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Vitae, vol. 18, núm. 2, 2011, pp. 144-152
Universidad de Antioquia
Medellín, Colombia

Available in: http://www.redalyc.org/articulo.oa?id=169822670005
CONJUGATED LINOLEIC ACID, FATTY ACID PROFILE AND PROCESS PROPERTIES IN KUMIS - FERMENTED MILK CONSUMED IN COLOMBIA

ACIDO LINOLEICO CONJUGADO, PERFIL DE ÁCIDOS GRASOS Y PROPIEDADES DEL PROCESO EN KUMIS - BEBIDA FERMENTADA CONSUMIDA EN COLOMBIA

Julián A. OSORIO¹,², Carolina RAMÍREZ², Carlos F. NOVOA², Luis-Felipe GUTIÉRREZ²*

Received: 01 December 2010 Accepted: 14 June 2011

ABSTRACT

In this study, we reported the concentration of conjugated linoleic acid of the main commercial kumis consumed and distributed in Colombia, as well as the concentration of conjugated linoleic acid of an artisanal kumis elaborated with two different types of milk (skim liquid and powder reconstituted). Conjugated linoleic acid (C18:2\text{c}9\text{t}11) contents, expressed as mg of rumenic acid/g fat, ranged from 7.63 ± 0.96 to 22.62 ± 3.85. Also, the main fatty acids of kumis samples were identified and quantified. pH value ranged between 3.84 ± 0.02 and 4.28 ± 0.01, and titratable acidity ranged between 0.69 ± 0.01 and 0.94 ± 0.02% of lactic acid. Consistence and flux indices presented values between 2.01 ± 0.05 and 7.08 ± 0.39 (Pa·s⁻¹) and from 0.43 to 0.26, respectively. These results indicate that kumis is a food product that could be used for supplying important amounts of conjugated linoleic acid in the human diet.

Keywords: Conjugated linoleic acid, fermented milk, kumis, fatty acids, rheological properties.

RESUMEN

En este estudio reportamos las concentraciones de ácido linoleico conjugado en algunos de los kumis comerciales de mayor distribución y consumo en Colombia, así como de kumis artesanal elaborado con dos tipos diferentes de leche (líquida semidescremada y leche en polvo reconstituida). Los contenidos de ácido linoleico conjugado (C18:2\text{c}9\text{t}11), expresadas en mg de ácido ruménico/g grasa, variaron de 7,63 ± 0,96 a 22,62 ± 3,85. Los principales ácidos grasos de las muestras de kumis fueron también identificados y cuantificados. Los valores de pH y la acidez titulable variaron entre 3,84 ± 0,02 y 4,28 ± 0,01, y entre 0,69 ± 0,01 y 0,94 ± 0,02% de ácido láctico, respectivamente. Los índices de consistencia y de flujo presentaron valores entre 2,01 ± 0,05 y 7,08 ± 0,39 (Pa·s⁻¹), y de 0,43 a 0,26, respectivamente. Estos resultados indican que el kumis es un alimento que podría ser utilizado para aportar cantidades importantes de ácido linoleico conjugado en la dieta humana.

Palabras clave: ácido linoleico conjugado, leche fermentada, kumis, ácidos grasos, propiedades reológicas.
INTRODUCTION

Conjugated linoleic acid (CLA) is a generic term used to describe the isomers of linoleic acid, which is an essential fatty acid found in appreciable amounts in food products derived from ruminant animals, especially in the lipid fraction of milk and meat (1). Most dairy products contain different CLA isomers, of which 85% to 95% consist of rumenic acid (cis-9, trans-11, octadecadienoic acid, C18:2n-11) in amounts ranging from 6 to 16 mg/g of total fat (2). The metabolic pathway proposed for the formation of CLA isomers includes the isomerization and biohydrogenation of unsaturated fatty acids by rumen bacteria (Butyribiofibrisolvens), and the desaturation of vaccenic acid (C18:1n-11) by Δ9-desaturase in the mammary gland (3). According to Ledoux et al., 2005 (4), the incomplete biohydrogenation of linoleic acid in the rumen provides the surplus of CLA found in milk fat.

Biologically active CLA isomers (cis-9, trans-11, octadecadienoic acid; C18:2n-11 and cis-10, trans-12, and octadecadienoic acid: C18:2n-12) have shown to have beneficial effects on human health, and can be considered as therapeutic nutrients with protective effects against various common diseases such as obesity, atherosclerosis, chronic inflammatory diseases and cancer (5-8). According to the published reports, the recommended CLA intake in order to achieve beneficial effects on health varies between 0.7 and 6.8 g CLA/day (9-11).

In the 90s decade, it was hypothesized that CLA concentration could increase during milk fermentation (12-13). Recent literature suggests that the CLA content in yogurts could be increased by using certain lactic acid bacteria. Different strains of Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium bifidum, Propionibacterium freudenreichii ssp. shermanii, Lactococcus lactis, and Lactococcus casei have been reported to enhance the CLA concentration during milk fermentation (14-18). According to Shantha et al., 1995 (13), typical values of CLA in fermented milks range from 3.41 to 9.12 mg/g of fat.

Kumis, a traditional fermented milk product widely consumed in Colombia, is produced in industrial and artisanal scales by fermenting whole or semi-skimmed milk with mesophilic cultures (Lactococcus lactis subsp. cremoris and Lactococcus lactis subsp. lactis) during 20 - 24 hours, at temperatures ranging from 26 to 28°C, until reaching a pH value varying between 4.00 and 4.25. The available data of the CLA concentration in milk and dairy products in Colombia are quite scarce; only two studies have been reported for milk products from the Sabana de Bogotá region (19-20). To the best of our knowledge, data of CLA content in kumis have not been still reported in Colombia. In this work, we report for the first time the CLA concentration and the fatty acid profiles of the most widely distributed and consumed kumis product brands in Colombia, in order to set a basis for further research focused on developing a CLA-enriched kumis. Some physicochemical properties such as the pH value, titratable acidity, and rheological parameters are also reported.

MATERIALS AND METHODS

The main characteristics of the kumis samples analyzed in this work are presented in table 1. Three samples of each brand from the same batch and date of production were obtained from local markets, and once arrived at the laboratory they were codified and stored at 6 - 8°C. Figure 1 shows the process followed for preparing samples of artisanal kumis.

Table 1. Main characteristics of the analyzed kumis samples.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Type</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Commercial</td>
<td>Hygienized milk, sucrose, and lactic cultures</td>
</tr>
<tr>
<td>B</td>
<td>Commercial</td>
<td>Pasteurized milk, sucrose, powder milk and lactic cultures</td>
</tr>
<tr>
<td>C</td>
<td>Commercial</td>
<td>Fluid milk, sucrose, powder milk, lactic cultures (Lactococcus lactis ssp. cremoris and spp. lactis, Streptococcus thermophilus)</td>
</tr>
<tr>
<td>D</td>
<td>Commercial</td>
<td>Partial skim milk, sucrose, lactic protein, lactic cultures (Lc. lactis, Lc. cremoris, L. casei)</td>
</tr>
<tr>
<td>E</td>
<td>Commercial</td>
<td>Hygienized milk, sucrose and lactic cultures</td>
</tr>
<tr>
<td>F</td>
<td>Commercial</td>
<td>Hygienized milk, sucrose and lactic cultures</td>
</tr>
<tr>
<td>G</td>
<td>Commercial</td>
<td>Hygienized milk, sucrose, lactic cultures (Streptococcus lactis or cremoris)</td>
</tr>
<tr>
<td>H</td>
<td>Commercial</td>
<td>Hygienized milk, sucrose and lactic cultures</td>
</tr>
<tr>
<td>EC1</td>
<td>Artisanal</td>
<td>Partial skim milk, sucrose, Lactococcus lactis ssp. cremoris and spp. lactis (Choozit MA11 LYO 25 DCU, Danisco)</td>
</tr>
<tr>
<td>EC2</td>
<td>Artisanal</td>
<td>Partial skim milk, sucrose, Lactococcus lactis ssp. cremoris and spp. lactis (Chr Hansen R-708®)</td>
</tr>
<tr>
<td>CC1</td>
<td>Artisanal</td>
<td>Water, powder milk, sucrose Lactococcus lactis ssp. cremoris and spp. lactis (Choozit MA11 LYO 25 DCU, Danisco)</td>
</tr>
<tr>
<td>CC2</td>
<td>Artisanal</td>
<td>Water, powder milk, sucrose, Lactococcus lactis ssp. cremoris and spp. lactis (Chr Hansen R-708®)</td>
</tr>
<tr>
<td>IC1</td>
<td>Artisanal</td>
<td>Water, powder milk, Lactococcus lactis ssp. cremoris and spp. lactis (Choozit MA11 LYO 25 DCU, Danisco)</td>
</tr>
<tr>
<td>IC2</td>
<td>Artisanal</td>
<td>Water, powder milk, sucrose, Lactococcus lactis ssp. cremoris and spp. lactis (Chr Hansen R-708®)</td>
</tr>
</tbody>
</table>
Figure 1. Procedure for the preparation of artisanal kumis samples.

Extraction and quantification of the fat content

The fat from the kumis samples was extracted following the methodology described by Folch et al., 1957 (21) with some modifications. In a typical extraction, samples (~6 g) were mixed with chloroform-methanol 2:1, and then centrifuged at 5000 rpm during 10 min. The mixture was transferred to a separatory funnel and then, 7 mL KCl (0.88% w/v) were added. After a vigorous agitation, the organic phase was separated, dried with anhydrous sodium sulfate, and after removing the solvent by vacuum evaporation, the obtained fat samples were collected, evaporated under nitrogen, weighed, and stored in sealed amber glass vials at -20°C until the analysis was performed.

Fatty acid analysis and quantification

Fat samples were converted into their methyl esters (FAME) by derivatization in alkaline media, using sodium methoxide 0.5 M as indicated by Christie et al., 2007 (22). FAMEs were analyzed through gas chromatography (GC), using an Agilent ® 7890A gas chromatograph (Agilent, USA). The oven temperature was programmed as follows: from 60°C (isothermal for 1 min) to 190°C at 20°C/min, and an isothermal period of 12.5 min at 190°C, for a total analysis time of 19 min. The injector and detector temperatures were set at 250°C. Helium was used as carrier gas at a flow rate of 2.0 mL/min. The separation of FAMEs was performed on a BPX-70 capillary column (60 m×0.25 mm i.d.×0.25 µm film thickness; SGE, Melbourne, Australia). Fatty acids were identified by comparing their retention times with those of the FAME standards (C4-C20 and CLA FAMEs), which were purchased from Sigma Aldrich (Sigma Aldrich, USA), under the same conditions. Peaks were integrated using Agilent ChemStation ® software. The quantification of the identified fatty acids was carried out following the internal standard method with calibration plots for each analyzed fatty acid.

Physicochemical characteristics

pH measurements of kumis samples were determined using an Orion® 420A+ pH-meter with automatic compensation of temperature at 25°C. Before the analysis, the pH-meter was calibrated using reference pH 4.0 and pH 7.0 buffer solutions, and the kumis samples were gently stirred. Titratable acidity was evaluated through titration with NaOH 0.1 M using phenolphthalein as indicator.

Rheological measurements

Rheological measurements were carried out at constant temperature (10 ± 0.5°C) using a rotational rheometer (Haake ROTOVISCO RV 20) with concentric cylinders, and a SV II sensor system. Shear rates ranging from 1.17 to 117.12 s⁻¹ were applied. The shear rate (s⁻¹) vs. shear stress (Pa) data were fitted to the following power law model:

\[ \tau = K \gamma^n \]

Equation 1.

where:
\( \tau \): Shear stress (Pa); \( K \): Consistency index (Pa·sⁿ); \( \gamma \): Shear rate (s⁻¹); \( n \): Flow index.

Experimental design and statistical analysis

A complete randomized experimental design was used to analyse the effects of the product, on CLA concentration, fatty acids profile, consistence index (\( K \)), flux index (\( n \)), pH and titratable acidity. All assays were made in triplicate. The obtained data were analyzed using the following model (23):

\[ Y_{ij} = \mu + a_i + e_{ij} \]

Equation 2.
where:
\( Y_{ij} \): Variable value; \( \mu \): Variable general mean; \( a \): Effect of product \((i=14)\); \( e_{ij} \): Experimental error.

The analysis of variance was used to identify differences in fatty acid concentration, consistence index \((K)\), flux index \((\eta)\), pH, and titratable acidity. The Shapiro Wilk test was used to verify the normality of the experimental error. The Tukey test was employed for the mean separation and correlation. A statistical analysis was carried out using the statistical analysis system software (SAS®).

Index of atherogenicity

The index of atherogenicity was calculated following the method described by Ulbricht and Southgate, 1991 (24), by means of the next equation:

\[
IA = \frac{C12:0 + 4C14:0 + C16:0}{\sum \text{Unsaturated fatty acids}}
\]

Equation 3.

Estimation of daily CLA intake in Colombia

The average of CLA intake in Colombia was evaluated from the data published by the National Survey of Nutritional Situation in Colombia 2005 (25), which provides the food intake (g/day) for different food products. We estimated the fat (%w/w) and CLA contents (mg/g fat) of each product from their typical available data (26-27).

RESULTS AND DISCUSSION

CLA concentrations and fatty acid profiles

Table 2 presents the results of CLA concentrations in the kumis samples, which ranged from 7.63 ± 0.96 to 22.62 ± 3.85 mg/g fat, which are amounts equivalent to 1.13% and 2.81% of fatty acid methylesters (FAMEs), respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CLA (mg/g fat)</th>
<th>CLA (g FA/100 FAME)</th>
<th>Portion (g)</th>
<th>CLA intake (mg/portion)</th>
<th>IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.29 ± 1.44ef</td>
<td>2.81 ± 0.20</td>
<td>200</td>
<td>77.55 ± 6.11</td>
<td>2.1</td>
</tr>
<tr>
<td>B</td>
<td>12.84 ± 1.39ef</td>
<td>1.86 ± 0.20</td>
<td>150</td>
<td>47.96 ± 5.19</td>
<td>2.3</td>
</tr>
<tr>
<td>C</td>
<td>15.47 ± 4.92ef</td>
<td>2.96 ± 0.70</td>
<td>150</td>
<td>43.16 ± 13.73</td>
<td>2.1</td>
</tr>
<tr>
<td>D</td>
<td>22.62 ± 3.85ef</td>
<td>2.90 ± 0.55</td>
<td>150</td>
<td>41.39 ± 7.04</td>
<td>2.2</td>
</tr>
<tr>
<td>E</td>
<td>13.13 ± 1.74ef</td>
<td>1.69 ± 0.25</td>
<td>210</td>
<td>59.56 ± 7.89</td>
<td>2.5</td>
</tr>
<tr>
<td>F</td>
<td>7.63 ± 0.96ef</td>
<td>1.13 ± 0.14</td>
<td>200</td>
<td>34.18 ± 4.30</td>
<td>2.5</td>
</tr>
<tr>
<td>G</td>
<td>10.59 ± 1.01ef</td>
<td>1.37 ± 0.14</td>
<td>150</td>
<td>41.62 ± 3.97</td>
<td>2.3</td>
</tr>
<tr>
<td>I</td>
<td>9.17 ± 0.70ef</td>
<td>1.31 ± 0.10</td>
<td>150</td>
<td>35.07 ± 2.68</td>
<td>2.6</td>
</tr>
<tr>
<td>EC1</td>
<td>20.83 ± 3.93ef</td>
<td>2.71 ± 0.56</td>
<td>230</td>
<td>66.11 ± 12.47</td>
<td>2.6</td>
</tr>
<tr>
<td>EC2</td>
<td>19.66 ± 1.41ef</td>
<td>2.68 ± 0.20</td>
<td>230</td>
<td>61.50 ± 4.41</td>
<td>2.7</td>
</tr>
<tr>
<td>CC1</td>
<td>16.63 ± 3.16ef</td>
<td>2.58 ± 0.45</td>
<td>230</td>
<td>93.71 ± 17.81</td>
<td>2.4</td>
</tr>
<tr>
<td>CC2</td>
<td>11.77 ± 2.21ef</td>
<td>1.31 ± 0.31</td>
<td>230</td>
<td>88.35 ± 16.57</td>
<td>2.9</td>
</tr>
<tr>
<td>IC1</td>
<td>10.56 ± 2.51ef</td>
<td>1.32 ± 0.36</td>
<td>230</td>
<td>85.01 ± 20.20</td>
<td>1.9</td>
</tr>
<tr>
<td>IC2</td>
<td>12.06 ± 1.98ef</td>
<td>1.78 ± 0.28</td>
<td>230</td>
<td>121.77 ± 19.99</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^a\) Values in the same column followed by different letters are significantly different, \(p \leq 0.05\).

\(^b\) Values are means ± standard deviation of triplicate determinations.
As it can be seen in table 2, the CLA concentration values are slightly higher than those reported in literature for fermented dairy beverages, which range from 0.35 to 3.95 FAMEs (%) (28-30). These results could be explained because pasture is the main component of the animal diet in Colombia, and various studies have concluded that the pasture feeding can enhance the CLA concentration in milk (3). Due to the fact that the production process of commercial kumis samples was unknown, it was not possible to identify the factors explaining the variation among their CLA concentrations. However, data for artisanal kumis indicate that kumis samples elaborated with liquid skim milk presented higher CLA concentration values than those produced with reconstituted whole powder milk. Differences between milk constituents, as well as the effects of the process for obtaining powder milk, could explain these results. No significant differences ($p > 0.05$) were found between the CLA content of kumis elaborated with different commercial lactic cultures. Our results are in agreement with those reported by Boylston and Beitz, 2002 (28) for yogurt produced from milk of cows fed with soy oil and conjugated linoleic acid, and with the ones published by Rico et al., 2007 (20) for milk fat from the Sabana de Bogotá region.

Figure 2 shows the concentrations of short chain saturated, medium chain (atherogenic), and long chain unsaturated and C18:0 fatty acids in kumis samples. As it can be observed in figure 2a, butyric acid (C4:0) was the predominant fatty acid in the group of short chain saturated fatty acids, varying between 18 and 69 mg/g of fat. Short chain fatty acids presented values varying between 9% and 15% of the total fatty acids. According to Parodi, 2004 (5), this group of fatty acids has no effects on blood cholesterol levels. Moreover, from this group of fatty acids, it is possible to highlight the antitumoral properties and the synergistic capacity in the treatment of hypercholesterolemia reported for butyric acid (29, 31-32). However, caproic (C6:0), caprylic (C8:0) and capric (C10:0) fatty acids have been reported to possess antibacterial and antiviral properties, presenting activity against HIV (33).

Concentrations of medium chain (atherogenic) fatty acids are presented in figure 2b. As it can be observed, palmitic acid (C16:0) was the most important fatty acid of this group, ranging from 98 to 280 mg/g of fat. These values are in agreement with those recently published by Collomb et al., 2002 (34) and Simionato et al., 2010 (35), who reported values for palmitic acid between 208 and 276 mg/g of fat. Myristic (C14:0) and lauric (C12:0) acids were found...
in amounts ranging between 39 and 81 mg/g of fat, and from 14 to 22 mg/g of fat, respectively.

Saturated and unsaturated C18 fatty acids found in kumis samples are shown in figure 2c. Oleic (C18:1) and stearic (C18:0) acids presented the highest values of this group. C18:1 ranged from 90 to 230 mg/g of fat, whereas C18:0 ranged between 64 and 163 mg/g of fat. Similar values have been reported in the literature for these fatty acids (34-35). According to Parthasarathy et al., 1990 (36), the stearic acid has no effects on the increase of serum cholesterol. This compound represents approximately 12% of the fatty acids in milk fat, and like oleic acid, which ranges between 15 and 23%, it is effective for reducing plasma cholesterol (36). Moreover, contrary to the hypercholesterolemic effects of atherogenic fatty acids, several studies indicate that long-chain saturated fatty acids produce a slow decrease of arterial occlusions and of platelet aggregation. Also, the presence of fatty acids such as linoleic and α-linolenic, having recognized beneficial effects on cardiovascular health, contribute to the biological potentialities of the kumis fat (37).

Physicochemical characteristics

Table 3 shows the physicochemical properties of the analyzed kumis samples. pH values and titratable acidity were in agreement with the reported data for fermented dairy beverages. pH values ranged between 3.84 and 4.28, lactic acid varied from 0.69% to 0.94%. The fat content of samples oscillated between 1.22 and 4.39% w/w, whereas the interval of values of the consistence and flow index were 2.0 and 7.0 Pas n, and 0.26 and 0.43, respectively. Due to the low formation of exopolysaccharides for the starter culture used in milk fermentation, the consistence index of kumis samples was lower than those reported for other fermented milks, such as the yogurt (38 - 40).

Table 3. Physicochemical characteristics of kumis samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Lactic acid (%)</th>
<th>Fat (%)</th>
<th>K (Pas n)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.18 ± 0.02a</td>
<td>0.69 ± 0.01b</td>
<td>2.12 ± 0.12c</td>
<td>3.08 ± 0.61d</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>B</td>
<td>4.08 ± 0.02c</td>
<td>0.74 ± 0.01d</td>
<td>2.49 ± 0.15e</td>
<td>2.23 ± 0.54f</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>C</td>
<td>4.11 ± 0.01e</td>
<td>0.74 ± 0.01f</td>
<td>1.86 ± 0.20g</td>
<td>2.48 ± 0.80h</td>
<td>0.37 ± 0.07</td>
</tr>
<tr>
<td>D</td>
<td>4.02 ± 0.04h</td>
<td>0.80 ± 0.01i</td>
<td>1.22 ± 0.30j</td>
<td>2.34 ± 0.99k</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>E</td>
<td>4.21 ± 0.01k</td>
<td>0.72 ± 0.01l</td>
<td>2.16 ± 0.25m</td>
<td>3.21 ± 1.11n</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>F</td>
<td>4.04 ± 0.02n</td>
<td>0.78 ± 0.01o</td>
<td>2.24 ± 0.15p</td>
<td>2.01 ± 0.05q</td>
<td>0.42 ± 0.01</td>
</tr>
<tr>
<td>G</td>
<td>4.01 ± 0.00r</td>
<td>0.75 ± 0.01s</td>
<td>2.62 ± 0.06t</td>
<td>4.56 ± 0.35u</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>H</td>
<td>3.84 ± 0.02v</td>
<td>0.81 ± 0.00w</td>
<td>2.55 ± 0.26x</td>
<td>2.47 ± 0.18y</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>EC1</td>
<td>4.17 ± 0.02z</td>
<td>0.81 ± 0.00a</td>
<td>1.38 ± 0.06b</td>
<td>6.35 ± 0.41c</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>EC2</td>
<td>4.20 ± 0.01d</td>
<td>0.83 ± 0.01e</td>
<td>1.36 ± 0.21f</td>
<td>7.08 ± 0.39g</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>CC1</td>
<td>4.19 ± 0.01h</td>
<td>0.94 ± 0.01i</td>
<td>2.45 ± 0.49j</td>
<td>5.91 ± 0.36k</td>
<td>0.43 ± 0.24</td>
</tr>
<tr>
<td>CC2</td>
<td>4.16 ± 0.01l</td>
<td>0.94 ± 0.01m</td>
<td>3.26 ± 0.14n</td>
<td>5.45 ± 0.33o</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>IC1</td>
<td>4.28 ± 0.01p</td>
<td>0.82 ± 0.01q</td>
<td>3.50 ± 0.46r</td>
<td>4.42 ± 0.22s</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>IC2</td>
<td>4.26 ± 0.01t</td>
<td>0.81 ± 0.01u</td>
<td>4.39 ± 0.33v</td>
<td>4.98 ± 0.67w</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

Values in the same column followed by different letters are significantly different, p ≤ 0.05.

Values are means ± standard deviation of triplicate determinations.

Index of atherogenicity

The index of atherogenicity (IA) of kumis samples is presented in table 2. This index allows comparing food products and diets, and it is related to the polyunsaturated/saturated fatty acids ratio. This index indicates the contribution of dietary factors that promote or protect against the development of coronary heart disease (CHD) subsequent to the onset of atherosclerosis. The equation proposed by Ulbricht and Southgate, 1991 (41) for calculating the atherogenic index, indicates that C12:0, C14:0, and C16:0 FA are atherogenic, and that n-3, n-6, and monounsaturated FA are antiatherogenic. Thus, the higher the IA, the more atherogenic dietary components are (41).
The IA of the kumis samples analyzed in this study ranged between 1.5 and 2.9. The Atherogenicity indices ranging between 1.35 and 2.80 were recently published by Huang et al., 2008 (42) for milk obtained from cows with soy oil supplemented diets, conjugated linoleic acid, or both. The lowest IA were obtained when supplementing with soy oil and CLA, and the highest value corresponded to milk sample control. Gagliostro et al., 2007 (30) reported IA of 2.32 in milk and yogurt with CLA concentrations of 1.04% and 1.09%, respectively, whereas for CLA-enriched milk products and yogurt, the IA ranged between 0.74 and 0.80 for CLA concentrations of 4.14% and 3.95%, respectively. Our results are in accordance with the available data, and the low IA observed in kumis samples indicates that kumis is a food product that could provide protection against coronary heart diseases.

Estimation of daily CLA intake in Colombia

Aiming to establish the contribution of CLA intake of kumis in the Colombian diet, it was necessary to estimate the average intake of CLA in Colombia because there was no available data. This estimation was made on the basis of the National Survey of Nutritional Situation in Colombia 2005 (25) as indicated above, and the results are presented in table 4.

From the data presented in table 2, the contribution of the kumis samples to the daily CLA intake, could reach values as higher than 47%, considering the average daily CLA intake to be 254.5 mg/day. These results suggest that at least one portion of kumis could be added to the daily diet, aiming to enhance the daily CLA intake.

Table 4. Estimated daily intake of CLA in Colombia.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>2 to 3</th>
<th>4 to 8</th>
<th>9 to 13</th>
<th>14 to 18</th>
<th>19 to 50</th>
<th>51 to 63</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA intake (mg/day)</td>
<td>293.5</td>
<td>281.1</td>
<td>274.5</td>
<td>263.2</td>
<td>262.2</td>
<td>195.1</td>
<td>254.5</td>
</tr>
</tbody>
</table>

However, when comparing the estimated daily CLA intake with the available data (Germany: 360-440; Switzerland: 160 ± 60; European Community: 380; Canada: 95 ± 41, and United States: 151 - 212 mg CLA/day) (27, 43-48), the daily CLA intake in Colombia could be higher than in Canada, similar to those reported for United States and Switzerland, and lower than the one registered for Europe.

CONCLUSIONS

The concentration of CLA in commercial samples of kumis has been evaluated for the first time in Colombia in this study. Results demonstrated that the CLA content of the analyzed kumis samples was slightly higher than the ones previously reported for various dairy products. The calculated values of CLA intake for each kumis portion indicated that this dairy product could provide up to 47% of the estimated daily CLA intake of 254.5 mg/day. This fact suggests that, in order to increase the daily CLA intake in the Colombian population, at least one kumis portion should be added to the daily diet. The index of atherogenicity of kumis samples were low, indicating that kumis consumption could contribute to the protection against coronary heart diseases. The estimated daily CLA intake in Colombia, also calculated for the first time, indicates that CLA consumption in Colombia is similar to that of United States, but lower than the one registered for Europe.

ACKNOWLEDGMENTS

This research was funded by the Ministerio de Agricultura y Desarrollo Rural of the República de Colombia and Instituto de Ciencia y Tecnología of the Universidad Nacional de Colombia Sede Bogotá. Additional support was provided by DANISCO® Colombia Ltda., and Productos Naturales de la Sabana S.A. ALQUERIA®. Martha S. Franco and Jairo Moreno are acknowledged for their technical assistance and collaboration.

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