Impact of processing on the healthy fatty acids in milk and other dairy products

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Abstract

Common dairy processing protocols can alter the composition of fluid milk and dairy foods in ways that affect the amount of healthy fats in the final product. The total amount of fat in dairy products is affected by controlling the fat content of the milk and making different dairy foods that concentrate the fat, for example, whole milk contains 3.25% fat, while butter contains 80% fat. The profile of the fatty acids that make up the fat in milk can be altered by some processing conditions and through animal feeding practices. A comparison of fatty acids in milk and dairy foods coming from pasture-fed or confined dairy herds illustrates the differences in levels of healthy fats. Milk from pasture-fed cows typically exhibits higher levels of the healthy fatty acids and is preferably used in products where the fats will contribute to the daily total of healthy fats in the diet.

Introduction

With increasing consumer interest in sustainable agriculture, organic foods, and grass-fed dairy products, many farmers have backed away from the conventional practices of feeding high grain total mixed rations to confined cows and have opted for practices that incorporate significant amounts of grazing. Certified organic herds must obtain a minimum average of 30% of their dry matter intake from pasture during the grazing season and can include some silages and grains (all grown according to organic guidelines), while the diet of grass-fed herds cannot include grains. Of the 3.8 billion gallons of milk sold in the U.S. in 2016, organic and grass-fed milk accounted for 4.9 and 0.04% of the sales, respectively (DMI, 2016).

Inclusion of grazing as a major source of nutrients for the milking herd has been found to have a tremendous effect on the distribution of fatty acids (**FAs**) that make up the fat found in milk. Milk from grazing cows contains higher levels of the specific FAs that are beneficial to human health, such as the omega-3 poly-unsaturated fatty acids (**PUFA**) and the conjugated linoleic acids (**CLA**). However, milk must be processed to some degree before it reaches the consumer and there is limited, and often contradictory, information on the processing stability of the healthy fats found in milk. The question we address

in this paper is: Once milk leaves the farm, what are the effects of common dairy processing practices on the amount and distribution of the healthy FAs in milk and dairy products?

Fatty acids (FAs) in milk

Almost all (98%) of the fat in milk is in the form of triglycerides with three FAs bound to a glycerol backbone (McGibbon and Taylor, 2006). Over 400 individual FAs have been identified in bovine milk fat so the combinations of the 3 FAs in the triglyceride molecule can be quite extensive (Mansson, 2008). There are 20 FAs that contain 2-18 carbons (C2 - C18) in length that make up 90% of the total FAs present in milk fat, while the rest of the FAs are present in trace amounts and require sophisticated isolation methods for identification. A typical FA profile identifies and quantifies the FAs. Their distribution can be correlated to a variety of factors, from changes in diet to the health of the animal.

Although many of the FAs are known to have specific functions in the body, the healthy fats targeted in this work are the longer chain C18 PUFA. The major omega-3 FA in milk is alpha-linolenic acid, C18:3, with one of its three double bonds located at the 3rd carbon from the end of the chain. Linoleic acid, C18:2, is the predominant omega-6 FA in milk with one of its two double bonds found at the 6th carbon from the end. Isomers of linoleic acid, the CLAs, also designated as C18:2, have a single bond between the two double bonds. The predominant CLA in milk is rumenic acid (70 - 90%) and is the one most beneficial to human health out of the C18:2 isomers. Vaccenic acid, C18:1, contains only one double bond, and is tracked because it is a precursor of rumenic acid. See other papers in this proceedings that describe the FA in more detail and summarize their functions in human health.

Milk processing

Raw milk undergoes many processing steps en route to its final product, which may include altering the fluid product, separating or concentrating the fat, or concentrating the protein and fats (Figure 1). One of the most common steps alters the amount of components in milk by removing or adding proteins, fats, sugar (lactose), and water.

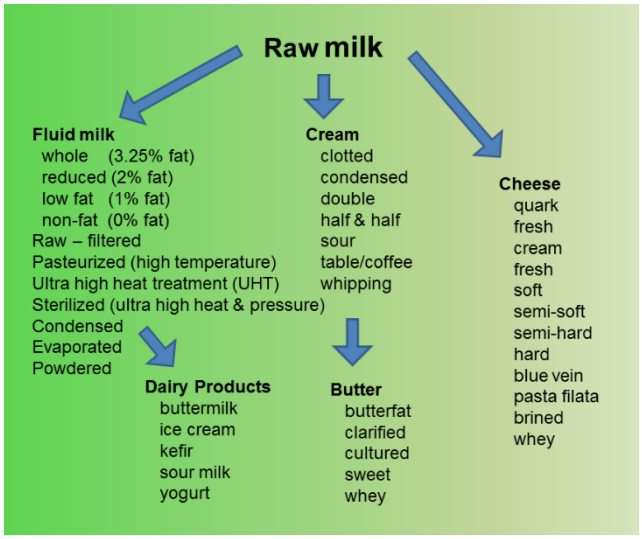

Figure 1. Flow chart of some of the many products manufactured from raw milk

Another step is to expose milk to a variety of stresses, such as heat, pressure, shear, and vacuum. Other modifications, including the addition of enzymes, cultures, and other ingredients. All approaches can affect the specific properties of the dairy food, such as texture, flavor/aroma, and functionality. Two common heat treatments expose milk to high temperatures: high temperature, short time (HTST) pasteurization at 161°F (72°C) for a minimum of 15 seconds and ultra high temperature (UHT) heating at 280°F (138°C) for 2 seconds (21CFR131.3, 2016). Cream, which must contain at least 18% milk fat, requires slightly higher pasteurization temperatures (5°F or 3°C higher). Homogenization is usually conducted in one or two stages, passing milk through a small orifice at pressures between 10 and 25 MPa. This removes the membrane around the fat droplets and breaks them into smaller spheres that stay suspended in the milk. Of the 3.8 billion gallons of fluid milk sold in the U.S. in 2016, raw (not pasteurized) and non-homogenized milk accounted for only 0.01 and 0.03% of sales, respectively (DMI, 2016).

The fat content of dairy products has significant impact on the amount of healthy fats present. Non-fat or skim milk versions contain no fat of any kind and do not contribute to the daily total of healthy fats consumed. In low fat products, the quantity of the healthy fats, although lower than full fat products, still contributes to the daily total in the diet.

There is limited information on the impact of processing on the profile of healthy FAs in milk. One study reported that heating milk at 85°C for 16 seconds or 95°C for 5 minutes did not alter the level of CLA in milk, while UHT heating (140°C for 4 seconds) and microwaving for 5 min decreased CLA content by 15 and 20%, respectively (Herzallah et al., 2005). Batch pasteurization (63°C for 30 min) and microwave heating of milk also increased the distribution of CLA *trans* isomers. Heating milk fat to 200-225°C, slightly higher than typical baking and frying temperatures, decreased CLA by 20-32%, while cooking at >300°C, slightly higher than typical of broiling and commercial pizza oven temperatures, led to isomerization and oxidation of FAs and up to a 60% decrease in CLA levels (Precht et al., 1999). Fermented skim milk products fortified with omega-3 FAs from milk fat were fairly heat stable at 80°C for 30 min (Luna et al., 2004), while fortifying with CLA from sunflowers decreased CLA content by 10% after heating at 73°C for 15 seconds

(Campbell et al., 2003). Unfortunately, not all researchers handle the milk samples the same way before measuring the initial level of FAs before processing. Some studies specifically state that they conducted FA analysis using fresh or frozen raw milk, others heated the milk to 60-100°C to extend shelf life and destroy bacteria, and still others do not mention how the milk was handled. Many of the survey studies report the FA profiles in dairy products without any information of the fat profile in the starting milk. Therefore, a better understanding of the impact that the processing of milk has on the healthy fatty acid profile of milk is needed.

ARS-DFFRU Processing Study

In a recent study conducted in the Dairy & Functional Foods Research Unit (**DFFRU**), fresh raw milk was collected from neighboring grazing certified organic (**ORG**) and confined non-grazing conventional (**CONV**) herds in Berks County, Pennsylvania, over an 8-week period during the grazing season (Van Hekken et al., 2017). Raw milk was standardized to 3.25% fat (Wr) and 1) homogenized (Wh), 2) HTST pasteurized (Wp), 3) homogenized and HTST pasteurized (Whp), or 4) homogenized and UHT heated (Whu). Quantities of the healthy FAs determined in the milk before (Wr) and after processing (Whr, Wp, Whp, and Whu) are shown in Table 1. Compared to the CONV milk, ORG milk contained higher levels of vaccenic acid (C18:1), linolenic acid (C18:3), and CLA. Milk from both farms contained similar amounts of linoleic acid (C18:2). The mean omega-6:omega-3 ratio (linoleic acid: linolenic acid) was lower for ORG milk than CONV milk, 4.9 and 7.3, respectively, and was closer to the ratio of <5 targeted by Simopoulos (2008) to aid in the prevention of many chronic diseases such as heart disease, arthritis, diabetes, and inflammatory disorders. Compared to the starting raw milk, processing did not significantly alter the quantities of the healthy FAs in the milk, therefore, ORG milk continued to contain higher levels of C18:1, C18:3, and CLA as well as lower ratios of omega-6:omega-3 FA. Results indicated that the healthy FAs were stable under common dairy processing conditions of homogenization, HTST pasteurization, and UHT heating.

Table 1. Levels of healthy fatty acids in whole milk fat from grazing organic and confined conventional herds before and after processing. *Samples were raw (Wr), raw homogenized (Whr), HTST pasteurized (Wp), homogenized and HTST pasteurized (Whp), and homogenized and UHT heated (Whu).*

Fatty acids	Vaccenic C18:1 *trans*	Linoleic C18:2	Conjugated Linoleic Acid C18:2 isomers	Linolenic C18:3	omega-6: omega-3 FA ratio
	(g fatty acid/100 g milk fat)				
Grazing organic					
Wr	3.52 [a]	3.31 [a]	0.90 [a]	0.72 [a]	4.6
Whr	3.29 [ab]	3.61 [a]	0.95 [a]	0.75 [a]	4.8
Wp	3.49 [a]	3.58 [a]	0.99 [a]	0.75 [a]	4.8
Whp	3.39 [ab]	3.56 [a]	1.01 [a]	0.68 [a]	5.2
Whu	3.43 [a]	3.51 [a]	0.92 [a]	0.69 [a]	5.1
Non-grazing conventional					
Wr	2.72 [c]	3.60 [a]	0.74 [b]	0.43 [b]	8.4
Whr	2.55 [c]	3.71 [a]	0.74 [b]	0.57 [b]	6.5
Wp	2.83 [bc]	3.51 [a]	0.91 [a]	0.50 [b]	7.0

Whp	2.62 [c]	3.50 [a]	0.81 [ab]	0.53 [b]	6.6
Whu	2.67 [c]	3.36 [a]	0.70 [b]	0.41 [b]	8.2

[a-c] *Means not sharing the same letter within a column are significantly different (P < 0.05).*

We also examined the digestibility of raw and processed milk from the grazing organic and confined conventional herds using *in vitro* digestion techniques (Van Hekken et al., 2017). Overall, milk was digested for one hour in gastric conditions [pepsin enzyme, pH 1.5, 98°F (37°C)] and two hours in intestinal conditions [phosphate buffer, bile salts, mix of enzymes for fats and proteins, 98°F (37°C)]. At the initial pH adjustment and addition of pepsin (G-0), milk formed large protein clots that entrapped the fat. The clots quickly broke down into smaller clots within 15 min of gentle shaking and steadily decreased in size as the *in vitro* digestion progressed. After 3 hours in a simulated gastrointestinal system, 85-94% of the proteins had been digested.

Milk fat was not hydrolyzed during gastric digestion, but the fat droplets tended to coalesce into larger ones as the protein clots broke apart and hydrolyzed. Once intestinal conditions were in place (lipases were included in the enzyme mix), lipolysis occurred as measured by the release of free fatty acids (FFA) from the milk triglycerides (Figure 2). Based on the total amount of FFA measured at 120 min (I-120), 50-60% of the FFA were released within the first 15 min. The rate slowed then stabilized as FFA accumulated and inhibited the lipase activity by blocking the enzyme access to new substrate. This inhibition would not occur in the body where the FFA would be absorbed or moved farther

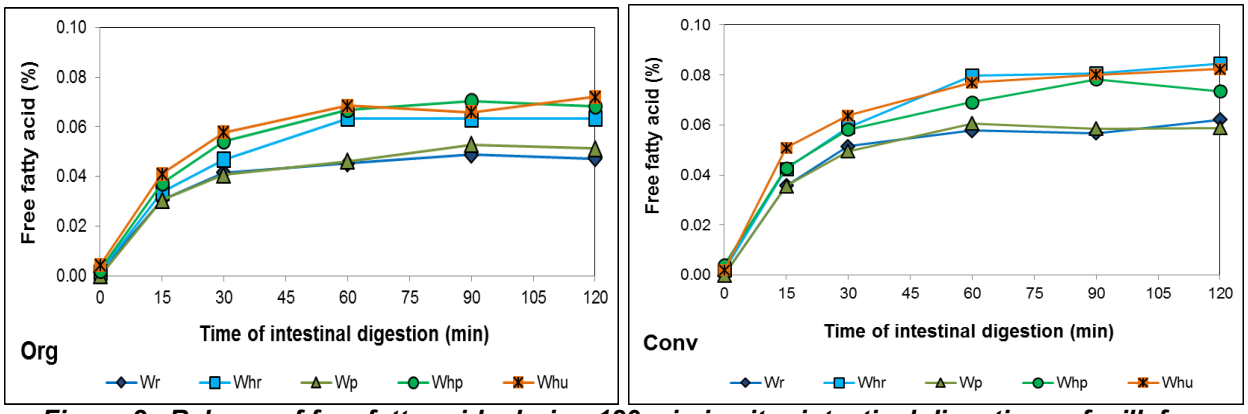

Figure 2. Release of free fatty acids during 120 min in vitro intestinal digestions of milk from grazing organic (ORG) and confined conventional (CONV) herds.

down the digestive tract. Homogenized samples (Whr, Whp, and Whu) released more FFA than the Wr and Wp samples, because homogenization removed the membrane surrounding the lipid droplets and shattered the large droplets into smaller spheres, thus increasing the surface area and accessibility of the lipase to the milk fat substrate. Compared to the ORG samples, the CONV samples released more FFA, in part because they contained higher amounts of C16:0 and C18:0 than the ORG samples, 43.1 and 40.5 mg FA/100 g milk fat, respectively. These saturated FAs are located at the first and third positions of the triglyceride, which are preferred sites for lipase activity. The size and quantity of the fat droplets in the digested sample decreased with time. More research is needed to understand the release and digestion of the healthy fatty acids in the intestinal tract.

Processing of dairy products

Regular yogurt is similar in fat content to milk and can contain 0-3.25% fat. The milk is pasteurized at 203°F (95°C) for 10 min and homogenized before cultures are added and the mix fermented at 107°F (42°C) for several hours. Once the yogurt reaches pH 4.5, it is chilled to 45°F (7°C). Depending on the final product, the yogurt base may have sweeteners and fruit mixed in before packaging.

Research has shown that the basic yogurt processing steps do not alter the CLA levels or distribution of the isomers in the final product (Boylston and Beitz, 2003; Dave et al., 2002; Herzallah et al., 2005; Shantha et al., 1995). Most studies claimed that CLA and omega-3 FAs levels were stable after 7-42 days of refrigerated storage (Boylston and Beitz, 2003; Luna et al., 2004; Dave et al., 2002; and Shantha et al., 1995); only one study reported that CLA content decreased after 7 days (Herzallah et al., 2005).

Cheese is a dairy product that concentrates milk fat (15-45% of the final product) and protein (7-36% of the final product). Cheese making protocols are as diverse as the hundreds of different varieties and styles of cheese made around the world. Briefly, milk (raw or pasteurized, seldom homogenized) is inoculated with cultures to slightly ferment the milk before coagulating with enzymes, primarily chymosin. The milk gel is cut into cubes and cooked before the whey is drained and the curd is salted. Lastly, the curd is packed into molds for pressing and then aged.

Although cheeses have high fat contents, studies have reported that processing protocols had no effect on the distribution of CLA in the fat fraction (mg per gram of fat basis) of Cheddar (Shantha et al., 1995), French Emmental (Gnadig et al., 2004), Gouda, Mozzarella (Shantha et al., 1995), and Swedish-Swiss-type cheeses (Jiang et al., 1997). Studies involving three different varieties of Cheddar, using different starter cultures and slightly

different manufacturing protocols, reported that total CLA levels were stable after 13 months of aging but that the CLA isomer distributions were different among the different brands (Werner et al., 1992; Lin et al., 1999). Another study reported that the CLA content of processed cheese increased 14% during manufacture and was attributed to the cooking step (Garcia-Lopez et al., 1994). One study demonstrated the variety of approaches and issues involved in enhancing omega-3 FA levels in Cheddar, Mozzarella, and Queso Fresco by adding fish or plant oils at different stages of cheesemaking (Bermúdez-Aguirre, 2012).

Research has shown that some dairy cultures can convert free linoleic acid (C18:2) to its isomers (CLA) in media and even less can do so in a milk environment (Bisig et al., 2007). The primary limitation is that the linoleic acid in milk is bound within the triglycerides and the cultures can convert it only when it is released in its free form. Therefore, to increase the CLA content in cheese or yogurt, non-dairy oils from plants and fish that are rich in unbound C18:2 are added, which the dairy cultures then convert to CLA (Kim and Liu, 2002; Bisig et al., 2007). Research continues to screen dairy cultures to identify ones that can increase CLA in dairy products.

Butter is the most fat-dense dairy product and typically contains 80% fat. Processing starts by pasteurizing cream (38% fat) at 203°F (95°C) for 15 seconds, aging for 12 hours and then using mechanical stress (churning) to remove the milk fat globule mem-brane from the fat droplets and coalesce the fat.

Because of the higher fat content, butter contains the highest amounts of PUFAs of any dairy food but the distribution of the healthy fats does not change significantly during processing or storage (Butler, et a., 2011; Mallia et al., 2008; Shantha et al., 1995; Bisig et al., 2007). However, butter is prone to fat oxidation, which causes off-flavors (rancidity) and degradation of the C18 FAs.

Processing does not appear to alter the CLA distribution in sour cream, ice milk, and ice cream. No differences have been reported between the starting raw milk and finished products (Shantha et al., 1995).

The only known process that can significantly alter the distribution of FAs in the final dairy product is cold fractionation. This process uses the low melting properties of the C18s, to concentrate PUFAs into a soft fraction. The method takes melted anhydrous milk fat from 140 to 50°F (60 to 10°C) to get hard and soft fractions, with the soft fraction containing 63% more CLA and 28% more vaccenic acid than the starting fat fraction (O'Shea et al 2000). Another approach is to manipulate the pressure and temperature within a supercritical carbon dioxide chamber to fractionate anhydrous milk fat. At 3500 psi (24 MPa) and 104°F (40°C), anhydrous milk fat yielded five fractions with one fraction containing 89% of the CLA (Romero et al., 2000).

Summary

Processing can alter the amount of total fat in a product, which will determine the total quantity of healthy fats in the food. Most of the processing steps used to make dairy products will not affect the distribution of the fatty acids on a mg/g of fat basis found in the starting milk. However, microwaving and extreme heat processing can alter the distribution of isomers of the C18:2. There are still only two ways to increase the distribution of healthy fats within the FA profile of dairy products:

- Manipulate the cow's diet to increase the level of naturally-occurring healthy fats in the milk.
- Add healthy fatty acids from plant or fish sources to milk or dairy products, which introduces other issues involving incorporation, stability, added cost, and off-flavors.

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