Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Retention of bioactive lipids in heated milk: Experimental and modelling

*Sergio I. Martínez-Monteagudo, Marleny D.A. Saldaña**

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5

A B S T R A C T

The retention kinetic of conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA) in milk thermally treated was investigated in a temperature range of 90–120 °C. Weibull distribution function was used to fit the experimental data and the results were evaluated through joint confidence regions. The shape parameter (β) was found to be independent of the temperature for both CLA and TVA, ranging from 0.48 to 0.76. The scale parameter (α) was affected by the temperature and its behaviour was described by an Arrhenius-type equation. The estimated activation energy (E_a) values were within the range of 151.3 ± 13.1 and 99.6 ± 12.3 kJ mol^{-1} for CLA and TVA, respectively. This kinetic information can be further used to design thermal processes, achieving high retention of bioactive lipids and identifying possible thermal treatment indicators.

Keywords: Conjugated linoleic acid; *trans*-Vaccenic acid; Weibull model; Kinetic parameters

1. Introduction

In the last two decades, the consumption per capita of fluid milk has declined (Stewart et al., 2012) due to the proliferation of sugar-sweetened beverages (Haug et al., 2007). Investigations on milk and soft drink intakes revealed that carbonated and sugar-sweetened beverages have displaced milk from the diet (Fisher et al., 2000; Nikpartow et al., 2012). Unfortunately, high consumption of sugar-sweetened beverages creates a caloric imbalance and consequently a strong link with obesity in children and adolescent (Rae-Ellen, 2010; Mathias et al., 2013).

Dairy farmers and government are actively promoting the consumption of fluid milk through education and research (Stewart et al., 2012), highlighting the health benefits of bioactive lipids naturally found in milk fat (Molkentin, 2007; Merrill et al., 1997). Conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA) are bioactive lipids that have been associated with health-promoting and disease-preventing properties, such as reducing the risk of cancer and atherosclerosis as well as weight control, and bone formation (Cook and Pariza, 1998; Fritsche et al., 1999; Lock and Bauman, 2004; Park, 2009; Wang et al., 2012). CLA is a mixture of positional and geometrical isomers of linoleic acid, having a conjugated double bond system while TVA is a metabolic precursor of CLA (Jacome-Sosa et al., 2010; Wang et al., 2009). Unfortunately, CLA and TVA are considered minor components of milk fat (1 and 3% of the total milk fat, respectively), which limits the use of milk for delivering bioactive lipids in the human diet. The amounts of CLA needed to provide anticarcinogenic responses ranges from 55 mg to 3.5 g per day (Ip et al., 1994; Knekt et al., 1996).

The profile of fatty acids in milk can be manipulated by the feeding regime of the dairy cattle (Jenkins and McGuire, 2006). An increase of 10-fold in the concentration of CLA and TVA was obtained by Bell et al. (2006), who used a safflower oil sumpplemented diet. In addition, the proportion or ratio of unsaturated to saturated fatty acids increased from 0.69 ± 0.015 (normal milk) to 1.40 ± 0.013 (Martínez-Monteagudo et al., 2012).

Milk is thermally processed to eliminate pathogens and make it safe for human consumption (Goff and Griffiths, 2006). The effect of thermal treatments on lactose, vitamins, proteins and fat have been extensively studied (van Boekel, 1998; Chavan et al., 2011; Cluskey et al., 1997; Fenaille et al., 2006). However, a milk with high proportion of unstaruated

* Corresponding author at: 3-18A, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5. Tel.: +1 780 492 8018; fax: +1 780 492 8914.

E-mail addresses: Marleny.Saldana@ales.ualberta.ca, marleny@ualberta.ca (M.D.A. Saldaña).

Available online 30 March 2014
http://dx.doi.org/10.1016/j.fbp.2014.03.008

fatty acids would be more suceptible to oxidation and thermal degradation and the bioactivity of CLA and TVA might be lost or reduced by thermal treatments. Studies on the effect of thermal treatments of milk rich in CLA are scarce and those effects have been evaluated qualitatively after UHT, microwave heating and pasteurization (Campbell et al., 2003; Herzallah et al., 2005). One way to quantify the impact of thermal processing is through retention kinetics of heat-sensitive bioactive compounds. Kinetics of chemical reactions in foods and their mechanistic interpretation are discussed somewhere else (Peleg et al., 2012; Corradini and Peleg, 2006; Barsa et al., 2012). To date, there are no reports on the retention of CLA and TVA upon thermal processing. Thus, the objective of this study was to obtain experimental data and model the retention kinetics of CLA and TVA enriched milk treated by thermal processing.

2. Materials and methods

2.1. Enriched milk

A safflower oil sumpplemented diet was used to obtain milk rich in CLA and TVA. The protocol guidelines to obtain this diet are described by Bell et al. (2006). The feeding regime was conducted at the Dairy Research and Technology Centre of University of Alberta (Edmonton, AB, Canada). After 21 days of dieting, the milk was collected and stored at −20 °C unitl further used.

2.2. Standardization and homogenization of milk

Milk rich in CLA and TVA (CLA/TVA-enriched milk) was first thawed by warming the enriched milk up to 40 °C. Then, untreated or raw CLA/TVA-enriched milk was centrifuged in a Beckman Coulter apparatus (Avanti® J-E, Fullerton, CA, USA) at 15,500 × *g* and 4 °C for 40 min to obtain full-cream and skim-milk. The fat content of milk was standardized by adding cream into the skim milk in order to obtain 2.5% (w/v) of fat. The standardized milk was then homogenized using a two stage APV-2000 homogenizer (Concord, ON, Canada). After homogenization, the milk was cooled immediately to 10 °C. Then, the standardized and homogenized CLA/TVA-enriched milk was thermally processed within less than 3 h.

2.3. Thermal treatment

Raw CLA/TVA-enriched milk was transferred into polypropylene tubes (Cryogenic vial, Fisher Scientific, Canada) of ~3 mL. Then, the tubes were capped and shaken before heated at times up to 60 min at 90, 100, 110 or 120 °C in an oil bath (Lauda Proline RP 855). The heating time was recorded at 2–3 min after the samples were immersed in the oil bath. At the end of the heating time, the tubes were removed from the oil bath and cooled down with ice water to stop further CLA and TVA degradation.

2.4. Fatty acid determination

A GC Varian 3400 (Palo Alto, CA, USA) was used to determine the fatty acid profile, including CLA and TVA. The GC was coupled with a splitless injection port and a flame-ionization detector. The samples were run on a SP-2560 column (100 m length × 0.25 mm; fused-silica capillary column, Supelco Inc, Belfonte, PA, USA). All samples were run following the chromatographic conditions reported by Cruz-Hernandez et al. (2004). Fatty acid methyl esters were obtained using base-catalyzed methylation, as previously reported by Martínez-Monteagudo et al. (2012). Methyl heptadecanoate (1 mL of 1 mg mL^{-1}) was used as an internal standard (Fluka #51633 purity 99.5%, Sigma–Aldrich, St. Louis, MO, USA). All the GC data were processed with Galaxi software (version V1.19, Varian Inc, Walnut Creek, CA, USA) and the obtained peaks were identified with a milk fat reference standard (463-Nu Check Prep Inc, Elysian, MN, USA). The CLA content measured by GC represents the total CLA. Samples were analyzed in triplicates.

2.5. Data analysis

The retention of CLA and TVA was fitted using Weibull model, which is a convenient and flexible model (van Boekel, 2002), according to Eq. (1):

$$\frac{c_t}{c_0} = \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \quad (1)$$

where C_t is the concentration of CLA or TVA [mmol] at a given time; C_0 is the initial concentration of CLA and TVA [mmol]; t is the heating time (min); α is a scale parameter which is inversely proportional to the rate constant (k, min^{-1}); β is the shape constant, corresponding to first-order model when $\beta = 1$. The influence of temperature on α parameter can be described by an Arrhenius-type relationship (Eq. (2)):

$$\frac{1}{\alpha} = \frac{1}{\alpha_{ref}} \cdot \exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad (2)$$

where $1/\alpha_{ref}$ is the rate constant at a reference temperature (T_{ref}); E_a is the activation energy; R is the universal gas constant (8.314 J mol^{-1} K^{-1}). The influence of temperature on α was expressed in terms of a reference finite temperature, which improves the parameter estimation. The average temperature of the range tested was selected as the reference temperature (105 °C). In order to improve the parameter estimation, Eq. (2) was incorporated into Eq. (1), yielding an overall kinetic Equation (3). Then, a single non-linear regression analysis was used for the whole set of experimental data using an average β value (β_{avg}).

$$\frac{c_t}{c_o} = \exp\left[-\left(\frac{1}{\alpha_{ref}}\exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)_{\cdot t}\right)\beta_{avg}\right] \quad (3)$$

Once the parameters were calculated with Eq. (3), the relation between the kinetic parameters was evaluated through joint confidence interval (Claeys et al., 2001a) using Eq. (4):

$$SSQ \leq SSQ(\vartheta)\left[1 + \frac{p}{n-p}F(p, n-p, 1-\delta)\right] \quad (4)$$

SSQ is the error sum of squares at a specific parameter combination, SSQ(ϑ) is the error sum of squares associated with the least squares estimated at optimal parameter values, p is the number of parameters estimated simultaneously, n is the number of observations and F is the classic F distribution with $(1-\delta)$ the upper quartile.

All experimental data were conducted in triplicates and parameters in Eqs. (1)–(3) were obtained by non-linear least-squares regression using the Solver option in Excel (Microsoft). The predictive capability of the individual and global models