Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations

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ABSTRACT

Consumers are increasingly aware that food components have the potential to influence human health maintenance and disease prevention, and dietary fatty acids (FA) have been of special interest. It has been 25 years since the last survey of US milk FA composition, and during this interval substantial changes in dairy rations have occurred, including increased use of total mixed rations and byproduct feeds as well as the routine use of lipid and FA supplements. Furthermore, analytical procedures have improved allowing greater detail in the routine analysis of FA, especially trans FA. Our objective was to survey US milk fat and determine its FA composition. We obtained samples of fluid milk from 56 milk processing plants across the US every 3 mo for one year to capture seasonal and geographical variations. Processing plants were selected based on the criteria that they represented 50% or more of the fluid milk produced in that area. An overall summary of the milk fat analysis indicated that saturated fatty acids comprised 63.7% of total milk FA with palmitic and stearic acids representing the majority (44.1 and 18.3% of total saturated fatty acids, respectively). Unsaturated fatty acids were 33.2% of total milk FA with oleic acid predominating (71.0% of total unsaturated fatty acids). These values are comparable to those of the previous survey in 1984, considering differences in analytical techniques. Trans FA represented 3.2% of total FA, with vaccenic acid being the major trans isomer (46.5% of total trans FA). Cis-9, trans-11 18:2 conjugated linoleic acid represented 0.55% total milk FA, and the major n-3 FA (linolenic acid, 18:3) composed 0.38%. Analyses for seasonal and regional effects indicated statistical differences for some FA, but these were minor from an overall human nutrition perspective as the FA profile for all samples were numerically similar. Overall, the present study provides a valuable database for current FA composition of US fluid milk, and results demonstrate that the milk fatty acid profile is remarkably consistent across geographic regions and seasons from the perspective of human dietary intake of milk fat.

Key words: milk fat composition, retail milk survey, conjugated linoleic acid, trans fatty acid

INTRODUCTION

Milk and dairy products are recognized as important sources of nutrients in human diets, providing energy, protein, and a wide variety of essential amino acids, vitamins, and minerals (Huth et al., 2006). The composition of milk fatty acids (FA) is of interest for human dietary considerations, as consumers and health professionals are increasingly aware that fat and specific FA can affect human health and the prevention of chronic and acute diseases. Milk fat contains a variety of saturated, trans, and bioactive FA, some of which are included on food nutrition labels or product packaging. In addition, milk fat is responsible for many of the sensory and physical properties of milk and the FA composition can affect the manufacturing properties during the production of dairy foods, such as cheese, butter, and yogurt (Kaylegian et al., 1993; Palmquist et al., 1993).

Assessment of dietary intake is an important tool in monitoring the nutritional status of the US population and this includes the intake of fat and FA (Ervin et al., 2004). Since the last survey of US retail milk supply used for cheese making in 1984 (Barbano, 1990), several changes have occurred in industry practices that may affect the FA composition of milk fat (Palmquist et al., 1993; Jensen, 2002; Chilliard et al., 2007). The use of TMR and dietary lipid supplements have become commonplace, and byproducts from the human food and fiber industry are routinely used in least-cost ration formulations and these vary in fat content and FA composition. In addition, analytical procedures for lipid analysis have improved, allowing greater detail of the FA profile. Jensen (1999) emphasized it was “important to know the fatty acid profiles of dairy foods in the U.S.” and concluded “we cannot make reliable recom-
mendations about the health aspects of milk fat unless we know the kinds and amounts of fatty acids therein.” In a review, Jensen (2002) provided related information by summarizing the composition of bovine milk lipids from scientific papers published between January 1995 and December 2000. However, these publications represent results from experimental treatments rather than reflecting the FA composition of retail dairy products. Hence, our objective was to survey the FA composition of the US fluid milk supply and provide an updated FA profile of retail milk. We were also interested in possible seasonal and regional effects on the FA composition and these were included in our experimental design.

MATERIALS AND METHODS

Sampling Methods

Whole fluid milk samples were obtained from 56 fluid milk processing plants across the contiguous 48 United States. All milk samples were homogenized, pasteurized, and packaged for transport to retail stores. Milk samples were conventionally produced; milk labeled as HTST or ultrapasteurized and specialty labeled milk such as “rbST-free” or “organic” were excluded to minimize variation in production and processing methods. Processing plants were selected based on the criteria that they represented at least 50% of the volume of milk produced in that area, and samples were obtained from each plant every 3 mo during 2008 to capture seasonal variation. Milk was shipped on ice overnight to Cornell University and immediately processed for the analysis of FA composition.

Fatty Acid Analysis

Extraction of FA was based on the Mojonnier method (AOAC, 2000; method 995.19) as modified by Barbano et al. (1988). Briefly, milk fat was obtained from 10 mL of whole fluid milk by a sequence of 3 successive extractions. The first involved the addition of 10 mL of 95% alcohol and 25 mL of ethyl ether followed by vigorous mixing, the addition of 25 mL of petroleum ether followed by vigorous mixing, and then decanting of the ether layer. The second and third extractions were similar except the volume of solvents were decreased to 5 mL of 95% alcohol and 15 mL each of ethyl and petroleum ethers, and the third extraction omitted the 95% alcohol. Ether solutions from the 3 extractions were combined, dried, and resuspended in hexane. Methyl esters of the extracted fat were prepared using sodium methoxide as the methylation reagent, according to Christie (1982), as modified by Chouinard et al. (1999). Fatty acid methyl esters were quantified by GC (Hewlett Packard GCD system HP 6890+, Hewlett-Packard, Avondale, PA) utilizing a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2 μm film thickness; Varian Instruments, Walnut Creek, CA). The flow rate for hydrogen carrier gas was 1 mL/min, hydrogen flow to the detector was 25 mL/min, air flow was 300 mL/min, the nitrogen make-up gas flow was 40 mL/min, and the split ratio was 100:1. The initial oven temperature was set at 80°C and increased to 190°C at 2°C/min and maintained for 15 min. Fatty acid peaks were identified and recoveries quantified using pure methyl ester standards (GLC-569; NuChek Prep, Elysian, MN). In addition, a butter oil reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was periodically analyzed as a control to verify column performance and correction factors for individual FA.

Statistical Analysis

The FA profile of milk samples (n = 228) was used for statistical analysis. Data were analyzed using JMP 7.0 (SAS Institute Inc., Cary, NC); geographic region (Pacific, n = 12; Southwest, n = 13; Southeast, n = 5; Northeast, n = 8; Central, n = 19), season (February, May, October, December) and their interaction were included in the model as fixed effects, and plant nested in region as a random effect. Means and SEM are reported, and significance declared when P < 0.05. For regional and seasonal effects, least squares means and SEM are illustrated in figures.

RESULTS AND DISCUSSION

The FA composition summarized over all milk samples is presented in Table 1. Statistical differences were observed for geographical region and season for individual FA; however, differences were minor and the overall SEM ranged from 0.1 to 0.7% of the mean value for individual FA (Table 1). Thus, the overall FA composition was numerically similar for all regions and seasons, and the minor differences have little or no physiological or public health importance.

Across all the samples, saturated fatty acids (SFA) comprised 63.7 ± 0.09% of total milk FA (mean ± SEM), and this is almost identical to values reported from the 1984 survey (63.8%; calculated from Palmquist et al., 1993). Averages for SFA were similar among regions and across seasons, and when statistical differences did occur they were of a very small magnitude (Figure 1). These results contrast with the wide seasonal variation observed in SFA of milk fat in samples from Dutch dairy plants. Heck et al. (2009) reported that SFA ranged from about 73 to 74% of total milk FA in
winter samples to 68 to 69% in summer samples, and attributed the variation to the marked differences in the diets of the cows between the 2 seasons.

The public health interest in SFA relates to the positive correlation between dietary SFA and cardiovascular disease (CVD; Lichtenstein et al., 2006). Whereas 4:0, 6:0, 8:0, 10:0, and 18:0 are considered neutral, lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0) are considered an atherogenic risk because they increase plasma concentrations of low-density lipoprotein cholesterol (Kris-Etherton and Yu, 1997). However, these specific FA also increase plasma concentration of high-density lipoprotein cholesterol and this is indicative of a reduced risk for CVD. Mensink et al. (2003) conducted a meta-analysis of the effects of different FA using data from 60 clinical studies and expressed changes on the basis of the ratio of total cholesterol to high-density lipoprotein cholesterol; the ratios for 12:0, 14:0, and 16:0 provided no support that these FA represented an atherogenic risk when compared with an isoenergetic substitution with carbohydrate. Furthermore, several recent data summaries and large-scale clinical investigations have challenged the overall concept of saturated fat intake as a risk factor for CVD (Hooper et al., 2001; Taubes, 2001; Howard et al., 2006; Mente et al., 2009). Likewise, recent studies have failed to show any relationship between the intake of dairy products and a risk for CVD (Elwood et al., 2004; German et al., 2009; Parodi, 2009).

Monounsaturated FA (MUFA) represented 29.1 ± 0.08% of total milk FA (mean ± SEM), and this was comparable to the value observed in 1984 (33.5%; calculated from Palmquist et al., 1993). As with the SFA, averages were very similar for different geographic regions and across seasons, although small statistical differences were observed (Figure 1). Oleic acid was the predominant MUFA (23.6 ± 0.08% of total milk FA; mean ± SEM; Table 1), and it is also the primary FA in Mediterranean diets based on olive oil. Diets having a greater content of MUFA are associated with the prevention of cardiovascular disease as reviewed by López-Miranda et al. (2006), and a meta-analysis of 60 controlled clinical studies demonstrated that intake of oleic acid was positively associated with a reduction in the risk for CVD based on effects on plasma cholesterol biomarkers (Mensink et al., 2003). Generally, the dietary intake of oleic acid by ruminants is low and would be extensively biohydrogenated in the rumen. Thus, the oleic acid in milk fat comes predominantly from Δ9-desaturase action on stearic acid (Chilliard et al., 2000). This enzyme is also responsible for the production of other MUFA in milk fat that have a cis-9 double bond, such as myristoleic acid (14:1) and palmitoleic acid (16:1).

### Table 1. Fatty acid (FA) composition of retail milk samples in the United States

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SEM</th>
<th>Effect, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Region</td>
</tr>
<tr>
<td>C4:0</td>
<td>4.15</td>
<td>0.017</td>
<td>NS</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.13</td>
<td>0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.19</td>
<td>0.006</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.59</td>
<td>0.015</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C12:0</td>
<td>2.87</td>
<td>0.018</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C14:0</td>
<td>9.53</td>
<td>0.039</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.82</td>
<td>0.007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.89</td>
<td>0.004</td>
<td>0.0005</td>
</tr>
<tr>
<td>C16:0</td>
<td>28.08</td>
<td>0.078</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.48</td>
<td>0.011</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.52</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>C18:0</td>
<td>11.68</td>
<td>0.078</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:1, trans-6</td>
<td>0.32</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:1, trans-9</td>
<td>0.29</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:1, trans-10</td>
<td>0.55</td>
<td>0.007</td>
<td>0.005</td>
</tr>
<tr>
<td>C18:1, trans-11</td>
<td>1.48</td>
<td>0.013</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:1, trans-12</td>
<td>0.54</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:1, cis-9</td>
<td>23.58</td>
<td>0.074</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:2, cis-9, cis-12</td>
<td>3.19</td>
<td>0.019</td>
<td>0.0001</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.09</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.38</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C18:2, cis-9, trans-11</td>
<td>0.55</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:2, trans-10, cis-12</td>
<td>ND²</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>3.09</td>
<td>0.021</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹Probability of significant differences among corresponding regions, seasons, and their interaction. NS: P > 0.05.
²ND = not detected (<0.01% of total fatty acids).
Polyunsaturated fatty acids (PUFA) are considered beneficial to human health and represented 4.1 ± 0.02% of total milk FA (mean ± SEM), which is greater than the value reported in 1984 (2.8% of PUFA in total milk FA). However, it is important to note that analytical techniques were not as advanced to identify 18:3 unsaturated FA or longer chain FA. This would likely result in an under-reporting of PUFA and a corresponding overestimation of the proportion of SFA and MUFA in 1984 values. Nevertheless, linoleic acid (18:2) was the predominant PUFA isomer at 3.1% of total milk FA and this is similar to the PUFA value from 1984. We observed no seasonal differences in the PUFA content of milk fat, but very small statistical differences did occur among geographic regions (Figure 1).

Trans FA (TFA) represented 3.2% of milk FA, and this value was not reported in the earlier 1984 study due to limitations in the analytical techniques that were used. However, Precht and Molkentin (1996) combined argentation thin-layer chromatography and gas-liquid chromatography to analyze TFA in the milk fat from Germany; this milk fat, derived by melting commercial butter from over 1,700 samples, had an average trans-18:1 FA content of 3.6% of total FA. In the present study, vaccenic acid (trans-11 18:1) was the main positional isomer, representing 46.5 ± 0.22% of total TFA (mean ± SEM) with trans-10 and trans-12 next at 17.4 ± 0.15% and 16.8 ± 0.08% of total TFA, respectively. Vaccenic acid was also the major trans-18:1 isomer in the study by Precht and Molkentin (1996),

Figure 1. Fatty acid composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in retail milk samples by region (A) and season (B) in the United States. Standard deviation bars are shown, but in some cases are too small to display properly. Mean bars with different letters are different within each fatty acid grouping (P < 0.05).
averaging 47.5% of the total \textit{trans}-18:1 FA. Regional and seasonal differences for the distribution of TFA isomers in the present study are illustrated in Figure 2; although some statistical differences were observed, in all cases the differences were minor.

The TFA content of milk fat and the TFA isomer distribution can be markedly affected by diet (Chilliard et al., 2007). The minor differences in the present study suggest that any diet variation among regions and across seasons must have had only very modest effects on rumen biohydrogenation. Interestingly, Precht and Molkentin (1996) observed substantial shifts in TFA related to season that they attributed to diet; a greater total TFA content of milk fat and a higher proportion of \textit{trans}-11 18:1 occurred in summer pasture feeding as compared with that in winter barn feeding. Heck et al. (2009) reached similar conclusions in their study of the seasonal variation in the milk supply from Dutch dairy plants, as availability of pasture directly correlated with increases in \textit{trans}-11 18:1 and other FA known to be affected by lush pasture grazing.

The consumption of monounsaturated TFA has been associated with a greater risk of CVD (Mensink et al., 2003; Mozaffarian et al., 2006). Two dietary sources for TFA exist in the food supply: industrial sources originating from the use of partially hydrogenated vegetable oils and natural sources that are found in ruminant-derived food products. The relationship between TFA intake and risk of CVD is based on industrial sources of TFA. However, several data summaries of epidemiological data have demonstrated that naturally occurring TFA from ruminant-derived foods were not

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**Figure 2.** Isomer distribution of the \textit{trans} 18:1 fatty acids (FA) in retail milk samples by region (A) and season (B) in the United States. Standard deviation bars are reported, but in some cases are too small to show properly. Mean bars with different letters are different within each FA grouping ($P < 0.05$).
correlated to incidence of CVD (see reviews by Lock et al., 2005; Jakobsen et al., 2008; and Stender et al., 2008). This important distinction may be explained, at least in part, by differences in the trans isomer distribution. Ruminant-derived TFA are primarily trans-11 18:1, whereas industrial TFA consist of a Gaussian distribution centered on trans-9 and trans-10 18:1. Of the TFA, trans-11 18:1 has been extensively investigated for potential health benefits related to its conversion to the cis-9, trans-11 isomer of conjugated linoleic acid (CLA; Bauman and Lock, 2006).

The CLA content of milk fat is of special interest because in human diets it predominantly originates from ruminant milk and meat products (approximately 95%). When consumed as a natural component of the diet, CLA has been shown to have potent anticarcinogenic and antiatherogenic effects in biomedical studies using animal models of human disease (see review by Bauman et al., 2006). Conjugated linoleic acid is often expressed as milligrams per gram of FA and we found an average of 5.5 mg/g of FA as the overall mean for the retail milk samples (Table 1). Similar values were reported for milk samples from Dutch dairy plants (5.4 mg/g of FA; Heck et al., 2009) and US specialty labeled retail milk samples (5.7 to 7.0 mg/g of FA; O’Donnell et al., 2010). Whereas we observed only very minor geographic or seasonal differences (Table 1), Precht and Molkentin (2000) observed the CLA content was about doubled in German retail samples obtained during summer pasture feeding as compared with those obtained during winter barn feeding. The CLA content in bovine milk is affected by several physiological factors such as breed and stage of lactation, but the major factor is diet (Bauman and Lock, 2006; Chilliard et al., 2007). Over the last decade, researchers have worked to develop methods to sustain an enhanced CLA content in milk fat to a value that may be physiologically effective, approximately 3- to 5-fold greater than the level reported in the current study (Bauman et al., 2006). Recently, milk and meat foods have appeared on grocery shelves and restaurant menus with label claims of an enhanced CLA content. Our results provide a formal baseline value for the current CLA content of US retail milk.

The n-3 FA are also considered beneficial to human health for the prevention of chronic and acute diseases. In milk fat, α-linolenic acid (ALA) is the predominant n-3 FA and in the present study it was present at 0.38% of total milk FA. Other studies with retail milk fat samples from Germany and the Netherlands and specialty labeled retail milk from the United States have observed a similar low level of ALA in milk fat (Precht and Molkentin, 2000; Heck et al., 2009; O’Donnell et al., 2010). Longer chain n-3 FA that are derived from ALA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were present in minimal quantities and not reported in the current study. The human health benefits from n-3 FA relate to EPA and DHA and their involvement in the prevention of CVD and other chronic diseases (Yashodhara et al., 2009). Unfortunately, the ability to convert ALA to longer chain n-3 FA is very limited in humans (Arterburn et al., 2006; Brenna et al., 2009) and cows (Hagemeister et al., 1991). Thus, the results from our analysis indicate that dairy products provide little contribution to the n-3 FA requirement of humans because the n-3 FA concentration in milk fat is very low and the particular isomer that is present (ALA) is poorly used for the synthesis of EPA and DHA.

Diet is a major factor affecting the FA composition of milk fat. Although feeding management systems and feed ingredients have changed in the last 25 years, milk FA composition was remarkably similar to that in 1984. Likewise, milk FA composition was relatively similar among geographic regions and across seasons, thereby implying that increased use of TMR, lipid supplements, and byproduct feeding must be well established in all regions and in routine use across all seasons. Some differences among individual farms would be expected, but the pooling of milk at the processing plant would dilute milk from farms implementing unique feeding or management practices. Our focus was on retail milk and for this reason we purposefully obtained processed and packaged milk samples to more accurately reflect milk available to the consumer. Improvements in analytical techniques and identification of CLA and trans FA have allowed for reporting of specific isomers of interest related to human health. The present study provides an updated report of the FA composition of US retail milk that can serve as a reference for estimating dietary intake of FA from dairy products and for future studies.

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