International Dairy Journal 43 (2015) 70–77

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Oxidative stability of ultra high temperature milk enriched in conjugated linoleic acid and *trans*-vaccenic acid

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ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form
18 November 2014
Accepted 18 November 2014
Available online 9 December 2014

ABSTRACT

Milk naturally enriched in conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA) was ultra high temperature (UHT)-treated at 125–145 °C for 2–20 s and stored at 4 and 25 °C for up to 120 d. The oxidative stability of treated enriched milk was evaluated in terms of changes on the contents of CLA and TVA, dissolved oxygen, hydroperoxides, thiobarbituric acid reactive substance (TBARS) and formation of volatiles. After UHT treatment, more than 78% of CLA and 87% of TVA remained. After 15 d of storage at 25 °C, the CLA and TVA were relatively stable with values in the range of 67–75 and 63–73%, respectively. During storage, CLA oxidized faster than TVA, independently of the UHT treatment and storage conditions. Heptanal was the most abundant volatile resulting from UHT processing and a potential suitable marker for heat treatment of milk rich in CLA and TVA.

1. Introduction

In many countries, bovine milk is considered to be an important part of a healthy diet, and a source of many beneficial bioactive compounds (Molkentin, 2007). One group of fatty acids of particular interest is conjugated linoleic acid (C18:2, CLA), which is a mixture of over 15 different positional and geometrical isomers of linoleic acid. The conjugated double bond can be *cis*-/*trans*-, *cis*-/*cis*- or *trans*-/*trans*- from position 6 to 14 (Lock & Bauman, 2004). The *cis*-9/*trans*-11 isomer is the most abundant (≥90%) and therefore the health-promoting and disease-preventing properties of CLA have been attributed to *cis*-9/*trans*-11 isomer. Minor isomers, such as *trans*-9/*trans*-11 have inhibited platelet aggregation (Al-Madaney, Kramer, Deng, & Vanderhoek, 2003) and *trans*-10/*cis*-12 isomer showed the ability to quench free radicals, which reduced lipid peroxidation of triglycerides rich in unsaturated fatty acids (Fagali & Catala, 2008). Another fatty acid of interest is *trans*-vaccenic acid (TVA; C18:1 t11), which is an endogenous precursor of *cis*-9/*trans*-11 (CLA) through delta-9 desaturase. In addition, TVA supplementation showed substantial hypotriglyceridemic benefits in obese rats (Wang et al., 2008). The concentration of CLA found in milk is only 1% of the total milk fat (Creamer & MacGibbon, 1996), which is below the estimated amount needed to provide anticarcinogenic effects, 55 mg to 3.5 g per d (Knekt, Jarvinen, Seppanen, Pukkala, & Aromaa, 1996). On the other hand, TVA represents 2% of the total milk fat and its recommended daily intake has not been estimated.

Strategies leading to increases in the concentration of CLA in dairy products have become a topic of industrial interest (Rodriguez-Alcala, Villar-Tajadura, Juarez, & Fontecha, 2013). Current approaches to increase the concentration of CLA include: (i) fermentation of dairy products with lactic acid culture, (ii) fortification by the addition of synthetic CLA, and (iii) enrichment of milk through nutritional management of dairy cattle. Enhancing the concentration of CLA through dietary manipulation of dairy cattle has created new opportunities for development of functional drinks (Jenkins & McGuire, 2006). The concentration of CLA in milk fat increased up to 10-fold in dairy cattle fed with a diet supplemented with safflower oil and vitamin E (Bell, Griinari, & Kennelly, 2006). Furthermore, the diet reduced the concentration of the major saturated fatty acids (C14:0 and C16:0), which was considered by some authors as an additional benefit since the consumption of saturated fatty acids increased risk of cardiovascular disease (Bell et al., 2006; Martínez-Monteagudo, Khan, Temelli, & Saldaña, 2014). However, this diet increased the concentration of C18:0 by 40–57% and lowered the milk fat content by 27–30%. Modifications

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http://dx.doi.org/10.1016/j.idairyj.2014.11.009
0958-6946/

of milk fat to achieve elevated levels of unsaturated fatty acids, such as CLA and TVA might also negatively impact the oxidative stability of milk upon thermal processing and subsequent storage (Rafalowski, Zegarska, Kuncewicz, & Borejszo, 2014). This was demonstrated by Martínez-Monteagudo and Saldaña (2014), who heated milk rich in CLA and TVA (90–120 °C for 15 min) and found that the remaining amounts of both fatty acids were reduced to 80 and 60% of the total CLA and TVA, respectively, as temperature increased. Similarly, 85% of CLA remained in non-enriched milk treated at 140 °C for 4 s (Herzallah, Humeid, & Al-Ismai, 2005).

Milk pasteurization under high-temperature-short-time (HTST, 72–75 °C for 15–20 s) provides a shelf life of 15 d at 6 °C. UHT treatment of milk (130–150 °C for 2–10 s) allows processors to achieve commercial sterilization that extends the shelf life up to 180 d at room temperature (Rysstad & Kolstad, 2006). It is well known that lipid oxidation of milk leads to the development of unpleasant flavors, loss of nutrients and the formation of potentially toxic compounds (Frankel, 1991). Information on the effect of UHT processing conditions on the oxidation of milk enriched in CLA is limited to one study of Herzallah et al. (2005). The objective of our study was to evaluate the oxidative stability of milk rich in CLA and TVA, following UHT treatments and during storage. The oxidative stability was evaluated in terms of changes on the CLA and TVA contents, dissolved oxygen content and the formation of primary oxidation products (hydroperoxides) and secondary oxidation products (aldehydes and thiobarbituric acid reactive substance, TBARS).

2. Materials and methods

2.1. Milk rich in conjugated linoleic acid and trans-vaccenic acid

A diet supplemented with safflower oil and vitamin E was fed to dairy cattle for 21 d. The guidelines of diet supplementation are provided elsewhere (Bell et al., 2006). The final concentrations of CLA and TVA obtained at the end of the feeding regime were 43 ± 2 and 106 ± 2 mg g^{-1} fat, respectively. The milk enriched in CLA and TVA was collected and handled following the methodology reported by Martínez-Monteagudo, Saldaña, Torres, and Kennelly (2012b).

2.2. Ultra-high-temperature treatment

Raw milk enriched in CLA and TVA was standardized and homogenized following the protocol reported by Martínez-Monteagudo and Saldaña (2014). The standardized and homogenized milk was then processed using an indirect UHT unit Armfield FT74P (Ringwood, Hampshire, UK) with a plate heat exchanger. The unit is coupled with a flow rate meter and either a 2 or 15 s holding tube for short (<5 s) and for long (>8 s) holding times. Six UHT conditions were used to treat the CLA/TVA-enriched milk (125 °C for 2 s and 15 s, 135 °C for 3 s and 10 s, and 145 °C for 4 s and 20 s). The selected processing conditions correspond to typical conditions used to extend the shelf life of milk. Before pumping the enriched milk, the unit was run with water until the desirable flow rate, temperature and pressure (4 bar) were reached. Then, the milk enriched in CLA and TVA was pumped and the first 20 mL milk was discarded. Afterwards, the UHT milk was collected and packed in amber glass vials, leaving a headspace of one quarter of the vial. Samples treated at 125 °C for 15 s, 135 °C for 10 s and 145 °C for 20 s were stored at 4 and 25 °C and analyzed at 0, 7, 15, 30, 60 and 120 d, but samples treated for volatile analysis were stored up to 30 d at 4 and 25 °C.

2.3. Conjugated linoleic acid and trans-vaccenic acid determination

The concentrations of CLA and TVA were determined by gas chromatography (GC; Varian 3400, Palo Alto, CA, USA) with an SP-2560 column (100 m length × 0.25 mm; fused-silica capillary column, Supelco Inc, Bellefonte, PA, USA) and a flame-ionization detector. The GC was operated following the conditions reported by Cruz-Hernandez et al. (2004). The extraction of fatty acids from milk and their further methylation was performed according to the methodology reported by Martínez-Monteagudo et al. (2012b). One milliliter of internal standard, methyl heptadecanoate (Fluka #51633 purity 99.5%, Sigma–Aldrich, Saint Louis, MO, USA), was added to each methylated sample. The CLA content measured by GC represents the total CLA.

2.4. Hydroperoxide determination

Lipid hydroperoxides were determined spectrophotometrically according to the methodology reported by Smet et al. (2009). The lipid hydroperoxide concentration was calculated using a calibration curve of cymene hydroperoxide (Sigma–Aldrich, Oakville, Canada), obtained within the range of 5–150 μM. Samples outside this range were diluted accordingly.

2.5. Thiobarbituric acid reactive substance determination

Secondary oxidation products were evaluated through thiobarbituric acid reactive substances (TBARS) as reported elsewhere (King, 1962). The TBARS was calculated using a calibration curve of tetraethoxypropene (TPE) (Sigma–Aldrich, Oakville, Canada) obtained within the range of 5–250 μg mL^{-1}. Samples outside this range were diluted accordingly.

2.6. Dissolved oxygen determination

Dissolved oxygen (DO_2) in milk was measured using an OM-4 oxygen meter (Microelectrodes Inc., Bedford, NH, USA). The oxygen meter was calibrated using two-point calibration at 0 and 100% of oxygen saturation in water. The 0% oxygen in water solution was obtained by boiling and subsequently cooling water under nitrogen purge. The 100% saturated solution of oxygen in water was obtained by bubbling oxygen through the water for 45 min at room temperature. For each sample, the electrode was allowed to stabilize to the sample temperature before the DO_2 measurement was recorded. All measurements of DO_2 were performed in duplicate.

2.7. Volatiles determination

A 2-cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coated StableFlex SPME fiber (Supelco Analytical, Bellemonte, Canada) conditioned at 270 °C for 1 h was used to extract hexanal, heptanal and octanal. Treated milk enriched in CLA and TVA (5 g) was transferred to a 20 mL amber headspace glass vial with a silicon/polytetrafluoroethylene (PTFE) septum (Canadian Life Science, Peterborough, Canada). Samples were equilibrated at 35 °C for 5 min with an agitation speed of 250 rpm and the SPME fiber was exposed to the glass vial headspace for 30 min. The fiber was then desorbed into the GC (250 °C for 5 min) using a CombiPAL system injector autosampler (CTC Analytics, Zwingen, BL, Switzerland) attached to an Agilent 7890A GC and 7975C series mass spectrometer (MS; Agilent Technologies, Mississauga, Canada), using an Rxi-5ms capillary column (29 m × 250 μm × 0.25 μm; Restek, Bellefonte, PA, USA) with liquid N_2 (Praxair, Edmonton, Canada) cryogenic coolant.