Chemical composition and seasonal changes in the fatty acid profile of Uruguayan “Colonia” Cheeses

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SUMMARY: The basic chemical composition and the fatty acid (FA) profile of Uruguayan Colonia cheese, consumed in the Mercosur region, were determined. 24 cheeses from different dairy farms: 10 elaborated in autumn and 14 in spring, were analyzed. Those cheeses are made from cow’s milk, whose diet was composed of 50% pasture system, 25% concentrate, and 25% silage reserves. The cheese making and ripening time were similar. Seasonal differences emerged in the profiles of saturated FA (SFA), unsaturated FA (UFA) and polyunsaturated FA (PUFA). The seasonal comparison of polyunsaturated FA (PUFA) as well as the CLA isomer such as C18:2 t10 c12 showed significant differences (p < 0.05). CLA isomer such as C18:2 c9 t11, showed no differences (p > 0.05). The atherogenesis index (AI) = 2.21 and thrombogenicity (IT) = 2.84, were calculated and discussed.

KEYWORDS: Cheese; CLA – Conjugated Linoleic Acid; Fatty Acid; IA – Index of Atherogenicity; IT – Index of Thrombogenicity

RESUMEN: Composición química y cambios estacionales en el perfil de ácidos grasos de los quesos uruguayos “Colonia”. Se determina la composición química básica y el perfil de ácidos grasos (FA) de los quesos Colonia, originarios de Uruguay, que se consumen en la región del Mercosur. Se analizaron 24 quesos: originarios de diferentes establecimientos 10 elaborados en otoño y 14 en primavera. Los quesos fueron elaborados con leche de vacas, alimentadas sobre la base de un sistema pastoril al 50%, concentrados al 25% y reservas de ensilaje al 25%. Las etapas de fabricación del queso y el tiempo de maduración fueron similares. Surgieron diferencias estacionales en los perfiles de FA saturada (SFA), FA insaturada (UFA) y FA poliinsaturada (PUFA). La comparación estacional de FA poliinsaturado (PUFA) al igual que el isómero de CLA C18:2 t10 c12 mostró diferencias (p< 0.05). El isómero C18:2 c9 t11, no mostró diferencias significativas (p>0.05). Se discuten los índices calculados de aterogénesis (IA) = 2.21 y de trombogénesis (IT) = 2.84.

PALABRAS CLAVE: Ácidos Grasos; CLA – Ácido Linoleico Conjugado; IA – Índice de Aterogénesis; IT – Índice de Trombogénesis; Queso

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1. INTRODUCTION

Cheese constitutes an important source of essential nutrients and health-promoting compounds in the human diet, and its nutritional value depends on the milk characteristics and the cheese making conditions, which determine the unique and distinct nutritional properties of each cheese. Milk fat is an important compound which influences the nutritional value and sensory attributes of cheese. Consumers perceive cheese as a food product with a high content in fat, rich in nutritionally controversial saturated fatty acids (SFAs): myristic (C14:0) and palmitic (C16:0). These fatty acids, may be responsible for increased blood plasma cholesterol concentration and for a growing incidence in coronary heart disease (Adamska et al., 2017). However, cheese fat also contains health-promoting components associated with unsaturated fatty acids (UFA) including conjugated linoleic acid (CLA) and palmatic (C16:0). These fatty acids, may be found to be the primary source of CLA (Bauman et al., 2001). CLA refers to a group of polyunsaturated FA (PUFA) that exists as positional and stereoisomers of octadecadienoic acid (C18:2).

The factors that influence the CLA content in cow’s milk are diet (type of pasture and quantity consumed, dietary restrictions, oil supplements, amount and type of concentrate), the production system used (animals free in fields, pens, etc.), race and age, and the season in which the milk is collected, which may involve accessibility restrictions to pastures rich in PUFA (Stanton et al., 1997).

Two CLA isomers, 18:2 c9t11 (rumenic acid) and 18:2 t10c12, have shown beneficial biological effects in animal models (Collomb et al., 2006). The health promoting properties of rumenic acid include anticarcinogenesis, immunomodulation, and antiatherosclerosis, whereas the 18:2 t10c12 CLA isomer has lean body mass-enhancing properties (Kelley et al., 2007). The FA profile of cheese is a result of composition of raw milk and the elaboration process (Gaglioni, 2004).

The consumption of dairy products with lower atherogenic index values leads to a decrease in the total cholesterol, and the LDL-cholesterol in human blood plasma (Poppitt et al., 2002).

The milk fat with a high content in UFA is characterized by a lower atherogenic index. The high prevalence of cardio-vascular diseases in humans had promoted the study of lipid sources in different foods. As a result of this works, scientist had determined that dairy products contain a bigger quantity of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, and UFA in low concentrations (Sacks and Katan, 2002). Ulbricht and Southgate (1991) had proposed the term Atherosogenesis Index (AI) and Thrombogenicity Index (TI), for the lipids as a nutritional index for the risk of cardio-vascular diseases.

The AI indicates the relationship between the sum of the main SFAs and that of the main classes of UFAs. The first is considered proatherogenic (favoring the adhesion of lipids to the cells of the immune and circulatory systems) and, the last antiatherogenic (inhibiting the aggregation of plaque and decreasing the levels of esterified FA, cholesterol and phospholipids, thus avoiding the occurrence of micro and macro coronary diseases). The TI shows the tendency to form clots in the blood vessels. It is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, PUFAs n-6 and PUFAs n-3).
The aim of this study is to determine if there are any differences in the basic composition and fatty acid profile between Colonia cheeses elaborated in autumn and spring and to calculate the AI and TI index to compare them. Finally, it is intended to contribute to information on the nutritional quality of this cheese, which does not hold a protected designation of origin (PDO) as of yet, and to give knowledge for its promotion.

2. MATERIALS AND METHODS

This study was conducted at COLAVECO laboratory in Colonia Suiza, Uruguay using 24 different artisanal Colonia cheeses produced from cow’s milk, elaborated in the spring (14) and autumn (10).

The cheeses had similar technological processes of production and ripening. The producers treated the milk thermally, added liquid rennet, and used commercial lyophilized ferments with lactic acid and propionic bacteria, and cooked the curds at 45-48 °C, for up to 20 min., and put them in a cold saltwater brine for 48 Hs. Ripening took place in cellars, at a temperature between 5 and 10 °C, for 30 days.

2.1. Cheese sampling

Samples were taken following the procedure recommended by the International Standard Organization ISO:707:2008.

2.2. Chemical analyses

A compositional analysis (moisture, protein, fat, and pH) of the cheese was conducted according to standard methods. For pH measurement, 10 g of cheese sample were weighed and diluted with 20 mL of distilled water and then measured using a digital pH-meter equipped with an electrode for solid surfaces (WTW 340-1). The dry matter content was evaluated through moisture determination using a gravimetric method by drying a sample to constant weight in an oven at 102 °C (ISO 5534:2004). Protein was estimated by measuring the N content of cheese using the Dumas method, and multiplying this value by a conversion factor (6.38) using a LECO Instrument Model FP-628. (Uncertainty = 0.37 g/100g). Fat determination was made by the Van Gulik Fat method (ISO 3433:2008), (Uncertainty = 0.91 g/100g). All determinations were performed in duplicate. The results were expressed as percentages (wt/wt of cheese).

2.3. Determination of fatty acid composition

Lipid extraction. 40 grams of ground cheese were transferred into a 50 mL polycarbonate tube (29 × 102 mm) and directly submitted to centrifugation at 35 °C at 27,000g. The clear supernatant was transferred to a screw-cap bottle and immediately stored at -20 °C. (De Noni and Battelli, 2008). No solvent was used for lipid extraction of the cheese samples.

Preparation of fatty acid methyl esters. An aliquot of 50 mg of the fat extracted was dissolved in hexane, followed by esterification with 2N potassium hydroxide in methanol to obtain Fatty acid methyl esters (FAMEs).

Gas chromatography. FAMES were separated and quantified using a gas chromatograph (Agilent 7890A GC System; Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an electron impact (70 eV) mass spectrometer detector (Agilent 5975C MSD; Agilent Technologies Inc., Santa Clara, CA, USA) and a 60-m 70% cyanopropyl (equiv) polysilphenylene-siloxano capillary column (250-µm i.d., 0.25-µm film thickness; Thermo Scientific Inc, Bellefonte, PA). Helium was used as the carrier gas and the flow rate through the column was 1.0 mL/min. The temperature of the injector (with a split ratio of 100:1) was set at 250 °C. The following temperature gradient program was used: the initial column temperature (40 °C) was held for 0.5 min, then increased at 25 °C/min to 175 °C, held for 10 min, then increased at 5 °C/min to 210 °C, held for 5 min, and then increased at 5 °C/min to 230 °C, and held for 5 min. Fatty acids were identified by comparing their retentions times with the following FAME standards: 37 Component FAME Mix (47885, Supelco, Bellefonte, PA, USA), trans-11-Octadecenoic acid methyl ester (46905-U, Supelco, Bellefonte, PA, USA), and Octadecadienoic acid, conjugated, methyl ester (O5632, Sigma-Aldrich, Saint Louis, MO, USA).

2.4. Index of atherogenicity (AI) and index of thrombogenicity (TI)

The AI and TI of Colonia cheeses were obtained according to the formulas proposed by Ulbricht and Southgate, 1991:

\[ AI = \frac{(C12:0 + 4 \times C14:0 + C16:0)}{\sum MUF A + \sum (n-6) + (n-3)} \]

These constants are related to the atherogenic or antiatherogenic potential of each fatty acid or fatty acid group.

\[ TI = \frac{(C14:0 + C16:0 + C18:0)}{0.5 \times \sum MUF A + 0.5 \times (n-6) + 3 \times (n-3) / (n-6)} \]

2.5. Statistical analysis

Data were analyzed using the SAS statistical package (SAS Institute Inc., Cary, NC, USA). The FA profile of the cheese was analyzed using the
MIXED procedure with the FAs as a fixed effect, and the season as a variable effect. Least square means (SEM), were reported with their respective standard errors; they were considered significantly different when \( p \leq 0.05 \).

3. RESULTS

Table 1 presents the values of the chemical composition parameters such as pH, as well as the amount of moisture, fat, and protein present in the analyzed cheeses. These values were as expected for this type of cheese in terms of moisture and fat averages expressed and recognized as “semi” and “semi-fat” cheese, included in the Mercosur Technical Regulation on Identity and Quality of Cheese, (Mercosur, 1994), which is produced throughout the country and which Uruguayan consumers are likely to buy.

The cheeses were analyzed in duplicate for each season and no significant difference were found in the four chemical parameters (moisture, protein, fat, and pH), when the two periods were compared, as shown in table 1. Thus, the samples may be considered as a single population exhibiting similar characteristics.

Table 2 shows the different amounts of FA, with the different ratios between them, obtained from the chromatograms of Colonia cheeses.

In the same table it can be seen that the SFA C8:0, C10:0, C12:0, C14:0, C18:0 and C20:0 appear in appreciable concentrations and present significant differences \(( p \leq 0.05 \)) between the two seasons in which the cheeses were prepared.

These results are consistent with other reports on different cheeses made with milk from other ruminants (Prandini et al., 2007).

Meanwhile, the SFA of shorter chains: C4:0 and C6:0; and long chain C16:0 showed no significant changes \(( p \geq 0.05 \)) between the two seasons under study.

In the MUFA group, the C18:1 \( c9 \) is the one with the highest concentration \((24.39 \text{ g/100 g fat})\), followed by C18:1 \( t11 \) \((3.20 \text{ g/100 g fat})\), C16:1 \((1.92 \text{ g/100g fat})\), C14:1 \((1.92 \text{ g/100g fat})\) and finally, in a very low concentration, C10:1 \((0.30 \text{ g/100g fat})\) and C20:1 \((0.10 \text{ g/100g fat})\).

In the PUFA group, the five components of FAs were found, except for C18:2 \( c9c12 \), which showed no significant differences \(( p \geq 0.05 \)) between the cheeses elaborated in the two seasons.

The average CLA content for both seasons was \(1.38 \text{ g/100 g of fat with 94.20\% corresponding to the C18:2 c9t11 isomer, 0.055 g/100 g fat with 3.98\% corresponding to the 18:2 r10c12 isomer, and 0.025 g/100 g fat with 1.81\% corresponding to the 18:2 c10c12 isomer.} \)

The C18:3 \( c9c12c15 \)-octadecatrienoic acid \((\omega3)\) and C18:2 \( c9c12 \) octadecadienoic acid \((\omega6)\) values, and the ratio identified in this document are considered relevant in the same way, as many studies conducted in recent years by many different authors, with regards to the average human’s intake of fatty acids (WHO/FAO, 1994).

The effects of FA and the ratio of lipids consumed have an impact on health, given that the quantities and their relationships could put human health at risk. There is evidence that if the replacement of SFA by PUFA is carried out, the risk of coronary heart disease in humans is reduced.

4. DISCUSSION

The objective of this study was to provide information about the main chemical characteristics and FA profile of the Colonia cheese produced in Uruguay and sold in Argentina and Brazil. The data emerging from this paper should help as a guide to the dairy industry to learn more about the desirable quality attributes (sensory and functional) of Colonia Cheese, and to inform consumers with the aim of learning to integrate this cheese in a healthy way.

The chemical characteristics were analyzed for cheeses of artisanal origin. The cheeses were made by small dairy farmers and agro-industries, and were elaborated with cow’s milk milked on their own farms.

The obtained pH values between the analyzed cheeses were in a fairly narrow range \((5.18-5.50)\) for each season. The obtained pH values between the analyzed cheeses were in a fairly narrow range \((5.18-5.50)\) for each season.

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**Table 1.** Chemical composition of Colonia cheese in autumn and spring

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Autumn</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(^1)</td>
<td>5.34±0.1(^a)</td>
<td>5.36±0.08c</td>
</tr>
<tr>
<td>Humidity(^2) (%)</td>
<td>41.4±2.25c</td>
<td>41.54±2.61c</td>
</tr>
<tr>
<td>Protein(^3) (%)</td>
<td>24.93±1.99e</td>
<td>24.64±2.08c</td>
</tr>
<tr>
<td>Fat(^4) (%)</td>
<td>30.28±3.37d</td>
<td>29.32±3.22c</td>
</tr>
</tbody>
</table>

Each result is the mean ± standard deviation (SD) of two replicates

\(^1\)Potenciometric Method; \(^2\)Gravimetric Method; \(^3\)Dumas Method; \(^4\)Van Gulik Method.

Different letters indicate significant difference between the seasons at \( p \leq 0.05 \)
across the ripening stage for all products. As for humidity, meaningful differences in values were obtained between cheeses, showing CV=5.44. The CV of the protein and fat parameters of all the analyzed cheeses presents a change between the products, and the reason could be associated with the differences in the composition of the milk on each farm, and the level of standardization of the elaboration process in the curd cutting phase.

Usually milk contains 2%-5% fat, 70% of which is comprised of SFA and 30% of UFA (Jensen, 2002). The fatty acid profile of cheese is a result of the raw milk composition and the elaboration process. Several reports on the FA in diverse types of cheese spreads, Sardo cheeses, and Tybo Argentino, which are made from heat-treated milk, showed an increase of 97% PUFA, particularly CLA (Gagliostro, 2004).

There have been several reports which have stated that the characteristics of the FA profiles would be associated with the type of grass pasture production used in the region, as well as the preparation and maturation characteristics that are similar to those of other previously reported cheeses (Luna et al., 2005; Croissant et al., 2007). Other authors suggest the existence of a relationship between the botanical composition of the forage and the composition of FA in triglycerides, especially with respect to the saturated/unsaturated FA ratio (Wyss and Collomb, 2008).

Examination of the chromatograms as a product of the samples analyzed in this study revealed that the most abundant FAs were SFA, as shown in Table 2 (64.59 g/100 g), while UFA were the second most abundant (35.15 g/100 g). The seasonal average of total C18:1 was 28.05 g/100 g fat, of which 3.20 g/100 g fat corresponded to C18:1 t11 or trans vaccenic acid (TVA) at 11.39%. The content of the C18:1 t9 isomer was 0.47 g/100 g fat, representing 1.68%, and finally the C18:1 t9 isomer was 24.39 g/100 g fat, representing 86.93%.

The comparison between seasons (autumn versus spring) showed significant differences for SFA (p=0.031), UFA (p=0.049), and MUFA (p=0.030). The same results were found for CLA, C18:2 t10c12 (P=0.015). However, the seasonal comparison for CLA C18:2 t9t11 did not show any differences (p=0.573).

The American Heart Association, in agreement with the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), have proposed to reduce the consumption of -generated trans-FA with the aim of preventing the development of cardiovascular disease. Nutrition studies developed after 1990 indicated that there was a relatively marked negative effect on blood cholesterol transport by trans-FA, as compared with palmitic acid (16:0), and it was associated with increased blood levels of triacylglycerols (TAGs) and low-density lipoprotein (LDL) cholesterol, while lowering high-density lipoprotein (HDL) cholesterol.

Analysis of the data obtained by ANOVA test indicated significant differences (p ≤ 0.05) between seasons for FA C8:0 and C10:0, with higher amounts observed in the spring.

In relation to human consumption, SFA integrated 4-10 carbon atoms (C4:0 to C10:0), representing 7%–10% of total milk FA. Several authors mentioned that this pattern does not result in increases in circulating cholesterol, nor does it affect cardiac function in those who consume SFA. The chromatographic profile of the Colonia cheeses analyzed showed no significant differences (p ≥ 0.05) between seasons for this group of Short-chain FA = C4:0-C6:0, as seen in Table 2. At the same time, several authors reported that FA are important for health and they have antiviral effects because of caprylic acid (C8:0) and capric acid (C10:0). Caprylic acid is also recognized as having a retarding effect on tumor growth (Haug et al., 2007).

The data analysis obtained for the Colonia cheese test yielded differences (p ≤ 0.05) for the FA C8:0 and C10:0 when the seasons were compared, with greater amounts observed in the spring.

However, the contraindicated FAs have 12 (C12:0), 14 (C14:0) and 16 (C16:0) carbon atoms, of which there is plenty evidence that when humans consume them in excess, they raise the levels of total cholesterol and bad cholesterol (LDL) (Gagliostro, 2004). In addition to being insoluble in the bloodstream, these FA (also known as lauric, myristic, and palmitic acids) are not metabolized in any other substance, which is the reason why they are associated with a greater potential for the development of clogged arteries in humans who consume them (Park, 2009).

Notably, between the two seasons analyzed, it is possible to find significant differences (p ≤ 0.05), in spring, the SFA C12:0 and C14:0 appear to be increased. Furthermore, with the SFA C16:0 (palmitic acid), no significant changes were found between seasons.

Several factors may influence FA content, including geographical origin and seasonal variations, as well as variations in the grass fodder used for free-fed animals, which gives these cheeses an increasing amount of MUFA, which in this work was 29.72 g/100 g and 33.47 g/100 g between spring and autumn, respectively.

The concentration range for UFA, MUFA, and PUFA in Colonia cheese elaborated with milk from cow’s fed from permanent pastures was found to be similar to those reported in other studies on driving opencast conditions and pastoral animal feed.

As some authors mentioned, the increased consumption of products rich in PUFA and CLA brings beneficial actions to the health of consumers. The cheese elaborated in autumn with milk

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from cows that consume a diet where the main component corresponds to the pastures grown in the same period, showed a significant increase in C18:2 t10c12 (p ≤ 0.05). For equal conditions is not possible to say the same when referring to the C18:2 c9t11 (p = 0.055).

According to the available information, CLA is generally regarded as a safe nutritional substance for humans, exercising many potential health benefits (Whigham et al., 2004). The mechanisms underlying the multifunction CLA is still under investigation.

Based on the information described above, the content of CLA in Colonia cheeses made with cow’s milk, from cows that are feed under grazing conditions can be useful when it is consumed...
consistently to improve human metabolic syndromes and to exert anti-obesity, antidiabetic and antihypertensive effects. Several surveys on the level of CLA in dairy products reported values ranging from 2 to 20 mg/g of fat for different cheeses (Prandini et al., 2007), so the values found in the Colonia cheeses, as shown in Table 2, are promising.

The values of C18:2 found in this study were 3.13 g/100 g and 3.32 g/100 g between both seasons according to the clear definitions obtained from the chromatograms, where three clear peaks correspond to the isomeric forms observed in Table 2.

This study shows that there is a good amount of C18:2 and C18:1 in Colonia cheeses, including CLA and TVA; as such, this is a promising avenue for further investigation. TVA undergoes conversion in the body to CLA; both are found in very appreciable amounts in dairy products from Uruguay (Vieitez et al., 2013). This author indicated that the trans-FA content of milk is in the order of 7% in fat, of which 79% and 21% are TVA and CLA, respectively.

In this study TVA, which is shown in Table 2, yields an average value of 3.20 g/100 g during both seasons; its level does not show significant changes (P ≥ 0.05) between seasons.

Regarding the average total Trans FFA (TFA) content (5.32 g/100 g), 63.22% corresponds to TVA, c9t11-CLA, and t10c12-CLA, which fall under the beneficial FA category.

Recommendations for a ω6/ω3 ratio in the diets of several countries were proposed to have a profound influence in terms of health effects, showing a coverage range from 4 to 5:1. This group of FA cannot be synthesized by a de novo pathway and it must be provided by food.

In Colonia cheese, the acids C18:2c9c12 (ω6) and C18:3c9c12c15 (ω3) showed in both season an average ratio of 3.88. According to the ω6/ω3 ratio in the human diet, the average intake of fatty acids is favorable as a result of ratios of 5:1 to 10:1 for the WHO and FAO (WHO/FAO, 1994). Therefore, due to the values found in this study for Colonia cheese, the authors believe they should extend the number of samples over time of cheese elaborated in all seasons, to understand whether the values achieved would be beneficial for human consumption.

In an investigation of seven dietary factors related to cardiovascular disease, Ulbricht and Southgate (1991) indicated that dairy products like milk, butter, and cheese have a high AI, equal to 2.03. The AI was also calculated for Colonia cheese for each season, and the resulting values were 2.21 for both seasons. The TI displayed a value of 2.84. Both calculated indices fall within the range of values for healthy nutrition, and they serve as a minimal risk factor for human health.

5. CONCLUSIONS

This study conducted with Colonia cheese, which does not yet have a protected designation of origin (PDO), could provide enough insight to help this cheese achieve this designation in the future. In summary, the conditions under which the Colonia cheese is produced from a milk production system that uses improved pasture, in a high proportion collected directly by animals, should help the Uruguayan artisan cheese makers to take advantage of the benefits of these systems. The particularity of the production gives a FA profile more “desirable” for human health, and improves the added value of these products for trade.

The data emerging from this paper should help as a guide to the dairy industry to learn more about the desirable quality attributes (sensory and functional) of Colonia Cheese, and to inform consumers with the aim to learn to integrate these cheeses in a healthy way.

CONFLICTS OF INTEREST

There are no conflicts of interest of researchers for the preparation and publication of this manuscript.

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