk	fresh cheese	whey
Butter	bulk butter cream	butter	butter milk	-
Sour cream	bulk cream	standardized and pasteurized cream	sour cream	-
UHT milk	raw milk	standardized milk	UHT milk	-

*Each sample was taken and analyzed in triplicate.

2.2. Lipid Extraction

The lipid separation from liquid samples (milk, cream, buttermilk, whey) was done by centrifugation. The lipids from cheese were extracted using hexane: 10 g of sample were dispersed with 15 ml hexane using a homogenizer (IKA T25 digital ULTRA TURAX) for 3 min, shaken mechanically and then centrifuged at 5000 rpm for 20 min. The upper solvent was removed and the sediment extracted again twice. The solvents with dissolved fats were combined and evaporated with a rotary evaporator (IKA, RV 10 basic) under vacuum [19].

2.3. Fatty Acids Analysis

The FA were converted into fatty acid methyl esters (FAME) as follows [20]: 60 mg of concentrated fat was mixed with 4 ml of hexane and 200 µl of 2 $molL^{-1}$ KOH in methanol, then 1 min intensively vortexed and after 10 min of standing the top layer was collected and filtered in to chromatography vial. FAME were quantified using a GC Clarus 680 (Perkin Elmer) equipped with flame ionisation detector (FID) and a capillary column SP-2560, 100 m x 0.25 mm id x 0.20 µm. Conditions for chromatographic analysis were as following: the injector and detector temperatures were maintained at 250 °C. FA profiles were determined by injection of 1 µL, with a split ratio of 1:19. Oven temperature was held at 100°C for 4 min, increased to 240°C (4°C/min) and held for 70 min. Carrier gas (He) flow rate was 20 mL/min. FA peaks were identified using using Supelco® 37 Component FAME Mix. Each FA was expressed in g/100 g of total FAME content.

FA were divided into four main groups depending on the presence and the number of double or triple bonds: saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Indices of atherogenicity (AI) and thrombogenicity (TI) were counted according following formulas [8]:

$$AI = \frac{(C12:0+(4xC14:0)+C16:0)}{(PUFA+MUFA)} \quad (1)$$

$$TI = \frac{(C12:0+C16:0+C18:0)}{\left[\begin{array}{l}(0.5\ x\ MUFA)+(0.5\ x\ n6PUFA)\\ +(3\ x\ n3PUFA)+(n3PUFA/n6PUFA)\end{array}\right]}. \quad (2)$$

The hypocholesterolaemic and hypercholesterolaemic (h/H) index was counted as follows (9):

$$h/H = \frac{(C18:1+PUFA)}{(C14:0+C16:0)}. \quad (3)$$

Desirable fatty acids (DFA) were expressed as the sum of unsaturated fatty acids (UFA) and C18:0 (stearic acid) according this formula [10]:

$$DFA=UFA+C18:0. \quad (4)$$

2.4. Statistical Analysis

The experiment was designed as a factorial experiment with product and processing treatment as the main factors. Statistical analysis was performed by SPSS statistical package (Chicago, SPSS Inc., SPSS 17). The data were analysed using Descriptive Statistics (Explore) and Analysis of Variance (ANOVA) methods. The

significance of interactions among the groups assessed was determined by the Tukey HSD test. The differences were considered as significant at P<0.05.

3. Results and Discussion

Analysis of summer and winter raw milk samples confirmed other author's findings [21,22]: summer milk had significantly (p<0.05) more UFA and MUFA and less SFA than winter milk (Figure 1). The PUFA level was similar in milk fat of both seasons.

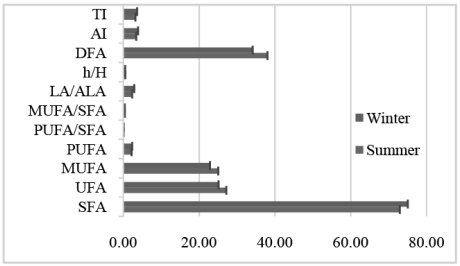

Figure 1. The health lipid indices and percentage composition of main groups of FA in raw milk samples

The PUFA/SFA ratio was much lower (0.03±0.00) than recommended value (0.45) in both seasons milk. The LA/ALA ratio was 2.25±0.10 for raw summer milk and 3.02±0.13 for raw winter milk. Although the LA/ALA ratio was significantly lower in summer milk, the milk fat of both seasons was consistent with and even exceeded recommendations.

In addition, summer milk had significantly higher content of DFA and higher h/H index as well. Obviously, that AI and TI were lower (p<0.05) in summer milk. Despite very low ratio of PUFA/SFA, summer milk is a healthier option for human nutrition. On the other hand, not all SFA are harmful to human health. The most unwanted and related to cardiovascular disease FA (C12:0, C14:0 and C16:0) [6] were accounted for 53% of all SFA in raw milk samples in this study.

The seasonal impact, which is most closely associated with cow's feeding ration [13], is evident on milk FA composition and HLI. Since the cow's breed has an influence on FA composition and HLI [9] it is necessary to mention that analyzed raw milk data largely represent the Lithuanian black and white and Lithuanian red cows' breeds. These two cows' breeds make up 56.71% and 21.40%, of dairy cows in Lithuania, respectively.

Fresh cheese selected for the study was manufactured from standardized, pasteurized and cooled milk by adding mesophilic lactic acid bacteria (LAB, Lactobacillus lactis subsp cremoris, subsp. lactis, subsp. lactis biovar diacetylactis and Leuconostoc subs). After 12 hours of fermentation, curd body was sliced and warmed to 65°C to separate the whey. Milk fermentation and whey separation could be identified as the most important stages in production of fresh cheese. However, milk fermentation did not have any impact on main FA groups during both seasons. And the similar amount of FA were transferred from curd body to whey. Hence, the HLI and ratio of PUFA/SFA and LA/ALA remained stable during whole fresh cheese production. The 2005 study did not showed a significant differences in FA profile between raw sheep milk and fresh cheese/ricotta fats. Authors state that concentrations of FA in fresh cheese fat were primarily dependent on the FA content of the raw milk [24]. That is in accordance with our findings with bovine milk. Fresh cheese made during summer period had healthier ratio of FA, more DFA acids and better AI and TI than cheese produced during winter (Table 2).

Unsalted 82 % fat butter was chosen for our study. Churning was the main stage in the butter-making process that destroyed fat globule's membranes. Very little data available on FA profile changes during butter production, but 2007 study showed that butter-making process had no significant influence on the conjugated linoleic acid and FA content of cream processed into butter [25]. The data of current study presented similar results: the profile and concentrations of FA remained similar during all technological stages. But the difference between analogous samples in a different season was confirmed (Table 3). Since summer butter had less SFA and more UFA and MUFA, the AI and DFA values were rated as healthier.

Table 2. The concentration of main groups and ratios of FA and HLI indices during fresh cheese production in diferent season

	Summer				Winter			
	Raw milk	Standardized milk	Fresh cheese	Whey	Raw milk	Standardized milk	Fresh cheese	Whey
	72.88±0.55^a	72.93±0.05^a	73.50±0.56^a	72.33±0.91^a	76.51±0.27^b	75.16±1.19^b	76.85±0.19^b	77.91±1.30^b
UFA	27.12±0.54^a	27.07±0.04^a	26.50±0.53^a	27.67±0.89^a	23.49±0.25^b	24.84±1.17^b	23.15±0.18^b	22.09±1.28^b
MUFA	25.09±0.48^a	24.79±0.13	24.51±0.49^a	25.50±0.87^a	21.33±0.11^b	22.88±0.94	21.24±0.26^b	20.08±1.26^b
PUFA	2.03±0.07	2.28±0.12	1.99±0.00	2.18±0.08	2.16±0.17	1.96±0.25	1.91±0.08	2.01±0.18
PUFA/SFA	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.02±0.00	0.03±0.00
MUFA/SFA	0.34±0.01^a	0.34±0.00	0.33±0.01^a	0.35±0.02^a	0.28±0.00^b	0.30±0.02	0.28±0.00^b	0.26±0.02^b
LA/ALA	2.00±0.12	2.03±0.32	2.01±0.16	2.14±0.25	2.88±0.84	2.57±0.37	2.86±0.79	2.81±0.27
h/H	0.46±0.02^a	0.46±0.00^a	0.49±0.01^a	0.48±0.03^a	0.37±0.01^b	0.40±0.02^b	0.35±0.01^b	0.37±0.01^b
DFA	37.95±1.11^a	37.93±0.20	38.26±0.29^a	39.18±0.66^a	32.32±0.26^b	34.24±1.10	31.91±0.47^b	30.45±1.45^b
AI	3.35±0.11^a	3.37±0.04	3.46±0.05^a	3.20±0.19^a	4.14±0.11^b	3.78±0.31	4.23±0.06^b	4.44±0.25^b
TI	3.12±0.09^a	3.07±0.13	3.11±0.13^a	3.10±0.16	3.75±0.18^b	3.69±0.19	3.93±0.08^b	4.39±0.55

Values are mean value±SEM g/100 g of FAME; means with diferent lowercase letters within the same row show significant (P<0.05) difference between analogous sampes in diferent seasons; SFA-saturated fatty acids, UFA-unsaturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; PUFA/SFA-ratio of polyunsaturated fatty acids and saturated fatty acids; MUFA/SFA-ratio of monounsaturated fatty acids and saturated fatty acids; LA/ALA-ratio of 18:2n6 (linoleic acid) and 18:3n3 (α-linolenic acid); h/H- hypocholesterolaemic and hypercholesterolaemic index; DFA-desirable fatty acids; AI-atherogenicity index; TI-thrombogenicity index.

Table 3. The concentration of main groups and ratios of FA and HLI indices during butter production in diferent season

	Summer			Winter		
	Bulk butter cream	Butter	Butter milk	Bulk butter cream	Butter	Butter milk
	70.49±0.70[a]	70.63±0.75[a]	71.21±0.89[a]	75.27±1.39[b]	74.12±0.42[b]	73.42±0.50[b]
UFA	29.51±0.69[a]	29.37±0.72[a]	28.79±0.84[a]	24.73±1.36[b]	25.88±0.40[b]	26.58±0.49[b]
MUFA	27.35±0.56[a]	27.11±0.68[a]	26.51±1.03[a]	22.21±1.48[b]	23.45±0.43[b]	23.71±0.29[b]
PUFA	2.16±0.14	2.26±0.07	2.27±0.14	2.52±0.09	2.43±0.00	2.87±0.21
PUFA/SFA	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.04±0.00
MUFA/SFA	0.39±0.01[a]	0.38±0.01[a]	0.37±0.02[a]	0.30±0.04[b]	0.32±0.01[b]	0.32±0.01[b]
LA/ALA	2.60±0.02	2.56±0.18	2.72±0.34	2.69±0.05	2.52±0.26	2.33±0.08
h/H	0.56±0.01	0.53±0.03	0.49±0.01	0.41±0.06	0.43±0.01	0.47±0.01
DFA	40.60±0.32[a]	41.33±0.37[a]	39.25±0.38[a]	35.21±0.68[b]	35.38±0.70[b]	36.17±.0.71[b]
AI	2.90±0.06[a]	2.91±0.18[a]	3.16±0.08[a]	3.93±0.53[b]	3.64±0.09[b]	3.33±0.07[b]
TI	2.77±0.01	3.04±0.08	3.00±0.02	3.45±0.37	3.31±0.10	2.97±0.09

Values are mean value±SEM g/100 g of FAME; means with diferent lowercase letters within the same row show significant ($P<0.05$) difference between analogous sampes in diferent seasons; SFA-saturated fatty acids, UFA-unsaturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; PUFA/SFA-ratio of polyunsaturated fatty acids and saturated fatty acids; MUFA/SFA-ratio of monounsaturated fatty acids and saturated fatty acids; LA/ALA-ratio of 18:2n6 (linoleic acid) and 18:3n3 (α-linolenic acid); h/H- hypocholesterolaemic and hypercholesterolaemic index; DFA-desirable fatty acids; AI-atherogenicity index; TI-thrombogenicity index.

Sour cream chosen for this study was manufactured by fermentation of standardized, homogenized and pasteurized cream using LAB strains. During both seasons, FA profile did not change during sour cream processing. Partially this comes in agreement with other findings - no pasteurization effect on milk fat profile [25,26], no homogenization effect on cream [28] or milk [29] FA profiles were found. Meantime, results from previous surveys with milk and cream fermentation were very controversial [29,30,31] pointing out the importance of LAB strains used for fermentation, with probiotic strains contributing to significant changes in FA distribution. However, the cream fermentation with LAB in our study did not reveal any significant changes in FA profile, therefore HLI and ratio of PUFA/SFA and LA/ALA remained stable during sour cream production. In contrast to other products analysed in this study, significant seasonal effect between summer and winter sour cream was not found either.

The milk treatment with ultra high temperature (135°C for 3 sec) showed a slight change in the amount of individual FA during UHT milk processing. For instance, the amounts of short chain FA (except C4:0) and midle chain FA tended to decrease, while long chain FA (except C16:0, C17:0) slightly increased after high temperature treatment. However, the significance of these changes in FA profile has not been statistically confirmed. The SFA, UFA, MUFA and PUFA groups remained stable during UHT milk prossesing, therefore, the HLI and FA ratios were not affected. Some authors refer to the significant impact on short chain FA in UHT milk [26,32], but the different heat treatment applied in their study (140°C for 3 sec) could have had a significant influence on this change.

4. Conclusion

The data analysis showed that technologies such as milk pasteurisation, homogenization, ultra-high temperature treatment, milk or cream fermentation by LAB, cream churning process had no significant effect on the FA profile or the corresponding FA ratio and HLI. In the by-products (whey and butter milk), the FA ratio remained similar. The FA composition and HLI in the final milk products were directly dependent on composition of the raw material.

Conflict of Interest

The authors declare no conflict of interest.

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CURRICULUM VITAE

Name, surname: Lina Laučienė
Address: Department of Food Safety and Quality, Lithuanian University of Health Sciences, Tilžės 18, LT-47181 Kaunas, Lithuania
E-mail: lina.lauciene@lsmuni.lt

Education:

1998–2005	Master degree of Veterinary Medicine (Lithuanian Veterinary Academy; present Lithuanian University of Health Sciences)
2015–2019	PhD student, Agricultural sciences, Veterinary (02A) (Lithuanian University of Health Sciences, Department of Food Safety and Quality

Work experience:

2016–present	Assistant/visiting lecturer/lecturer (Lithuanian University of Health Sciences, Department of Food Safety and Quality)
2018–present	Junior researcher (Lithuanian University of Health Sciences, Department of Food Safety and Quality, Project No. 01.2.2-LMT-K-718)
2014–2016	Coordinator of infrastructure project (Lithuanian University of Health Sciences)
2007–2014	Administrator/project coordinator (Center of Veterinary Continuing Education and Counseling, Lithuanian University of Health Sciences)

Other skills: State license for veterinary practice (No. VP-1763)

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