J. Dairy Sci. 97:7307–7315
http://dx.doi.org/10.3168/jds.2013-7849

Stability of fatty acid composition after thermal, high pressure, and microwave processing of cow milk as affected by polyunsaturated fatty acid concentration

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ABSTRACT

Interest has been increasing to enhance the contents of healthy polyunsaturated fatty acid (PUFA) in milk. However, *trans* fatty acids and conjugated linoleic acid (CLA) can be altered after thermal processing and high pressures disrupt the milk fat globule membrane, exposing the lipid core and helping its oxidation. The objective of the present research was to study whether processing can alter the fatty acid composition of milk and if these changes are affected by PUFA concentration as previous studies suggest. Two cow milk batches (500 L each), one naturally enriched in PUFA, were processed to obtain pasteurized; high temperature, short time; UHT; high pressure; and microwave pasteurized samples. The detailed fatty acid composition was analyzed with special attention to *trans* fatty acids and CLA isomers. Results showed that after high temperature, short time processing, total CLA content increased in both milk batches, whereas sterilization resulted in a sigmatropic rearrangement of C18:2 *cis*-9,*trans*-11 to C18:2 *trans*-9,*trans*-11. The extent of these effects was greater in milks naturally enriched in PUFA.
Key words: milk processing, conjugated linoleic acid isomers, *trans* fatty acids, unsaturated fatty acids

INTRODUCTION

Because of the relatively high amount of SFA and *trans* fatty acids (**TFA**) in dairy fat and the role of those compounds in the risk of chronic and other metabolic diseases, food industries are committed to decrease SFA, cholesterol, and TFA contents as much as is possible (Nishida et al., 2004; Haug et al., 2007). The high susceptibility of milk fat profile modulation to diet composition has led to the carrying out of an extensive number of research works supplementing forage/ensilage diets with high content PUFA oils or oilseeds and resulting in dairy products naturally enriched in polyunsaturated bioactive lipids (from 3.6 to 4.8 g of PUFA/100 g of fat with control diets to 9 g of PUFA/100 g of fat with supplemented feeding) and lower SFA concentrations (from 65 to 68 g of PUFA/100 g of fat with control diets to 55–47 g of PUFA/100 g of fat with supplemented feeding; Jones et al., 2005; Lynch et al., 2005; Jenkins and McGuire, 2006).

Furthermore, PUFA have been demonstrated to exert important beneficial activities in human health: linoleic acid (C18:2 *cis* (**c**)-9c12) is a precursor of arachidonic acid, involved in the synthesis of molecules associated to inflammatory processes (Calder, 2006), and linolenic acid (C18:3 c9c12c15) is a precursor of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids (Williams, 2000). The rate of these 2 FA (n-6/n-3) in human erythrocytes is related to cardiovascular risk (Harris, 2008). Even more, CLA has been extensively associated with anticancer (Hagen et al., 2013) and body weight properties (Rodríguez-Alcalá et al., 2013b).

In milk, oxidation of lipids produces strong off-flavors and deterioration of the nutritional and health quality, affecting both the shelf-life and acceptability of the product by consumers (Rafalowski et al., 2014). These reactions are affected by the degree of unsaturation of the FA, and PUFA are highly prone to oxidation, mainly when radical starters are present. Because bioactivity of these compounds is associated with a dose [e.g., 3 g of CLA/d according to Ip et al. (1996)], any possible degradation would result in a daily intake below the amount needed to obtain the beneficial effect. Thus, in dual-homogenized (20,000 kPa) skim milk fortified with CLA oil, total *trans trans* CLA isomers concentration increased, whereas total double-bond *cis cis* decreased after industrial atomization processing (Rodríguez-Alcalá and Fontecha, 2007). Studies focused on the effects of pasteurization (homogenization at 13,789 kPa; 77.2°C, 12 s) reported that rumenic acid but not C18:2 *trans* (**t**)-10,*cis*-12 CLA decreased within 24 h after treatment and refrigerated storage at 5°C (Campbell et

Received December 18, 2013.
Accepted August 5, 2014.
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al., 2003). Herzallah et al. (2005b) found that low pasteurization (63°C, 10 min) and microwaving (96.8°C, 5 min) processing of milk caused losses of TFA whereas HTST after 3 d at 5°C (85°C, 16 s), UHT after 5 d (140°C, 4 s), and microwave processing decreased the total concentration of CLA.

As a solution to this, new emerging technologies such as high pressure processing and homogenization (50–1,000 MPa) are being studied to evaluate their potential as alternative or complementary process to thermal pasteurization as 400 MPa, 15 min are conditions able to kill most bacteria in milk (Urala and Lahteenmaki, 2007; Chawla et al., 2011). However, it has not been found modifications of the lipid fraction when human (0–600 MPa) or cow, goat, and sheep milk (0–350 MPa) were processed by high pressure or high pressure homogenization (Rodríguez-Alcalá et al., 2009; Moltó-Puigmartí et al., 2011). It is known that those treatments can result in partial whey protein denaturation, casein micelle dissociation, and disruption of the milk fat globule membrane (Pereda et al., 2007; Zamora et al., 2007). There is also evidence that 200 MPa, 30 min and inlet temperature of 60°C increased the native lipase activity when compared with raw milk (Datta et al., 2005).

The aim of the present work is to study the possible effects of conventional heating (pasteurization, UHT, and sterilization), high pressure, and microwave processing on the fatty acid composition of naturally PUFA-enriched milk with special attention to CLA isomers and TFA.

MATERIALS AND METHODS

Chemicals

Hexane, methanol, and chloroform were purchased from LabScan (Dublin, Ireland); potassium hydroxide, sodium sulfate-1 hydrate, and 20% aqueous solution of $AgNO_3$ from Panreac (Barcelona, Spain); CLA standards from Nu-Chek Prep (Elysian, MN); stearic FAME (C18:0), elaidic FAME (C18:1 t9), rumenic acid (C18:2 c9,t11; **RA**), 1,2,3-tritridecanoylglycerol, and sodium azide from Sigma (St. Louis, MO); and high CLA concentration oil (Tonalin) was obtained from Cognis (Illertissen, Germany). All reagents were GC or HPLC grade. Reference milk fat butter BCR-164 (EU Commissions; Brussels, Belgium) was purchased from Fedelco Inc. (Madrid, Spain).

Samples

Two raw milk batches of 500 L labeled as B1 (batch 1) and B2 (batch 2), collected from different cows herds (Holstein breed) from the Castilla-La Mancha region (Spain) were kindly donated by a Spanish dairy company (Leches Pascual, Aranda de Duero, Spain). Animals producing the B2 samples were fed with linseed. Milk was processed to obtain pasteurized (72°C, 30 s), HTST (85°C, 30 s), and UHT (**UHT1**, 135°C, 30 s, and **UHT2**, 150°C, 5 min) samples using an aseptic sterilizer (Rossi & Catelli, Parma, Italy) as follows: 30 L of raw milk per assay were preheated to 65°C and then homogenized at 18 MPa. The temperature was raised to pasteurization conditions and then cooled to an output value of 20°C. In UHT processing, the preheating step was carried out after homogenization (85°C). Sterilization (**STR**, 121°C, 15 min) was carried out using 1 L of raw milk from each batch in a laboratory autoclave (Selecta Autotester E DRY-PV, Barcelona, Spain). As control samples 4 L of raw milk were collected from the 2 batches. For high pressure processing (400 MPa, 25°C, 15 min), a laboratory-scale high pressure machine (ACB, GEC, Alsthom, Nantes, France) was used with 200 mL of raw milk fitted in pressure-resistant packages. Microwaving (650 W, 1.30 min) was performed using 100 mL of raw milk in a domestic apparatus (Moulinex AET1, Ecully Cedex, France). All samples were placed into amber-glass bottles and sodium azide (0.06 g/mL) added to avoid microbial growth. All samples were placed in refrigeration after processing. Assays were performed in duplicate.

Basic Parameter Analysis

Fat content, protein, lactose, and dried extract were measured using a Milkoscan (Foss, Hillerød, Denmark).

Lipid Extraction and FA Derivatization

Milk fat extraction was carried out according to standard methods (ISO-IDF, 2001). The fat residue was collected into amber vials and stored at −20°C until analysis. Fatty acid methyl esters were prepared by base-catalyzed methanolysis (2 *N* KOH in methanol) according to ISO-IDF (2002). As internal standard, 1,2,3-tritridecanoylglycerol, was added to samples (200 μL, 1.24 mg/mL). All analyses of samples were carried out in duplicate.

Fractioning by $AgNO_3$-Thin Layer Chromatography of FAME

Fatty acid methyl esters were fractionated according to the number and geometry of double bounds by thin layer chromatography (**TLC**) according to Alonso et al. (1999). The TLC glass plates (20 × 20 cm) with silica gel (0.25 mm; Merck, Darmstadt, Germany) were