



CONCLUSIONS AND RECOMMENDATIONS FROM THE EXPERT WORKING GROUP

Review of available literature and information and gaps in knowledge on issues related to the characteristics of Canadian butter

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INTRODUCTION

Reports from media and consumers have raised questions about the consistency of butter and the use of animal feed supplements containing palm by-products. Specifically, it has been asserted that the use of palm-derived supplements has made retail butter harder in texture. In response to these questions, the Dairy Farmers of Canada (DFC) put in place an Expert Working Group to assess these reports and develop fact-based explanations to address these questions. The overarching goal of the Expert Working Group is to deliver evidence-based conclusions on the hardness of butter and any relation to the use of palm-derived supplements for cows, conclusions informed by the best available peer reviewed science.

Explanatory Notes

- For technical and scientific portions of the report (including literature reviews and scientific analysis), chapter authors have been listed. Additionally, references have been left in their original form.
- To ensure this work contributes to the scientific body of knowledge, the content is technical in nature. For this reason, a glossary of key terms and technical definitions has been developed (Please see Appendix 1). In addition to each chapter summary, please consult the comprehensive Executive Summary for key findings.
- In the literature review and scientific analysis sections of this report, all sources are listed at the end of each chapter.
- For the section on butter samples, specific brand names have been redacted to respect commercial confidentiality and privacy.



Scope of work

The Expert Working Group set the scope of its work and established the following key objectives:

- Confirm whether there are or have been changes in the characteristics of butter;
- Review the literature to assess current science as it relates to:
 - » Feeding of palm-derived lipids to cows;
 - » Milk composition;
 - » Milk handling and processing techniques; and
 - » Health and safety of supplements;
- Identify any gaps in data or research;
- Review of the level of sustainability of palm-derived feed supplements
- Assess the role and nutritional value of palm-derived feed supplements for dairy cows.

Establishing an Expert Working Group

The Expert Working Group includes prominent academics and experts from across Canada, with a diverse range of expertise. All are recognized as leaders in their fields, with specializations in areas such as dairy nutrition, animal health, sustainability, food science, and human nutrition. The group also includes representation from the Consumers' Association of Canada. The Expert Working Group also includes participation from dairy processors and farm level experts. The group met seven times from March to November and received presentations and reports from discussions with several outside experts.

The Group also commissioned two data collection efforts:

- Analysis, compilation and statistical analysis of the fatty acid composition of raw milk from across Canada
- Collection of samples of retail butter from across Canada and subsequent analysis of the fatty acid profile and physical properties.

Please see Appendix 2 for a summary of the findings presented to the Working Group.

Please see Appendix 3 for a full list of Expert Working Group members and their biographies



EXECUTIVE SUMMARY

It is generally known that milk naturally contains about 4% milkfat when it comes from the cow's udder, though they likely stop short of considering the specific mix of saturated and unsaturated fatty acids in their milk. Furthermore, there are several types of saturated fats and unsaturated fats- or about 400 different fatty acids in milk. Therefore, when people first heard the term "palmitic acid", most people had limited background available to understand what it means in the broader scientific context.

Palmitic acid (C16:0) is a saturated fat, and the predominant fatty acid in milk, regardless of what cows eat. It is also the most common saturated fatty acid in nature. Cows produce palmitic acid naturally, along with hundreds of other fatty acids in their milk. Ingredients in animal feed also contain such fatty acids. However, feeding cows palm-derived feed supplements is not the main factor contributing to palmitic acid in milk. Most of the C16:0 present in milk is derived from the cow eating traditional feed ingredients (mainly hay, silage, grass, cereal grains, etc.) and making C16:0 naturally in her udder (de novo synthesis).

Additionally, the fatty acid composition of milk is influenced by a variety of other factors including season, stage of lactation, diet (influenced by geographic region) and a range of other variables.

With respect to processing, the literature review indicates that cream handling, temperature of storage and churning are key factors that may affect the rheological properties of final products (e.g., the melting points of butter or its firmness and perceived 'spreadability').

Extensive consultations with processors found that while there has been a significant shift in demand from the restaurant and hospitality industry to the retail sector (due to pandemic-related restaurant closures and restrictions), there has been no significant change in manufacturing processes and practices over the past year and a half.

A key aspect of this report was to test 40 samples of retail butter from across the country. The concentration of C16:0 of these samples varied between 32 and 39 g/100 g of fatty acids. As it has been extensively reported in the literature

before, due to the high melting point of C16:0, its concentration in butter is positively correlated with the percentage of solid fat in butter and its firmness at room temperature. However, this survey demonstrates that many other milk fatty acids are also associated positively or negatively with the percentage of solid fat in butter at room temperature and can also impact its firmness.

And while the content of palmitic acid in retail butter varies across the country, this variation could not be attributed to one single factor such as feeding cows supplements that contain palmitic acid.

Regarding human health, it should be clearly understood that any increases in palmitic acid (C16:0) content in butter induced by feeding changes will be modest and extremely unlikely to have human health implications.

According to the Animal Nutrition Association of Canada (ANAC), dairy farmers in Canada that use a palm-derived supplement typically feed between 200-600 grams/cow/day. Based on import data, approximately 35,000 metric tonnes of these supplements were imported into Canada in 2020 which is less than 0.1% of the estimated globally produced palm and palm kernel oil.

In fact, palm oil is widely and safely used in many products in the food industry because it is versatile and has many different functions, such as keeping spreads spreadable. It helps favour a longer shelf life, and is odourless (WWF).

There remain very legitimate concerns over the global production of palm oil and its environmental and social impacts. Concerns about deforestation, exploitation of workers, and general negative impacts on biodiversity, ecosystems and pollution remain issues that require global solutions.

Efforts to make palm oil production more sustainable are ongoing and are encouraging. Major Canadian feed suppliers that use palm by-products

source them from companies that espouse the ideals of the Round Table for Sustainable Palm Oil, or other existing sustainability systems and thus aim to increase the overall sustainability of palm oil production.

It is important to note that palm-derived products used in animal feed are by-products of palm oil processing and are not the primary driver for palm oil production. As stated above, the broader sustainability concerns regarding global palm oil production will require international cooperation. Again, this work should be encouraged across palm oil production as a whole.

Following the publication of an article (in February 2021), expressing concerns about the consistency of butter, consumer organizations in Canada received contacts from individuals expressing concerns about this issue; particularly as it related to the use of butter in baking. However, given other issues that were occurring at the time due to the pandemic, these contacts were not widespread.

The Expert Working Group was convened to review these issues and undertake a literature review and related analyses. In the end, it observed there is no data to confirm that there has been a change in the consistency of butter over time. As a result of this lack of data, it is not possible to test for a causal relationship, and therefore draw conclusions, on the link between the use of palm-derived supplements on Canadian farms and the consistency of butter in the last number of years. A key recommendation of this report is the need for better and more consistent time series data both when it comes to the evolution of the fatty acid profile of milk and butter, and butter hardness off retail shelves.

Consumers can and should remain confident in the comprehensive regulatory frameworks that underpin Canada's food systems, agriculture and agri-food practices and overall safety and nutrition of Canadian food products.

HEALTH AND NUTRITION

Chapter 1: An Overview of Animal Nutrition

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Overview

Milk fat is one of the most complex natural mixtures of lipids. It is comprised of 98% triglycerides: three fatty acid chains esterified to a glycerol molecule. Milk fatty acids are either synthesized de novo ("from scratch") in the mammary gland cell with acetate produced by rumen fermentation as the primary precursor or they are taken up from blood circulation. In the latter case, they are mostly long chain fatty acids (16-carbons or more) that come from the cow's diet or are mobilised from body fat reserves when cows are in negative energy balance (i.e. losing weight). De novo synthesized fatty acids range in length from 4 to 16 carbons. Regulation of milk fat synthesis is one of the most complex metabolic processes in mammals.

Livestock animals need a balanced diet containing the necessary nutrients, fluids, minerals, and vitamins. This ensures livestock animals have the vitality to grow, develop robust immune systems and reproduce and support economical production of high-quality food.

Dairy farmers work closely with animal nutrition experts to formulate dairy cattle diets to match nutrient supply with the animals' requirements to support production and maintain optimal health. Healthy animals are more productive. Beyond productivity, consumers are also increasingly conscious of the quality of the food they buy and the health and well-being of food-producing animals as well as the sustainability of food production systems.

Cattle are herbivores, meaning that their diet consists of plant matter. Like sheep and goats, they are ruminants: instead of having just one stomach like humans, they have four separate stomach compartments that allow specialized digestion of different components of their high-fibre diet. The symbiotic relationship with the microflora inhabiting their rumen that enables ruminants to digest high fiber feedstuffs, such as forage plants and by-products, that would be indigestible for non-ruminants and convert these feed resources in highly nutritious foods for humans.

Today's high producing dairy cows consume about 29 kg of feed dry matter every day. Diets are formulated to keep cows in good condition while supporting high milk production allowed by their genetic potential. Dairy cows' diets are typically composed of a combination of farm-grown feeds, food by-products and commercially manufactured supplements. Feed that is grown on-farm can include hay, fresh grass and pasture, silage, grains and oilseeds. Many food by-products, such as distillers' grain, oilseed meals, select food waste such as bakery waste and other food industry products, are also approved for use as feed sources for dairy cows. They can either be incorporated in cows' diets directly on the farm or in commercially manufactured supplements. Commercial feeds are used to provide cows with appropriate nutrition to complement nutrition derived from farm-grown feeds. This includes a balance of energy, protein, vitamins, minerals as well as other specialty feed additives supporting animal health and productivity.

To ensure dairy cows are receiving proper nutrition, the crops that are grown on farm and fed to cows are routinely tested. Diets are formulated based on cows' requirements for protein and energy (supplied by fibre, carbohydrates, and lipids), minerals and vitamins. Dairy cattle nutrition experts test the nutrient values of the crops grown and then use those results to help formulate diets that work best for specific groups of cows. For example, dietary needs will vary depending on their body size, production level and stage of lactation.

Farmers can store their forages wet and fermented as silage or dry as hay. This ensures year-round feed supply. These feeds are usually mixed in with vitamins, minerals and other supplements to create what farmers call a “ration”. This mixture is usually specific to each herd and classes of animals in that herd (i.e., cows in early lactation and cows that are in their dry period - not lactating- or replacement heifers) and may change based on the recommendations of cattle nutrition expert or veterinarian.

Farmers may add vitamins, minerals, and energy and protein supplements into the feed to make sure that their cows are healthy and productive. For some farms, supplements can include a small amount of lipids such as palm-derived products that are included in cow diets to increase energy density of the ration and help meet cows’ energy requirements. The level and use of palm-derived supplements vary from farm to farm, and on farms where it is used, it is typically less than 1% of a cow’s diet.

Feed crops grown on-farm are regionally dependant and this influences the cow’s diet composition. As a result, cows in different regions of Canada have different supplemental dietary requirements. For example, corn silage is widely grown in Eastern Canada and often constitutes the base forage of dairy cows’ diets but is not as widely available in Western Canada where barley silage is more common. Similarly, barley grain is more widely used than corn grain in Western Canada (Table 1). Considering that barley, either as silage or as grain, provides less digestible energy than corn, and that due to the shorter growing season, the corn silage that is grown in Western Canada contains less starch and energy, it is often more challenging to meet cows’ energy requirements with Western Canadian base rations (Table 2).

Table 1: Common feeds used in Eastern and Western Canada

INGREDIENT TYPE	EASTERN CANADA	WESTERN CANADA
Silage	Corn silage	Barley/corn silage (50/50)
Grains	Corn	Barley primarily
Other	Soybean meal, corn gluten meal, distillers’ grains	Canola meal

Source: ANAC

Table 2. Typical nutritional value of silages in Eastern and Western Canada

CVAS Global Equations						
Date Range: April 2016-April 2021						
	Region: QC & ON			Region: AB, SK, MB		
	Corn Silage*	Barley Silage	Oat Silage	Corn Silage	Barley Silage	Oat Silage
n	12,609	54	19	9,011	6,751	763
DM	38.2	34.1	32.8	35.2	33.1	32.7
CP	7.47	12.2	14.3	8.71	11.2	11.2
ADF	22.9	27.6	33.8	27.0	29.3	35.2
NDF	39.3	45.7	50.8	46.1	47.3	53.3
Ca	0.19	0.35	0.55	0.25	0.37	0.4
P	0.22	0.26	0.35	0.23	0.27	0.28
TDN	72.6	66.6	63.3	69.1	64.8	62.4
Starch	35.7	15.6	3.97	23.2	17.0	9.99
Nel Mcal/kg	1.702	1.546	1.456	1.580	1.480	1.420
Nem Mcal/kg	1.770	1.568	1.456	1.620	1.480	1.400
Neg Mcal/kg	1.142	0.963	0.851	1.020	0.890	0.810
*April 2018-2021						

The role of lipids in dairy cow diets

The modern dairy cow can produce large amounts of milk and milk components, such as milk protein and milkfat. High production comes with high energy requirements for cows to reach their genetic potential. Meeting energy requirements can be challenging, especially in early lactation, when milk production increases faster than the amount of feed consumed by the cow. If the energy supplied by the diet cannot meet a cow’s energy requirements, the cow enters negative energy balance. Cows attempt to compensate for the energy deficit by mobilizing energy reserves, mostly found in adipose tissue. This process is relatively normal for high-producing cows, which rebuild energy reserves later in lactation. Prolonged and acute energy deficit can lead to excessive body fat mobilisation, which can in turn cause metabolic problems and reduced fertility.

High energy requirements are typically met by increasing the proportion of concentrates, typically corn and cereal grains, which are generally more energy-dense than forages. However, there is a limit to this approach as excess grain in dairy cows’ rations can cause rumen acidosis and digestive disturbances.

Because fat is the most energy-dense nutrient available, containing almost two times more energy value than carbohydrates, supplemental fat is added to the ration to help meet energy requirements. Supplementing fat in dairy cows' diets has been extensively studied for decades. Practical fat sources to supplement cows' rations include oilseeds, vegetable oils, animal fats (lard, tallow) and commercially available dry fat supplements.

Ruminants have a very low tolerance for unsaturated fatty acids due to their deleterious effects on rumen microbes. For this reason, oilseeds and vegetable oils containing a significant proportion of unsaturated fatty acids (corn oil, soybean oil, canola oil, etc.) are not suitable for inclusion in dairy cows' diets at substantial levels. The toxicity of unsaturated fatty acids to the rumen microflora can be attenuated by the chemical transformation of fatty acids into their calcium salts.

Sources of saturated fatty acids are therefore preferred as fat supplements to increase the energy density of cows' rations. Other than animal fats, palm oil derivatives are the lipid source with the highest content of saturated fatty acids, and as such, presents very desirable properties as a supplemental source of fat in dairy cow rations.

Calcium salts of palm fatty acids have been approved and used in dairy cow rations in Canada for over thirty years. These supplements typically contain 50% of palmitic acid (C16:0) and 35% of oleic acid (C18:1) as the main fatty acids. Other sources of commercial dry fats include prilled hydrolysed fatty acids from tallow, which typically contains 45% stearic acid (C18:0) and 30% palmitic acid. High palmitic acid palm derivatives have come to the market more recently, in the last 15 years. These result from the fractionation and distillation of palm oil and contain over 80% of palmitic acid (C16:0).

C16:0 – Palmitic acid

Palmitic acid is the most common saturated fatty acid found in nature, in plants, animals and microorganisms. Common sources of C16:0 include palm oil, coconut oil and milk fat.

Palmitic acid is the predominant fatty acid found in milk fat. Feeding cows palm-derived feed supplements can increase butterfat percentage by 0.2-0.4 percentage points, depending on ration formulation. However, the cow also synthesizes most of the C16:0 in her mammary gland. A small proportion of all C16:0 found in milk is a result of feeding fat supplements.

A survey of 1585, Quebec dairy farmers conducted in 2018 by Valacta reported that 22% of farms included a palm-derived supplement in their cows' ration. The average inclusion rate was 236 grams per cow per day. According to Animal Nutrition Association of Canada (ANAC), dairy farmers in Canada who use a palm-derived supplement typically feed between 200-600 grams/cow/day. Imports of these feed supplements are estimated to be approximately 35,000 metric tonnes per year which is less than 0.1% of the estimated globally produced palm and palm kernel oil.

According to ANAC, palm-derived supplements are used in over 60 countries, including all the largest dairy producing countries in the world.

Use of palm-derived supplements in selected countries

Both New Zealand and the Netherlands had experiences in dealing with concerns over the use of palm supplements. Below is a summary of lessons learned based on discussions with stakeholders from these two countries:

New Zealand

In New Zealand, Palm Kernel Expeller (PKE) is fed to cows, especially during droughts, to provide protein and energy; It contains fat, but is not meant to give only fat to cows, unlike the palm-derived supplements used in the Northern hemisphere. Rather, PKE is a by-product of palm plantations that is used to provide various nutrients to cows, similar to why dairy farmers here feed oilseed meals or crushed grains from beer-making to cows.

In 2015, the farmer-owned co-op Fonterra reportedly put a limit of feeding a max of 3kg of PKE (out of 18 kg of feed) to cows. Over the years, they have changed tactics to inform farmers what results they expected from the milk and ways to achieve them. For example, Fonterra's Milk Fat Evaluation Index has become a multi-purpose tool to understand milk composition, and to understand which milk is suitable for various end products.

Furthermore, the industry has developed guidelines for feed management. A part of this, they noted that feeds high in sugar or starch influence the fat index. The seasonal production in New Zealand is an important factor that can cause short-term variability in the fat index of the milk. Other factors include stage of lactation, breed of cows and frequency in milking.

Additionally, Fonterra became engaged in the RSPO initiative as part of its commitment to contributing to the sustainable development of the industry.

The Netherlands

In the Netherlands, 70% of farmers are members of the Friesland Campina co-op. The co-op became a member of the RSPO to stay informed on sustainability issues. During hot summer months when cows were eating less, a palm-derived product was considered based on the advice of cow nutrition experts. However, it was expensive and it created tension between feed suppliers and dairy farmers.

And just as in Canada, farmers get feedback on their protein yield, fat yield, urea, temperature of the milk, point of freezing and overall milk yield. They also receive a full fatty acid profile of their milk, from infrared spectrometry analysis.

Overall, these examples demonstrate that dairy farmers around the world are constantly focused on maintaining the health and well-being of their animals, while maximizing efficiencies and looking for innovative ways to support sustainable farming practices.

The role of the Canadian Food and Inspection Agency in regulating animal feed

The manufacture, sale and import of livestock feeds, including dietary supplements, are regulated in Canada under the Feeds Act and its corresponding regulations and are administered by the Canadian Food Inspection Agency (CFIA).

The legislative authorities under which the CFIA regulates feeds include the following:

- Feeds Act
- Feeds Regulations
- Health of Animals Act
- Health of Animals Regulations
- Organic Products Regulations

All feeds must be safe for livestock; for humans (both the potential transfer of residues into meat, milk and eggs destined for human food, as well as worker/bystander exposure). The safety for the environment in Canada is also assessed.

Feeds must also be shown to be effective for their intended purpose. Approved feed ingredients are listed and defined in Schedules IV and V of the Feeds Regulations, with appropriate guarantees, standards and labelling requirements. All imported feeds must meet the same standards as domestic feeds.

Palm-derived supplements are approved as feed ingredients for animals by the CFIA.

Chapter Summary:

- Farmers work closely with nutrition experts to ensure that every ration meets an animal's essential dietary needs during its various stages of life.
- The manufacture, sale and import of livestock feeds, including palm-derived supplements, are regulated in Canada by the Canadian Food Inspection Agency (CFIA).
- What crops are grown on farm is regionally dependant. This influences the cow's diet which results in cows in Western Canada having different supplemental dietary needs than those in Eastern Canada.
- Because fat is the most energy-dense nutrient available, double the energy value of carbohydrates, supplemental fat is added to the ration to help cover energy needs of high-producing cows. This is particularly important during hot summer months when cows eat less or in instances where the nutrient and energy content of crops is less.
- Palmitic acid is the most common saturated fatty acid found in plants, animals and microorganisms.
- Palmitic acid is the predominant fatty acid naturally found in milk fat. The cow synthesizes C16:0 in her mammary gland. Feeding cows a palm-derived supplement, which is high in palmitic acid content can increase butterfat percentage by 0.2-0.4 percentage points, depending on ration formulation, but C16:0 found in milk is not only a result of feeding supplements.
- Palm-derived supplements are used in dairy cattle feeding in over 60 countries, including the largest dairy-producing countries in the world.

Chapter 2: Summary of the Literature on Potential Human Health Impact

Chapter authors:

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Overview

Dairy products have been extensively studied in terms of their potential impact on human health. Saturated fat content of dairy products is predominantly studied for association with obesity and associated cardiometabolic outcomes including type 2 diabetes (T2DM) and cardiovascular disease (CVD). This is the context in which this report is looking closely at issues related to dairy cow lipid supplementation and the hypothesis that it could impact the fat content of dairy products.

Although much of the public discussion and media coverage has focused on the impact of butter and potential health impacts of this specific dairy product, it is important to recognize that milk from dairy cows (regardless of lipid supplementation) is used in a range of widely consumed products including milk, cheese, yogurt and ice cream. Nonetheless, given recent consumer concerns, wherever possible we have highlighted relevant data that have addressed the health effects of butter specifically.

The impact of dairy consumption on changes in body weight and body fat distribution has been investigated in several observational studies and randomized controlled trials (1, 2). Despite the saturated fat content in dairy foods, accumulating evidence suggests that dairy products may have a substantial protective impact on body weight regulation (3). A recent meta-analysis of 22 prospective cohorts found that while total dairy intake was not associated with body weight changes, there was an inverse association of dairy intake with changes in abdominal obesity, waist circumference, and risk of incident overweight (1).

Similarly, a growing body of evidence suggests that higher intakes of dairy products may be associated with reduced risk of T2DM. Five of six recent meta-analyses of prospective observational studies have shown that total dairy intake was significantly inversely associated with T2DM risk (RR=0.85-0.97) (4-9). For example, a meta-analysis published in 2017 by Schwingshackl et al of 21 cohort studies found a 9% lower T2DM risk for high vs. low consumption of total dairy (RR: 0.91, 95% CI=0.85, 0.97) (9). None of these meta-analyses found any evidence of publication bias. In a 2020 umbrella review of observational studies, total dairy consumption had either neutral (coronary heart disease (CHD), CVD mortality) or inverse (CVD incidence, stroke, stroke mortality, and blood pressure) associations with CVD outcomes or intermediate CVD phenotypes (10).

Regarding dairy product fat content, meta-analyses of cohort studies have shown significant inverse associations of low-fat dairy with T2DM, and neutral associations with higher fat products (5, 9, 10). Results of meta-analyses of dairy product subtypes have been less consistent across studies, although collectively the data point to inverse or neutral associations of milk, cheese and yogurt with a range of cardio-metabolic outcomes (10).

Very few studies have analyzed data for butter individually. In a 2016 meta-analysis of 15 cohort studies (6.5 million person-years of follow-up, >28,000 total deaths, >9000 incident cases of CVD and >23,000 incident cases of T2DM), consumption of butter was weakly associated with all-cause mortality (RR = 1.01, 95%CI = 1.00-1.03, p=0.045) but was not significantly associated with CVD (RR = 1.00 (0.98-1.02), p=0.7), CHD (RR = 0.99 (0.96-1.03), p=0.5), or stroke (RR = 1.01 (0.98-1.03), p=0.7)(11). In this analysis, butter consumption was inversely associated with incidence of diabetes (RR = 0.96 (0.93-0.99), p= 0.02)(11). In an umbrella review of observational studies by Godosetal (10), butter consumption was not significantly associated with stroke, CHD or all cause mortality.

Fat content of dairy products

Dairy products contain a complex fat profile including saturated, polyunsaturated and monounsaturated fatty acids. The profile of fatty acids in dairy products is unique, as it includes species that are either specific to dairy or for which dairy is the most important dietary source. Examples include odd chain, branched chain, trans and conjugated fatty acids; indeed, many of these fatty acids are being increasingly used as biomarkers of consumption in human research studies, and some have been documented to have bioactive properties (12). Recently, a large meta-analysis from the FORCE consortium pooled the findings from 16 prospective cohort studies and reported that higher levels of 15:0, 17:0, and t16:1n-7 were associated with a lower risk of T2DM (13). More recently, branched chain fatty acids (BCFA, including several iso and anteiso species), have received increased scientific attention regarding their potential role in the etiology of cardiometabolic disease (14, 15).

Palmitate /palmitic acid in dairy products

Dairy products are an important source of palmitate intake in humans contributing about 23 percent of total saturates (16) [note this total includes other fatty acids beyond just palmitate]. Importantly, the predicted increase in the palmitate content of butter is between 6 to 17%. While it is not clear what percentage of dietary palmitate comes from butter intake in Canadians, even if we assume that total saturated fat intake from dairy of 23% increased by 6 to 17%, this would still represent a small increase in dietary intake. Importantly this number is an overestimate as butter only makes up a fraction of the total dairy intake and there are other saturated fatty acids in butter and dairy beyond palmitate. Thus, any increases total palmitate in milk or butter contributed by supplements of palm-derived lipids in the feed will be negligible and are extremely unlikely to have human health implications.

Chapter Summary

- Dairy products have been extensively studied in terms of their potential impact on human health.
- Dairy products are an important source of palmitate intake in humans contributing about 23 percent of total saturates (saturated fats intake).
- While it is not clear what percentage of dietary palmitate comes from butter intake in Canadians, it only makes up a fraction of the total dairy intake.
- Thus, any increases in total palmitate in milk or butter contributed by supplemental palm-derived lipids in the feed will be negligible and are extremely unlikely to have human health implications.

References

- » Schwingshackl L, Hoffmann G, Schwedhelm C, Kalle-Uhlmann T, Missbach B, Knuppel S, et al. Consumption of Dairy Products in Relation to Changes in Anthropometric Variables in Adult Populations: A Systematic Review and Meta-Analysis of Cohort Studies. *PLoS One*. 2016;11(6):e0157461.
- » Chen M, Pan A, Malik VS, Hu FB. Effects of dairy intake on body weight and fat: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2012;96(4):735-47.
- » Louie JC, Flood VM, Hector DJ, Rangan AM, Gill TP. Dairy consumption and overweight and obesity: a systematic review of prospective cohort studies. *Obes Rev*. 2011;12(7):e582-92.
- » Tong X, Dong JY, Wu ZW, Li W, Qin LQ. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. *Eur J Clin Nutr*. 2011;65(9):1027-31.
- » Gijsbers L, Ding EL, Malik VS, de Goede J, Geleijnse JM, Soedamah-Muthu SS. Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. *Am J Clin Nutr*. 2016;103(4):1111-24.
- » Aune D, Norat T, Romundstad P, Vatten LJ. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *Am J Clin Nutr*. 2013;98(4):1066-83.
- » Gao D, Ning N, Wang C, Wang Y, Li Q, Meng Z, et al. Dairy products consumption and risk of type 2 diabetes: systematic review and dose-response meta-analysis. *PLoS One*. 2013;8(9):e73965.
- » Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. *Lipids*. 2010;45(10):925-39.
- » Schwingshackl L, Hoffmann G, Lampousi AM, Knuppel S, Iqbal K, Schwedhelm C, et al. Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *Eur J Epidemiol*. 2017;32(5):363-75.
- » 1Godos J, Tieri M, Ghelfi F, Titta L, Marventano S, Lafranconi A, et al. Dairy foods and health: an umbrella review of observational studies. *Int J Food Sci Nutr*. 2020;71(2):138-51.
- » Pimpin L, Wu JH, Haskelberg H, Del Gobbo L, Mozaffarian D. Is Butter Back? A Systematic Review and Meta-Analysis of Butter Consumption and Risk of Cardiovascular Disease, Diabetes, and Total Mortality. *PLoS One*. 2016;11(6):e0158118.
- » Santaren ID, Watkins SM, Liese AD, Wagenknecht LE, Rewers MJ, Haffner SM, et al. Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, is inversely associated with incident type 2 diabetes and its underlying disorders. *Am J Clin Nutr*. 2014;100(6):1532-40.
- » Imamura F, Fretts A, Marklund M, Ardisson Korat AV, Yang WS, Lankinen M, et al. Fatty acid biomarkers of dairy fat consumption and incidence of type 2 diabetes: A pooled analysis of prospective cohort studies. *PLoS Med*. 2018;15(10):e1002670.
- » Mika A, Stepnowski P, Kaska L, Proczko M, Wisniewski P, Sledzinski M, et al. A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight. *Obesity (Silver Spring)*. 2016;24(8):1669-76.
- » Su X, Magkos F, Zhou D, Eagon JC, Fabbrini E, Okunade AL, et al. Adipose tissue monomethyl branched-chain fatty acids and insulin sensitivity: Effects of obesity and weight loss. *Obesity (Silver Spring)*. 2015;23(2):329-34.
- » Harrison S, Brassard D, Lemieux S, Lamarche B. Consumption and Sources of Saturated Fatty Acids According to the 2019 Canada Food Guide: Data from the 2015 Canadian Community Health Survey. *Nutrients*. 2019;11(9).

LITERATURE REVIEW AND SCIENTIFIC KNOWLEDGE SYNTHESIS

Chapter 3: Factors That Can Affect Milk Fatty Acid Composition/And Palmitic Acid Concentration In Dairy Cows

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Overview

Fat is the most variable component of milk. Both milk fat content and fatty acid (FA) composition of triglycerides vary from one lactating dairy cow to the next. This section provides a summary of the various factors that can affect FA composition, with an emphasis on the concentration of palmitic acid (16:0).

Animal factors

Genetic/Breed

Milk composition varies between breeds and even more between individuals within a breed (Soyeurt et al., 2006). Several factors may be involved in these variations, such as yields of major components of the individual breeds, different activity of desaturases (enzymes) and genetic polymorphism. Beyond the well-known difference in fat and protein concentrations in milk from Jersey and Holstein cows, a few experiments in the 1990s demonstrated that Jerseys have a lower oleic acid-to-stearic acid (c918:1-to-18:0) compared with Holsteins (Townsend et al., 1997), but that their milk fat contains higher proportions of short- and medium-chain FA ($\leq 14C$) (Beaulieu and Palmquist, 1995; Bitman et al., 1996), and lower concentrations of c918:1 (Beaulieu and Palmquist, 1995). As for the difference in milk fat concentration of palmitic acid (16:0) between Holsteins and Jerseys, results remain confusing. Whereas Beaulieu and Palmquist (1995) reported a greater concentration of 16:0 in milk fat from Holsteins compared with Jerseys, others have reported no significant difference (DePeters et al., 1995) or even a greater concentration of 16:0 in milk fat from Jersey cows (Stull and Brown, 1964). However, results from the different studies support the idea that type of breed only explains a limited proportion of the variability in milk FA composition.

According to Gibson (1991), it seems reasonable to assume that the molar proportion of FA components of total fat has a moderate heritability (around 0.3) with a coefficient of variation in the range of 0.05 to 0.2. Consequently, milk fat composition could respond to genetic selection. More recently, heritability coefficients have been established based on results from numerous milk samples originating from different countries, breeds, and animal populations (Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008; Arnould and Soyeurt, 2009; Mele et al., 2009). Stoop et al. (2008) evaluated that estimates of heritability for the individual FA are correlated with their origin: “de novo” synthesized FA (6:0 to 14:0) had higher heritability estimates (0.35–0.54) than FA originating from the diet and from body fat mobilization (long-chain and polyunsaturated FA) (0.09–0.21). These results are supported by Bastinet al. (2011). As other de novo FA, 16:0 has a greater heritability (0.31) than preformed FA (Stoop et al., 2008).

Stage of lactation

At the onset of lactation, dairy cows are in negative energy balance (energy expenditure exceeds intake) causing mobilization of FA from adipose tissue. The main FA contained in bovine adipose tissue are oleic acid (c9 18:1; $\approx 40\%$) and 16:0 ($\approx 30\%$; Christie, 1981). The mammary gland will take up these long-chain



performed FA and incorporate them into milk fat. The greater uptake of these long-chain FA during this period decreases the proportion of de novo synthesised FA in milk fat, due to both a dilution effect and inhibition of de novo synthesis of FA (Chilliard et al., 2000). Consequently, concentration of short- and medium-chain FA is relatively low at the onset of lactation and increases rapidly during the first two weeks and continues increasing at a slower rate until about 10 weeks into lactation, whereas the proportions of preformed long-chain FA essentially follow an opposite pattern with a progressive decrease in the first 10 weeks of lactation (Palmquist et al., 1993; Craninx et al., 2008; Bilal et al., 2014). Palmquist et al. (1993) established that concentration of de novo synthesized FA increased 117% to 200% in the first 16 weeks of lactation, whereas, during the same period, concentrations of preformed FA decreased by more than 30%. Because 16:0 is partly de novo synthesized in the mammary gland and is also released by mobilization of body fat when cows are in negative energy balance, concentration of this FA was not as affected by stage of lactation. Indeed, as for the other de novo synthesized FA, milk fat concentration of 16:0 increased in the first weeks of lactation, but because, during the first week of lactation, milk fat concentration of 16:0 was already at 75% of the concentration observed at week 16, the increase was occurring at a slower rate than what was observed for other de novo FA.

These results were later confirmed by Kay et al. (2005) who monitored differences in milk FA profile as lactation progressed in dairy cows of different genetic potential. In this experiment, milk fat concentration of 16:0 increased by 10% in the first 16 weeks of lactation compared to an increase varying between 20% and 114% for the other de novo synthesized FA. Also, in accordance with previous results, milk fat concentration of oleic acid (c918:1) decreased by 23% during the same period.

Contemporary data from Canadian cows follow the pattern reported in the literature cited above. Figure 1 shows the changes in the proportion of 16:0, 18:1, de novo and preformed fatty acids through lactation. Preformed fatty acids, including 18:1 are elevated in early lactation as a result of the mobilization of body reserves and decrease until about 100 days in milk. Conversely, the concentrations of de novo and 16:0 increase in parallel with the increase in dry matter intake in early lactation.

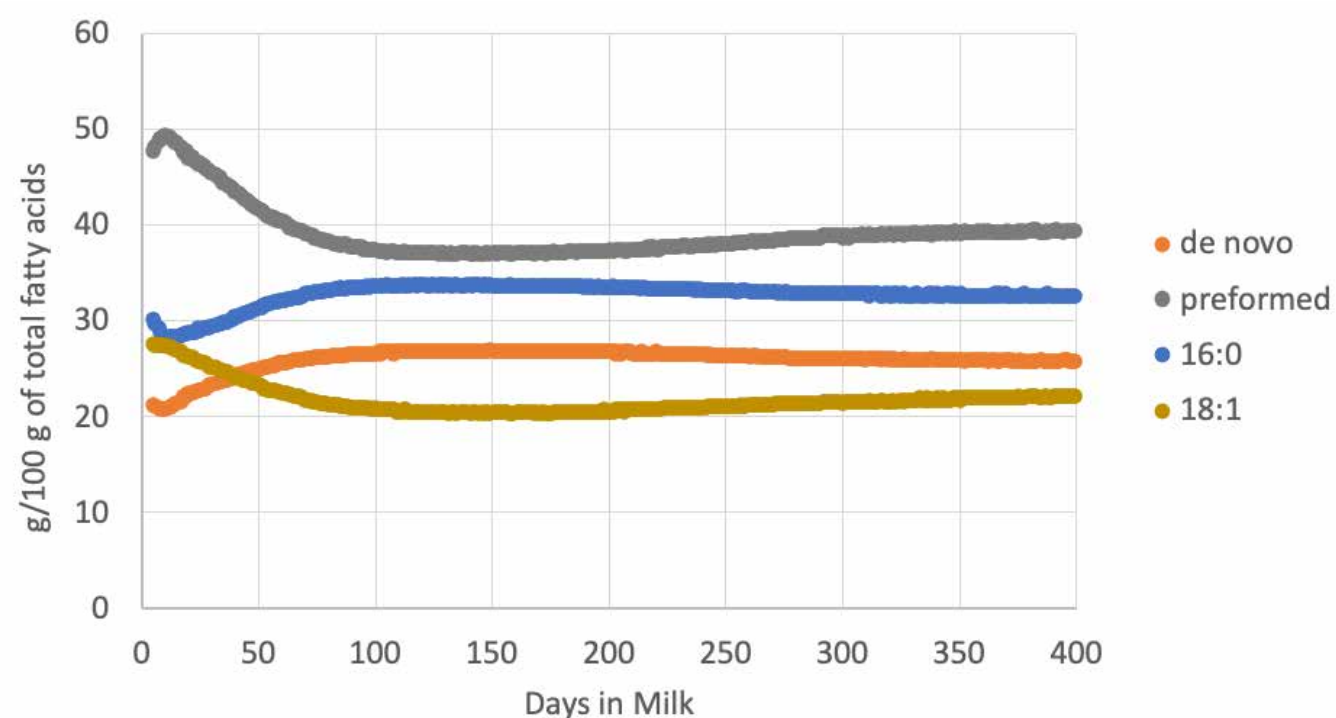


Figure 1. Changes in the proportion of fatty acids during lactation. Data collected from 345,786 cows in 3175 dairy herds in Quebec from February 2020 to October 2021. Lactanet (unpublished data).

Parity

Parity (the number of times a cow has had offspring) is another factor that can affect milk FA composition (Kelsey et al., 2003). Despite studies in cattle where no effect of parity was observed (Secchiari et al., 2003; Rani et al., 2011), results from many studies show that milk fat from primiparous cows (those bearing young for the first time) contains greater concentrations of unsaturated FA and lower proportion of saturated FA compared with multiparous cows (Thomson and Poel, 2000; Craninx et al., 2008; Bilal et al., 2014).

In 2014, Bilal et al. looked at the effects of parity on individual FA based on milk samples of Canadian Holsteins obtained from commercial herds. Their results show that in their first lactation, cows will produce a milkfat with relatively higher proportions of preformed FA such as c918:1, vaccenic acid (t1118:1), linoleic acid (18:2n6) and conjugated linoleic acid (c9t11CLA), whereas multiparous cows will produce a milkfat containing more de novo FA, namely 12:0, 14:0, and 16:0. These trends reported in the literature are consistent with the FA profile observed in cows on milk recording from Quebec herds as shown in figure 2.

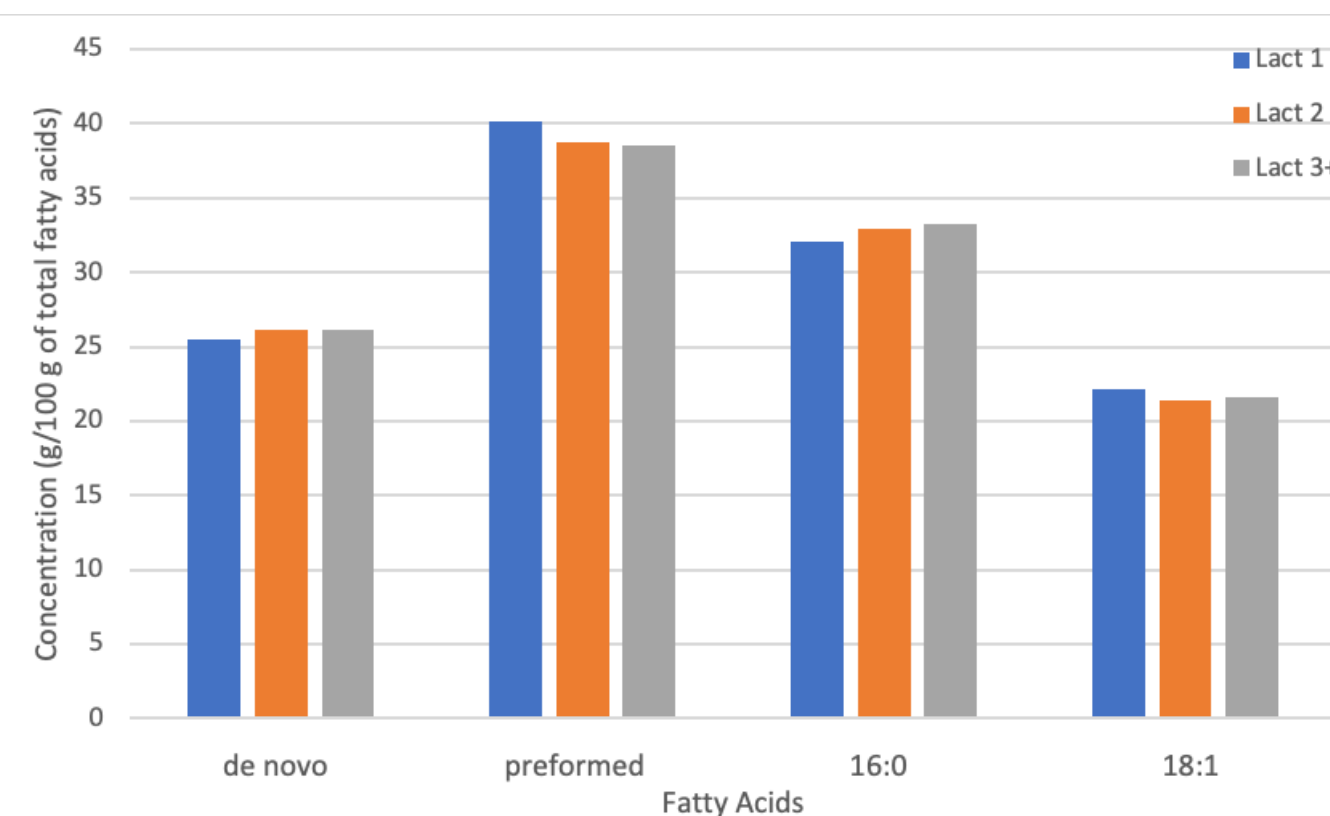


Figure 2. Average concentration of milk fatty acids by parity. Data collected from 345,786 cows in 3175 dairy herds in Quebec from February 2020 to October 2021. Lactanet (unpublished data).

The effect of parity on milk fat composition could be partly explained by the lower capacity of the mammary tissue for de novo FA synthesis in primiparous compared with multiparous cows. Miller et al. (2006) reported that the mammary expression of FA synthetase (FAS), a key enzyme implicated in mammary FA biosynthesis, was lower in primiparous cows.

Moreover, research suggested that parity can influence the pattern of changes in metabolic hormones and metabolites following calving which in turn can influence milk synthesis (Wathes et al., 2007), including lipogenesis by the mammary tissue.

Finally, because of the difference in dry matter intake and difference in nutrient requirements, primiparous and multiparous cows are commonly fed different diets, which, as described below, will affect milk FA composition. Therefore, the difference in diet composition could be an important factor explaining the difference observed in milk FA composition between primiparous and multiparous cows under commercial conditions (Bilal et al., 2014).

Environmental factors

Seasonal effects

In 2008, O'Donnell-Megaró et al. obtained samples of fluid milk from 56 milk processing plants across the U.S. every three months for one year to capture seasonal and geographical variations in U.S. milk FA composition (O'Donnell-Megaró et al., 2011). This survey demonstrated that season influences milk FA composition with each milk fat constituents being significantly affected. However, the authors concluded that differences were minor and that the overall FA composition was numerically similar for all seasons. They also concluded that the minor differences have little or no physiological or public health importance. Different studies have also been conducted in various countries, to establish seasonal effects on milk FA profile of retail milk fat or butter (Larsen et al., 2010; Kliem et al., 2013; Capuano et al., 2015; Paredes et al., 2018). From these studies, it is possible to observe that relative to summer, milk fat produced or sold during winter contains more saturated FA, mainly represented by an increase in 16:0 (+6 to +12 %) and lower cis-monounsaturated FA, mainly represented by c918:1 (-8 to -13%). These findings are similar to the data from Canada reported in the following section of this report. Nevertheless, Kliem et al. (2013) concluded that, when differences were applied to national dietary intakes, monthly variation in the FA composition of milk available at retail has limited influence on total dietary FA consumption by UK adults.

Organic versus conventional

Milk from organic farming is richer in 18:3n3 than milk from conventional farming, whereas a difference in c9t11CLA is less consistently observed (Jahreis et al., 1997; Kraft et al., 2003; Ellis et al., 2006). This high concentration in 18:3n-3 is very likely related to the larger use of legume plants in organic farming. Differences in the amounts of fresh forage and concentrates incorporated into diet can explain part of the differences reported in FA composition of milk fat produced on organic versus conventional dairy farms. However, Schwendel et al. (2015) observed similar differences in FA profile between production systems (organic versus conventional) even when both sets of cows were kept continuously on pasture. In contrast though, c9t11CLA concentration was greater in conventional milk compared with organic milk.

Feed factors

Diet is the main environmental factor influencing milk fat composition from lactating cows. Palmquist et al. (1993) offered a comprehensive review on what are the main feed factors that can affect milk FA composition. These factors will be revisited here, with a more specific emphasis on 16:0.

Grain intake / forage-to-concentrate ratio

Feeding starch from grains to dairy cows allows them to increase the energy density of their diet to support the energy demand required for milk production. However, when fed higher amounts (typically >50% of dry matter intake), starch from grains will inhibit milk fat synthesis and milk FA composition will be affected (Lock and Shingfield, 2004). Increasing the concentrate intake in the low range will increase different 18:1 isomers, as a result of incomplete rumen biohydrogenation, and at the same time favour milk 18:2n6 and mammary de novo FA, at the expense of 18:3n3 and c9t11CLA (Bargo et al., 2006). However, when concentrates are increased over 50-60%, ruminal biohydrogenation is altered leading to inhibition of milk fat synthesis (Bauman and Griinari, 2001; Lóor et al., 2005). Usually, this decrease in milk fat content, due to high grain diets, will be accompanied by a decrease in de novo FA, saturated FA, including 16:0, and an increase in 18:1 isomers and 18:2n6 (Palmquist et al. 1993).

However, no clear effect of grain intake can be drawn from the literature as the effect of starch intake on milk FA composition ultimately depends on the forage type included in the basal diet and dietary FA composition, among other factors (Lóor et al., 2005; Bargo et al., 2006; Sterk et al., 2011; Saliba et al., 2014).

Starch degradability

Regardless of the proportion of starch, the starch source itself may also affect milk FA composition, mainly due to the impact of starch degradability on ruminal biohydrogenation. In an experiment, Jurjanz et al. (2004) fed dairy cows total mixed rations with two sources of starch: slowly degradable (potatoes) or rapidly degradable (wheat). Cows receiving rapidly degradable starch produced a milk with a lower fat content, higher concentrations of 18:1 and with reduced proportions of de novo FA, including 16:0, compared with cows fed a slower degradable starch. Mohammed et al. (2010) observed that the greater fermentability of barley compared with corn resulted in an increased milk fat concentration in total saturated FA and total short-chain FA (4:0-10:0). However, in this experiment, starch degradability did not affect the concentrations of 16:0 and 14:0, whereas that of 18:0 was greater for a corn than for a barley-based diet.

Pasture

Fresh grass dry matter contains 13% FA, with the highest values during spring and autumn, and about conservely 50 to 75% of these FA as 18:3n3 (Bauchart et al., 1984; Boufaïed et al., 2003; Elgersma et al., 2006). Typically, milk fat from cows fed on pasture contains greater concentrations of 18:0, 18:1, 18:3, and CLA, whereas concentrations of 10:0, 12:0, 14:0, and 16:0 are lower compared with milk fat from cows receiving the forage portion of their diet as silage or hay (Chilliard et al., 2007; Villeneuve et al., 2013). These differences will increase as the portion of pasture in the total diet increases (Couvreur et al., 2006). On the other hand, the relatively high concentrations of 18:0, 18:1, 18:3 and CLA observed in milk fat from cows fed on pasture will decrease as the pasture matures, due to the decrease in FA and 18:3 concentrations in mature compared to young growing grass (Dewhurst et al., 2006; Ferlay et al., 2006).

Forage conservation method

Hay and grass silage

Wilting of the crop to a higher dry matter content is known to decrease FA and 18:3n3 concentrations due to oxidative loss and leaf shatter when harvesting forages. Consequently, lower concentrations of 18:3n3 are observed in grass hay compared to grass silage (Shingfield et al., 2005). However, Villeneuve et al. (2013) observed that, even though 18:3n3 concentration was almost twice higher in timothy silage, milk fat content of 18:3n3 was significantly lower for cows fed this silage compared with milk fat from cows fed timothy from the same field but harvested as hay. This result can be explained by a greater ruminal bypass of this FA for hay than for silage (Boufaïed et al., 2003).

Legume silage

Legume silage increases milk 18:2n6 and 18:3n3 concentrations due to legumes being rich in these polyunsaturated FA; the transfer efficiency of 18:3n3 from diet to milk being higher with red clover compared to grass silage (9% vs. 4.5%); passage rate through the rumen being higher for white clover; and red clover having a much lower lipolysis in the rumen than grass due to its polyphenol oxidase activity (Dewhurst et al., 2006).

Corn silage

Due to the important proportion of grain (30-40%) found in corn silage, this forage is rich in 18:2n6 and c918:1 but contains low concentrations of 18:3n3. Results from different experiments show that, compared with other forages, feeding corn silage to dairy cows increases the n6-to-n3 ratio of milk fat but has a limited impact on c918:1 and 18:0 concentrations (Chilliard et al., 2001). Other effects related to milk fat composition when corn silage is incorporated in the forage fraction of the diet are mostly related to ruminal biohydrogenation intermediates of 18:3n3 and 18:2n6 and the respective proportions of these dietary FA (Chilliard et al., 2001).

Amount and composition of dietary fat

Effects of dietary fat on milk fat composition have been studied comprehensively and continue to be. It has been long established that feeding lipid supplements to dairy cows inhibits key enzymes of de novo FA synthesis by the mammary tissue (Grummer, 1991). This inhibition results in a decrease in milk fat concentration of de novo FA, which is more extensive as the chain length of these milk FA increases (up to 12:0).

Another well established fact is that the effect of dietary lipid supplementation on milk FA composition is directly affected by i) the amount of lipid included in the diet; ii) the FA profile of lipid supplement; iii) the type of lipid supplement (processing, inertness, etc.); and iv) the nature of the basal diet (Kliem and Shingfield, 2007).

Plant oils or oilseeds

Increasing plant oils or oilseeds typically decreases concentrations of saturated FA and increases unsaturated FA in milk fat (see Table 1; adapted from Kliem and Shingfield, 2016). More specifically, when plant oils or oilseeds are added to the diet, a decrease in the milk fat concentration of de novo FA, including 16:0, is observed, whereas the concentration of 18:0 will be increased. Feeding plant oils or oilseeds will also elevate milk c918:1, due to both ruminal escape of c918:1 and an increased uptake of 18:0 which will serve as substrate for delta-9 desaturase in the mammary tissue, leading to endogenous synthesis of c918:1 (Kliem and Shingfield, 2016).

As reported by Kliem and Shingfield (2016), even though plant oils and oilseeds fed to ruminants contain high concentrations of polyunsaturated FA, mainly 18:2n6 and 18:3n3, the transfer of these dietary lipids to milk fat is very limited due to extensive biohydrogenation of dietary polyunsaturated FA in the rumen, and once absorbed, utilisation by other tissues. Consequently, even when oilseeds are fed in large amounts or over a long period, milk fat concentration is limited to 4% for 18:2n6, and 1.20% for 18:3n3 (Shingfield et al., 2013). Accordingly, feeding plant oils or oilseeds to dairy cows increases milk fat concentration in biohydrogenation 18:1, 18:2, and 18:3 intermediates, among which t1118:1 is typically the most abundant (Roy et al., 2006). This isomer will also serve as substrate for the endogenous synthesis of c9t11CLA by the delta-9 desaturase in mammary tissue. Increases in 18:1 isomers in milk fat in cows fed dietary lipid supplements is dependent on the amount and source of lipid fed and the composition of the basal diet (Table 1). Shift towards the t10 biohydrogenation pathway can occur when lipid supplements rich in 18:2n6 are included in high starch-containing diets (Shingfield et al., 2005).

The effects of dietary lipid supplements have been reported to be greater when dietary lipids are fed to early lactation cows compared with cows in established lactation, despite their greater mobilisation of FA from adipose tissue (Grummer, 1991; Chilliard et al., 2000).

Changes in milk fat 12:0, 14:0, 16:0, and, to a lesser extent, 18:0 concentrations in response to dietary lipid supplements are relatively stable, but this is not the case for isomers of 18:1 and CLA, for which increases are transitory when unsaturated FA are fed to dairy cows, because of shifts in biohydrogenation pathways (Bauman et al., 2000; Dhiman et al., 2000).

Saturated fatty acid supplements

The addition of fat supplements designed to have minimal effects on rumen fermentation to diets is a common practice in dairy nutrition to increase dietary energy content, feed efficiency, and the yields of milk and milk components (Rabiee et al., 2012) (see Chapter 5 for more details). In this regard, calcium salts from palm oil distillate have been used for many years to minimize the negative effect of unsaturated FA on ruminal fermentation (Palmquist, 1991). In a recent meta-analysis regrouping results from 33 published studies, dos Santos Neto et al. (2021) evaluated the effects of calcium salts of palm FA supplementation on nutrient digestibility and production responses of lactating dairy cows. Using meta-regression techniques, these researchers established that each 1 percentage unit increase of calcium salts of palm FA in diet dry matter decreased milk fat concentration in de novo FA by 2.05 g/100 g of FA, and increased concentrations of 16C FA by 0.51 and preformed FA by 1.85 g/100 g of FA.

In recent years, an increase interest has been observed for saturated FA supplements because of their rumen inertness, their reduced impact on dry matter intake compared with other lipid supplements (Allen, 2000), and their positive effect on milk and milk fat and protein yield (Hu et al., 2017). Most saturated FA supplements commercially available contain different proportions of two main FA's, namely 16:0 and 18:0. The proportion of each of these FA in the dietary lipid supplement can modulate the responses in intake, production and nutrient digestibility, but also on milk FA composition. However, quantifying the transfer of each FA from diet into milk fat is a challenge due to de novo synthesis of 16:0, and desaturation of 18:0 to c918:1 in the mammary tissue. Moreover, incorporation of these FA appears to be selectively limited by the required balance between saturated and unsaturated FA to maintain milk fluidity and ensure secretion by the mammary tissue (Loften et al., 2014).

Typically, supplements commercially available can be categorized into five groups based on their saturated FA composition:

- i) a mix of 16:0 + 18:0
- ii) 16:0 (85% pure)
- iii) 18:0 (85% pure)
- iv) 16:0 (99% pure)
- v) 18:0 (99% pure)

Moreover, some of these FA supplements contain varying levels of c918:1, which can affect their digestibility, and consequently, their impact on cow performance and milk composition.

Only a few studies looked at the effect of feeding a pure (>85%) 18:0 fat supplement to dairy cattle. Recently, Shepardson and Harvatine (2021) observed a 20% increase in milk fat concentration of 18:0, and of 10% in c918:1 when feeding enriched-18:0 supplement at 2%. These results, though of less amplitude, are consistent with the previous work from Noble et al. (1969) where a 165% increase in 18:0, a 64% increase in 18:1, and a decrease of about 23% in 16:0 was observed when feeding 5% of 18:0-enriched supplement. In both studies, milk fat concentration of short-chain FA also decreased.

Rico et al. (2014) compared feeding highly purified 16:0 and 18:0 (>97%) to early lactation cows at a rate of 642 and 646 g/d respectively and found that milk FA proportions of 6:0 to 14:0 were significantly lower for the 16:0 treatment compared with the 18:0 treatment. On the other hand, milk fat concentration of 16:0 was significantly increased by feeding 16:0. These results agree with the earlier work of Noble et al. (1969), and Steele and Moore (1968) and indicate that lipid supplements enriched in 16:0 have a stronger inhibiting effect on de novo synthesis compared with supplements enriched in 18:0.

The current interest in the commercial dairy industry in feeding a concentrated form of 16:0 (>85%) to lactating dairy cows is due to the more consistent stimulating effect of these dietary supplements on milk fat content (Loften et al., 2014). As reported in Table 2, incorporating these supplements in dairy rations increased milk fat concentration in 16:0 from 5.5% to 12.3%, for an average of approximately 8.1% for each 1-percentage-unit increase of 16:0-enriched supplement in dry dairy matter.

Table 1. Effect of plant oils and oilseeds in the diet on the milk fatty acid composition of lactating cows. Adapted from Kliem and Shingfield (2016)

Lipid source	Intake ^a (g/d)	Forage ^b	F:C ^c	Milk fatty acid composition (g/100 g of fatty acids)											Reference		
				4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	c9-18:1	trans-18:1	18:2n-6		CLA ^d	18:3n-3
Control	0			2.95	2.02	1.18	2.68	3.06	9.79	30.7	9.12	21.2	2.26	3.59	0.46	0.5	
Palm oil by-product	476	LH/LS (55:45)	44:56	3.09	1.88	0.99	2.12	2.42	8.63	39.1	6.83	19.3	1.79	3.17	0.4	0.41	Mosley et al., 2007
Palm oil by-product	887			3.09	1.77	0.89	1.85	2.11	7.96	44	5.79	17.9	1.54	2.99	0.34	0.36	
Palm oil by-product	1248			3.03	1.72	0.85	1.78	2.04	7.89	45.6	4.95	17.4	1.44	3.11	0.3	0.36	
Control	0	MS/LS/LH (66/19/16)	39:61	2.95	2.11	1.31	3.43	4.11	12.4	33	8.77	17.1	1.97		NR		Plantoni et al., 2013
Prilledpalmitticacid	556			2.94	2.02	1.21	3.1	3.68	11.3	37.6	7.77	16.4	1.81		NR		
Control	0	MS/LS/ LH/GH (66/19/8/8)	49:51	2.96	1.95	1.14	2.97	3.51	11.7	35.2	8.67	19	1.65	2.11	0.3	0.27	Lock et al., 2013
Palm oilfattyacids	NR			2.93	1.8	0.99	2.43	2.81	10.4	41.8	7.41	18	1.39	1.89	0.26	0.23	
Control	0	LH/LS/BS (26:48:26)	53:47	3.29	2.13	1.3	3.25	4.08	12.3	32.6	9.22	14.8	2.83	2.26	0.23	0.4	Hristov et al., 2009
Coconutoil	530			3.14	1.9	1.15	2.93	8.54	14.7	30.8	6.94	12.4	3.79	1.87	0.25	0.28	
Ca salts of palm oil	950			3.3	2.3	1.3	2.7	2.9	10	34.5	9.8	18.6	4.1	2.25	0.57	0.25	
Wholerapeseed	1186	MS/GS (75:25)	50:50	3.3	2.5	1.5	3.3	3.6	11.7	31.1	10.8	17	3.2	1.76	0.44	0.23	Givens et al., 2009
Milledrapeseed	1147			3.1	2.2	1.2	2.4	2.6	9.6	21.6	15.5	23	6.4	1.73	0.86	0.27	
Rapeseedoil	1044			2.7	1.8	0.9	1.9	2.2	8.7	19.8	14.6	24.3	10	1.78	1.31	0.22	
Ca salts of palm oil	826			3.5	2.4	1.2	2.4	2.5	9.2	33.7	9.1	21.1	3.3	2.56	0.6	0.23	
Milledrapeseeds	847			3.2	2.4	1.3	2.6	2.9	10.3	24.3	13.3	22.7	4.8	1.97	0.58	0.29	
Milledrapeseeds	1127			3	2.3	1.2	2.5	2.7	9.9	21.2	15.2	24.8	5.2	1.87	0.66	0.29	
Milledrapeseeds	1345	MS/ GS(75:25)	50:50	3.2	2.2	1.1	2.1	2.3	8.8	20.2	16.1	26.9	5.7	1.82	0.71	0.28	Kliem et al., 2011
Milledoleicrapeseeds	865			3.3	2.4	1.2	2.4	2.6	9.7	21.9	14.8	25.1	4.9	1.85	0.57	0.22	
Milledoleicrapeseeds	1113			2.8	2.3	1.2	2.4	2.6	9.2	20	17	26.1	5.1	1.67	0.64	0.19	
Milledoleicrapeseeds	1345			2.9	1.9	0.9	1.9	2.1	8.3	19.2	16.7	28.9	5.9	1.61	0.61	0.17	
Control	0			2.76	1.64	0.99	2.39	2.93	10.5	24.9	11.2	20.7	6.64	2.79	0.67	0.36	
Rapeseedexpeller	609	MS/LH/GH (78:14:8)	57:43	2.58	1.46	0.84	1.93	2.38	9.26	23.4	12.5	22.7	8.17	2.58	0.68	0.36	Hristov et al., 2011
Oleicrapeseedexpeller	515			2.71	1.5	0.86	1.94	2.4	9.13	22.9	11.9	23.5	8.34	2.45	0.91	0.3	
Erucicacidrapeseedexpeller	648			2.56	1.47	0.86	1.94	2.39	8.96	21	11.6	23.1	6.43	2.52	0.49	0.36	
Control	0			NR	1.82	1.21	2.99	3.9	13.2	35	10.4	17.3	2.9	2.02	1.92	0.38	
Soybeanoil	352	MS/Haylage (60:40)	53:47	NR	1.62	1.06	2.5	3.24	12.3	33.3	11.1	18.8	4.32	2.4	2.24	0.42	Alzahal et al., 2008
Soybean oil	732			NR	1.24	0.78	1.82	2.5	10.9	30.9	11.5	21.6	6.41	2.57	2.35	0.4	
Control	0	MS/LS/WS (62/32/6)	56:44	2.89	2.13	1.35	3.68	4.55	13.2	34.3	7.29	0.47	1.69	2.23	0.35	0.37	Boerman and Lock, 2014
Soybean oil	534			2.99	2.02	1.22	3.02	3.54	11.5	26.6	10.2	0.52	4.52	2.99	0.83	0.45	
Control	0	MS/GH	48:52	3.35	2.67	1.51	3.46	3.94	12.1	32.3	8.63	16.6	2.8	2.19	0.55	0.21	
Sunflower oil	957			2.31	1.21	0.54	1.2	1.65	7.06	18.9	13.6	28.3	11.5	2.34	0.93	0.2	Roy et al., 2006
Control	0	MS	27:73	3.28	2.7	1.62	4.26	5.14	12.8	28.7	5.77	14.9	5.19	2.96	0.6	0.09	
Sunflower oil	755			1.84	1.03	0.48	1.16	1.81	7.36	19.1	6.27	19.4	23.7	4.59	1.17	0.15	
Control	0			4.12	2.37	1.19	2.53	2.87	11.6	30.6	9.76	17.7	4.58	1.75	0.68	0.41	
Safflower oil	1125	BS/LS/LH	60:40	2.77	1.39	0.63	1.26	1.53	8.1	18.7	11.4	17.7	17.6	2.89	4.12	0.32	Bell et al., 2006 ^e
Linseed oil	1066			3.23	1.56	0.7	1.38	1.64	8.48	17.9	11.1	19.2	14.3	2.01	2.8	0.73	
Control	0			1.87	1.4	0.97	2.33	2.83	10.2	24.1	14.3	22.1	4.95	1.12	1.19	0.6	
Rapeseed oil	500	Pasture	(5)	1.58	0.99	0.59	1.29	1.63	6.9	18	17.3	30.6	7.51	0.98	1.14	0.38	
Sunflower oil	500			1.38	0.87	0.53	1.15	1.52	6.61	18.2	16.8	29.6	8.78	1.25	1.61	0.42	Rego et al., 2009
Linseed oil	500			1.67	1.06	0.65	1.41	1.75	7.09	17	16.8	26.5	9.67	0.99	1.54	0.53	

Lipid source	Intake ^a (g/d)	Forage ^b	F:C ^c	Milk fatty acid composition (g/100 g of fatty acids)												Reference	
				4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	c9-18:1	trans-18:1	18:2n-6	CLA ^d		18:3n-3
Control	0		100	2.72	2.13	1.42	3.16	3.91	11.4	29.4	9.1	19.1	3.18	1.49	0.64	0.55	
Rapeseed oil	408	MS/GS (81:19)	64:36	2.52	1.8	1.14	2.49	3.17	10.5	27.6	8.75	21.3	6.08	1.49	0.93	0.35	Jacobs et al., 2011
Soybean oil	389			2.57	1.86	1.12	2.36	2.84	10.2	26.6	10.91	21.2	6.27	1.9	1.09	0.45	
Linseed oil	392			2.48	1.93	1.22	2.65	3.27	10.5	23.8	9.71	21.3	6.69	1.47	1.07	0.66	
Ca salts of palm oil	NR			0.64	1	0.77	1.57	2.07	8.59	36.2	8.76	22.6f	3.49	2.15	0.53	0.19	
Crushed sunflower seeds	600	Barley silage	45:55	0.48	1.05	0.83	1.69	2.19	8.65	22.3	14.32	28.0f	5.2	2.82	0.99	0.18	Mohammed et al., 2011
Crushed linseed	594			0.58	1.15	0.91	1.91	2.43	8.88	22.6	13.54	26.6f	4.36	2.42	0.7	1.01	
Crushed canola	640			0.48	1.2	0.93	2.05	2.59	9.75	22.8	13.94	28.0f	3.62	2.14	0.63	0.3	
Control	0			3.35	1.76	1.23	3.19	3.91	13	32.4	7.63	13.2	4.02	2.08	0.44	1.1	
Rapeseed oil	310			3.54	1.72	1.14	2.75	3.24	11.8	27.3	10.4	17.3	5.2	1.99	0.56	1.02	
Sunflower oil	280	RCS	55:45	3.58	1.71	1.14	2.76	3.24	11.7	26.5	10.9	16.6	5.55	2.55	0.64	0.99	Halmemies-Beauchet-Filleau et al., 2011
Camelina oil	300			3.57	1.69	1.14	2.72	3.2	11.6	27.1	9.86	16.5	4.91	2.1	0.57	1.17	
Camelina expeller	210			3.67	1.69	1.09	2.57	3.08	11.9	26.8	7.33	13.5	8.28	1.98	1.02	1.06	
Control	0			2.44	1.95	1.34	3.33	4.13	13	37	6.61	16.4	2.95	1.86	0.82	0.2	
Camelina seeds	630	MS	58:42	2.38	1.84	1.27	3.16	4.04	13	32.2	6.09	17.3	6.93	2.2	0.93	0.32	Hurtaud and Peyraud, 2007
Camelina expeller	630			1.4	0.99	0.61	1.59	2.61	11.8	31.9	3.4	14.1	16.35	1.89	1.48	0.36	
Control	0	GS	60:40	3.1	1.9	1.12	2.66	3.24	12.1	34.4	8.78	16.4	2.23	1.33	0.29	0.45	Bayat et al., 2015
Camelina oil	930			3.18	1.56	0.79	1.55	1.84	8.1	21.3	12.9	23.5	6.71	1.09	0.73	0.49	
Control	0	GH	64:36	2.96	2.31	1.43	3.54	4.22	13.1	34.9	6.99	14.2	2.11	1.58	0.54	0.74	Roy et al., 2006
Linseed oil	1050			2.82	1.76	0.84	1.77	1.96	8.29	17.1	12.8	20.6	1.22	1.16	2.89	0.74	
Control	0	MS/LH (88:12)	69:31	2.69	2.16	1.33	2.74	3.95	11.4	33.6	9.4	18.7	3.18	1.92	0.59	0.27	
Whole linseed	1311			2.69	2.01	1.23	2.37	3.15	9.15	24.4	14.2	21.8	6.61	1.7	0.84	0.95	Akram et al., 2007
Extruded linseed	943			2.57	1.82	1.06	2.06	2.54	9.95	23.3	14.4	22	8.91	1.62	1.12	1.2	
Control	0	Pasture		1.72	1.59	0.94	1.69	1.87	7.91	23.3	12.1	25.4	6.19	1.93	1.25	0.59	
Linseed oil	170			1.72	1.53	0.92	1.64	1.75	7.48	22.7	12.4	25.7	6.76	1.96	1.36	0.78	Flowers et al., 2008 ^g
Linseed oil	340			1.57	1.42	0.83	1.51	1.69	7.38	21.2	12.3	25.1	8.17	1.97	1.6	1.01	
Linseed oil	510			1.4	1.22	0.71	1.31	1.51	6.71	19.8	12.2	25.8	9.43	1.95	1.88	1.03	
Control	0	MS/GH (90:10)	65:35	3.13	2.24	1.41	3.37	4.22	12.6	29.1	8.32	17.4	3.49	1.69	0.77	0.67	
Whole linseed	559			3.11	2.14	1.24	2.74	3.22	10.8	25	13.7	23.5	2.13	1.28	0.44	0.65	Chilliard et al., 2009
Extruded linseed	497			2.78	1.64	0.89	1.89	2.36	8.83	19.6	11.7	22.4	9.95	1.61	1.27	1.2	
Linseed oil	721			2.05	1.06	0.54	1.09	1.52	5.88	15.9	11.3	26.3	10.6	1.53	0.65	0.54	
Control	0	MS/GH (88:12)	69:31	2.57	2.25	1.5	3.77	4.5	13.9	38.1	6.92	13.3	2.26	1.35	0.38	0.22	Ferlay et al., 2010
Extruded linseed	670			2.58	1.96	1.2	2.69	2.95	9.66	20	14.1	21	9.61	1.22	1.51	1.05	

» CLA, conjugated linoleic acid; NR, not reported.

» ^a Intake of oil from dietary lipid supplements. ^b BS, barley silage; GH, grass hay; GS, grass silage; LH, lucerne hay; LS, lucerne silage; MS, maize silage; RCS, red clover silage, WS, wheat straw. ^c Forage:concentrate ratio of the diet (on a dry matter basis). For studies in grazing cows the amount of concentrate supplements fed (kg/d) is reported in parentheses. ^d Sum of t7c9 CLA, t8c10 CLA and c9t11 CLA. ^e Fatty acid concentrations reported as g/100 g fatty acid methyl esters. ^f Co-eluted with c10 and t15 18:1. ^g Cows consumed 7 kg concentrates per day (fresh weight basis).



Table 2. Milk fatty acid concentration of 16:0 (g/100 g of total fatty acids) of cows fed non-fat supplemented control diets (CON) or diets supplemented with 16:0 enriched lipid supplement (PALM).

REFERENCE	TREATMENT	DRY MATTER INTAKE, KG/D	INTAKE OF DIETARY 16:0 SUPPLEMENT		MILK FATTY CONCENTRATION OF 16:0 (G/100 G OF FATTY ACIDS)	% OF INCREASE ^A
			% OF DRY MATTER	G/D		
de Souza et al., 2017	CON	26.6	0	0	35.1	+8.2%
	PALM	26	1.7	442	40.8	
de Souza and Lock, 2018a	CON	28.4	0	0	33.7	+12.3%
	PALM	30.3	1.2	368	39.6	
de Souza and Lock, 2018b	CON	29.5	0	0	32.7	+7.7%
	PALM	29.6	1.2	342	35.9	
de Souza and Lock, 2019	CON	29.2	0	0	30.1	
	PALM-FA	29.1	1.9	560	36.3	+8.9%
	PALM-TG	27.8	1.8	500	33.4	+5.5%
Piantoni et al., 2013	CON	27.8	0	0	33.0	+6.2%
	PALM	27.8	2.0	550	37.6	
Average						+8.1%

» ^A Percentage of increase in milk fat concentration of 16:0 for each 1-percentage-unit increase of 16:0-enriched supplement in diet dry matter.

Ionophores

Feeding ionophores such as monensin to dairy cows has been reported to reduce the efficiency of ruminal biohydrogenation of long-chain FA, resulting in an inhibition of milk fat synthesis (Alzahal et al., 2008). However, Odongo et al. (2007) reported that long-term feeding of monensin reduced the percentage of some short and medium chain saturated FA, including 16:0. On the other hand, long-term feeding of monensin increased the percentage of long-chain saturated FA, biohydrogenation isomers, as well as n6 and n3 polyunsaturated FA. A meta-analysis by Duffield et al. (2008) summarized that monensin was associated with a reduction of short-chain FA (from 1% to 12% reduction) and 18:0 (-7.8%). The impact of monensin on 18:2 and 18:3 was variable, but monensin increased CLA (22%). Based on these results, we can conclude that feeding monensin limits rumen biohydrogenation of unsaturated FA, but these effects appear to be changing over time, and interactions with other factors are possible.

Other factors

Research is currently exploring the impact of feed management practices such as crowding at the bunk, stall stocking density, feed mixing, and feeding frequency on milk fatty acid composition. Despite that management practices, such as higher stall stocking density and lower feeding frequency have been related to lower de novo FA content in bulk tank milk (Woolpert et al., 2016, 2017), no clear effect of management has yet been identified with clear variations in milk fat concentration of 16:0.

Chapter Summary

- Palmitic acid (16:0) is the predominant fatty acid in milk, irrespective of what a cow is fed. It occurs naturally.
- Proportion of FA components of total fat has a moderate heritability. As other de novo FA, 16:0 has a greater heritability than preformed FA. Consequently, milk fat composition could respond to genetic selection.
- Milk fat concentration of de novo synthesized FA increases during the first 16 weeks of lactation, and so is concentration of 16:0. However, due to its dual origin (de novo and preformed), the increase in 16:0 at the onset of lactation occurs at a slower rate than what is observed for other de novo FA.
- Due partly to the lower capacity of the mammary tissue for de novo FA synthesis in primiparous compared with multiparous cows, milk fat concentration in 16:0 usually increases with parity.
- Research from around the globe has established that relative to summer, milk fat produced or sold during winter typically contains more 16:0 and less c18:1. However, when differences are applied to national dietary intakes, monthly variation in the FA composition of milk available at retail has limited influence on total dietary FA consumption.
- Diet is the main environmental factor influencing milk fat composition from lactating cows.
- Usually, high grain diets will reduce milk fat concentration and this decrease will be accompanied by a decrease in de novo and saturated FA, including 16:0. However, the effect of starch intake on milk FA composition will depend on the forage type included in the basal diet, dietary FA composition and starch degradability, among other factors.
- Typically, milk fat from cows fed on pasture contains greater concentrations of 18:0, 18:1, 18:3n-3, and CLA, whereas concentrations of de novo FA, including 16:0, are lower.
- Feeding lipid supplements to dairy cows inhibits key enzymes of de novo FA synthesis which results in a decrease in milk fat concentration of de novo FA, which is more extensive as the chain length of these milk FA increases (up to 12:0).

- The effect of dietary lipid supplementation on milk FA composition is directly affected by i) the amount of lipid included in the diet; ii) the FA profile of lipid supplement; iii) the type of lipid supplement; and iv) the nature of the basal diet.
- Saturated FA supplements are considered as an interesting source of energy for dairy cows because of their rumen inertness, their reduced impact on dry matter intake compared with other lipid supplements, and their positive effect on milk and milk fat and protein yield.
- Most of saturated FA supplements commercially available contain different proportions of two main FA, namely 16:0 and 18:0.
- Milk fat concentration in 16:0 increases of 8.1% for each 1-percentage-unit increase of 16:0-enriched supplement in diet dry matter, on average when a concentrated form of 16:0 is incorporated in dairy ration.
- Feeding cows supplements that contain palm by-products is not the main factor contributing to 16:0 in milk.

References

- » Akraim, F., M.-C. Nicot, P. Juaneda, and F. Enjalbert. 2007. Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma and milk fat of cows fed raw or extruded linseed. *Animal* 1:835–843. doi:10.1017/S175173110700002X.
- » Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science* 83:1598–1624. doi: 10.3168/jds.S0022-0302(00)75030-2.
- » Alzahal, O., N.E. Odongo, T. Mutsvangwa, M.M. Or-Rashid, T.F. Duffield, R. Bagg, P. Dick, G. Vessies, and B.W. McBride. 2008. Effects of monensin and dietary soybean oil on milk fat percentage and milk fatty acid profile in lactating dairy cows. *Journal of Dairy Science* 91:1166–1174. doi:10.3168/jds.2007-0232.
- » Arnould, V.M.-R., and H. Soyeurt. 2009. Genetic variability of milk fatty acids. *Journal of Applied Genetics* 50:29–39. doi:10.1007/BF03195649.
- » Bargo, F., J.E. Delahoy, G.F. Schroeder, and L.D. Muller. 2006. Milk fatty acid composition of dairy cows grazing at two pasture allowances and supplemented with different levels and sources of concentrate. *Animal Feed Science and Technology* 125:17–31. doi:10.1016/j.anifeedsci.2005.05.010.
- » Bastin, C., N. Gengler, and H. Soyeurt. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. *Journal of Dairy Science* 94:4152–4163. doi:10.3168/jds.2010-4108.
- » Bauchart, D., R. Vérité, and B. Remond. 1984. Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. *Canadian Journal of Animal Science* 64:330. doi:10.4141/cjas84-285.
- » Bauman, D.E., D.M. Barbano, D.A. Dwyer, and J.M. Griinari. 2000. Technical note: Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. *Journal of Dairy Science* 83:2422–2425. doi:10.3168/jds.S0022-0302(00)75131-9.
- » Bauman, D.E., and J.M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livestock Production Science* 70: 1529. doi:10.1016/S0301-6226(01)00195-6.
- » Bayat, A.R., P. Kairenius, T. Stefański, H. Leskinen, S. Comtet-Marre, E. Forano, F. Chaucheyras-Durand, and K.J. Shingfield. 2015. Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae*) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *Journal of Dairy Science* 98:3166–3181. doi:10.3168/jds.2014-7976.
- » Beaulieu, A.D., and D.L. Palmquist. 1995. Differential Effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. *Journal of Dairy Science* 78:1336–1344. doi:10.3168/jds.S0022-0302(95)76755-8.

- » Bell, J.A., J.M. Griinari, and J.J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. *Journal of Dairy Science* 89:733–748. doi:10.3168/jds.S0022-0302(06)72135-X.
- » Bilal, G., R.I. Cue, A.F. Mustafa, and J.F. Hayes. 2014. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. *Canadian Journal of Animal Science* 94:401–410. doi:10.4141/CJAS2013-172.
- » Bitman, J., D.L. Wood, R.H. Miller, H.F. Tyrrell, C.K. Reynolds, and H.D. Baxter. 1996. Comparison of milk and blood lipids in Jersey and Holstein cows fed total mixed rations with or without whole cottonseed. *Journal of Dairy Science* 79:1596–1602. doi:10.3168/jds.S0022-0302(96)76522-0.
- » Bobe, G., J.A. Minick Bormann, G.L. Lindberg, A.E. Freeman, and D.C. Beitz. 2008. Short communication: Estimates of genetic variation of milk fatty acids in US Holstein cows. *Journal of Dairy Science* 91:1209–1213. doi:10.3168/jds.2007-0252.
- » Boerman, J.P., and A.L. Lock. 2014. Effect of unsaturated fatty acids and triglycerides from soybeans on milk fat synthesis and biohydrogenation intermediates in dairy cattle. *Journal of Dairy Science* 97:7031–7042. doi:10.3168/jds.2014-7966.
- » Boufaïed, H., P.Y. Chouinard, G.F. Tremblay, H. v Petit, R. Michaud, and G. Bélanger. 2003. Fatty acids in forages. II. In vitro ruminal biohydrogenation of linolenic and linoleic acids from timothy. *Canadian Journal of Animal Science* 83:513–522. doi:10.4141/A02-099
- » Capuano, E., R. Gravink, R. Boerrigter-Eenling, and S.M. van Ruth. 2015. Fatty acid and triglycerides profiling of retail organic, conventional and pasture milk: Implications for health and authenticity. *International Dairy Journal* 42:58–63. doi:10.1016/j.idairyj.2014.11.002.
- » Chilliard, Y., A. Ferlay, and M.D. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science* 70.1-2 (2001): 31-48. doi:10.1016/S0301-6226(01)00196-8.
- » Chilliard, Y., A. Ferlay, R.M. Mansbridge, M. Doreau, and Y. Chilliard. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. In *Annales de zootechnie* (Vol. 49, No. 3, pp. 181-205). EDP Sciences. Doi:10.1051/animres:2000117.
- » Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* 109:828–855. doi:10.1002/ejlt.200700080.
- » Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. *Journal of Dairy Science* 92:5199–5211. doi:10.3168/jds.2009-2375.
- » Christie, W.W. 1981. The composition, structure and function of lipids in the tissues of ruminant animals. *Lipid metabolism in ruminant animals*: 95-191. doi:10.1016/B978-0-08-023789-3.50008-8.
- » Couvreur, S., C. Hurtaud, C. Lopez, L. Delaby, and J.L. Peyraud. 2006. The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *Journal of Dairy Science* 89:1956–1969. doi:10.3168/jds.S0022-0302(06)72263-9.
- » Craninx, M., A. Steen, H. van Laar, T. van Nespen, J. Martin-Tereso, B. de Baets, and V. Fievez. 2008. Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. *Journal of Dairy Science* 91:2662–2677. doi:10.3168/jds.2007-0656.
- » DePeters, E.J., J.F. Medrano, and B.A. Reed. 1995. Fatty acid composition of milk fat from three breeds of dairy cattle. *Canadian Journal of Animal Science* 75:267–269. doi:10.4141/cjas95-040.
- » de Souza, J., J.L. Garver, C.L. Preseault, and A.L. Lock. 2017. Short communication: Effects of prill size of a palmitic acid-enriched fat supplement on the yield of milk and milk components, and nutrient digestibility of dairy cows. *Journal of Dairy Science* 100:379–384. doi:10.3168/jds.2016-11610.
- » de Souza, J., and A.L. Lock. 2018a. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. *Journal of Dairy Science* 101:3044–3056. doi:10.3168/jds.2017-13946.
- » de Souza, J., and A.L. Lock. 2018b. Short communication: Comparison of a palmitic acid-enriched triglyceride supplement and calcium salts of palm fatty acids supplement on production responses of dairy cows. *Journal of Dairy Science* 101:3110–3117. doi:10.3168/jds.2017-13560.
- » de Souza, J., and A.L. Lock. 2019. Milk production and nutrient digestibility responses to triglyceride or fatty acid supplements enriched in palmitic acid. *Journal of Dairy Science* 102:4155–4164. doi:10.3168/jds.2018-15690.
- » Dewhurst, R.J., K.J. Shingfield, M.R.F. Lee, and N.D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* 131:168–206. doi:10.1016/j.anifeedsci.2006.04.016.
- » Dhiman, T.R., L.D. Satter, M.W. Pariza, M.P. Galli, K. Albright, and M.X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *Journal of Dairy Science* 83:1016–1027. doi:10.3168/jds.S0022-0302(00)74966-6.
- » dos Santos Neto, J.M., J. de Souza, and A.L. Lock. 2021. Effects of calcium salts of palm fatty acids on nutrient digestibility and production responses of lactating dairy cows: A meta-analysis and meta-regression. *Journal of Dairy Science*. doi:10.3168/jds.2020-19936.
- » Duffield, T.F., A.R. Rabiee, and I.J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. *Journal of Dairy Science* 91:1347–1360. doi:10.3168/jds.2007-0608.
- » Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. *Animal Feed Science and Technology* 131:207–225. doi:10.1016/j.anifeedsci.2006.06.012.
- » Ellis, K.A., G. Innocent, D. Grove-White, P. Cripps, W.G. McLean, C. v Howard, and M. Mihm. 2006. Comparing the fatty acid composition of organic and conventional milk. *Journal of Dairy Science* 89:1938–1950. doi:10.3168/jds.S0022-0302(06)72261-5.
- » Ferlay, A., B. Martin, S. Lerch, M. Gobert, P. Pradel, and Y. Chilliard. 2010. Effects of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning v. evening milking on milk fatty acid profiles in Holstein and Montbéliarde cows. *Animal* 4:627–640. doi:10.1017/S1751731109991224.
- » Ferlay, A., B. Martin, P. Pradel, J.B. Coulon, and Y. Chilliard. 2006. Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbéliarde cow breeds. *Journal of Dairy Science* 89:4026–4041. doi:10.3168/jds.S0022-0302(06)72446-8.
- » Flowers, G., S.A. Ibrahim, and A.A. AbuGhazaleh. 2008. Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. *Journal of Dairy Science* 91:722–730. doi:10.3168/jds.2007-0410.
- » Gibson, J.P. 1991. The potential for genetic change in milk fat composition. *Journal of Dairy Science* 74:3258–3266. doi:10.3168/jds.S0022-0302(91)78511-1.
- » Givens, D.I., K.E. Kliem, D.J. Humphries, K.J. Shingfield, and R. Morgan. 2009. Effect of replacing calcium salts of palm oil distillate with rapeseed oil, milled or whole rapeseeds on milk fatty-acid composition in cows fed maize silage-based diets. *Animal* 3:1067–1074. doi:10.1017/S175173110900442X.
- » Grummer, R.R. 1991. Effect of feed on the composition of milk fat. *Journal of Dairy Science* 74:3244–3257. doi:10.3168/jds.S0022-0302(91)78510-X.
- » Halmemies-Beauchet-Filleau, A., T. Kokkonen, A.-M. Lampi, V. Toivonen, K.J. Shingfield, and A. Vanhatalo. 2011. Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. *Journal of Dairy Science* 94:4413–4430. doi:10.3168/jds.2010-3885.
- » Hristov, A.N., C. Domitrovich, A. Wachter, T. Cassidy, C. Lee, K.J. Shingfield, P. Kairenius, J. Davis, and J. Brown. 2011. Effect of replacing solvent-extracted canola meal with high-oil traditional canola, high-oleic acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science* 94:4057–4074. doi:10.3168/jds.2011-4283.

- » Hristov, A.N., M. vander Pol, M. Agle, S. Zaman, C. Schneider, P. Ndegwa, V.K. Vaddella, K. Johnson, K.J. Shingfield, and S.K.R. Karnati. 2009. Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *Journal of Dairy Science* 92:5561–5582. doi:10.3168/jds.2009-2383.
- » Hu, W., J.P. Boerman, and J.M. Aldrich. 2017. Production responses of Holstein dairy cows when fed supplemental fat containing saturated free fatty acids: A meta-Analysis. *Asian-Australasian Journal of Animal Sciences* 30:1105–1116. doi:10.5713/ajas.16.0611.
- » Hurtaud, C., and J.-L. Peyraud. 2007. Effects of feeding camelina (seeds or meal) on milk fatty acid composition and butter spreadability. *Journal of Dairy Science* 90:5134–5145. doi:10.3168/jds.2007-0031.
- » Jacobs, A.A.A., J. van Baal, M.A. Smits, H.Z.H. Taweel, W.H. Hendriks, A.M. van Vuuren, and J. Dijkstra. 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. *Journal of Dairy Science* 94:874–887. doi:10.3168/jds.2010-3511.
- » Jahreis, G., J. Fritsche, and H. Steinhart. 1997. Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutrition Research* 17:1479-1484. doi:10.1016/S0271-5317(97)00138-3.
- » Jurjanz, S., V. Monteils, P. Juaneda, and F. Laurent. 2004. Variations of trans octadecenoic acid in milk fat induced by feeding different starch-based diets to cows. *Lipids* 39:19-24. doi:10.1007/s11745-004-1196-4.
- » Kay, J.K., W.J. Weber, C.E. Moore, D.E. Bauman, L.B. Hansen, H. Chester-Jones, B.A. Crooker, and L.H. Baumgard. 2005. Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. *Journal of Dairy Science* 88:3886–3893. doi:10.3168/jds.S0022-0302(05)73074-5.
- » Kelsey, J.A., B.A. Corl, R.J. Collier, and D.E. Bauman. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *Journal of Dairy Science* 86:2588–2597. doi:10.3168/jds.S0022-0302(03)73854-5.
- » Kliem, K.E., and K.J. Shingfield. 2016. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. *European Journal of Lipid Science and Technology* 118:1661–1683. doi:10.1002/ejlt.201400543.
- » Kliem, K.E., K.J. Shingfield, D.J. Humphries, and D.I. Givens. 2011. Effect of replacing calcium salts of palm oil distillate with incremental amounts of conventional or high oleic acid milled rapeseed on milk fatty acid composition in cows fed maize silage-based diets. *Animal* 5:1311–1321. doi:10.1017/S1751731111000310.
- » Kliem, K.E., K.J. Shingfield, K.M. Livingstone, and D.I. Givens. 2013. Seasonal variation in the fatty acid composition of milk available at retail in the United Kingdom and implications for dietary intake. *Food Chemistry* 141:274–281. doi:10.1016/j.foodchem.2013.02.116.
- » Kraft, J., M. Collomb, P. Möckel, R. Sieber, R., and G. Jahreis. 2003. Differences in CLA isomer distribution of cow's milk lipids. *Lipids* 38:657-664. doi:10.1007/s11745-003-1111-z.
- » Larsen, M.K., J.H. Nielsen, G. Butler, C. Leifert, T. Slots, G.H. Kristiansen, and A.H. Gustafsson. 2010. Milk quality as affected by feeding regimens in a country with climatic variation. *Journal of Dairy Science* 93:2863–2873. doi:10.3168/jds.2009-2953.
- » Lock, A.L., C.L. Preseault, J.E. Rico, K.E. DeLand, and M.S. Allen. 2013. Feeding a C16: 0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. *Journal of Dairy Science* 96:6650–6659. doi:10.3168/jds.2013-6892.
- » Lock, A.L., and K.J. Shingfield. 2004. Optimising milk composition. *BSAP Occasional Publication* 29 (2004): 107-188. doi:10.1017/S0263967X00040076
- » Loften, J.R., J.G. Linn, J.K. Drackley, T.C. Jenkins, C.G. Soderholm, and A.F. Kertz. 2014. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. *Journal of Dairy Science* 97:4661–4674. doi:10.3168/jds.2014-7919.
- » Loo, J.J., A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard. 2005. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. *Journal of Dairy Science* 88:726–740. doi:10.3168/jds.S0022-0302(05)72736-3.
- » Mele, M., R. Dal Zotto, M. Cassandro, G. Conte, A. Serra, A. Buccioni, G. Bittante, and P. Secchiari. 2009. Genetic parameters for conjugated linoleic acid, selected milk fatty acids, and milk fatty acid unsaturation of Italian Holstein-Friesian cows. *Journal of Dairy Science* 92:392–400. doi:<https://doi.org/10.3168/jds.2008-1445>.
- » Miller, N., L. Delbecchi, D. Petitclerc, G.F. Wagner, B.G. Talbot, and P. Lacasse. 2006. Effect of stage of lactation and parity on mammary gland cell renewal. *Journal of Dairy Science* 89:4669–4677. doi:10.3168/jds.S0022-0302(06)72517-6.
- » Mohammed, R., J.J. Kennelly, J.K.G. Kramer, K.A. Beauchemin, C.S. Stanton, and J.J. Murphy. 2010. Effect of grain type and processing method on rumen fermentation and milk rumenic acid production. *Animal* 4:1425–1444. doi:10.1017/S175173111000039X.
- » Mohammed, R., S.M. McGinn, and K.A. Beauchemin. 2011. Prediction of enteric methane output from milk fatty acid concentrations and rumen fermentation parameters in dairy cows fed sunflower, flax, or canola seeds. *Journal of Dairy Science* 94:6057–6068. doi:10.3168/jds.2011-4369.
- » Mosley, S.A., E.E. Mosley, B. Hatch, J.I. Szasz, A. Corato, N. Zacharias, D. Howes, and M.A. McGuire. 2007. Effect of varying levels of fatty acids from palm oil on feed intake and milk production in Holstein cows. *Journal of Dairy Science* 90:987–993. doi:10.3168/jds.S0022-0302(07)71583-7.
- » Noble, R.C., W. Steele, and J.H. Moore. 1969. The effects of dietary palmitic and stearic acids on milk fat composition in the cow. *Journal of Dairy Research* 36:375–381. doi:10.1017/S0022029900012887.
- » Odongo, N.E., M.M. Or-Rashid, R. Bagg, G. Vessie, P. Dick, E. Kebreab, J. France, and B.W. McBride. 2007. Long-term effects of feeding monensin on milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science* 90:5126–5133. doi:10.3168/jds.2007-0242.
- » O'Donnell-Megaro, A.M., D.M. Barbano, and D.E. Bauman. 2011. Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. *Journal of Dairy Science* 94:59–65. doi:10.3168/jds.2010-3571.
- » Palmquist, D.L. 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. *Journal of Dairy Science* 74:1354–1360. doi:10.3168/jds.S0022-0302(91)78290-8.
- » Palmquist, D.L., A. Denise Beaulieu, and D.M. Barbano. 1993. Feed and animal factors influencing milk fat composition. *Journal of Dairy Science* 76:1753–1771. doi:10.3168/jds.S0022-0302(93)77508-6.
- » Paredes, C.L.L., M. Werteker, B. Rossmann, J. Keplinger, I.L. Olschewski, and M. Schreiner. 2018. Discrimination of haymilk and conventional milk via fatty acid profiles. *Journal of Food Measurement and Characterization* 12:1391–1398. doi:10.1007/s11694-018-9753-0.
- » Piantoni, P., A.L. Lock, and M.S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. *Journal of Dairy Science* 96:7143–7154. doi:10.3168/jds.2013-6680.
- » Rabiee, A.R., K. Breinhild, W. Scott, H.M. Golder, E. Block, and I.J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. *Journal of Dairy Science* 95:3225–3247. doi:<https://doi.org/10.3168/jds.2011-4895>.
- » Rani, Z.T., M. Chimonyo, A. Hugo, U. Marume, and V. Muchenje. 2011. Effect of parity on the proximate composition and fatty acid profile of milk from Nguni cattle grazing on natural pastures. *African Journal of Biotechnology* 10:8647–8653. doi:10.5897/ajb10.2384.
- » Rego, O.A., S.P. Alves, L.M.S. Antunes, H.J.D. Rosa, C.F.M. Alfaia, J.A.M. Prates, A.R.J. Cabrita, A.J.M. Fonseca, and R.J.B. Bessa. 2009. Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. *Journal of Dairy Science* 92:4530–4540. doi:10.3168/jds.2009-2060.
- » Rico, J.E., M.S. Allen, and A.L. Lock. 2014. Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. *Journal of Dairy Science* 97:1057–1066. doi:10.3168/jds.2013-7432.
- » Roy, A., A. Ferlay, K.J. Shingfield, and Y. Chilliard. 2006. Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on trans-C18: 1 fatty acids and isomers of conjugated linoleic acid. *Animal Science* 82:479–492. doi:10.1079/ASC200658.
- » Saliba, L., R. Gervais, Y. Lebeuf, and P.Y. Chouinard. 2014. Effect of feeding linseed oil in diets differing in forage to concentrate ratio: 1. Production performance and milk fat content of biohydrogenation intermediates of α -linolenic acid. *Journal of Dairy Research* 81. doi:10.1017/S0022029913000691.

- » Schwendel, B.H., P.C.H. Morel, T.J. Wester, M.H. Tavendale, C. Deadman, B. Fong, N.M. Shadbolt, A. Thatcher, and D.E. Otter. 2015. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *Journal of Dairy Science* 98:1411–1425. doi:10.3168/jds.2014-8322.
- » Secchiari, P., M. Mele, A. Serra, A. Buccioni, F. Paoletti, and M. Antongiovanni. 2003. Italian Journal of Animal Science Effect of breed, parity and stage of lactation on milk conjugated linoleic acid content in Italian Friesian and Reggiana cows. *Journal of Animal Science* 2:269–271. doi:10.4081/ijas.2003.11675982.
- » Shepardson, R.P., and K.J. Harvatine. 2021. Effects of fat supplements containing different levels of palmitic and stearic acid on milk production and fatty acid digestibility in lactating dairy cows. *Journal of Dairy Science* 104:7682–7695. doi:10.3168/jds.2020-19665.
- » Shingfield, K.J., M. Bonnet, and N.D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal* 7(s1) 132-162. doi:10.1017/S1751731112001681.
- » Shingfield, K.J., P. Salo-Väänänen, E. Pahkala, V. Toivonen, S. Jaakkola, V. Piironen, and P. Huhtanen. 2005. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. *Journal of Dairy Research* 72:349–361. doi:10.1017/S0022029905000919.
- » Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2006. Variation in fatty acid contents of milk and milk fat within and across breeds. *Journal of Dairy Science* 89:4858–4865. doi:10.3168/jds.S0022-0302(06)72534-6.
- » Soyeurt, H., A. Gillon, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2007. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. *Journal of Dairy Science* 90:4435–4442. doi:10.3168/jds.2007-0054.
- » Steele, W., and J.H. Moore. 1968. The effects of a series of saturated fatty acids in the diet on milk-fat secretion in the cow. *Journal of Dairy Research* 35:361–370. doi:10.1017/S0022029900019099.
- » Sterk, A., B.E.O. Johansson, H.Z.H. Taweel, M. Murphy, A.M. van Vuuren, W.H. Hendriks, and J. Dijkstra. 2011. Effects of forage type, forage to concentrate ratio, and crushed linseed supplementation on milk fatty acid profile in lactating dairy cows. *Journal of Dairy Science* 94:6078–6091. doi:10.3168/jds.2011-4617.
- » Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk fatty acids and milk production traits of dutch Holstein-Friesians. *Journal of Dairy Science* 91:385–394. doi:10.3168/jds.2007-0181.
- » Stull, J.W., and W.H. Brown. 1964. Fatty acid composition of milk. II. Some differences in common dairy breeds. *Journal of Dairy Science* 47:1412. doi:10.3168/jds.S0022-0302(64)88928-1.
- » Thomson, N.A., and W. Poel. 2000. Seasonal variation of the fatty acid composition of milkfat from Friesian cows grazing pasture. In *Proceedings of the New Zealand Society of Animal Production* (Vol. 60, pp. 314-317).
- » Townsend, S.J., B.D. Siebert, and W.S. Pitchford. 1997. Variation in milk fat content and fatty acid composition of Jersey and Friesian Cattle. *Proceedings of the Association Advancement of Animal Breeding and Genetics* 12: 283-291.
- » Villeneuve, M.-P., Y. Lebeuf, R. Gervais, G.F. Tremblay, J.C. Vuilleumard, J. Fortin, and P.Y. Chouinard. 2013. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *Journal of Dairy Science* 96: 7181-7194. doi:10.3168/jds.2013-6785.
- » Wathes, D.C., Z. Cheng, N. Bourne, V.J. Taylor, M.P. Coffey, and S. Brotherstone. 2007. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. *Domestic Animal Endocrinology* 33:203–225. doi:10.1016/j.domaniend.2006.05.004.
- » Woolpert, M.E., H.M. Dann, K.W. Cotanch, C. Melilli, L.E. Chase, R.J. Grant, and D.M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. *Journal of Dairy Science* 99:8486–8497. doi:10.3168/jds.2016-10998.
- » Woolpert, M.E., H.M. Dann, K.W. Cotanch, C. Melilli, L.E. Chase, R.J. Grant, and D.M. Barbano. 2017. Management practices, physically effective fiber, and ether extract are related to bulk tank milk de novo fatty acid concentration on Holstein dairy farms. *Journal of Dairy Science* 100:5097–5106. doi:10.3168/jds.2016-12046.

Chapter 4: Regional and Seasonal Variation in FA Composition of Canadian Milk

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Overview

Given that there is little published information about the regional and seasonal variation in FA composition of milk leaving Canada's 10,095 dairy farms, an observational study was undertaken to utilize existing data to describe the FA composition of bulk tank milk, and its variability across seasons and regions of Canada. With the cooperation of provincial milk boards and Lactanet, we were able to assemble and analyze two data sets.

The first data set (Data Set A) included analysis of a sample of milk from every farm pick-up from all Ontario and Quebec dairy farms between October 2019 and April 2021 (n=1,810,450), which represents 80% of all dairy farms in Canada and 70% of all dairy cows in Canada, based on 2020 dairy industry statistics. This data set was used to describe changes in FA composition of milk over time (18 months).

The second data set (Data Set B) included analysis of bulk tank milk samples from Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Alberta and British Columbia collected between May and July 2021 (n=191,000 samples). All samples were analysed for fatty acid profile by Lactanet. This data set was used to describe differences in FA composition in milk across the country with 9,743 of 10,095 (96.5%) of dairy farms represented.

Analysis of Data Set A

Overall, there have not been any remarkable changes in 14:0, 16:0, 18:0 or 18:1 composition in Quebec and Ontario milk over the 18-month period (Figures 1-4). There is a small numerical increase in 14:0 and 16:0, and a small numerical decrease in 18:0 and 18:1 in the last 6 months (November 2020 to April 2021) in comparison to the same six months in the previous year (November 2019 to April 2020) but given the large variation among farms in each month, the significance of this small average increase is not clear. It is clear from these data that there is tremendous between farm variation, suggesting that there are many farm-level factors (described above) that influence the FA composition of milk, and that the between farm variation is far greater than the overall seasonal or temporal variation.

Analysis of Data Set B

While there are small differences in overall FA composition of milk among provinces, the western provinces (Manitoba, Alberta and British Columbia) have slightly higher levels of 16:0 and slightly lower levels of 18:1 than Ontario and the eastern provinces (Nova Scotia and New Brunswick) (Figures 5-8). Given the large variation among farms in each province, the significance of these small differences is not clear. Here again, the variation among farms within a region is substantially greater than any overall differences among the provinces or regions. The summary statistics for the major FAs for each province are presented in Table 1.

Table 1: Summary statistics by province for major fatty acids (C:14, C:16, C:18_0 and C:18_1) based on analysis of bulk tank milk samples collected in late spring and early summer 2021.

PROV	FATTY ACID (G/100 G TOTAL FA)	N	MEAN	STD	MIN	MEDIAN	MAX
NS	C:14	191	10.63	1.03	7.17	10.79	12.70
NB	C:14	330	10.53	1.06	7.50	10.62	13.05
QC	C:14	149243	11.24	0.80	5.80	11.32	14.27
ON	C:14	99362	11.09	0.80	6.09	11.14	14.42
MB	C:14	303	10.74	0.79	8.39	10.71	12.95
AB	C:14	3060	10.97	0.78	5.95	10.99	13.47
BC	C:14	529	10.96	0.81	6.14	11.03	13.22
NS	C:16	191	30.62	2.53	21.58	31.17	35.23
NB	C:16	330	30.37	2.34	23.85	30.53	36.31
QC	C:16	149243	31.52	1.88	21.77	31.68	39.97
ON	C:16	99362	31.14	2.07	19.60	31.23	41.57
MB	C:16	303	32.04	1.93	26.33	32.10	37.43
AB	C:16	3060	33.27	2.09	22.12	33.31	47.62
BC	C:16	529	31.96	2.16	21.89	31.86	37.78
NS	C:18_0	191	10.27	0.98	7.44	10.29	13.12
NB	C:18_0	330	10.78	0.94	8.28	10.79	13.09
QC	C:18_0	149243	10.47	0.87	0.05	10.42	21.69
ON	C:18_0	99362	10.28	0.86	5.07	10.27	14.68
MB	C:18_0	303	10.54	0.95	7.90	10.53	13.08
AB	C:18_0	3060	10.62	1.00	0.04	10.63	15.18
BC	C:18_0	529	10.75	0.86	8.49	10.75	15.22
NS	C:18_1	191	22.50	1.84	18.32	22.36	28.18
NB	C:18_1	330	22.87	1.94	17.96	22.69	28.31
QC	C:18_1	149243	22.14	1.63	13.64	21.95	35.14
ON	C:18_1	99362	23.61	2.08	15.70	23.50	35.78
MB	C:18_1	303	22.42	1.43	18.37	22.43	26.81
AB	C:18_1	3060	21.91	1.55	13.12	21.84	32.27
BC	C:18_1	529	22.26	1.74	17.28	22.22	34.35

Figure 1: Monthly average 14:0 per 100 g total FA for the period October 2019 to April 2021 for all Quebec and Ontario dairy farms (n=8,133)

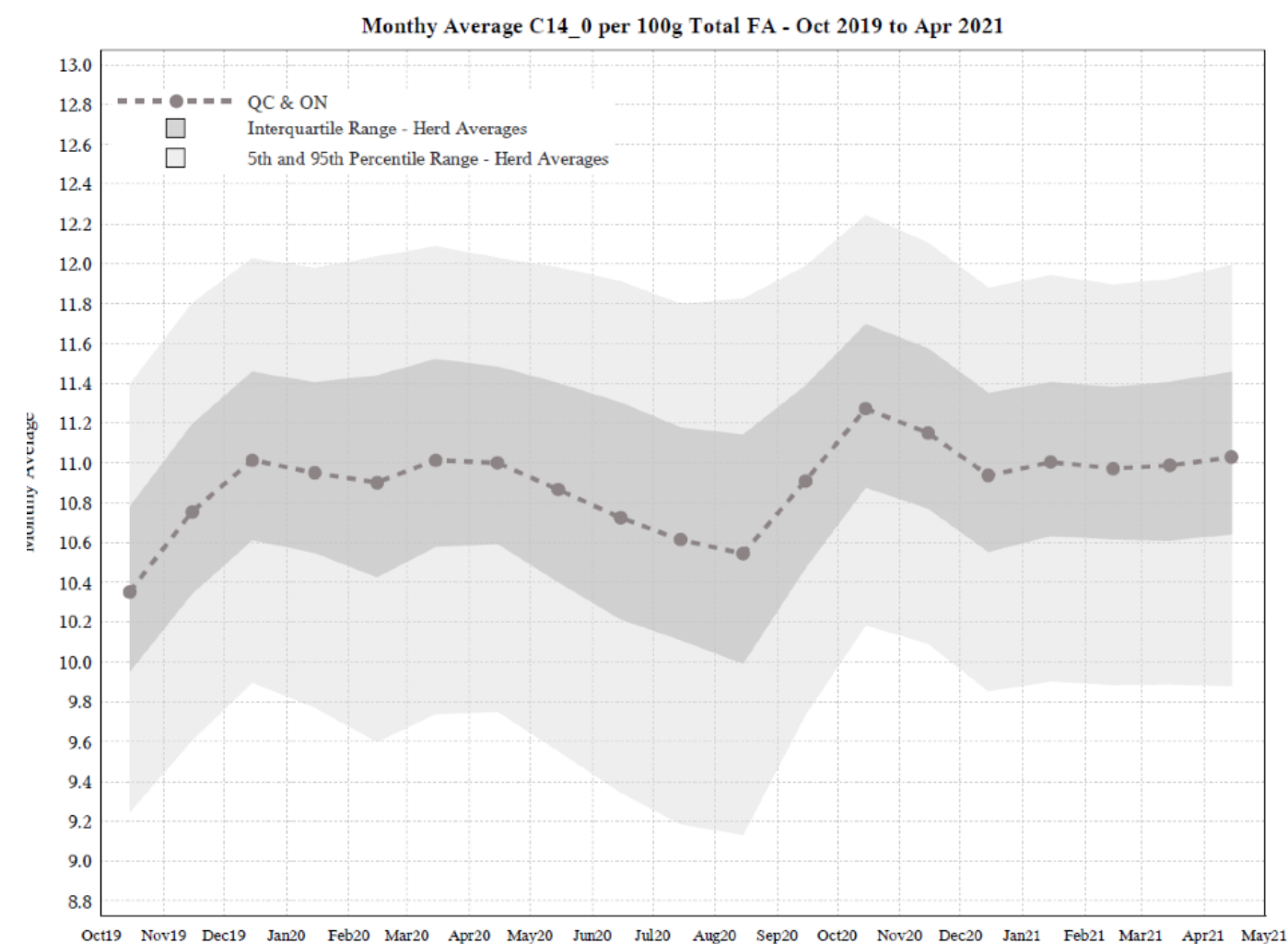


Figure 2: Monthly average 16:0 per 100 g total FA for the period October 2019 to April 2021 for all Quebec and Ontario dairy farms (n=8,133).

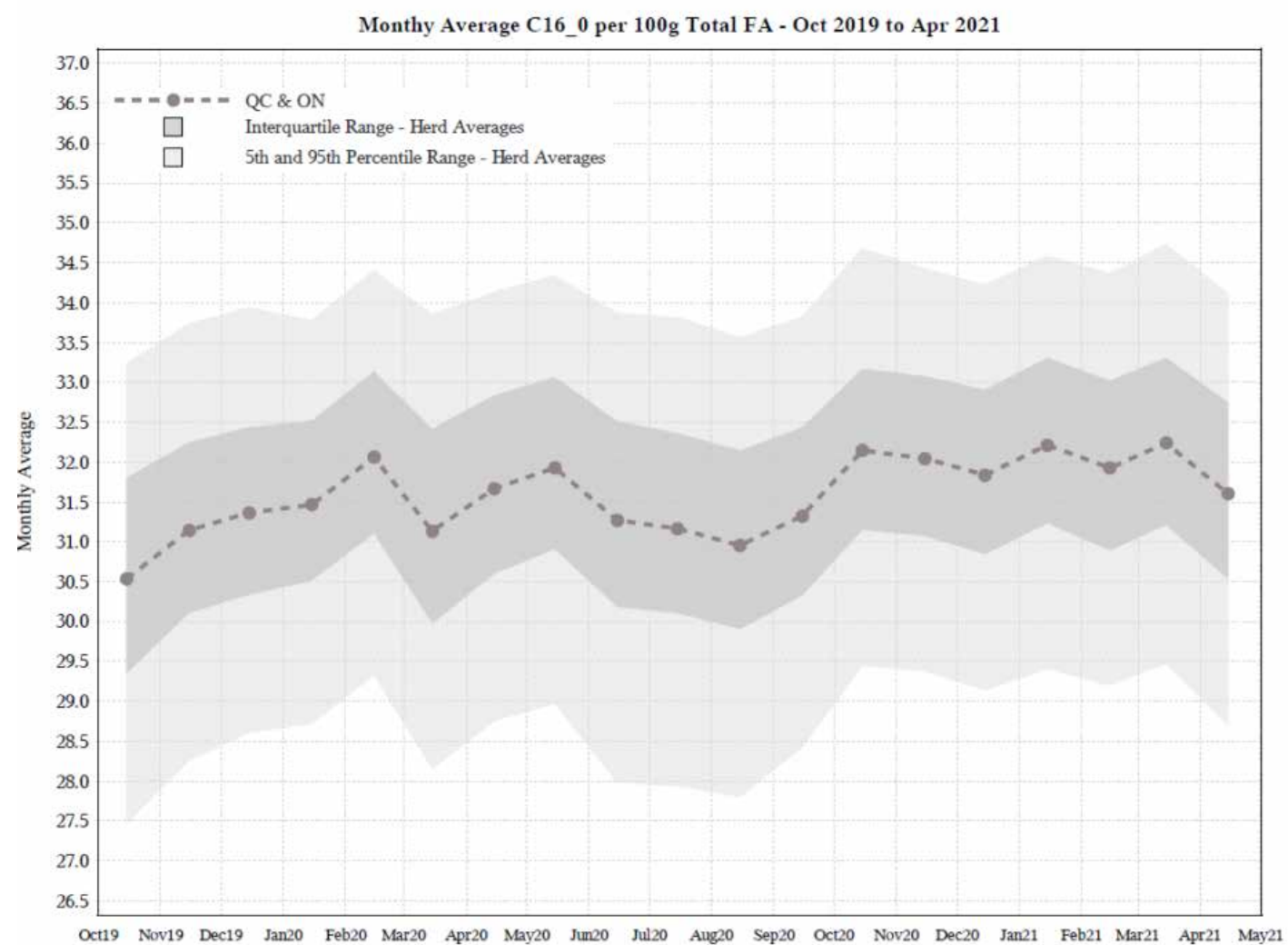


Figure 3: Monthly average 18:0 per 100 g total FA for the period October 2019 to April 2021 for all Quebec and Ontario dairy farms (n=8,133).

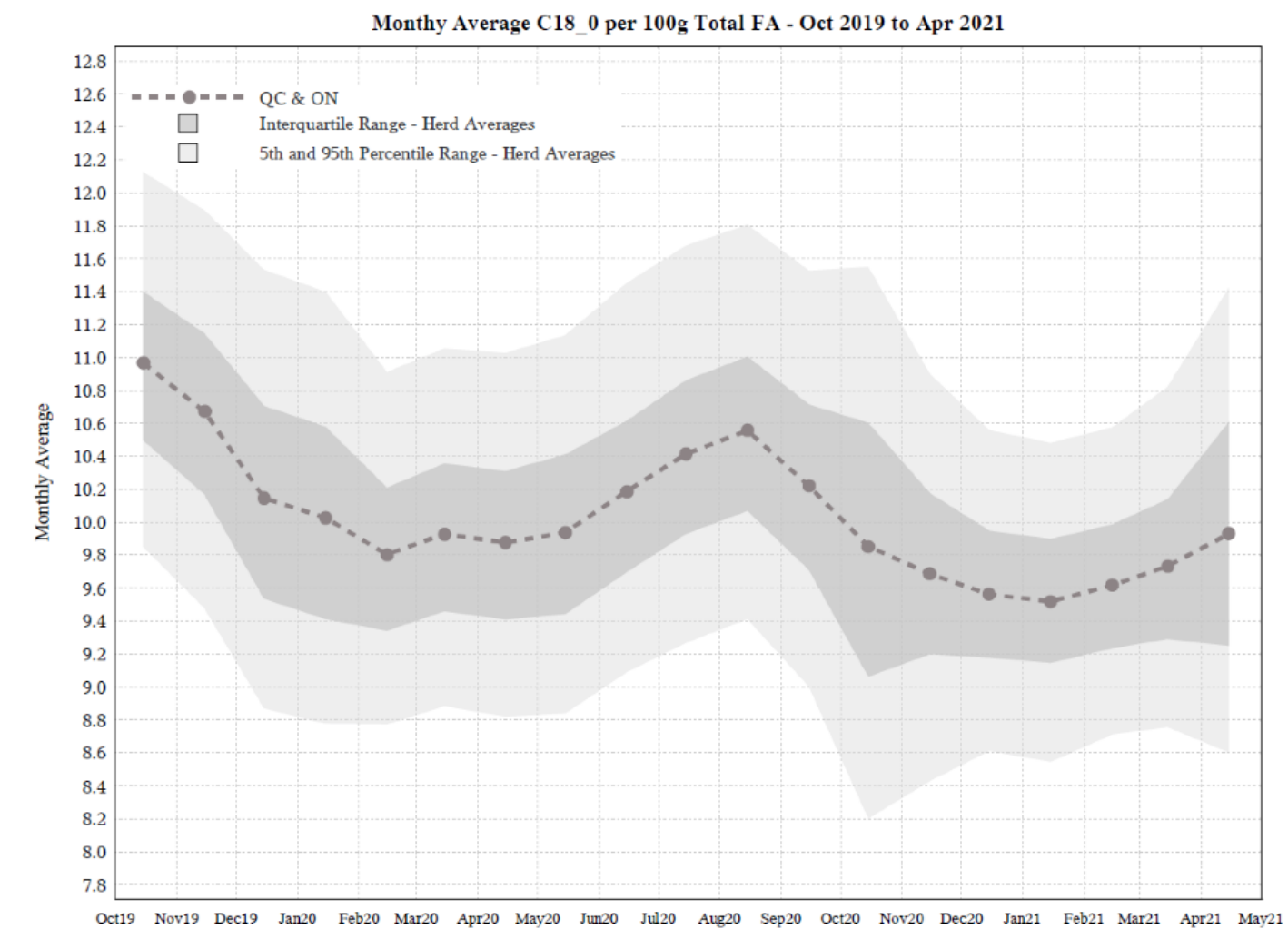


Figure 4: Monthly average 18:1 per 100 g total FA for the period October 2019 to April 2021 for all Quebec and Ontario dairy farms (n=8,133).

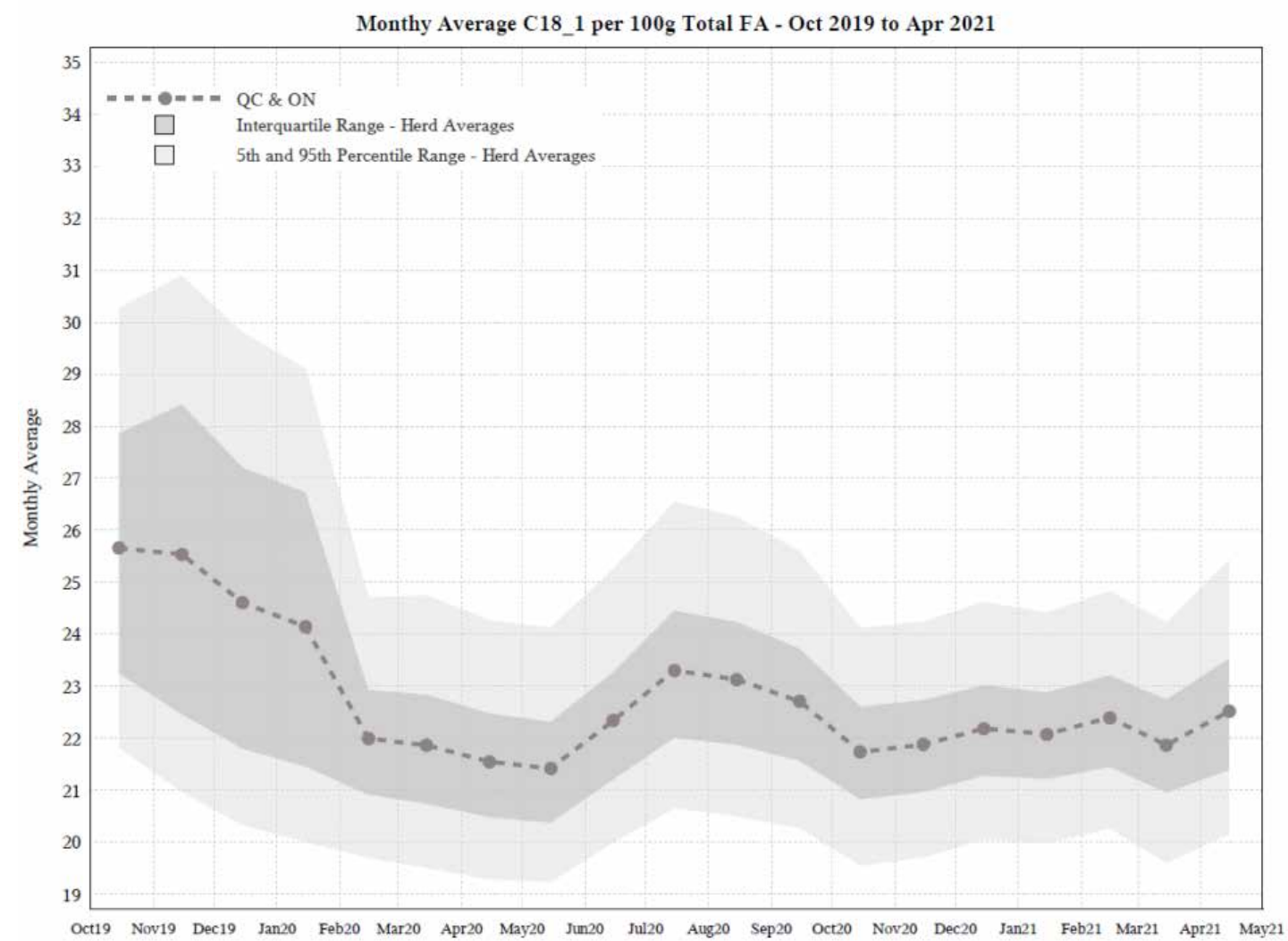


Figure 5: Provincial average 14:0 per 100 g total FA for the period May 2021 to July 2021 for all dairy farms in Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Alberta and British Columbia (n=9,743).

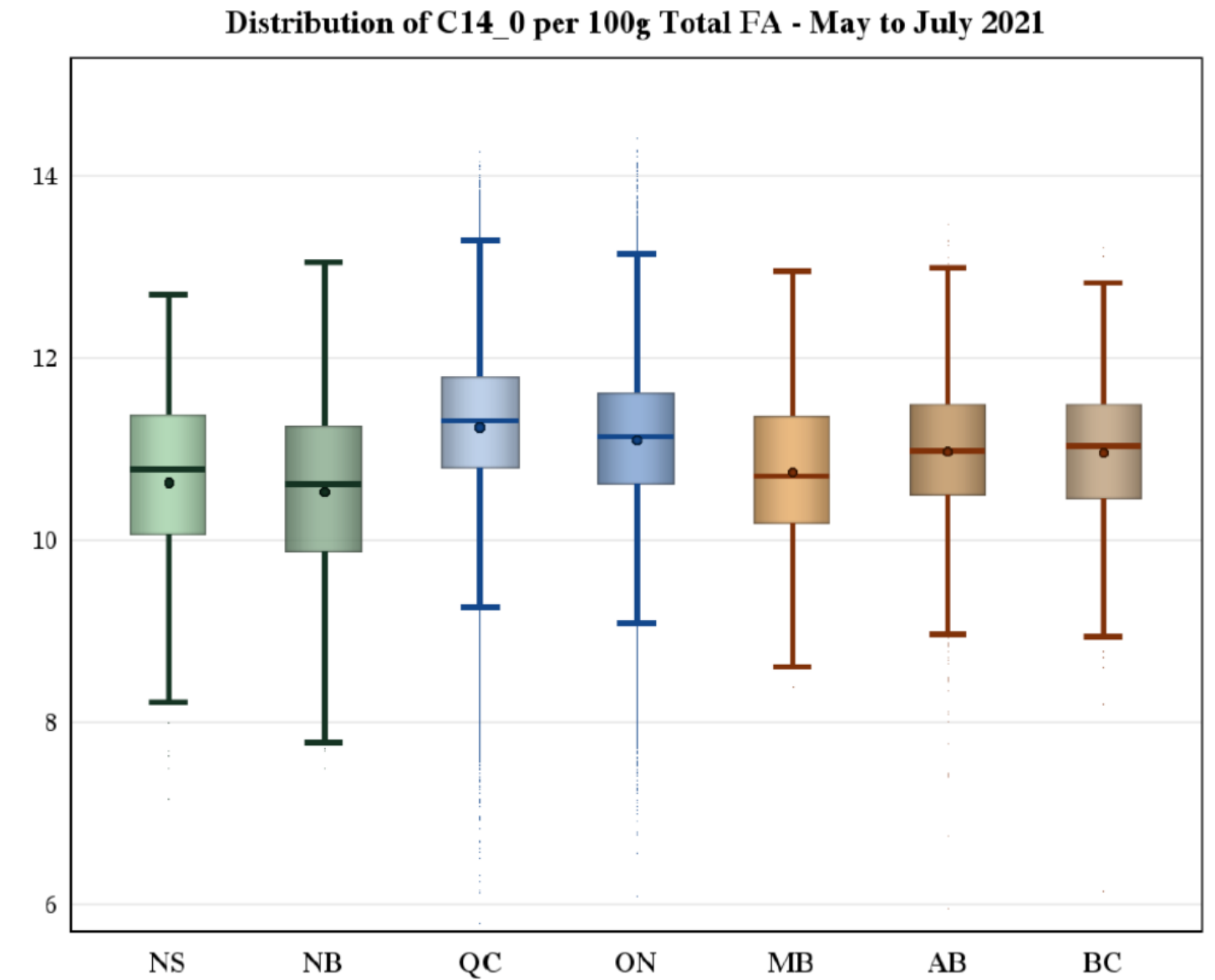


Figure 6: Provincial average C:16 per 100 g total FA for the period May 2021 to July 2021 for all dairy farms in Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Alberta and British Columbia (n=9,743).

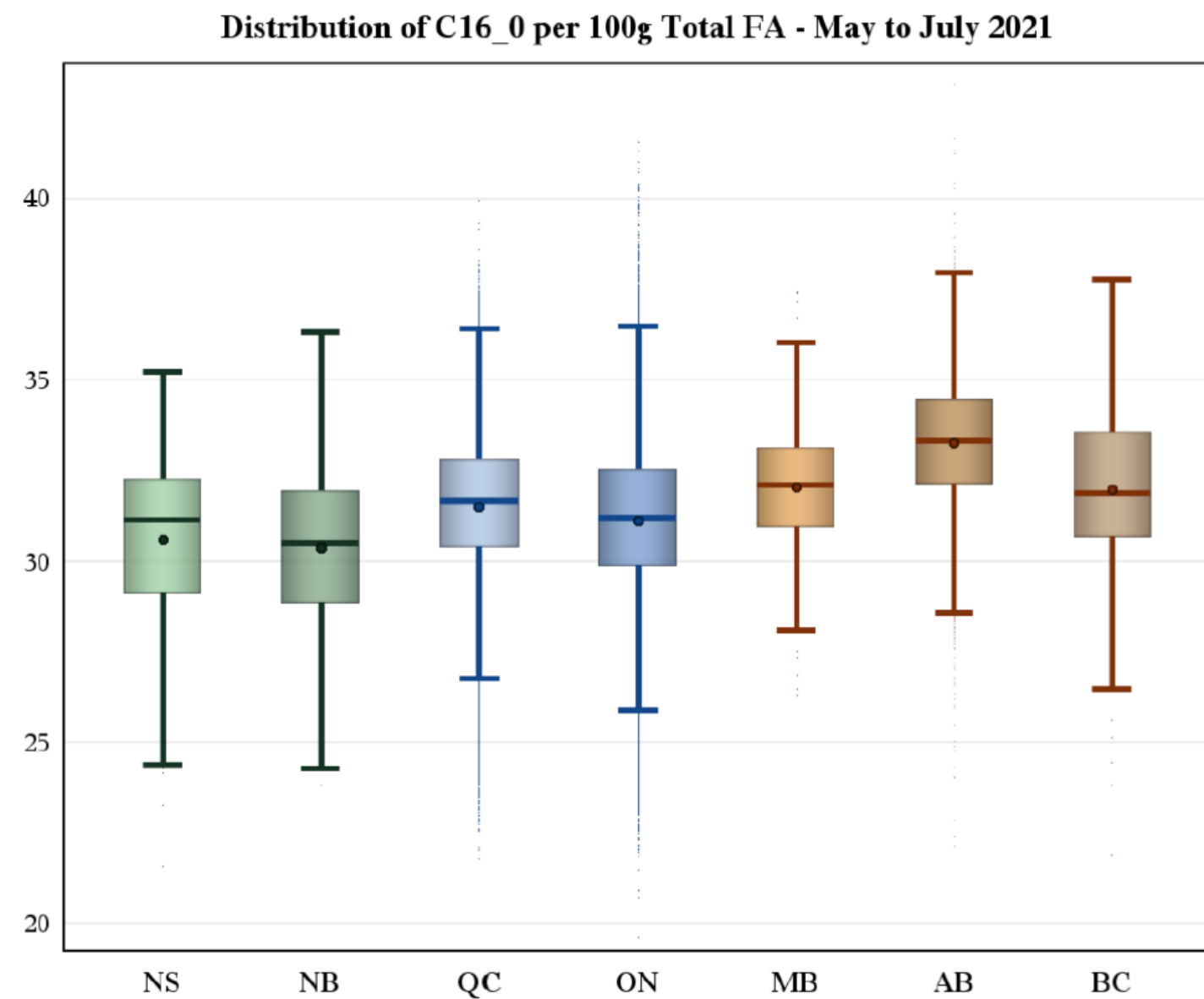


Figure 7: Provincial average 18:0 per 100 g total FA for the period May 2021 to July 2021 for all dairy farms in Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Alberta and British Columbia (n=9,743).

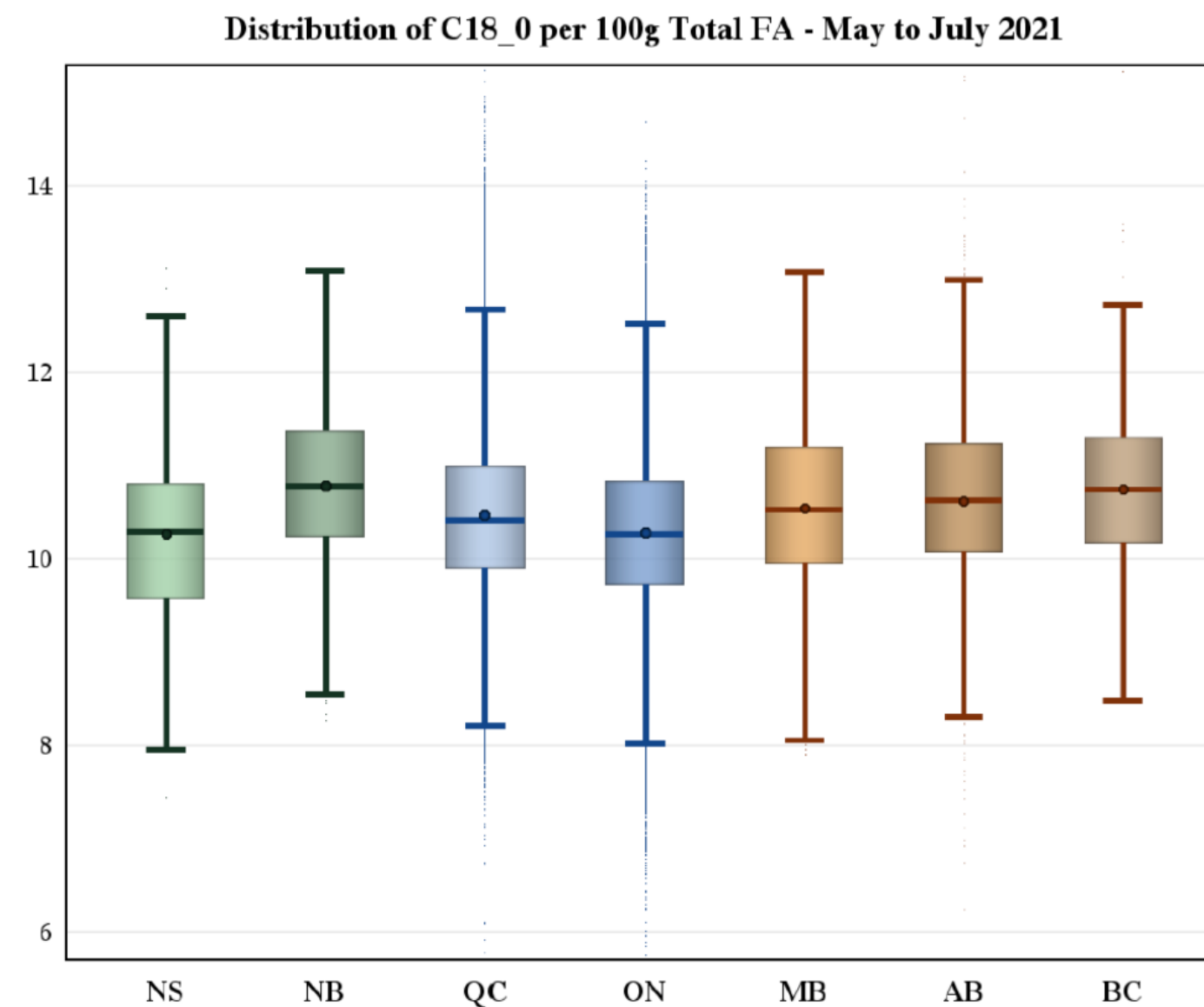
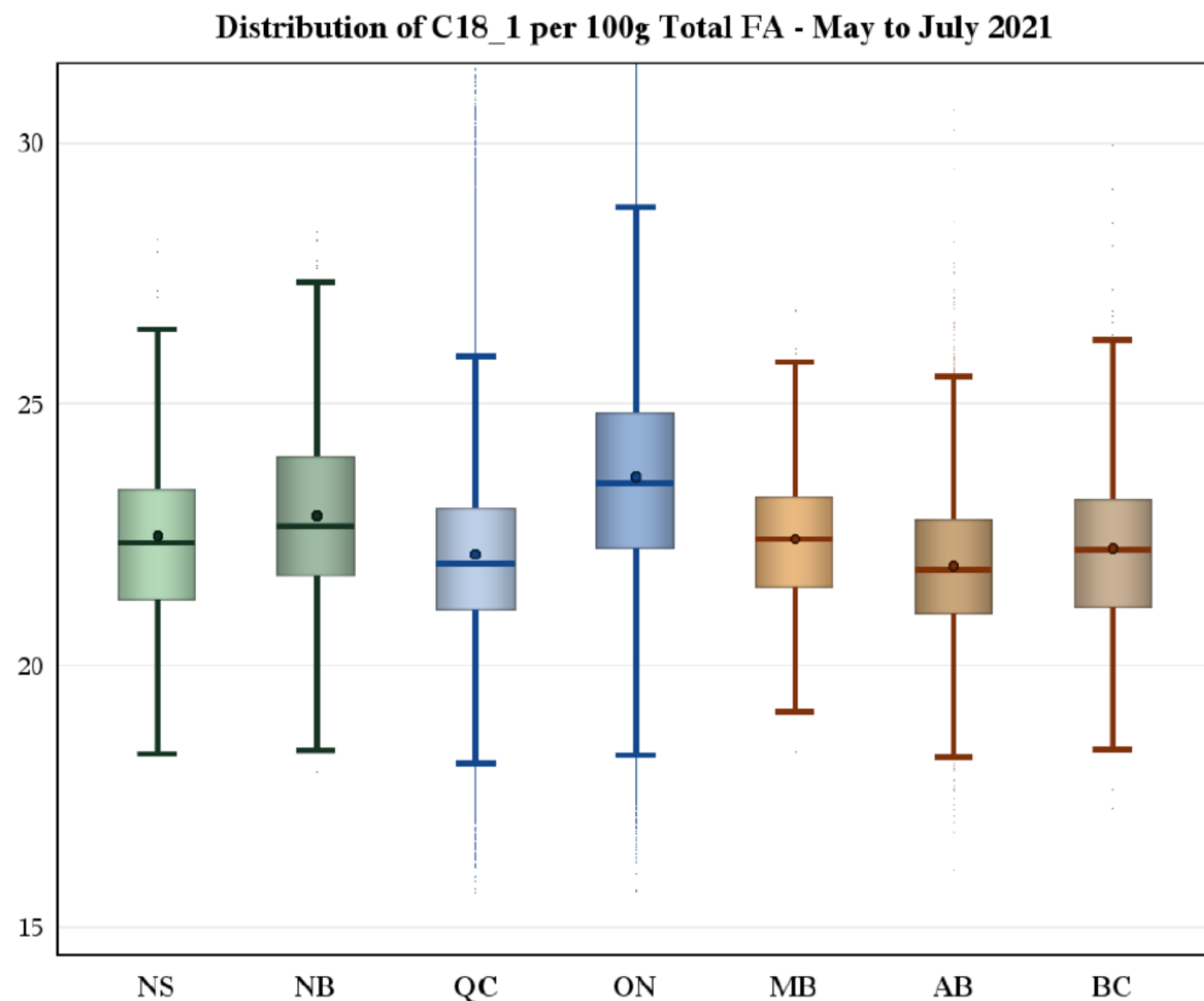


Figure 8: Provincial average 18:1 per 100 g total FA for the period May 2021 to July 2021 for all dairy farms in Nova Scotia, New Brunswick, Ontario, Manitoba, Alberta and British Columbia (n=4,977)



Chapter Summary

- Based on analysis of bulk tank milk samples from most dairy farms in Canada, there has been very little change in FA composition of milk, and there are only small seasonal and regional differences in FA composition.
- The between farm variation is much greater than any differences over time or among regions, supporting the fact that there are many on-farm factors that determine the FA composition of milk produced on each farm. Despite that, the overall composition of the pooled (overall) milk supply has remained quite consistent.

IMPACT OF MANUFACTURING PROCESSES ON THE CHARACTERISTICS OF BUTTER

Chapter 5: Process-Related Factors Determining Butter Hardness

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Overview

Textural attributes, which are the sensory and functional manifestation of the structural, mechanical, and surface properties of foods, can be correlated with rheological properties. Several terms have been defined to assess the texture of butter (Macias-Rodriguez and Marangoni 2020). The most common properties of interest are “firmness” or “hardness” and “spreadability”, which are indicators used as sensory and quality control attributes (Mattice, Wright et al. 2020). These two properties are inversely related (Wright, Scanlon et al. 2001). The spreadability of butter, or the ease with which it spreads over another substrate, reflects the state of dispersion of the fat crystals and liquid oil. The term “consistency” can also be used to describe the butter spreadability (Schäffer, Szakály et al. 2001). It is affected by several factors, such as the ratio of solid to liquid fat, the size and shape of fat crystals, the mechanical treatments and the temperature (Vélez-Ruiz, Barbosa Cánovas et al. 1997).

To be spreadable, butter must typically possess a solid fat content (SFC) below 45% or more precisely between 20% and 40% at temperatures between 11°C and 20°C (Frede 2002, Mattice, Wright et al. 2020). The hardness of butter, or resistance to deformation or penetration, is related to its structural response to some external forces. In other words, it represents the elastic capacity of the fat to respond to compression forces (Vélez-Ruiz, Barbosa Cánovas et al. 1997). Some authors use the terms “firmness” and “hardness” interchangeably, while others suggest the use of “firmness” for recoverable viscoelastic deformation and the term “hardness” for non-recoverable plastic deformation (Macias-Rodriguez and Marangoni 2020). The term “**hardness**” will be used in this chapter.

Characterisation and relation with textural properties

The tests conducted on butter to evaluate the rheological properties can be classified into three main categories: imitative, empirical, and fundamental tests. Imitative tests, such as machines that imitate spreading of butter on bread by measuring the shear imposed by a knife edge, show some correlation with sensory scores or empirical methods, but they lack methodology, control of deformations and quantitative measurements. Empirical tests more closely imitate the basic notions of deformations applied during processing and product usage. Their measurements correlate well with sensory assessment of texture. They are useful for quality control and product development. Fundamental tests are rigorously defined in physical and mathematical terms and aim to measure true or apparent bulk properties. They are used for research and development purposes and require a certain degree of expertise (Macias-Rodriguez and Marangoni 2020).

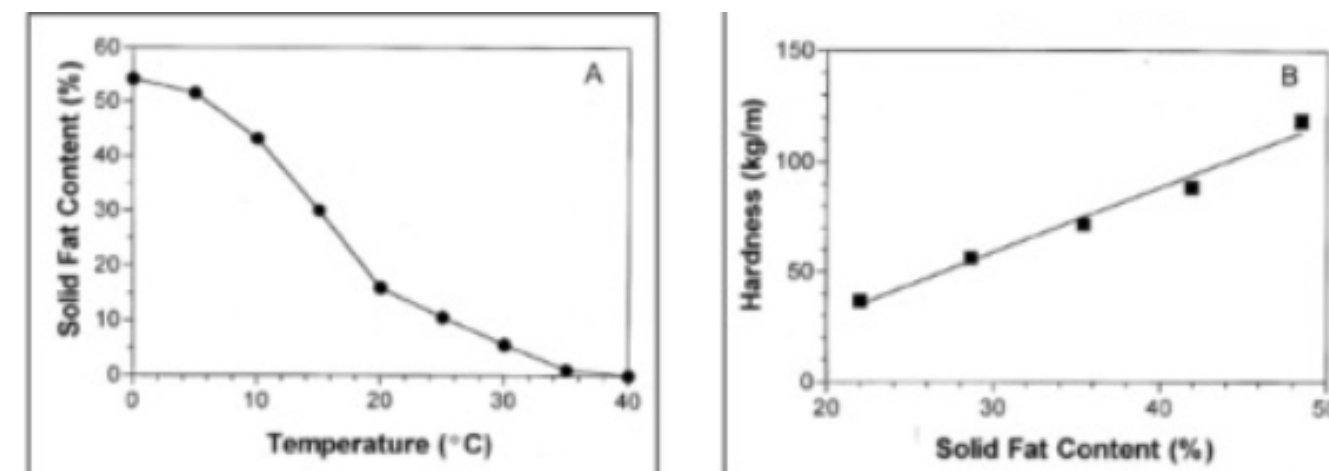
Four empirical and fundamental objective methods are commonly used for evaluating butter: penetration, cutting, extrusion and compression (Vélez-Ruiz, Barbosa Cánovas et al. 1997):

- **Penetrometry** is the most common method used to evaluate butter texture. Penetration tests are based on the resistance of a material to be “pierced” or indented by a test body: rod, cone, sphere, needle, etc. Results are reported as penetration depths or converted into yield stress values, hardness or spreadability indexes, using various equations dependent on the testing body and test conditions (Van Aken and Visser 2000, Macias-Rodriguez and Marangoni 2020).
- **Sectility test**, also called wire cutting tests is a measure of the force required to cut the butter using a taut steel wire at a constant speed. A universal testing machine (UTM) type rheometer can be used (McCarthy and Wong 2020). The latest standard for measuring butter hardness is based on sectility measurement at a cutting speed of 1.0 mm s^{-1} (International Standards Organization 2005). Excellent correlations of sectility tests with firmness and viscosity of butter have been reported.
- **Compression** tests are one of the most popular tests for determining fundamental rheological, fracture properties and empirical textural attributes. They involve deforming a specimen of known dimensions at constant force or at constant crosshead speed for a standardized time. A combination of apparent shear modulus (G) and viscosities (η) provides a measure of ‘firmness’ (Macias-Rodriguez and Marangoni 2020).
- **Extrusion** is used to mimic the flow of butter during spreading. It involves measuring the thrust of a piston required to extrude butter through the orifice of a nozzle. The force of extrusion correlates well with spreadability attribute (Macias-Rodriguez and Marangoni 2020).

The main fundamental rheological properties of interest in butter, milk fat and milk fat blends include their elastic and viscous properties and yield stress. These parameters are used to assess butter texture such as firmness, hardness and spreadability. The properties of the fat crystal network can be quantified using rheological analysis in which storage/elastic modulus (G') and loss/viscous modulus (G'') have been used to relate whether the sample behaves as a solid or liquid. The G' is shown to be directly correlated to a product’s hardness (Macias-Rodriguez and Marangoni 2020). These values are obtained with a small deformation rheometer over a predetermined range of increasing shear strain, frequency, temperature or time (Mattice, Wright *et al.* 2020). In butter and milk fat derivatives, the yield stress refers to the critical value beyond which the material transitions from purely elastic deformations to plastic deformations, a measure of resistance to the applied shear stress.

Temperature is a key factor that influences the solid fat content and rheological properties of butter (Vithanage, Grimson *et al.* 2009). As shown in figure 1, the temperature dictates the solid fat content, which has a direct impact on butter hardness. At refrigerator temperature (4°C), the butter may be unspreadable while at room temperature it may exhibit oiling off depending on the milkfat FA composition. The temperature at which the rheological test is performed is critical and should always be the same for all the samples evaluated.

Figure 1: A. Solid fat content (%) of milk fat as a function of temperature. B. Milk fat hardness (kg/m) determined from cone penetrometry as a function of solid fat content (%). Source: (Wright, Scanlon et al. 2001)



Milk composition and its influence on the rheological properties of butter

Impact of the chemical composition (fatty acids, triglycerides) of milk fat

Milk fat contains potentially thousands of different triacylglycerols (TAGs) species, each with their own melting point that depend on their fatty acid (FA) composition. Also, the distribution of FA on the different positions of the TAG modifies the crystallization and liquefaction patterns of the fat (Hawke and Taylor 1983). Milk fat composition is often discussed in terms of the three different fractions of TAGs, which are chemically and physically distinct. These main TAG fractions are distinguished by their melting behaviour: the low-melting fraction (LMF) (from -25°C to 10°C), the middle-melting fraction (MMF) (from 10°C to 19°C) and the high-melting fraction (HMF) (above 20°C) (Mattice, Wright et al. 2020, Waldron, Hoffmann et al. 2020). The overall melting point of milk fat is approximately 34°C (Wright, Scanlon et al. 2001). The LMF is liquid at room temperature, due to its substantial content of long-chain unsaturated and short-chain saturated FAs.

Conversely, MMF melts at temperatures greater than room temperature, resulting from many long-chain saturated FAs and a much lower content of long-chain unsaturated and short-chain saturated FAs. Accordingly, the MMF is characterized by an intermediate melting temperature (Mattice, Wright et al. 2020).

In milk fat, the ratio of high melting TAGs to medium and lower melting TAGs is a significant determining factor for hardness (Mattice, Wright et al. 2020) and it is well accepted that the texture and spreadability of butter is influenced by FA melting points.

Milk FAs, depending on their chain length, arise from two sources. The short to medium chain fatty acids (C4:0 to C14:0) are derived from de novo synthesis in the epithelial cells of the mammary gland of the cow. Long chain fatty acids, on their end, are sourced from diet of the cow. The palmitic acid (C16:0) can be sourced from both diet and de novo synthesis (Knutsen, Olsen et al. 2018).

Supplementation with specific dietary lipids is a way to improve cows’ energy balance, and in some cases, can increase milk fat output. Feeding lipids high in palmitic acid (C16:0) to lactating cows can influence the FA composition of milk lipids. Chamberlain et al (2016) were able to increase the C16:0 proportions in milk fat, and an increase in melting temperature was observed, which induced changes in butter hardness. However, this pilot experiment was performed with only 12 cows.

Conversely, lower concentrations of palmitic acid in pasture-derived butters lead to lower hardness of butter (O'Callaghan, Faulkner et al. 2016, Magan, O' Callaghan et al. 2021). Increasing the proportion of fresh grass in the diet induces a linear increase in unsaturated FA percentages at the expenses of saturated FAs such as palmitic acid (Couvreur, Hurtaud et al. 2006).

In Ireland and New Zealand, the alteration between winter-indoor silage and summer-outdoor fresh grass grazing has a significant effect on the FA composition. In summer (April- June), the increase in unsaturated fatty acid (UFA) such as oleic (18:1), α -linolenic (18:3) and palmitoleic(C16:1) acids, and the decrease in palmitic acid (C16:0) leads to a softer butter (hardness of 137 kPa at 4 °C, 37 kPa at 15 °C) compared with the firmer winter (December- February) butter (hardness of 412 kPa at 4 °C, 137 kPa at 15 °C) (Cullinane, Condon et al. 1984). Butter was evaluated by a sensory panel for spreadability and by a texture analyzer at both 5 and 23°C for hardness and adhesiveness.

Milk and butter samples from cows with a more unsaturated milk FA composition were more spreadable, softer, and less adhesive (Bobe, Hammond et al. 2003).

As mentioned, modifying the FA composition of butter by decreasing the proportions of saturated fatty acids 12:0, 14:0, 16:0, and stearic acid (18:0) and increasing the proportions of unsaturated and short-chain fatty acids improves its spreadability. Such changes can be achieved by processing technologies such as milk fat fractionation, by cow nutrition, or by cow selection within a herd or breeding program. Feeding cows supplemental high in unsaturated FA such as fish oil or soybean products have been used as nutritional approaches to achieving less saturated and more spreadable butters (Bobe, Zimmerman et al. 2007).

Impact of the macroscopic fat composition (crystallin structure) and its processing ability

Fat crystallization

Milk fat is semi-solid in nature due to the presence of a large proportion of high melting triacylglycerols (TAGs). These TAGs form crystalline structures at room temperature, resulting in a network that confines the lower melting TAGs in liquid state within (Mattice, Wright et al. 2020).

The rheological behaviour of fats is governed by interactions between fat crystals in an aggregated three-dimensional, solid-liquid matrix. The liquid portion of the fat, integrated throughout the fat network, serves as a continuous phase and, in conjunction with the solid fraction, is responsible for viscoelastic behaviour. Of primary importance to the rheological behaviour of fat are the amount of crystalline fat and the type of crystals (polymorphic form) present in the fat crystal network (Herrera and Hartel 2000).

Fat crystallization involves nucleation (initial nucleation sites), crystal growth (the conditions present determine the number and size of the crystals formed) and crystal rearrangements. The temperature at which a fat is crystallized is a major determinant of the reaction kinetics and resultant structure. The cooling rate has a major impact on the crystallization of milk fat. As an example, during rapid cooling of cream, crystallization of fat occurs more quickly and nucleation events predominate over crystal growth, resulting in a high number of small crystals formed. The formation of many small crystals increases milk fat hardness, as the greater surface area allows for a greater proportion of liquid fat to be absorbed and immobilized (Mattice, Wright et al. 2020).

Polymorphism, on the other hand, arises due to geometric packing arrangement of the long hydrocarbon chains within the fatty acids. The packing arrangement is characterized by the sub-cell concept. The three major sub cell packing arrangements in fats, which define a polymorphic form, are α (hexagonal), β' (orthorhombic) and β (triclinic), listed in increasing order of melting point, density and stability. The polymorphic forms have a direct influence on the melting point of a fat and have also been correlated to macroscopic rheological properties. Nucleation of milk fat typically occurs in the metastable α -form (for

example in cream <20 °C) (Waldron, Hoffmann et al. 2020) as these crystals require a lower activation energy for nucleation. This unstable form rapidly converts to the β' form during ageing. Milk fat is still considered to be a β' tending fat, as crystals have a tendency to transform to the β' form and remain in this conformation even after prolonged storage (Mattice, Wright et al. 2020).

In cream, the presence of fat crystals in the fat globules of the emulsion and partial coalescence phenomenon are required to obtain the desired product and occur as per mechanism of churning. The partial coalescence phenomenon occurs when a crystal present at the interface of the fat globule penetrates the milk fat globule membrane and binds another fat globule. When partial coalescence has started, oil will be released out of the fat globules, continuing until the formed network is wetted by oil (Lopez, Bourgaux et al. 2002). Thereafter, a network made of aggregated fat globules will be formed. Fat globules retain most of their original shape but are linked by a semi solid connection; therefore, the process is called partial coalescence (Buldo, Kirkensgaard et al. 2013).

Crystallization proceeds at a more rapid rate when the minor components are removed from milkfat. Minor components include partial acylglycerols, free fatty acids (FFA), cholesterol and phospholipids (Mattice, Wright et al. 2020).

Milk fat globules size

Fat in milk is organized as spherical lipid droplets ranging in size from 0.1 to 10 μm , with a mean diameter of around 4 μm . Milk fat globules can be separated into small (less than 1 μm), intermediate (1-8 μm), and large (more than 8 μm). The small fat globules encompass for about 80% of the fat globules base on their numbers (Michalski, Briard et al. 2001).

The core of the fat droplet is comprised mainly of triglycerides (98%) and smaller quantities of diglycerides, monoglycerides and cholesterol esters, and is stabilized by an outer biological membrane, the milkfat globule membrane (MFGM), composed of polar lipids, cholesterol and proteins (Waldron, Hoffmann et al. 2020).

However, there is little information available on the relationship between globule size, crystallization behaviour and processability/quality of butter and the role of fat globule size has not been much explored. Nevertheless, it has been reported that butter made from cream containing small milk fat globules (MFG) (obtained from cows fed with unsaturated fatty acid enriched meal) had shorter churning time, higher fat loss during churning, and greater water retention. It was also softer, more spreadable and had better 'mouthfeel' and melting properties than the control butter. At the same measuring and storage temperature, liquid fat tended to be more concentrated in smaller fat globules (Hurtaud, Faucon et al. 2010). Panchal et al. 2021 also found that smaller fat globules lead to butter considerably softer (0.24 μm compared to 3.49 μm) (Panchal, Truong et al. 2021).

Also, the decrease in milk globule fat (MGF) size caused an increase in the moisture content of butter as the proportion of milk fat lost in buttermilk increased, which resulted in reduced hardness values for butter and an increased spreadability (Magan, O' Callaghan et al. 2021). Small milk fat globules fraction obtained by a microfiltration process was also shown to have a delayed crystallization compared to larger ones (Michalski, Camier et al. 2004). It has also been reported that smaller fat globules obtained by microfiltration fractionation tend to be more oily and greasy (Goudédranche, Fauquant et al. 2000).

Influence of milk collection practices on milk composition

Impact of milk pumping on the free fatty acid composition

The usage of automatic milking systems (AMS) on dairy farms can affect the free fatty acids (FFAs) levels in milk as well as the fat globules size. AMS leads to a small increase in FFA levels in the milk (de Koning, Slaghuis et al. 2003), which can be caused by the increase of the milking frequencies. The air inlet in the teat cups, excessive air intake and a too long post run time of the milk pump can also affect the FFA level (de Koning, Slaghuis et al. 2003). Another explanation for the higher FFA content of milk from cows milked more than twice daily is the fat globule size.

Shorter milking intervals are associated with larger fat globules which are more susceptible to lipolysis than smaller ones (Truong, Palmer et al. 2016, De Marchi, Penasa et al. 2017, SimõesFilho, Lopes et al. 2020). As mentioned in the previous section, a bigger milk globule fat size can contribute to an increase of butter hardness.

The temperature of the milk at pumping also influences the formation of FFAs. The results of a study conducted by Wiking et al. (2005) indicated that instant cooling of raw milk to 4°C or up to 15 min after milking and prior to pumping reduced the formation of FFAs. In comparison, the formation of FFAs increased significantly in milk pumped at 31°C. However, when the milk was incubated at 4°C for 60 min after cooling and then subjected to pumping, a significant increase in the formation of FFAs was observed. It is suggested that this increase in FFAs is caused by transition of polymorphic crystal forms or higher level of attached lipoprotein lipases to the milk fat globule before pumping (Wiking, Bertram et al. 2005). Higher concentration of FFA is also observed in milk moved by a pump turning at higher speed (rpm) (Escobar and Bradley Jr 1990).

Impact of milk storage time

The storage time of milk has an impact on TAG lipolysis. Lipolysis in milk derives from two different enzymatic processes. The first one is caused by the natural milk lipase secreted by the animal udder. The increase of the lipolytic activity is dependent on the time elapsed between milking and heating treatment (pasteurization) and the storage temperature of the raw milk before processing. The second one is due to the microbial lipase, produced by psychrotrophic bacteria that can grow at milk temperature storage (4°C). These lipases are thermoresistant at pasteurization temperatures and to UHT treatments. The increase in FFAs from lipolytic activity, leads to a rancid taste (Antonelli, Curini et al. 2002).

As shown in the figure below, the FFAs begin to increase significantly after 3 days of storage, and even more after 5 days.

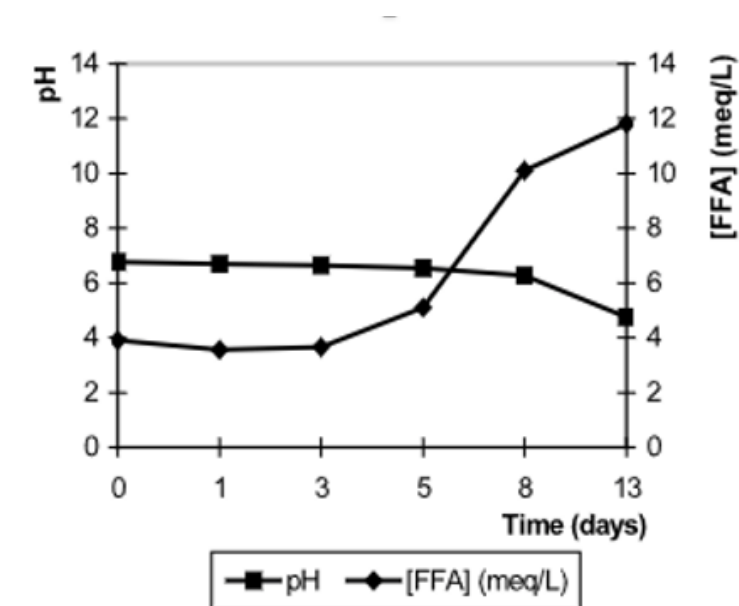


Figure 3: Variation of the FFA concentration and pH, in function of time, in raw milk samples. Source: (Antonelli, Curini et al. 2002)

FFAs are part of the indigenous minor components of milk, along with monoacylglycerols (MAG), diacylglycerols (DAG), and phospholipids, and they can influence fat crystallization at the nucleation stage, the crystal growth and the polymorphic behaviour. With respect to nucleation, it can be alteration in nucleation time, shift of nucleation temperature, or change in the number and the nature of the nuclei formed (Panchal and Bhandari 2020). Short chain FFAs and monounsaturated FFAs have a clear slowing effect on crystallization, without affecting the number of crystals formed. However, the addition of long chain saturated fatty acids either as FFAs or triglycerides accelerates crystallization (Bayard, Leal-Calderon et al. 2017).

Influence of Manufacturing Parameters on the Rheological Properties of Butter

The production of butter results from a phase inversion from an oil-in-water emulsion (cream) to a water-in-oil emulsion (butter) caused by the destabilization and aggregation (partial coalescence) of the milk fat globules. Some processing conditions, such as cooling rate, crystallization temperature, ripening rate, as well as the physicochemical matrix of milk fat globules and milk fat fatty acid composition, affect milk fat crystallization and rheological properties of milk fat (Ceylanand Ozcan 2020).

Manufacturing methods of butter (regular, cultured)

Butter can be produced by the churning of fresh (sweet cream) or cultured cream. Butter made from cultured cream has certain advantages over the butter made from sweet cream: the aroma is richer; the butter yield is higher and there is a lower risk of re-infection after temperature treatment as the bacterial culture suppresses undesirable microorganisms (Tetra Pak 2021). Usually, such cream is pasteurized at a higher temperature than sweet cream, then cooled to ripening temperature (20 to 27 °C) and inoculated with starter cultures (1% to 2%) (Fearon 2011).

A study reported that butter made from cream inoculated with *Lactobacillus helveticus* (LH-butter) was softer than the non incubated butter at room temperature. This correlated well with the fatty acid composition. A higher proportion of total unsaturated fatty acids (55%) was detected in LH-butter compared to control (36%). The triacylglyceride (TAG) composition of the products could be accountable for the differences between the network strength of LH-butter and control (Ewe and Loo 2016). Ripening cream with butter culture addition also influences the crystallization of milk fat. The presence of lactic acid bacteria (LAB) within the microstructure of butter could have affected the crystal arrangement of the fat, resulting in a different functionality between the two butters. Indeed, the fat crystal network of LH-butter could be small and randomly organized, resulting in a weak and random network that can be easily distorted by external forces. On the other hand, the crystal matrix formed upon the crystallization process in the control butter could be highly ordered, thus stronger, resulting in lower spreadability than the LH-butter (Ewe and Loo 2016).

Increasing acidification and agitation rates promote the formation of more and smaller initial crystals. This is because agitation can break up the crystals already formed, which can act as nucleation sites promoting secondary nucleation and resulting in a higher number of crystals (Ceylan and Ozcan 2020).

Ingredients (water, salt, surfactants)

The consistency of butter can be adjusted by varying its moisture content as the strength of the fat crystal network depends on the size of the water droplets, which can change the contact points between the crystals. The increase of water content changes the ratio between solid and liquid phases, so that less fat contributes to the fat crystals network (Panchal and Bhandari 2020).

The presence of water droplets tends to weaken the structure (Mattice, Wright et al. 2020). Increasing moisture content from 12% to 15% imparts softer texture to butter at both 5 and 15 °C. A further increase in moisture content up 35% completely changes the rheological properties of butter (Wright, Scanlon et al. 2001).

It has also been reported that incorporating air into butter significantly reduced its hardness, and the decrease in hardness was greater than incorporating a similar amount of water into butter (Panchal and Bhandari 2020). Salt is added for flavor and preservation of the butter. It is necessary to use very fine-grained salt to avoid insufficient dissolving of large salt grains which would attract moisture during storage (Mortensen 2011).

The addition of surfactants to cream or directly to butter also has an impact on the texture of butter. For example, addition of 1% monoglycerides to cream before churning increases spreadability of butter by 30% (Wright, Scanlon et al. 2001).

Butter Making Steps

Cream separation

The first step in butter making aims to increase the fat content of the cream to about 40% by involving centrifugation and separation. The separation of milk to skimmed milk and cream can cause some damage, resulting in loss of surface proteins and destabilizing the MFGM (Waldron, Hoffmann et al. 2020).

Pasteurization and cooling

During pasteurization of the cream, all of the fat becomes liquid. The subsequent cooling rate of the cream has a major influence on fat crystallization by affecting the number of nucleation sites and size of the crystals and consequently influencing butter rheological properties. Upon fast cooling (strong super-cooling), fat crystallization equilibrium is not attained, and nucleation

predominates over crystal growth. Consequently, many small homogenous crystals form, primarily of α -polymorph that subsequently transforms into β' -polymorph. The formation of many small crystals upon fast cooling provides a larger surface area facilitating the adsorption of more liquid fat onto the crystal surfaces. As a result, less liquid fat remains free to form the continuous oil phase during churning and working, resulting in a firmer butter (Wiking, De Graef et al. 2009, Panchal and Bhandari 2020, Tetra Pak 2021). However, care must be taken to ensure that there is enough liquid oil left to act as bridges between the solid fat crystals to create butter grains during churning.

A study conducted by Ronholt et al. (2014A) compared butter made from fast-cooled cream (7.5°C/min) and slow-cooled cream (0.4°C/min). The storage modulus (G'), which is related to the hardness of the product, was higher during the first 14 days of storage in butter made from fast-cooled cream. After 21 days of storage, no difference was observed depending on the cooling rate of the cream (Rønholt, Kirkensgaard et al. 2014A). Butter produced from slow cooled cream had fewer crystals with a wider size distribution, whereas butter produced from fast-cooled cream had more uniform crystals (Rønholt, Kirkensgaard et al. 2012).

Cream maturation

The maturation, or ripening of the cream, is the most time-consuming processing step in the manufacture of butter (Panchal, Truong et al. 2021). This is an important step, as it governs the milk fat crystallization in the fat globules, either by separating or mixing high- and low-melting fractions of the milk fat, consequently affecting crystal microstructure and rheological behaviour. This heat treatment of the cream influences the extent and the rate of partial coalescence of milk fat globules (Buldo, Kirkensgaard et al. 2013). Generally, cream held before churning has more free liquid fat and a softer texture than cream that is churned immediately after cooling.

It is common to apply different cream ripening methods to influence the consistency of butter (Schäffer, Szakály et al. 2001). A type of ripening called Alnarp or cold-warm-cold (CWC) (for example 8/20/14 °C) method can decrease the hardness of butter. During the first cooling, numerous crystal nuclei are formed, then upon warming, crystals of high melting TAGs melt and further re-crystallize upon cooling. It appears that with this method, the butter produced will have a higher amount of liquid fat and hardness reduced by up to 25% compared to butter produced from cream cooled directly to a lower temperature (Rønholt, Mortensen et al. 2013). The melting of the high-melting fat crystals during the warming process is responsible for the observed reduction in hardness (Wright, Scanlon et al. 2001).

Cream aging is an effective way to influence and normalize the consistency of butter between winter and summer. “Hard” winter cream requires cooling to 8 °C to induce as few crystals as possible, before heating to 20 °C to melt the bulk oil phase leaving only the hard-fat crystals. This cream is cooled back to 16 °C where any crystallizing fat will adhere to the existing crystals. This yields a higher volume of free liquid oil to soften the resulting butter (Waldron, Hoffmann et al. 2020).

Agitation during aging can also have an impact on fat crystallization. At a constant temperature (10°C), harder butters were obtained when the cream was aged with weak agitation (40 rpm) versus strong agitation (240 rpm), which promoted partial coalescence in the cream due to air incorporation leading to larger fat crystals that would retain less water (Lee and Martini 2018).

Churning

The churning step involves agitation of the cream, leading to a partial phase inversion and agglomeration of the crystalline fat and ruptured fat globules. For example, the formation of fat crystals is essential for the destabilization of the emulsion. As the cream cools, fat crystals grow and pierce the interface between two globules leading to aggregation and eventual phase inversion. This process is called partial coalescence because it leads to the formation of aggregated, irregularly shaped clumps (Lopez, Bourgaux et al. 2002).

Usually, a longer churning time promotes the formation of smaller crystals due to longer exposure to shear. The churning process (conventional (batch) or continuous) has a major impact on the butter consistency. In conventional process, phase inversion is rather slow (30-60 min) while during the continuous process it happens within seconds (1-2 s) (Mortensen 2011). Differences between conventional and continuous butter making process have been specifically attributed to differences in the degree of crystallinity of the fat and in fat crystal morphologies. In addition, in part because of the differences in the structural integrity of the fat globules, continuously churned butter is typically harder than that made by the conventional process (Panchal and Bhandari 2020).

While conventionally made butter is mechanically treated after the fat has crystallized, in the continuous process most of the mechanical treatment takes place before crystallization. This is a possible explanation for some of the differences observed in the butters made by each process. The fat crystals in conventionally made butter are larger and more irregular in shape than those resulting from a continuous process (Wright, Scanlon et al. 2001). Moreover, the design of the continuous cream churner could also affect the churning efficiency and hardness of the butter.

Usually, the churning temperature employed is in a range from 10 °C to 15 °C. By increasing the churning temperature from 10 °C to 22 °C, Rønholt, Madsen, et al. (2014B) found that despite differences in crystal stability, solid fat content, and water droplet size, no significant changes were observed in the microstructure and hardness or brittleness of butter during 28 days of isothermal storage. Moreover, crystal polymorphism was also similar as all butters mainly contained β' -crystals with traces of α - and β -polymorphs (Rønholt, Madsen et al. 2014B).

Washing and salting

The washing step involves mixing the butter grains with cold water, after which the water is drained off. This removes any residual buttermilk and milk solids. Formerly, washing was done to improve the quality of the butter, but nowadays it is only done to control the temperature, if needed (Walstra, Geurts et al. 2006). The addition of salt has no particular impact on the rheological properties of the butter. If the butter grains are not too large, their firmness can be affected to some extent by washing, via the temperature of the wash-water.

Mechanical working

This step, occurring just before packaging, aims to uniformly disperse the salt and the water in the continuous oil phase of butter. The working of the butter causes a strong reduction of its firmness because the shear applied during the working breaks down the crystal bridges within the crystal network (Herrera and Hartel 2000), decreasing the hardness to about a quarter of the original value of fat spreads (Heertje, Van Eendenburg et al. 1988). So, the major effect of the working is to disrupt the fat crystal matrix without changing the solid fat content. The intense working, however, destroys a large number of fat globules resulting in a more crystalline inter-globular phase and consequently a harder consistency.

Hence, to obtain a firm butter, the packaging must be done immediately after manufacture while the butter is still very soft (the packaging itself involves intensive working); the packaged butter then can set fully, especially if it is not stored too cold. If it is desirable to make soft spreadable butter, the best policy is to first let the butter set for a considerable amount of time after manufacture and to package it afterward (Walstra, Geurts et al. 2006).

The temperature of mixing is another parameter that can be used to modify the rheological properties of butter. Applying a high temperature during the mixing of milk fat-based products induces the melting of the low- and eventually medium-melting fractions of the triglycerides, followed by re-crystallization during the cooling and the storage of the products. Such re-crystallization will however rebuild a less dense crystal network because a fraction of the milk fat is solubilized in the oil phase. Consequently, mixing at high temperature results in soft products (Buldo and Wiking 2012).

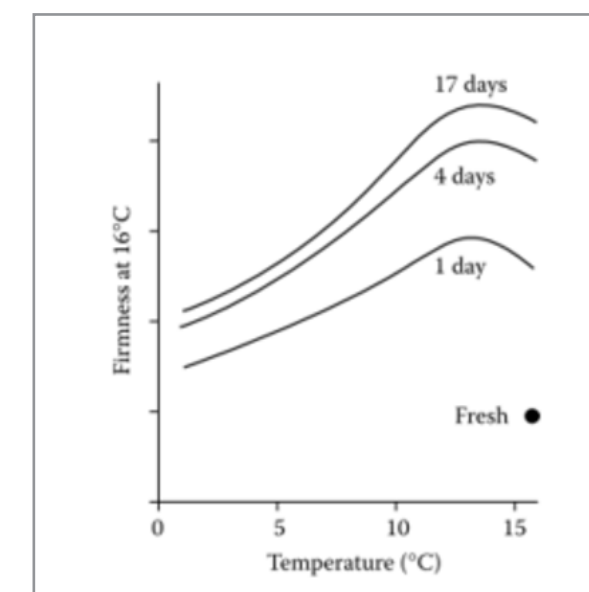
Conditioning and storage

Post manufacturing storage and handling have a considerable effect on product hardness (Walstra, Geurts et al. 2006). The time and temperature of storage affect the setting (post-crystallization) of butter. Setting refers to the increase in firmness over storage time due to continued crystallization and crystal aggregation, which causes an increase in the viscoelastic moduli during storage (Macias-Rodriguez and Marangoni 2020). The setting occurs faster at a higher temperature because more liquid fat is available (Walstra, Geurts et al. 2006).

Temperature fluctuation during storage can cause differences in the rheological behaviour of butter. When the temperature is temporarily raised, a part of the triglycerides melts and then adsorb on the surface of the remaining crystals upon cooling, leading to larger crystals. Further crystal growth induces strong crystal-crystal interactions and a more solid structure corresponding to an increase in hardness. The hardness of the butter has been shown to increase by 25% with a temporary increase in storage temperature from 8 to 20 °C, regardless of the cooling rate of the cream and the fat composition (Rønholt, Mortensen et al. 2013).

The storage time also has a considerable effect on firmness, as shown in the Figure 5. Firmness increases between day 1 and day 17.

Figure 4: Effect of temperature and time of storage on the firmness of butter at 16°C. Source: (Walstra, Geurts et al. 2006)



The initial differences in rheological behaviour between the samples due to either different cooling rates of the cream (different size and number of crystals), water content, or cream aging decrease during storage (Rønholt, Kirkensgaard et al. 2012, Rønholt, Kirkensgaard et al. 2014A) as a result of the continuous formation of fat crystal networks until critical solid fat content (Panchal and Bhandari 2020).

Chapter Summary

- This analysis of scientific knowledge has identified many factors that contribute to the rheological properties of butter. These factors are related either to the milk composition, the steps in the butter manufacturing process or the butter composition.
- Some steps in the butter manufacturing process to increase butter hardness:
 - » Cooling of the cream after pasteurization: a faster cooling rate leads to an increased butter hardness.
 - » Maturation of the cream: a butter that is churned immediately after pasteurization and cooling, with no maturation of the cream has less liquid fat, resulting in a firmer texture.
 - » Mechanical working before packaging: if butter is packaged immediately after manufacture, with no or limited mechanical working, the fat crystal interactions are stronger, leading to a firmer texture.
- A difference in the storage conditions of butter, for example, a longer storage time before butter consumption, or a fluctuation in the storage temperature can lead to an increase in butter hardness.
- The increasing usage of automatic milking systems can potentially increase butter hardness as it leads to larger fat globules.

However, considering the limited amount of information available at the present time, including the lack of data on the actual evolution of butter hardness, it is not possible to link specific process-related factors that would have evolved over the recent years period with variations in butter hardness.

References

- » Antonelli, M., et al. (2002). "Determination of free fatty acids and lipase activity in milk: quality and storage markers." *Talanta*58(3): 561-568.
- » Bayard, M., et al. (2017). "Free fatty acids and their esters modulate isothermal crystallization of anhydrous milk fat." *Food chemistry*218: 22-29.
- » Bobe, G., et al. (2003). "Texture of Butter from Cows with Different Milk Fatty Acid Compositions1." *Journal of Dairy Science*86(10): 3122-3127.
- » Bobe, G., et al. (2007). "Butter Composition and Texture from Cows with Different Milk Fatty Acid Compositions Fed Fish Oil or Roasted Soybeans." *Journal of Dairy Science*90(6): 2596-2603.
- » Buldo, P., et al. (2013). "Crystallization mechanisms in cream during ripening and initial butter churning." *Journal of Dairy Science*96(11): 6782-6791.
- » Buldo, P. and L. Wiking (2012). "The role of mixing temperature on microstructure and rheological properties of butter blends." *Journal of the American Oil Chemists' Society*89(5): 787-795.
- » Ceylan, O. and T. Ozcan (2020). "Effect of the cream cooling temperature and acidification method on the crystallization and textural properties of butter." *LWT*132: 109806.
- » Chamberlain, M. B., et al. (2016). "Feeding lactating Holstein cows a lipid source high in palmitic acid changes the fatty acid composition and thermal properties of lipids in milk and butter." *The Professional Animal Scientist*32(5): 672-680.
- » Couvreur, S., et al. (2006). "The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties." *Journal of Dairy Science*89(6): 1956-1969.
- » Cullinane, N., et al. (1984). "Influence of season and processing parameters on the physical properties of Irish butter." *Irish Journal of Food Science and Technology*: 13-25.
- » de Koning, K., et al. (2003). "Robotic milking and milk quality: effects on bacterial counts, somatic cell counts, freezing point and free fatty acids." *Italian Journal of Animal Science*2(4): 291-299.
- » De Marchi, M., et al. (2017). "Comparison between automatic and conventional milking systems for milk coagulation properties and fatty acid composition in commercial dairy herds." *Italian Journal of Animal Science*16(3): 363-370.
- » Escobar, G. and R. Bradley Jr (1990). "Effect of mechanical treatment on the free fatty acid content of raw milk." *Journal of Dairy Science*73(8): 2054-2060.
- » Ewe, J.-A. and S.-Y. Loo (2016). "Effect of cream fermentation on microbiological, physicochemical and rheological properties of *L. helveticus*-butter." *Food chemistry*201: 29-36.
- » Fearon, A. M. (2011). *Butter and Butter Products. Dairy Ingredients for Food Processing*: 199-223.
- » Frede, E. (2002). *Butter and Other Milk Fat Products | Properties and Analysis. Encyclopedia of Dairy Sciences (Second Edition)*. J. W. Fuquay. San Diego, Academic Press: 506-514.
- » Goudédranche, H., et al. (2000). "Fractionation of globular milk fat by membrane microfiltration." *Le Lait*80(1): 93-98.
- » Hawke, J. and M. Taylor (1983). *Influence of nutritional factors on the yield, composition and physical properties of milk fat. Developments in Dairy Chemistry—2*, Springer: 37-81.
- » Heertje, I., et al. (1988). "The effect of processing on some microstructural characteristics of fat spreads." *Food structure*7(2): 9.
- » Herrera, M. and R. Hartel (2000). "Effect of processing conditions on physical properties of a milk fat model system: Rheology." *Journal of the American Oil Chemists' Society*77(11): 1189-1196.
- » Hurtaud, C., et al. (2010). "Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties." *Journal of Dairy Science*93(4): 1429-1443.
- » International Standards Organization (2005). "ISO 16305:2005 butter – Determination of firmness." from <https://www.iso.org/standard/29865.html>.
- » Juriaanse, A. and I. Heertje (1988). "Microstructure of shortenings, margarine and butter—a review." *Food structure*7(2): 8.
- » Knutsen, T. M., et al. (2018). "Unravelling genetic variation underlying de novo-synthesis of bovine milk fatty acids." *Scientific reports*8(1): 1-13.
- » Lee, J. and S. Martini (2018). "Effect of cream aging temperature and agitation on butter properties." *Journal of Dairy Science*101(9): 7724-7735.
- » Lopez, C., et al. (2002). "Thermal and Structural Behavior of Milk Fat: 3. Influence of Cooling Rate and Droplet Size on Cream Crystallization." *Journal of Colloid and Interface Science*254(1): 64-78.
- » Macias-Rodriguez, B. A. and A. G. Marangoni (2020). *Rheology and Texture of Cream, Milk Fat, Butter and Dairy Fat Spreads. Dairy Fat Products and Functionality: Fundamental Science and Technology*. T. Truong, C. Lopez, B. Bhandari and S. Prakash. Cham, Springer International Publishing: 245-275.
- » Magan, J. B., et al. (2021). "Compositional and functional properties of milk and dairy products derived from cows fed pasture or concentrate-based diets." *Comprehensive Reviews in Food Science and Food Safety*.
- » Mattice, K. D., et al. (2020). *Crystallization and Rheological Properties of Milk Fat. Advanced Dairy Chemistry, Volume 2: Lipids*. P. L. H. McSweeney, P. F. Fox and J. A. O'Mahony. Cham, Springer International Publishing: 219-244.
- » McCarthy, O. J. and M. Wong (2020). *Physical Characterization of Milk Fat and Milk Fat-Based Products. Advanced Dairy Chemistry, Volume 2: Lipids*. P. L. H. McSweeney, P. F. Fox and J. A. O'Mahony. Cham, Springer International Publishing: 375-442.
- » Michalski, M.-C., et al. (2001). "Optical parameters of milk fat globules for laser light scattering measurements." *Le Lait*81(6): 787-796.
- » Michalski, M.-C., et al. (2004). "The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese." *Le Lait*84(4): 343-358.
- » Mortensen, B. K. (2011). *Butter and other milk fat products | The Product and Its Manufacture. Encyclopedia of Dairy Sciences (Second Edition)*. J. W. Fuquay. San Diego, Academic Press: 492-499.

- » O'Callaghan, T. F., et al. (2016). "Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems." *Journal of Dairy Science*99(12): 9441-9460.
- » Palmquist, D. (1991). "Influence of source and amount of dietary fat on digestibility in lactating cows." *Journal of Dairy Science*74(4): 1354-1360.
- » Panchal, B. and B. Bhandari (2020). *Butter and Dairy Fat Spreads. Dairy Fat Products and Functionality: Fundamental Science and Technology*. T. Truong, C. Lopez, B. Bhandari and S. Prakash. Cham, Springer International Publishing: 509-532.
- » Panchal, B., et al. (2021). "Influence of fat globule size, emulsifiers, and cream-aging on microstructure and physical properties of butter." *International Dairy Journal*117: 105003.
- » Rønholt, S., et al. (2014A). "Effect of cream cooling rate and water content on butter microstructure during four weeks of storage." *Food Hydrocolloids*34: 169-176.
- » Rønholt, S., et al. (2012). "Polymorphism, microstructure and rheology of butter. Effects of cream heat treatment." *Food chemistry*135(3): 1730-1739.
- » Rønholt, S., et al. (2014B). "Effect of churning temperature on water content, rheology, microstructure and stability of butter during four weeks of storage." *Food structure*2(1-2): 14-26.
- » Rønholt, S., et al. (2013). "The effective factors on the structure of butter and other milk fat-based products." *Comprehensive Reviews in Food Science and Food Safety*12(5): 468-482.
- » Schäffer, B., et al. (2001). "Melting Properties of Butter Fat and The Consistency of Butter. Effect of modification of cream ripening and fatty acid composition." *Journal of Thermal Analysis and Calorimetry*64(2): 659-669.
- » Simões Filho, L. M., et al. (2020). "Robotic milking of dairy cows: a review." *Semina: Ciências Agrárias*41(6): 2833-2850.
- » Staniewski, B., et al. (2021). "The effect of triacylglycerol and fatty acid composition on the rheological properties of butter." *International Dairy Journal*114: 104913.
- » Tetra Pak (2021). "Dairy Processing Handbook." from <https://dairyprocessinghandbook.tetrapak.com/chapter/butter-and-dairy-spreads>.
- » Tomaszewska-Gras, J. (2013). "Melting and crystallization DSC profiles of milk fat depending on selected factors." *Journal of Thermal Analysis and Calorimetry*113(1): 199-208.
- » Truong, T., et al. (2016). *Effect of milk fat globule size on the physical functionality of dairy products*, Springer.
- » Van Aken, G. and K. Visser (2000). "Firmness and crystallization of milk fat in relation to processing conditions." *Journal of Dairy Science*83(9): 1919-1932.
- » Vélez-Ruiz, J. F., et al. (1997). "Rheological properties of selected dairy products." *Critical Reviews in Food Science and Nutrition*37(4): 311-359.
- » Vithanage, C. R., et al. (2009). "The effect of temperature on the rheology of butter, a spreadable blend and spreads." *Journal of texture studies*40(3): 346-369.
- » Waldron, D. S., et al. (2020). *Role of Milk Fat in Dairy Products. Advanced Dairy Chemistry, Volume 2: Lipids*. P. L. H. McSweeney, P. F. Fox and J. A. O'Mahony. Cham, Springer International Publishing: 245-305.
- » Walstra, P., et al. (2006). *Dairy science and technology*. Boca Raton, CRC/Taylor & Francis.
- » Wiking, L., et al. (2005). "Evaluation of cooling strategies for pumping of milk-Impact of fatty acid composition on free fatty acid levels." *The Journal of dairy research*72(4): 476.
- » Wiking, L., et al. (2009). "Relations between crystallisation mechanisms and microstructure of milk fat." *International Dairy Journal*19(8): 424-430.
- » Wright, A., et al. (2001). "Rheological properties of milkfat and butter." *Journal of Food Science*66(8): 1056-1071.

Chapter 6: Insights from Processors

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Overview

Five butter manufacturers were consulted to gather information about butter processing, identify existing data, and survey possible factors impacting butter consistency. The paragraphs below provide a summary of those consultations.

Data availability

None of the butter manufacturers consulted have consistently tested for the level of palmitic acid (C16:0) in cream used to make butter, nor for the iodine value¹ in the past several years. Although spot tests may have been done by some, those were conducted in different seasons on a limited sample which means that results cannot be compared through time. Therefore, there are no time series available regarding the iodine value of milk fat at the processing level.

One set of data that was possible to collect from four butter manufacturers is the number of complaints from consumers related to butter texture (soft, hard, crumbly, spreadability, etc.). The chart below summarizes those data. For most butter makers, complaints related to butter texture represent typically less than 10% of the total number of complaints in any given year.

As can be seen on the graph on the previous page, there is no clear trend toward a higher level of complaints pertaining specifically to butter texture (including hardness) from 2017 to 2020.

Factors impacting butter consistency

Numerous factors could have an impact on butter consistency at the processing level. According to consultations, the most significant of those factors is the cooling treatment of cream after pasteurization. Cream is subjected to a program of cooling designed to control the crystallization of the fat so that the resultant butter has the right consistency². All other factors remaining the same, a more rapid cooling treatment of the cream could produce a harder butter, while a slower cooling program will produce a softer butter. In theory, it is possible to adjust the cooling treatment to account for the iodine value of cream. However, as we have seen in the previous section, the iodine value is not tested for, and therefore, the cream cooling program tends to be standardized irrespective of the fatty profile of the cream. It should be noted that decades ago, there were very significant differences in milk composition (including iodine value) between "Summer milk" and "Winter milk". This difference was so important back then that to ensure a butter with similar organoleptic properties throughout the year, the cream cooling program was likely different between the Summer months and the Winter months. However, seasonal adjustment in the cream cooling program was likely discontinued given that the difference between "Summer milk" and "Winter milk" had largely diminished.

Of note, the cream cooling program is not a factor on which butter manufacturers have complete control as some of the cream to make butter is sourced from other dairy processors. Anecdotally, it would appear that cream coming from fluid milk plants could produce a butter with a different texture than cream from cheese plants.

A factor also mentioned as having an impact on butter texture is the churning temperature. As is the case with the cream cooling process, churning temperature tends to be standardized irrespective of the fatty profile of the cream.

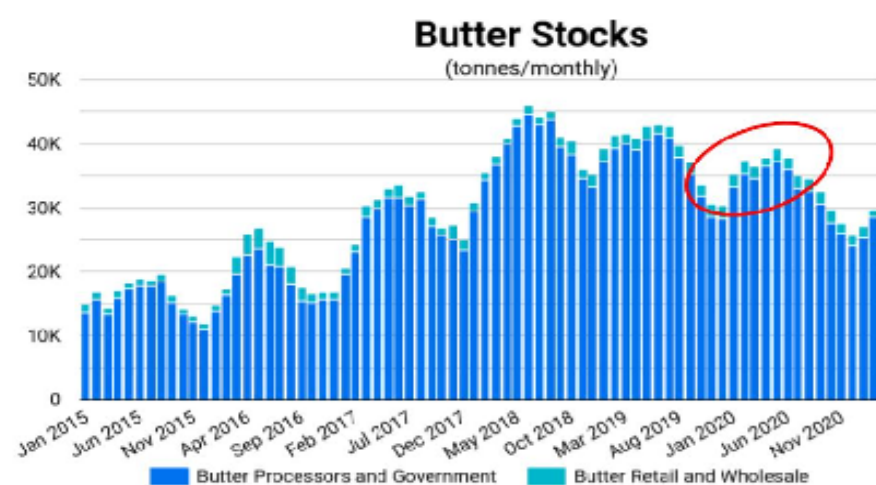
¹ Iodine value, also called Iodine number, measures the degree of unsaturation of a fat, with a higher value meaning a higher level of unsaturated fat as compared to saturated fat.

² Butter manufacture, <https://www.uoguelph.ca/foodscience/book/export/html/1687>

Increasing churning speed and/or decreasing churning time

It has been pointed out in the media in the past year that strong market demand for butter during the pandemic may have induced butter makers —faced with capacity constraints— to increase throughput through changes to their production process, such as modifications to the churning speed and/or churning time. The problem with this claim is that it fails to recognize the interconnection between churning time, churning speed, churning temperature, butter overrun, and moisture and butterfat content. For example, the milk fat content of butter cannot be less than 80% as per the [Food and Drug Regulations](#). For obvious cost competitiveness reasons, manufacturers do not want to produce a butter with 85% butterfat when the regulations set the minimum at 80%. Therefore, they tend to align at all times as closely as possible with the minimum 80% regulated content when it comes to regular butter. Increasing churning speed and/or reducing churning time could cause undesirable effects on butterfat and humidity content, in addition to impacting butter texture. Therefore, simply stepping on the “churning gas pedal” to respond to an increase in butter demand is not something that is done because increasing throughput this way will come with its own costs, including possible inventory write downs if the regulated minimum butterfat content is not met.

Rather than tweaking churning speed and/or churning time, shifts in market demand for butter (and demand for dairy products in general) and change in milk production have traditionally been managed in the Canadian dairy industry through management of butter stocks, in particular government inventories. For more than 40 years, the Canadian Dairy Commission has had butter storage programs in place to help the industry navigate shifts in supply and demand. It is therefore instructive to examine how the sudden increase in demand for butter in grocery stores during the early months of the pandemic (Spring 2020) impacted butter stocks. As we can see on the graph on the next page, butter stocks experienced a very typical seasonal pattern in 2020 with increasing stocks in the Spring and declining stocks in the Fall. While very unusual market conditions prevailed in the first half of 2020 for dairy products with the closure of the foodservice market, inventory data certainly do not suggest that the butter market was under significant stress.



Source: Canadian Dairy Commission, Compilation by DPAC.

Chapter Summary

- Butter making process is generally standardized and has not changed on the basis of cream fatty acid composition nor on the basis of shift in market demand.
- Notwithstanding, a renewed interest in butter research and development (R&D) has emerged recently given strong market demand. From consultations with butter manufacturers and academics, it is evident that there are emerging areas of R&D in butter manufacturing that may bode well for product and process innovation in the future.



SECTION 4 – BUTTER TESTING AND SCIENTIFIC ANALYSIS

Chapter 7: Functional Properties And Fatty Acid Composition Of Canadian Retail Butters

Chapter Authors:

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- With the collaboration of Micheline Gingras

Overview

In conjunction with the literature review, presentations from experts and related scientific analyses, a comprehensive review of butters from across Canada was conducted as part of the Working Group’s activities. This chapter presents the summary findings from this aspect of the Working Group’s examination of the characteristics of butter.

Methodology

Sample collection

Salted butters from various brands were purchased in different grocery stores located in British Columbia (n = 7), Alberta (n = 6), Ontario (n = 8), Quebec (n = 11), and Prince-Edward Island (n = 8) between the 15 and the 17th of March 2021, except for one butter sample in Quebec, which was purchased on March 28th 2021.

Butter Oil

Butter oil was prepared by melting butter samples (10 g) at 70°C for 15 min and centrifuging at 3 500 × g for 5 min to separate the aqueous phase. The top layer was then recovered, melted at 70°C for 10 min, and centrifuged again at 3 500 × g for 5 min. The top layer was recovered at 70°C, transferred into microtubes and stored at -20°C prior to differential scanning calorimetry (DSC; see below).

Physical and textural evaluation of butter

Heating thermograms were obtained by using DSC (DSC Q1000; TA Instruments Inc., New Castle, DE). Calibration of the instrument was performed using an indium standard and a reference empty aluminum pan. Samples of butter oil (10 ± 1 mg) were melted at 70°C and transferred into hermetically sealed aluminum pans (TA Instruments Inc.). Samples were held at 60°C for 5 min to melt any crystal present, cooled to -60°C at 5°C/min, held for 5 min, and then heated to 60°C at 5°C/min to obtain a melting profile. Solid fat content was calculated by dividing the partial area under the melting curve by the total area from -20 to 50°C multiplied by 100. Solid fat content from -20 to 50°C was calculated at 1°C intervals and solid fat concentration at 5, 8, 20, 30 and 35°C are reported.

Hardness of butter samples was determined by constant-speed penetrometry, which involved measurements of the force required to push a conical probe moving at constant speed of 1 mm/s for a penetration depth of 12 mm and withdrawn at the same speed. Hardness was measured with a texture analyzer equipped with a 5 kg load cell (model TA-XT2; Texture Technologies Corp., Scarsdale, NY) fitted with a TA-15, 45° conical probe. Samples were kept at room temperature (20°C) overnight before analysis. Three penetration tests were conducted in each butter samples. The penetration force was reported as hardness and expressed in Newton (N).

Fatty acid composition

Lipid extraction of milk samples and methylation of fatty acids were performed according to procedures described by Rico et al. (2021). Butter fatty acid composition was determined according to the procedure described by Boivin et al. (2012) by GC (Agilent 7890A; Agilent Technologies Canada Inc.; Mississauga, ON) using a 100-m CP-Sil-88 capillary column (0.25 mm i.d., 0.20 mm film thickness; Agilent Technologies Canada Inc.) and a flame ionization detector.

Statistical analysis

Pearson correlations (PROC CORR of SAS) were used to evaluate associations between fatty acid composition and rheological properties of butter samples.

Results

Table 2. Descriptive statistics on fatty acid composition of Canadian retail butter samples purchased in March 2021

FATTY ACID	AVERAGE	MINIMUM	MAXIMUM	STANDARD DEVIATION
	G/100 G OF FATTY ACIDS			
4:0	3.50	2.97	4.46	0.51
6:0	2.17	1.74	2.77	0.31
8:0	1.24	0.98	1.51	0.15
10:0	2.77	2.18	3.22	0.27
12:0	3.24	2.61	3.65	0.29
14:0	10.7	9.3	11.8	0.7
14:1 c9	0.95	0.83	1.04	0.06
15:0	1.10	0.98	1.17	0.06
16:0	34.8	32.2	39.1	2.2
16:1 c9	1.59	1.46	1.79	0.10
17:0	0.51	0.44	0.56	0.03
18:0	9.24	8.06	10.40	0.55
18:1 t11	0.90	0.63	1.41	0.17
18:1 c9	16.7	16.0	17.9	0.5
18:1 c11	0.62	0.52	0.75	0.06
18:2 c9c12	1.78	1.59	1.96	0.09
18:3 c9c12c15	0.37	0.30	0.48	0.04
18:2 c9t11	0.37	0.27	0.52	0.06

Table 3. Fatty acid composition (average ± standard deviation) of butter samples based on the province of purchase

FATTY ACID	BRITISH COLUMBIA	ALBERTA	ONTARIO	QUEBEC	PRINCE-EDWARD ISLAND
	----- G/100 G OF FATTY ACIDS -----				
4:0	3.55 ± 0.68	3.58 ± 0.53	3.75 ± 0.59	3.41 ± 0.43	3.27 ± 0.33
6:0	2.11 ± 0.37	2.16 ± 0.36	2.36 ± 0.34	2.18 ± 0.28	2.05 ± 0.19
8:0	1.16 ± 0.16	1.19 ± 0.18	1.34 ± 0.14	1.27 ± 0.13	1.2 ± 0.09
10:0	2.53 ± 0.22	2.58 ± 0.31	2.98 ± 0.19	2.88 ± 0.22	2.75 ± 0.17
12:0	2.92 ± 0.17	2.96 ± 0.27	3.47 ± 0.12	3.4 ± 0.22	3.29 ± 0.19
14:0	9.81 ± 0.34	9.93 ± 0.55	11.21 ± 0.13	11.16 ± 0.56	11.03 ± 0.28
14:1 c9	0.87 ± 0.01	0.88 ± 0.05	0.98 ± 0.02	0.99 ± 0.05	0.97 ± 0.03
15:0	1.03 ± 0.02	1.03 ± 0.05	1.13 ± 0.03	1.12 ± 0.04	1.13 ± 0.04
16:0	37.2 ± 1.5	36.9 ± 2.5	33.1 ± 0.6	34 ± 1.6	33.8 ± 1.0
16:1 c9	1.7 ± 0.07	1.67 ± 0.11	1.52 ± 0.02	1.56 ± 0.08	1.53 ± 0.03
17:0	0.47 ± 0.02	0.47 ± 0.03	0.52 ± 0.02	0.53 ± 0.02	0.53 ± 0.02
18:0	8.73 ± 0.4	8.85 ± 0.49	9.35 ± 0.49	9.33 ± 0.39	9.73 ± 0.49
18:1 t11	0.71 ± 0.08	0.75 ± 0.11	0.98 ± 0.09	0.94 ± 0.1	1.03 ± 0.21
18:1 c9	16.7 ± 0.4	16.7 ± 0.3	16.7 ± 0.4	16.8 ± 0.5	16.9 ± 0.6
18:1 c11	0.7 ± 0.03	0.66 ± 0.03	0.58 ± 0.04	0.58 ± 0.05	0.59 ± 0.04
18:2 c9c12	1.84 ± 0.08	1.82 ± 0.05	1.78 ± 0.11	1.75 ± 0.06	1.72 ± 0.1
18:3 c9c12c15	0.33 ± 0.04	0.34 ± 0.03	0.38 ± 0.02	0.39 ± 0.05	0.39 ± 0.03
18:2 c9t11	0.3 ± 0.03	0.31 ± 0.05	0.39 ± 0.03	0.39 ± 0.05	0.41 ± 0.07

Table 4. Descriptive statistics on functional properties of Canadian retail butter samples purchased in March 2021

FUNCTIONAL PROPERTY	AVERAGE	MINIMUM	MAXIMUM	STANDARD DEVIATION
	----- G/100 G OF FAT -----			
SOLID FAT CONTENT* AT				
5°C	84.8	83.4	85.5	0.46
8°C	78.9	77.1	79.9	0.59
20°C	41.0	39.4	44.1	1.34
30°C	16.0	13.7	20.4	2.02
35°C	3.47	1.59	7.37	1.75
Hardness at 20°C, N**	3.33	1.25	5.23	0.87

» *Determined on butter oil. Butter is a semi-solid emulsion and the extent of solid fat at a specific temperature will determine rheological properties such as hardness and fluidity.

» **Hardness is determined by measuring the force required to push a conical probe moving at constant speed of 1 mm/s for a penetration depth of 12 mm and withdrawn at the same speed when butter is maintained at room temperature.

Table 5. Functional properties (average ± standard deviation) of butter samples based on the province of purchase

FUNCTIONAL PROPERTY	BRITISH COLUMBIA	ALBERTA	ONTARIO	QUEBEC	PRINCE-EDWARD ISLAND
	----- G/100 G OF FAT -----				
Solid fat content* at					
5°C	84.5 ± 0.7	85.0 ± 0.3	84.7 ± 0.4	84.9 ± 0.4	84.7 ± 0.4
8°C	78.3 ± 0.8	79.0 ± 0.3	79.0 ± 0.5	79.2 ± 0.5	78.9 ± 0.5
20°C	42.1 ± 1.2	42.6 ± 1.4	40.1 ± 0.7	40.2 ± 0.8	40.7 ± 0.7
30°C	18.2 ± 1.3	18.3 ± 2.2	14.5 ± 0.6	14.9 ± 1.3	15.3 ± 0.8
35°C	5.31 ± 1.11	5.55 ± 1.91	2.18 ± 0.45	2.55 ± 1.20	2.87 ± 0.60
Hardness at 20°C, N	4.01 ± 0.64	3.87 ± 1.30	3.11 ± 0.62	3.09 ± 0.82	2.86 ± 0.49

» *Determined on butter oil

Table 6. Correlations among functional properties of butter*

	SOLID FAT CONTENT AT					HARDNESS AT 20°C, N
	5°C	8°C	20°C	30°C	35°C	
	SOLID FAT CONTENT AT					
5°C	-	0.93** <0.01	0.38 0.01	0.19 0.25	0.18 0.26	0.27 0.10
10°C	0.93 <0.01	-	0.03 0.83	-0.17 0.30	-0.17 0.29	-0.03 0.87
20°C	0.38 0.01	0.03 0.83	-	0.97 <0.01	0.96 <0.01	0.82 <0.01
30°C	0.19 0.25	-0.17 0.30	0.97 <0.01	-	0.99 <0.01	0.83 <0.01
35°C	0.18 0.26	-0.17 0.29	0.96 <0.01	0.99 <0.01	-	0.81 <0.01
Hardness at 20°C, N	0.27 0.10	-0.03 0.87	0.82 <0.01	0.83 <0.01	0.81 <0.01	-

» *Within a cell, r value is presented at the top and p value at the bottom.

**Significant positive correlations are highlighted in green.

Table 7. Correlations between functional properties of butter and fatty acid composition

FATTY ACID	FUNCTIONAL PROPERTY					
	SOLID FAT CONTENT AT					HARDNESS AT 20°C, N
	5°C	8°C	20°C	30°C	35°C	
4:0	-0.42**	-0.31	-0.32	-0.24	-0.25	-0.23
	<0.01	0.05	0.04	0.13	0.12	0.15
6:0	-0.36	-0.18	-0.50	-0.45	-0.46	-0.41
	0.02	0.26	<0.01	<0.01	<0.01	<0.01
8:0	-0.28	-0.03	-0.67	-0.65	-0.66	-0.57
	0.08	0.85	<0.01	<0.01	<0.01	<0.01
10:0	-0.10	0.20	-0.81	-0.85	-0.86	-0.70
	0.52	0.21	<0.01	<0.01	<0.01	<0.01
12:0	0.09	0.41	-0.82	-0.91	-0.91	-0.71
	0.58	<0.01	<0.01	<0.01	<0.01	<0.01
14:0	0.14	0.46	-0.81	-0.92	-0.92	-0.70
	0.38	<0.01	<0.01	<0.01	<0.01	<0.01
14:1 c9	0.14	0.44	-0.79	-0.88	-0.89	-0.66
	0.38	<0.01	<0.01	<0.01	<0.01	<0.01
15:0	0.14	0.42	-0.67	-0.80	-0.81	-0.61
	0.39	<0.01	<0.01	<0.01	<0.01	<0.01
16:0	0.18	-0.16	0.91	0.96	0.94	0.85
	0.26	0.32	<0.01	<0.01	<0.01	<0.01
16:1 c9	0.10	-0.23	0.86	0.93	0.92	0.83
	0.54	0.15	<0.01	<0.01	<0.01	<0.01
17:0	0.17	0.42	-0.60	-0.71	-0.70	-0.63
	0.29	<0.01	<0.01	<0.01	<0.01	<0.01
18:0	0.09	0.25	-0.39	-0.48	-0.45	-0.44
	0.58	0.11	0.01	<0.01	<0.01	<0.01
18:1 t11	-0.01	0.20	-0.61	-0.67	-0.65	-0.56
	0.93	0.21	<0.01	<0.01	<0.01	<0.01
18:1 c9	-0.27	-0.21	-0.15	-0.10	-0.04	-0.28
	0.10	0.20	0.35	0.52	0.79	0.08
18:1 c11	-0.30	-0.55	0.60	0.71	0.71	0.55
	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
18:2 c9c12	-0.36	-0.42	0.13	0.22	0.24	-0.02
	0.02	<0.01	0.41	0.17	0.13	0.90
18:3 c9c12c15	0.01	0.20	-0.56	-0.62	-0.64	-0.62
	0.96	0.22	<0.01	<0.01	<0.01	<0.01
18:2 c9t11	-0.08	0.16	-0.67	-0.72	-0.71	-0.64
	0.62	0.32	<0.01	<0.01	<0.01	<0.01

* Within a cell, r value is presented at the top and p value at the bottom.

** Significant positive and negative correlations are highlighted in green and red, respectively.

Figure 1. Correlation between solid fat content at 20°C and palmitic acid (16:0) concentration of butter

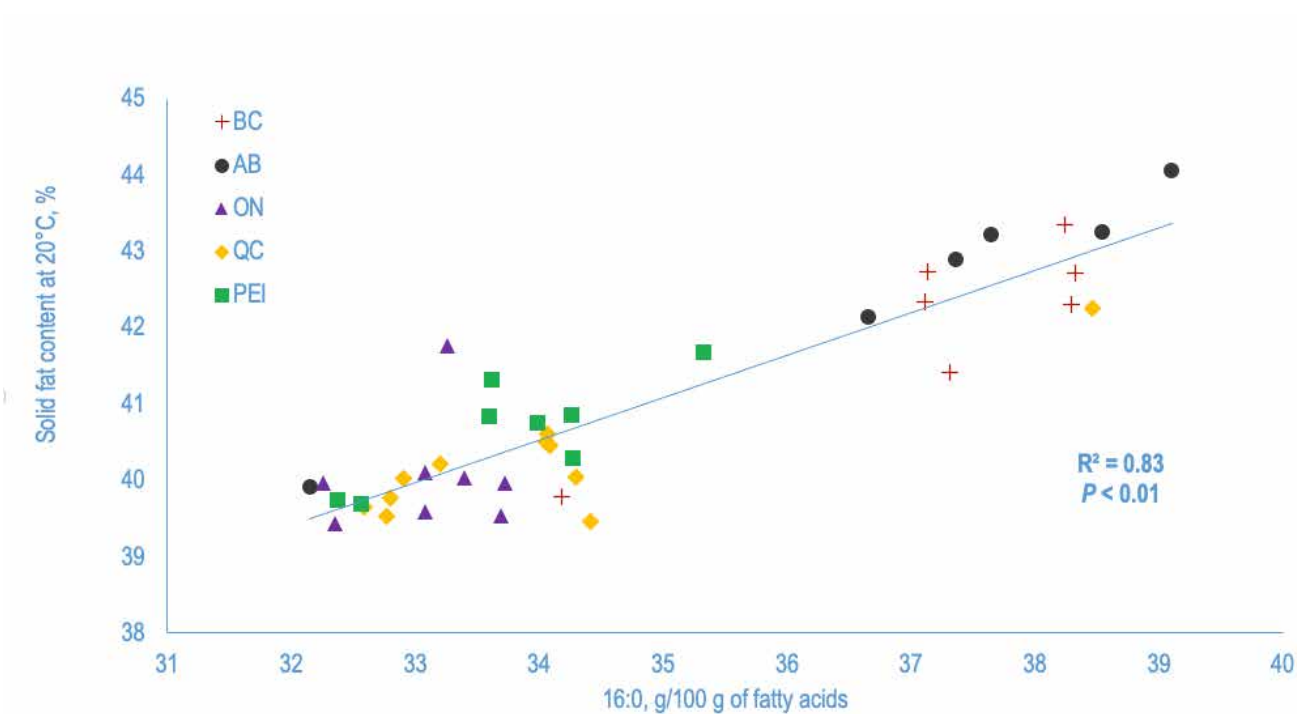
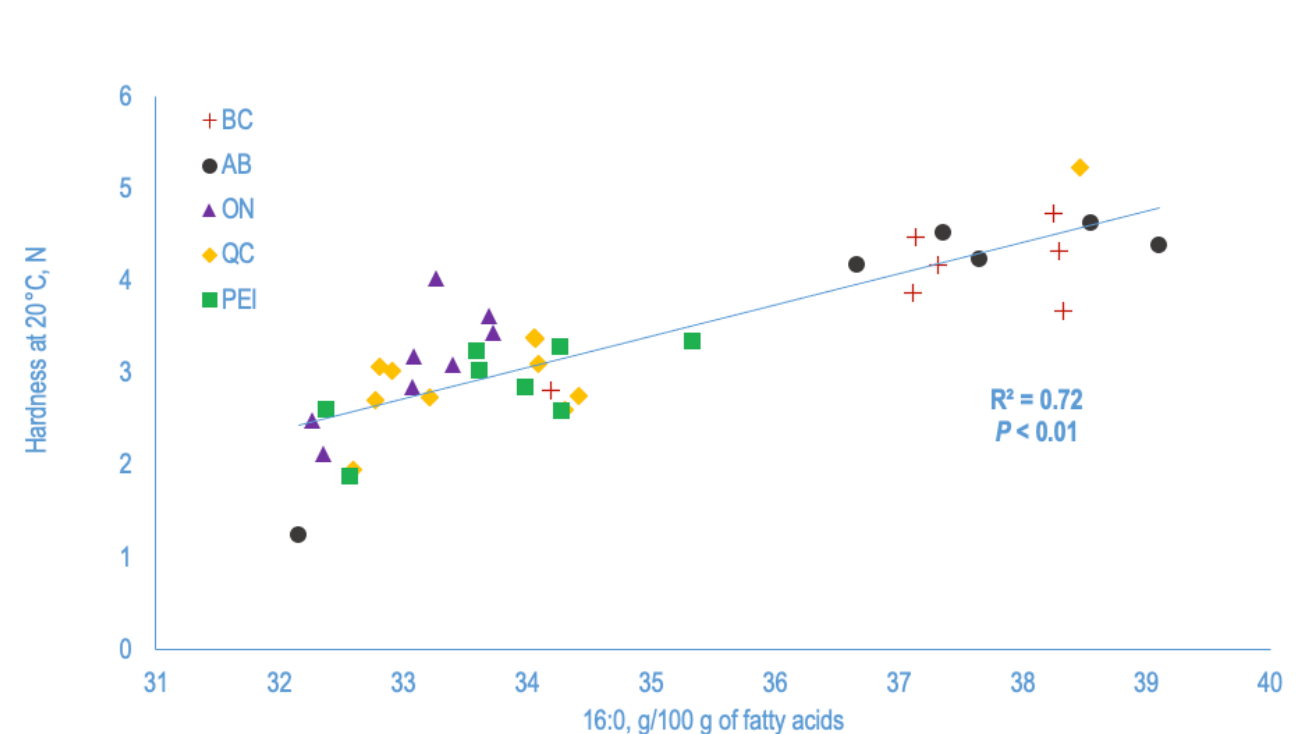


Figure 2. Correlation between hardness at 20°C and palmitic acid (16:0) concentration of butter



Chapter Summary

The information provided in this report is strictly descriptive and cannot be submitted to a proper statistical analysis due to the nature of the sampling procedure. However, results from the current survey provide useful descriptive statistics on the rheological properties of Canadian retail butters and correlations with fatty acid composition.

- One important observation is that milk fatty acid composition is reported here as g/100 g of FA. Comparisons with data from the literature should take this into consideration as milk fatty acid composition can be reported as g/100 g of milk, g/100 g of FA methyl esters (FAME), g/100 g of milk fat, and g/100 g of butter. To better illustrate the differences between these units, if, for example, a milk fatty acid concentration of 16:0 is estimated to be 33.6 g/100 g of FA, this concentration will correspond to approximately 33.4 g/100 g of FAME, 29.6 g/100 g of milk fat, 1.22 g/100 mL of milk, and 24.1 g/100 g of butter. From that, one can easily understand that products need to be compared on a similar basis.
- The 16:0 concentration of Canadian retail butters collected varied between 32 and 39 g/100 g of fatty acids.
- The current survey has demonstrated a relationship between hardness of butter and fatty acid composition.
- As expected, based on previous studies (Chamberlain et al., 2016; Enjalbert et al., 2000) and a recent technical note published by Marangoni and Ghazani (2021), due to the high melting point of 16:0, its concentration in butter is positively correlated with the percentage of solid fat in butter at room temperature and its hardness. However, this survey demonstrates that many other milk fatty acids are also associated positively or negatively with the percentage of solid fat in butter at room temperature and can also impact hardness of butter.
- As mentioned in chapter 4, the packaging of the different fatty acids in milk triacylglycerols is another factor that will have a major impact on the hardness of butter, an aspect that was not covered by the current survey.

References

- » Boivin, M, R Gervais, and PY Chouinard. 2013. Effect of grain and forage fractions of corn silage on milk production and composition in dairy cows. *Animal* 7: 245-254
- » Chamberlain, MB, BC Veltri, SJ Taylor, JW Pareas, R Jimenez-Flores, SO Juchem, G Getachew, and EJ DePeters. 2016. Feeding lactating Holstein cows a lipid source high in palmitic acid changes the fatty acid composition and thermal properties of lipids in milk and butter. *Prof. Anim. Sci.* 32: 672-680
- » Enjalbert, F, MC Nicot, C Bayourthe, and R Moncoulon. 2000. Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. *J. Dairy Sci.* 83: 1428-1433
- » Marangoni, AG, and SM Ghazani, 2021. Perspective: A commentary on elevated palmitic acid levels in Canadian butter and their relationship to butter hardness. *J. Dairy Sci.* 104: 9380-9382.
- » Rico, DE, R Gervais, L Schwebel, Y Lebeuf, and PY Chouinard. 2021. Production performance and oxidative stability of milk enriched with n-3 fatty acids in Holstein cows fed flaxseed meal. *Can. J. Anim. Sci.* 101: 329-341



SECTION 5 – GLOBAL PALM OIL PRODUCTION

Chapter 8: Palm oil and its uses

Chapter Author:

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A primer palm

Palm oil is extracted from the mesocarp of the fruit of oil palms. When harvesting and handling fresh palm fruit bunches, normal bruising occurs causing the fat in the fruit to start degrading. Once the fresh palm fruit bunches are crushed to obtain crude palm oil, these degraded fats (free oleic, stearic and palmitic fatty acids) need to be removed (by distillation, thus Palm Fatty Acid Distillates or PFAD), along with other impurities (e.g., gums) and pigments (e.g., beta-carotene, giving crude palm oil its reddish color), during the refining process to improve the taste, odor, color, and shelf life of the obtained refined, bleached and deodorized palm oil (RBDPO) (Neste, 2021).

Why the use of palm oil is under debate

Oil palms grow best in low lying, wet tropical areas, where rainforests grow. Most palm oil is produced in Malaysia and Indonesia, as global demand for palm oil grows, more and more rainforests are being cleared to make place for oil palm plantations – not just in Southeast Asia, but also in Africa and Latin America. Such expansion has negatively impacted biodiversity, including critically endangered species (e.g., orangutans, elephants and tigers). It has also threatened freshwater ecosystems and caused soil erosion and air pollution. The burning of forests and peatlands to clear and manage land important amounts of greenhouse gases, driving climate change. Finally, expansion has also often occurred at the expense of the rights and interests of local communities and indigenous peoples.

Palm oil use in Canada

Palm oil is widely used in Canada in a number of food products and consumer packaged goods including cookies, chocolates, snack foods, granola bars, baked goods, margarines, peanut butters, well known hazelnut spreads and vegan food products. According to Earthsave Canada, palm oil is reportedly in about 40% of the foods in Canadian grocery stores. It's also in soaps, shampoos, lotions, pet food and toothpastes. Based on Statistics Canada data, (2021), about 120,000 tonnes of palm and palm kernel oil, classified by Stat Can as crude oil and its refined but not chemically modified fractions, were directly imported in Canada in 2020. This does not include palm oil coming into Canada indirectly through the imported transformed goods mentioned previously.

Efforts to make palm oil production more sustainable

Given the sustainability concerns outlined above, several structures were put in place to encourage change and make sustainable production of palm oil a norm. For example, the Roundtable on Sustainable Palm Oil (RSPO), established in 2004 through the collaboration of industry and civil society, has developed guidelines, updated in 2018, for the sustainable production of palm oil, addressing prosperity (for a competitive, resilient, and sustainable sector), people (for sustainable livelihoods and poverty reduction) and planet (for conserved, protected and enhanced ecosystems that provide for the next generation) (RSPO, 2018). The RSPO and other sustainability structures foster strategies and activities to trigger the transformation of the palm oil sector in a sustainable way.

The RSPO has two certification systems to ensure, through transparency and traceability:

- 1) Palm oil is produced sustainably
- 2) The integrity of the trade in sustainable palm oil, i.e., that palm oil sold as sustainable palm oil has indeed been produced by certified plantations and mills.

Both systems involve third-party certification bodies. Such rigorous certification systems considerably reduce the risk for consumers to use palm oil that is not sustainable (RSPO, 2021a). Four levels of certification are available to palm oil users, in decreasing order of assurance (and cost):

- 1) Identity preserved, where sustainable palm oil from a single identifiable certified source is kept separately from ordinary palm oil throughout the supply chain;
- 2) Segregated, where sustainable palm oil from different certified sources is kept separately from ordinary palm oil throughout the supply chain;
- 3) Mass Balance, where sustainable palm oil from certified sources is mixed with ordinary palm oil throughout the supply chain; and
- 4) RSPO Credits/Book & Claim, where the supply chain is not monitored for the presence of sustainable palm oil but manufacturers and retailers can buy Credits from RSPO-certified growers, crushers and independent smallholders.

RSPO certified palm oil only presently represents a small fraction, about 19%, of total palm oil production (RSPO, 2021b), while it is estimated that close to 80% of palm oil refiners are involved in structures to improve sustainability, with focus on three main areas: in line with the RSPO – no deforestation, no peat fire, no exploitation (NDPE) (Chain Reaction Research, 2020).

It is currently difficult to assess the ability to supply certified palm-derived supplements to the Canadian dairy sector. However, major companies supplying the Canadian feed market are members of the RSPO or other similar programs; and have adopted internal NDPE policies in line with the RSPO (ANAC, 2021).

To strengthen Canadian consumers' confidence in the sustainability of the continued use of palm-derived supplements, these sourcing efforts should be encouraged and supported, leading to the ability to choose RSPO certified products (one of the four levels mentioned above) by feed mills and dairy farmers who use such supplements.

Supplementation for cows

Not all dairy farmers use palm-derived supplements. When they do, there are generally two types used: low palmitic acid palm fatty acid distillates (PFAD) calcium salts and high palmitic acid fractionates of palm stearin, the second being more used in the last 10 to 15 years (ANAC, 2021). The first type, PFAD, are a by-product of crude palm oil refining and are used as raw material by many industrial sectors, e.g., to produce soaps and detergents, biodiesel and animal feed (Neste, 2021).

Although refined bleached deodorized palm oil (RBDPO) is widely used for cooking in Asia, it can also be further processed, through fractionation, hydrogenation, interesterification and glycerolysis, into a wide range of products used in the food, cosmetic, personal care products, and animal feed sectors. Through fractionation, RBDPO can be split into olein (liquid) and stearin (solid) fractions (GreenPalm). The most important product obtained from palm stearin is triple pressed stearic acid and soap raw material fatty acids. High palmitic acid fractionates, the second type of product that has been available for dairy cows in the last 10 to 15 years, are a by-product of the soap raw material fatty acids (Green & Natural Industries, 2021).

Palm-derived supplements are then essentially by-products of the refining and transformation of crude palm oil, as sawdust is a by-product of lumber production. Just as the demand for sawdust is not the reason why trees are being cut down, the demand for these supplements is not the driver for palm oil production.

No data are available for the global palm oil production in 2020, the closest are for 2018 with about 79 million tons of palm and palm kernel oil produced (FAOSTAT, 2021). Approximately 35,000 tons of high palmitic acid supplements were imported in Canada by the dairy sector in 2020 (ANAC, 2021). Those are not included in the tariff line/code for "palm oil" imports reported by Statistic Canada but are classified under animal feedcode. This represents less than 0.1% of the globally produced palm and palm kernel oil. It is then reasonable to think that whether Canadian dairy farmers stop using palm-derived supplements or not, it would have no effect on global palm oil production.

For dairy farmers who use palm-derived supplements, it is not clear if they make a significant contribution to the milk's environmental footprint.

A life cycle assessment^{3[1]} was completed in 2018 for Dairy Farmers of Canada by Groupe AGÉCO (DFC, 2018), using 2016 data. It showed that between 2011 and 2016, the carbon footprint of milk decreased by 7.3 %, mainly due to increased productivity (the amount of milk produced by a cow increased by 12.8%).

During the same five-year period, better farming practices leading to the increased productivity were more widely adopted. Such practices include optimization of ration formulation and feeding, improved forage management and feed quality, more frequent emptying of manure storage, composting of manure, reduction of conventional tillage, diversified crop rotation and the use of precision agriculture technologies.

However, at the time of the LCA study, the best estimate for on-farm use of palm-derived lipids was about 0.14% of all the feeds used. At that level, their contribution was not considered material in terms of the LCA assessment.

³ [1] Life cycle assessment is an environmental assessment methodology that holistically evaluates the environmental performance, or footprint, of a production system by considering the complete supply chain and a wide range of environmental issues.

Chapter Summary

- Palm oil is extracted from the mesocarp of the fruit of oil palms. It is used throughout the world as a cooking oil, additive in food manufacturing and in various industrial and consumer packaged goods.
- Approximately 35,000 tons of high palmitic acid supplements were imported in Canada by the dairy sector in 2020. This represents less than 0.1% of the globally produced palm and palm kernel oil.
- The palm-derived supplements that are used by Canadian dairy farmers, and not all farmers use them, are made from palm oil by-products and are therefore not drivers of global palm production.
- Palm-derived supplements represents a small share of all palm oil used in products consumed in Canada.
- Encouraging efforts to improve sustainability and sourcing RSPO certified products when possible are some of the efforts underway in Canada and abroad to make the production, processing and use of palm oil more sustainable.

References

- » *Neste (2021), PFAD residue from palm oil refining*, <https://www.neste.com/products/all-products/raw-materials/pfad-residue-palm-oilrefining#:~:text=Although%20linked%20through%20supply%20chains%2C%20palm%20oil%20and,meets%20the%20EU%20RED%20definition%20of%20%22processing%20residues%22%3A>, consulted 22-08-2021
- » *RSPO (2018), Principles and Criteria for the Production of Sustainable Palm Oil*, <https://www.rspo.org/resources/archive/1079>, consulted on 21-09-2021
- » *RSPO (2021a), RSPO Supply Chains*, <https://www.rspo.org/certification/supply-chains>, consulted on 21-09-2021
- » *RSPO (2021b), The Impact of RSPO*, <https://rspo.org/impact#certification-figures,%20consulted%20on%2021-09-2021>
- » *ANAC (2021), personal communication from Melissa Dumont, Executive Director of Animal Nutrition Association of Canada (ANAC)*
- » *Stat Can (2021), Canadian International Merchandise Trade Database*, <https://www5.statcan.gc.ca/cimt-cicm/home-accueil?lang=eng>, consulted on 22-08-2021
- » *Green & Natural Industries (2021), personal communication from Adrian Ding, Managing Director of Green & Natural Industries SdnBhd, Malaysia*
- » *FAOSTAT (2021), Crops and livestock products*, <https://www.fao.org/faostat/en/#data/QCL>, consulted on 22-08-2021
- » *DFC (2018), Canadian milk production LCA Update*, https://www.dairyfarmers.ca/content/download/6327/56092/version/2/file/LCA_ExecutiveSummary.pdf
- » *Chain Reaction Research (2020), NDPE Policies Cover 83% of Palm Oil Refineries, Implementation at 78%*; <https://chainreactionresearch.com/wp-content/uploads/2020/04/NDPE-Policies-Cover-83-of-Palm-Oil-Refining-Market.pdf>
- » *GreenPalm* - <https://greenpalm.org/resources/infographics>

SECTION 6 – EXPERT WORKING GROUP RECOMMENDATIONS TO THE DAIRY SECTOR

After a careful review of the existing scientific literature and undertaking new testing and consultations with various industry and academic experts, the Expert Working Group has concluded there are gaps in the body of knowledge that should be addressed. These recommendations will help the dairy sector better understand issues related to the characteristics of butter while also ensuring that industry is better equipped to meet consumer expectations.

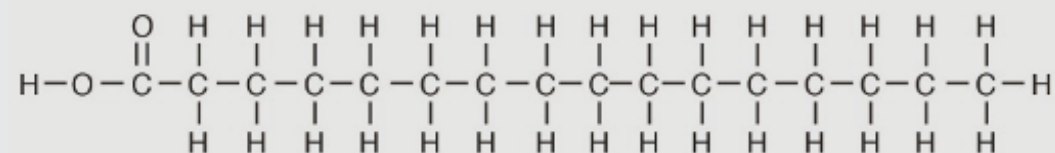
Please note the following recommendations are in no particular order and have been agreed to by all members of the Expert Working Group.

1. In support of science and progress, testing over time and across regions for both milk and butter to understand fatty acid profile in addition to testing for butter hardness off retail shelves should continue and be done in a consistent manner.
2. The dairy sector's innovation and technological advancement continues to evolve rapidly, especially when it comes to R&D in manufacturing and processing. The above testing will help facilitate product and process innovation and greater collaboration between producers, processors and academia. Most importantly, consumers will inevitably benefit from a sector that is working together to enhance and embrace practices that support innovation, sustainability, health and quality.
3. Scientific methods exist to determine firmness of butter, but scientific knowledge to understand what threshold consumers can perceive is sparse. We recommend undertaking research to better understand how consumers perceive texture differences in dairy products, such as hardness of butter.
4. We understand that currently, very limited certified palm-derived supplements are available for the dairy sector to use, but that major Canadian feed mills have been sourcing from companies that are members of the RSPO or have adopted internal policies in line with the RSPO – no deforestation, no peat fire, no exploitation (NDPE) (ANAC, 2021). Efforts towards ethical sourcing and improving the palm oil production sector should be encouraged and supported.
5. Any new Life Cycle Assessment (LCA) study on the environmental footprint of cow milk should collect relevant and representative data on current and new feed practices. This will help understand the impact of various feeding strategies, including the use of any lipids or other supplements to the major crops used in feeds, and understand if there are differences on the environmental footprint of milk.
6. Industry should continue to stay in close contact with consumer groups to better understand consumer preferences as well as potential concerns. Ultimately, providing products that consumers want and expect is a key aspect of ensuring Canadians have trust and confidence in the products they buy and consume.

APPENDIX 1 – GLOSSARY OF KEY TERMS

Palmitic Acid/ palmitate

Palmitic acid (C16:0) is a saturated long-chain fatty acid. It is the most common saturated fatty acid found naturally in animals, plants and microorganisms. It is a major component of the oil from the fruit of oil palms. It is also the predominant fatty acid produced by the human body, and the predominant fatty acid produced by the cow in her milk, irrespective of what a cow is fed.



Palmitic Acid

Palm-derived supplements or palm fat

The use of palm fat in the dairy sector is limited to derivatives of palm oil industry, analogous to the use of sawdust as a by-product of lumber. Cows are not directly fed palm oil; they are fed derivatives of palm oil. Please see Chapter 7 for more information.

FA (s)

Fatty acids are carboxylic acids with long aliphatic chains, which can be saturated (containing only single carbon bonds) or unsaturated (containing multiple bonds between carbon atoms). Palmitic acid and steric acid are examples of saturated fatty acids while oleic acid and linoleic acid are examples of unsaturated fatty acids. There are over 400 different fatty acids in milk.

Rheological properties

This refers to the study of physics which examines how materials form (and deform) and flow in response to applied forces and pressure. With respect to looking at the characteristics of butter this aims to measure firmness or spreadability through various forms of applied forces, and the study of factors that have the potential to contribute to final product characteristics. Please see Section 3 for more details.

Milkfat and Milk fat globules

Milkfat is the fatty portion of milk and is commonly understood by how fluid milk is sold (2%, 4%, skim milk, etc.) Milkfat globules are the fatty structures that are created by the combination of triglycerides, cholesterol and retinol esters. This is important as how these milkfat globules from can be affected by the various factors that influence milk fatty acid composition.

Life Cycle Assessment (LCA)

A Life Cycle Assessment (LCA) is an environmental assessment methodology that holistically evaluates the environmental performance, or footprint, of a production system by considering the complete supply chain and a wide range of environmental issues.

Roundtable of Sustainable Palm Oil (RSPO)

The Roundtable of Sustainable Palm Oil (RSPO) is a global certification system formed in 2004 to define and set standards for the sustainable production of palm oil. While RSPO certified palm oil only presently represents a small fraction, about 19%, of total palm oil production, much of the non-certified palm oil is produced in line with core RSPO policies including no deforestation, no peat fire and no exploitation (NDPE), as about 80% of palm refineries participate in a structure like RSPO or other similar programs.

Palm oil/ Palm fat

Palm oil is extracted from the mesocarp of the fruit of oil palms. The fresh palm fruit bunches are crushed to obtain crude palm oil. Palm oil is used widely in the food industry and in a variety of consumer goods. The terms palm oil and 'palm fat' designate different products, one is the oil squeezed from the fruit, while the latter is a by-product of multiple processing steps and used to designate specific products given to farm animals.

Mammary gland/ udder

The mammary gland is located in the breasts of females and is responsible for lactation (production of milk). Cows have four mammary glands grouped into a structure known as an udder. Various fatty acids such as palmitic acid are synthesized in the mammary gland ('de novo') in the milk produced by lactating cows.

Canadian Food Inspection Agency (CFIA)

The CFIA is a federal government agency within Health Canada that is dedicated to safeguarding food, animals and plants as part of its mandate to protect the health and wellbeing of Canadians and the environment. It regulates and approves animal feed in Canada including the use of any supplement, including palm-derived products.

APPENDIX 2 – SUMMARY OF INFORMATION BY OUTSIDE EXPERTS AND PRESENTERS TO EXPERT WORKING GROUP

- Melissa, Dumont, Animal Nutrition Association of Canada
 - » Overview of cows' diets by region
 - » Why cow nutrition experts recommend lipid supplementation
- Dr. Alejandro Marangoni (University of Guelph)
 - » Presentation of butter testing data
 - » Discussion of various factors that affect fatty acid composition in milk
- David Svab and David Johnson, Canadian Food Inspection Agency (CFIA)
 - » Presented the process CFIA follows when approving animal feeds including supplements

Other discussions with industry and other experts included:

- Dr. Jeremy Hill and Sharon Mitchell, Fonterra (New Zealand)
- Tom Wright, Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)
- Wil Meulenbroeks, Chair of the LTO Dairy Farming Committee, Mr. Aebe Alberts, Sector Specialist Dairy Farming & Secretary of the LTO Dairy Farming Committee (Netherlands)
- Dr Jamie Jonker and Miquela Haselman, National Milk Producers Federation (É. U.)

APPENDIX 3 – EXPERT WORKING GROUP MEMBERS AND BIOGRAPHIES

The Expert Working Group includes prominent academics and experts from across Canada, with a diverse range of expertise. All are recognized as leaders in their fields, with specializations in areas such as dairy nutrition, animal health, sustainability, food science, and human nutrition. The Expert Working Group also includes representation from the Consumers' Association of Canada as well as participation from dairy processors and farm level experts.

Chair of the expert working group

Daniel Lefebvre, Ph. D., PAS, Dipl. ACAN, agr., Chef des opérations, Lactanet

Having grown up on a dairy farm in Montérégie, Québec, Daniel takes great pride in the family heritage that has sparked his passion for dairy production. He studied animal science at McGill University, where he graduated in 1989. Member of l'Ordre des agronomes du Québec, Daniel obtained a Ph.D. in 1998 in Dairy cow nutrition and physiology, also from McGill. Currently COO of Lactanet and Director of the Centre of Expertise in Dairy Production. He has been with the organization since 1993, first as Dairy Nutrition Specialist, then as Director of Research and Development. Daniel is certified by the American Registry of Professional Animal Scientists and the American College of Animal Nutrition. He has been awarded the Animal Industries Award for Science Extension and Service to the Public, by the Canadian Society of Animal Science in 2009 and 2018. He is currently the President of ICAR, the International Committee for Animal Recording.

Anthony Hanley, PhD

Professor, Department of Nutritional Sciences, University of Toronto

Dr. Hanley has a PhD in Epidemiology with expertise in the nutritional and metabolic factors that are related to the progression of type 2 diabetes and its underlying physiological traits, including obesity, insulin resistance and pancreatic beta cell dysfunction.

He also studies high-risk individuals who develop metabolic syndrome—a clustering of risk factors for both diabetes, heart disease and stroke including abnormal blood lipid levels, increased blood pressure, excess fat around the waist and high fasting blood sugar levels.

His research has been supported by the Canadian Institutes for Health Research, the Canadian Diabetes Association, Dairy Farmers of Canada and the University of Toronto Banting and Best Diabetes Centre. Dr. Hanley is also a member of Dairy Farmers of Canada's Expert Scientific Advisory Committee.

Richard Bazinet, PhD

Professor, Department of Nutritional Sciences, University of Toronto

Dr. Bazinet is an expert in lipid metabolism and a Canada research chair in brain lipids and metabolism. He serves as president of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) until March 31, 2021. His research interests include the role of fats in human health, particularly as it relates to brain health and disease. He is also studying the role of certain fatty acids in the development of diabetes and its related metabolic outcomes. Furthermore, he has studied the different lipid profile of grass fed and conventional milk and beef.

His research has been supported by the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes of Health Research, Bunge Ltd, Arctic Nutrition, Dairy Farmers of Canada, and Nestle Inc. and has provided complementary fatty acid analysis to farmers, food producers, and others involved in the food industry.

David Kelton, DVM, PhD, FCAHS

Professor, Department of Population Medicine, University of Guelph

Dr. Kelton is a veterinary epidemiologist and the Dairy Farmers of Ontario Dairy Cattle Health Research Chair at the Ontario Veterinary College. He has been working closely with dairy farmers and veterinary practitioners for over 25 years in carrying out field-based research that address practical issues of concern to the Canadian dairy industry, focusing on on-farm milk quality and safety, animal health and welfare and infectious disease control.

Dr. Kelton's research is supported by NSERC, Agriculture and Agri-Food Canada, the Ontario Ministry of Agriculture, Food and Rural Affairs, Dairy Farmers of Canada, Dairy Farmers of Ontario and the Ontario Research Excellence Fund.

Rachel Gervais, PhD., agr.

Professor, Department of Animal Sciences, Université Laval

Dr. Gervais completed her PhD studies in animal sciences at Université Laval. She completed her postdoctoral studies at Ghent University in Belgium. She is now a professor in the Department of Animal Sciences at Université Laval. Dr. Gervais's research focuses on the effects of diet and nutrition of dairy cows on milk composition and functional properties, mechanisms at work in dairy cows for the synthesis and secretion of fatty acids in milk, and the possibility of using fatty acids in individual cows' milk as a diagnostic tool.

She participates to the Centre de recherche en sciences et technologie du lait (STELA) and is an active member of Op+lait, regroupement pour un lait de qualité optimale.

Dr. Gervais' research is supported by Novalait, the MAPAQ, the Consortium de recherche et innovations enbioprocédésindustriels au Québec and the Natural Sciences and Engineering Research Council of Canada.

Yves Pouliot, PhD

Professor, Department of Food Sciences, Université Laval

As a member of STELA Dairy Research Center, Dr. Pouliot developed a research expertise on milk and dairy ingredients processing, more specifically on membrane separation processes. He has recently been Chair of the NSERC-Novalait industrial research chair on process efficiency in dairy technology. He also led numerous collaborative projects involving different processors from the Canadian dairy industry, Novalait inc., and the Dairy Farmers of Canada.

Jean-François Ménard B.Sc., B.Ing.

Senior Analyst, CIRAIG-Polytechnique Montréal, life cycle assessment (LCA) expert

Mr. Ménard has been involved in life cycle assessment (LCA) since 2002. He has participated in numerous LCAs for both the private and public sectors. As an LCA expert, he regularly conducts critical reviews of LCA done by third parties.

Elaine Scott M.Sc., M.Admin.

Consumers' Association of Canada

Ms. Scott participates in the Canadian Milk Supply Management Committee on behalf of the Consumers' Association of Canada. She holds a Master of Science degree in Human Nutrition from the University of British Columbia and a Master of Administration degree from the University of Regina. Elaine has held senior positions in the Government of Canada and the Government of Saskatchewan including the position of Provincial Nutritionist for the Government of Saskatchewan.

Mathieu Frigon, MSc, MBA, CPA,CMA

Dairy Processors Association of Canada

Mr. Frigon holds a master's degree in Agricultural Economics from Laval University, with over 10 years of experience working in the dairy industry. Mathieu is also a certified accountant and holds a MBA. Mathieu has been President and CEO of DPAC since 2018.

Ed Friesen

Board member, Dairy Farmers of Canada, Lactanet

Ed has been a DHI director for the past 13 years, serving nine of those years as Chair. Ed is currently a Director At Large for Lactanet, and represented Lactanet on the board of Dairy Farmers of Canada until July 2021. Ed recently completed his years of service on Dairy Farmers of Manitoba's board of Directors. He has also served 11 years on the Eastern Holstein Club, including 4 years as President.

Bitra Farhang, Ph.D.

Research and Development Manager at Dairy Farmers of Ontario

Dr. Farhang provides expertise to provincial and national programs related to research, Business and Product Development Programs, Niche market, Nutrition and Sustainability.

Ms. Farhang holds a Ph.D. in Food Science from the University of Guelph, with over 10 years of experience in the dairy industry, and a comprehensive scientific background and technical experience of the dairy science and dairy product manufacturing. She is a member of the IDF (International Dairy Federation) standing committees on Nutrition and Marketing and board of director of the FIL-IDF Canada.

Woody Siemens, P. Ag., B.Sc., MBA

BC Milk Marketing Board

Mr. Siemens currently works for the BCMMB (BC Milk Marketing Board) leading transportation, milk quality, animal welfare and business development functions; working closely with the entire dairy supply chain from the farm to the processor, and most places in between. Woody's career experience includes a range of food and agriculture sectors from feed and nutrition support for dairy farmers to supply chain within PepsiCo Canada. His experience is backed by a BSc in Food Nutrition and Health, a Professional Agrologist Designation, and recently completed an MBA at the University of Guelph specialising in Food and Agribusiness.

Chantal Fleury, agr

Assistant Director Economic Research—Agrology, Quebec Milk Producers

After growing up on a dairy farm in Centre-du-Québec, Ms. Fleury completed a Bachelor's degree in Animal Science at McGill University. She then worked six years for the Centre d'insémination artificielle du Québec (CIAQ) as a service development manager. She has been working for Les Producteurs de lait du Québec since 2013 as an Agrology Advisor and, most recently, as Assistant Director of Economic Research.

CONCLUSIONS AND RECOMMENDATIONS FROM THE EXPERT WORKING GROUP