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Influence of seasonal variation and ultra high temperature processing on lipid profile and fat globule structure of Swedish cow milk

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ABSTRACT

To investigate the effects of seasonal variations and processing on cow milk fat, raw milk collected in six individual months and corresponding ultra high temperature (UHT) milk were analyzed. Similar seasonal variations in lipid classes and fatty acid composition were found in raw and UHT milk.

Under commercial processing, lipid content was standardized to approximately 1.5% in UHT milk. Decreased diameter of fat droplets (around 1 μm) and thinner globule membranes were observed, as revealed using confocal laser scanning microscopy (CLSM). The distribution of lipid classes was modified with a decreased proportion of triacylglycerol accompanied by the increase of phospholipids and free fatty acids. Saturated fatty acids C12:0 and C14:0, trans-fatty acids including conjugated linoleic acid (CLA), polyunsaturated fatty acids C18:2(n-6) and C18:3(n-3), showed increased proportions in UHT milk.

These results provide an indication of the effect of UHT processing on milk lipid properties.

1. Introduction

Bovine milk is the main dairy product used for human consumption, since it is a rich source of essential nutrients. Several categories of milk with varying fat content are commercially available on the market. In particular, consumption of ultra high temperature (UHT) milk is growing due to high demands on safety and long shelf-life, although it is still not consumed frequently in the Nordic countries. A number of studies have examined whether UHT processing affects the constituents in milk and e.g., Ford, Porter, Thompson, Toothill, and Edwards-Webb (1969) reported a 20% decrease in ascorbic acid and folic acid during UHT processing of milk. Troise, Vitiello, Tsang, and Fiore (2016) suggested that the undesired color and odor of UHT milk might be attributable to Mallard reactions. Moreover, a substantial amount of research is currently being done on protein degradation associated with UHT processing (e.g., Enright, Patricia Bland, Needs, & Kelly, 1999; Marchand, Duquenne, Heyndrickx, Coudijzer, & De Block, 2017). However, little evidence is available on the influence of UHT processing on physical and chemical properties of fat, an important constituent of milk.

Novel microscopy techniques providing great potential to detect the microstructure of fat globules have been developed during the past 10 years (Lopez, 2005; Evers et al., 2008). Native milk fat is present in milk as spherical droplets with diameter ranging from 0.2 to 20 μm (Gallier, Gragson, Jiménez-Flores, & Everett, 2010). The droplets consist mainly of triacylglycerols (TAG) and are enveloped by a surface layer called milk fat globule membrane (MFGM), which protects the core of TAG (Zou et al., 2015). Heating of milk during UHT processing has been suggested to decrease the proliferation of micro-organisms, inactivate adverse enzymatic activity, and thereby extend shelf-life, but also to affect milk properties and composition (Chavan, Chavan, Khedkar, & Jana, 2011). In addition, homogenization causes a decrease in average globule size, and thus results in a corresponding increase in lipid surface area, leading to association of milk proteins to the newly formed surfaces (Ong, Dagastine, Kentish, & Gras, 2010). Taken together, this series of changes in the physical structure of native lipid globules and membranes might modify the chemical composition of lipids in UHT milk.

Lipids in milk are mainly composed of TAG, free fatty acids (FFA), phospholipids (PL), sterols and some trace components (carotenoids, vitamins, and flavor compounds). TAG have a complex composition and are mainly found in the core of globules (Jensen, 2002). PL play an important role in MFGM due to their highly surface-active properties, which have been suggested to contribute to the physical functions of MFGM in preventing aggregation of droplets and resisting mechanical force during processing to a certain degree. There are approximately 400 types of fatty acids (FA) in milk (Månsson, 2008). Today, there is an interest in

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milk lipid and fatty acid profile due to its nutritional value. According to some studies, PL may be involved in anti-cancer and anti-cholesterolemic functions (Spitsberg, 2005). Unsaturated fatty acids (UFA), especially conjugated linoleic acid (CLA), show anti-carcinogenic properties (Lock & Bauman, 2004). Changes in the lipid profile (e.g., seasonal variations) in raw milk have been reported in many studies (e.g., Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). In contrast, data on how the characteristics of raw milk influence its final lipid properties in UHT-processed milk are lacking.

Therefore, the aims of this study were to investigate i) seasonal variations in lipid and fatty acid composition in both raw and UHT milk samples and ii) the influence of processing on the lipid profile and microstructure of UHT milk fat globules and membranes.

2. Materials and methods

2.1. Raw milk and UHT processing

Unprocessed raw milk and the corresponding UHT-processed milk were collected at Norrmejerier dairy plant located in Luleå (Sweden) in March, May, June, July, September, and November 2015. The same samples as in Karlsson, Langton, Innings, Wikström, and Lundh (2017) were used, comprising pooled raw milk sampled from approximately 80 farms and transported to the Swedish University of Agricultural Sciences (SLU, Uppsala) at chill temperature (4 °C). The UHT milk was standardized to a fat content of 1.5%. The UHT treatment was performed with indirect tubular heat exchangers at 137 °C for 4 s on preheated, defatted, and re-fatted (1.5% fat added) milk. All samples were aliquoted and analyses were performed on the day after delivery. Raw and UHT milk samples were done in triplicates from March, May, June, July, September, November, respectively, resulting in 36 samples.

2.2. Confocal laser scanning microscopy

2.2.1. Sample preparation

Neutral lipids (mainly TAG) were stained with the lipid-soluble fluorescent dye Nile Red (5H-benzo aphenoxazine-5-one, 9-diethylamino; Sigma-Aldrich, St Louis, USA), by mixing 0.5 mL of milk with 5 μL of Nile Red stock solution (0.1% Nile Red in acetone) for 15 min. Rh-DOPE (1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl), Avanti Polar Lipids, USA) was used to localize phospholipids distributed on the surface of the milk fat globule membrane. About 8 μL Rh-DOPE solution (1 mg/mL in chloroform) were added to 2 mL milk samples in Eppendorf tubes for 30 min. Low melting point agarose (Sigma, St Quentin Fallavier, France) was prepared at 10 g/L, heated to 85 °C, and cooled to 45 °C prior to use. Then 100 μL agarose and 100 μL stained samples were mixed in an Eppendorf tube and 20 μL of the mixture were placed on a concave microscope slide. The function of agarose was to prevent milk structures from flowing during microscopy. During all staining procedures, the Eppendorf tubes were wrapped in aluminum foil to minimize loss of fluorescence signal.

2.2.2. CLSM analysis

A confocal laser scanning microscope (CLSM; Zeiss LSM 780, Jena, Germany), built on an inverted Zeiss Axio Observer and supersensitive GaASp detector, was used for the microstructural analysis. Nile Red was excited with an Argon laser (488 nm) and emission wavelengths between 500 nm and 530 nm were detected. A He-Ne laser operated at 543 nm excitation wavelength and emission wavelengths from 565 nm to 615 nm was used to detect Rh-DOPE. All images were acquired using a C-Apochromat 63x oil immersion objective (1.32 NA) with a resolution of 1024*1024 pixels.

2.3. Lipid analyses

2.3.1. Fatty acid composition

Lipid extraction from milk samples was based on the methods of Hara and Radin (1978), using a mixture of hexane and isopropanol (3:2, v/v). Total lipid content was extracted, dried under a stream of nitrogen and weighed. Fatty acid methyl esters (FAME) were obtained from methylation according to Appelqvist (1968) and analyzed by gas chromatography using a CP 3800 instrument (Varian AB, Stockholm, Sweden), fitted with a flame-ionization detector (GC-FID). Chromatographic separation was carried out using a fused silica capillary column BPX 70 (50 m × 0.25 mm inner diameter, 0.25 μm film thickness, SGE, Austin, TX, USA). Peak identification was performed by comparing retention time with that of available FA methyl ester standard mixture GLC-461 (Nu-chek Prep, Elysian, MN, USA). Galaxie chromatography software (version 1.9, Varian AB, Stockholm, Sweden) was applied for peak integration. Triplicates of each milk sample were measured. Each fatty acid in the samples was expressed as g/100 g FAME.

2.3.2. Lipid class composition of total lipids

Lipid class composition analysis was performed as previously reported by Mraz and Pickova (2009) with minor modifications, using high performance thin layer chromatography (HPTLC). According to the results in a pre-study (data not shown), extracted lipids (4 μg/μL) were applied on the pre-coated silica 60 HPTLC plates (20 × 10 cm, 0.20 mm layer; Merk, Darmstadt, Germany) using a Camag ATS4 automatic TLC sampler (Kovalent AB, Västra Frölunda, Sweden) and separated by a Camag ADC2 automatic developing chamber (Kovalent AB, Västra Frölunda, Sweden) with mobile phase hexane: diethyl ether: acetic acid (85:15:2, v/v/v). Phosphomolybdic acid in ethanol was used for derivatization and a Camag TLC scanner 3 (Kovalent AB, Västra Frölunda, Sweden) was used to scan the dried plates at 650 nm. Peak identification for lipid class was conducted by comparing with an external standard (TLC 18-5A; Nu-check prep Inc.) and expressed as percentage of height (Fig. S4). Each sample was displayed on three plates to minimize the variations.

2.4. Statistical analysis

The effects of season and process and their interactions on lipid profile, including six lipid classes, i.e., PL, 1-2 diacylglycerols (1-2 DAG), 1-3 diacylglycerols (1-3 DAG), sterols, free fatty acids (FFA), TAG, and 26 fatty acids, were analyzed using the General Linear Model (GLM) statement in SAS (SAS 9.3, SAS Institute, Cary, NC, USA). In terms of lipid classes, each class was calculated as percentage of total lipids. For FA, an individual FA was included in the analysis only when its area percentage exceeded 0.1% of total area. Eight uncertain FA (present as UNK) were also included. All FA were expressed as g per 100 g total FA for further analysis. Non-normally distributed data, assessed by the Anderson-Darling test, were log-transformed and re-tested. Tukey's post hoc test was used for pair-wise comparisons when a significant effect of interest was observed. False discovery rate (FDR, q value) were used to offset the problems of multiple comparisons, with q value < 0.5 set as significant. Least squares means (LSM) obtained from fitted models are presented throughout the paper.

Moreover, principal component analysis (PCA) was used to screen distinct groupings for lipid profile. Partial least squares discriminant analysis (PLS-DA) was used to investigate the effect of season and processing on lipid profile, while partial least squares regression (PLS) was used to evaluate the relationship between