Effect of cholesterol reduced and zinc fortification treatments on physicochemical, functional, textural, microstructural and sensory properties of soft cheese

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Running headline: Evaluation of functional soft cheese

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Abstract
The aim of this study was to produce and evaluate a soft cheese fortified with zinc and with cholesterol-reduced content. To meet this objective a cream base was prepared, from which Cholesterol was removed using β-cyclodextrin as extracting agent. Then, cholesterol-reduced content cheese with and without the addition of zinc salts (ZnSO₄ or ZnCl₂) was produced. Additionally, a cheese without any treatment was prepared. Furthermore, physicochemical, textural, functional, microstructural and sensory determinations were performed. As a result, 87-94% zinc fortified and 93% cholesterol-reduced cheese samples were obtained which had similar sensorial characteristics to the cheese without treatment.

Keywords: Soft cheese, Zinc Fortification, Cholesterol removal, β-cyclodextrin

INTRODUCTION
In the last years, the “functional foods” concept has achieved a great importance for consumers and between them dairy products are the most required. It has been reported that high cholesterol blood levels represent a major risk factor for developing coronary heart disease. In spite of the relationship between dietary cholesterol and plasma cholesterol levels it is being still investigated, the World Health Organization and the American Heart Association have recommended the reduction of dietary cholesterol consumption (Dias et al. 2010). Reduce cholesterol from fat dairy products is a way to avoid the risk of these diseases with keeping the rest of the milk fat, since is an excellent source of energy, vitamins (A, D and E), antioxidants and essential fatty acids (Rehm et al. 2015). In addition, it is important to highlight that fat removal from dairy products affects in a negative way their sensorial characteristics (Kim et al. 2009).
The methods to reduce cholesterol from dairy products include physical, chemical or biological strategies, between them could be mentioned the extraction with high-methoxyl pectins, plant sterols, adsorption with saponin and solvent. However, most of these methods are not suitable, since other molecules are removed at the same time than the cholesterol (Dias et al. 2010). The use of β-cyclodextrin (β-CD) is a strategy that effectively removes cholesterol from dairy products improving their nutritional characteristics, leaving the rest of milk fat. This method is selective and avoids the removal of flavours and nutritional components during the cholesterol extraction. β-CD has a central cavity which allows complex formation with the cholesterol molecule (Kwak et al. 2001, Kwak et al. 2002). In addition, β-CD is nontoxic, edible, non-hygroscopic, chemically stable and easy to separate. Furthermore, in 1998 it was introduced into the Generally Recognized As Safe list of compounds (GRAS), because it is not absorbed in the upper gastrointestinal tract, and it is completely metabolized by the colon microflora (Dias et al. 2010). Moreover, recent works have shown that the use of β-CD as a strategy to remove cholesterol from dairy products does not show significant effects on their chemical, rheological, and sensory properties (Dias et al. 2010). All these positives attributes make β-CD use a suitable strategy for cholesterol removal from food.

Zinc is one of the most important trace elements present in the body with a great nutritional importance and that has a recognized action on more than 300 enzymes, participating in either their structure or in their catalytic and regulatory action. Due to the great number of zinc-dependent biological processes, the deficiency states of this essential nutrient entails multiclinical consequences, which range from mild to serious dysfunctions (Salgueiro et al. 2000, Pomastowski et al. 2014).
Zn\(^{2+}\) deficiency states emerge as a consequence of two groups of causes. The first group includes several syndromes related to metabolic or genetic malfunctions and the second one comprises nutritional causes like decreased Zn\(^{2+}\) intake or consumption of poor Zn\(^{2+}\) content foods, which represent the most important and the most common (Salgueiro et al. 2000). The Recommended Dietary Allowance (RDA), according to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO), for Zn\(^{2+}\) is 3.9 mg day\(^{-1}\) for females and 4.2 mg day\(^{-1}\) for males, assuming a bioavailability of dietary zinc of 50\% (diet high in meat), and 9.8 mg day\(^{-1}\) for females and 14 mg day\(^{-1}\) for males, assuming a bioavailability of dietary zinc of 15\% (vegetarian diets) (WHO/FAO 2005, Kahraman and Ustunol 2012).

The available studies clearly show that food fortification with Zn\(^{2+}\) can increase the dietary intake and the total daily absorption of this trace element (Hess and Brown 2009). In addition, is extremely important to control the quantity of mineral supplementation in food to ensure the desired level in the final products does not exceed the critical limits for an adult man, which is 45 mg/day according to FAO/WHO (WHO/FAO 2005). The selection of an adequate vehicle is crucial for successful fortification. Milk and dairy products are a good option for Zn\(^{2+}\) fortification, not only due to their worldwide consumption, but also because of their high nutritional value, the buffer effect in the digestion and absorption processes and the low pH value (in fermented products) that enhances the Zn\(^{2+}\) solubilization increasing its bioavailability (Aquilanti et al. 2012). Zinc sulfate and zinc chloride salts belong to the list of compounds listed as GRAS (Kahraman and Ustunol 2012).

Previous works present strategies for Zn\(^{2+}\) fortification of different kinds of cheese and dairy products, thus supporting the use of fortification with Zn\(^{2+}\) as a method to counteract intake deficiency (El-Din et al. 2012, Kahraman and Ustunol 2012).
Cuartirolo cheese is a soft cheese defined by the Código Alimentario Argentino as a high (46-55%) or very high (>55%) moisture, fatty (45-60%), manufactured with raw milk, acidified by lactic cultures and coagulated by rennet or specific enzymes (ANMAT 2002). The treatments (homogenisation, heating) and the addition of different cosolutes (β-CD, zinc salts) in order to cholesterol-removal and Zn$^{2+}$ fortification may alter dairy products characteristics. The aim of this work is to evaluate physicochemical, sensory, functional, rheological, textural and microstructural characteristics of a cholesterol-reduced and Zn$^{2+}$ fortified Cuartirolo type soft cheese.

MATERIALS AND METHODS

Materials

The Cream (48% fat content) and the skim milk (< 0.05 % w/w fat content) used to obtained the cream base (CB) were purchased from Manfrey (Santa Fe, Argentine). Commercial β-CD (Kleptose®, Roquette, France) was the extracting agent used to remove cholesterol from the CB. The fortification agents, ZnSO$_4$.7H$_2$O and ZnCl$_2$ (anhydrous), were purchased from Novalquim (Santa Fe, Argentine) and Cicarelli (Santa Fe, Argentine), respectively. Salts belong to the list of compounds listed as GRAS.

Methods

Cholesterol-reduced and Zn$^{2+}$ fortified Cuartirolo soft cheese manufacture

Preparation of cholesterol-reduced content cream base

The quantities of cream (48 % w/w fat content) and skim milk (< 0.05 % w/w fat content) required to obtain a cream base (CB: 10 % w/w fat content) were mixed and homogenised using a two stages homogeniser ST2 (Simes S.A., Santa Fe, Argentine) with a total pressure of 100 atm at 70 °C in order to promote cholesterol extraction.
The homogenised CB was brought to 30 °C, where β-CD was added at a ratio of 2.85 % w/w after which the final mixture was left in mechanic agitation for 30 min. The system was centrifuged at 700 g (Mistral 4L, MSE, Crawley, UK) and 20 °C for 15 min in order to precipitate the β-CD/cholesterol complex, obtaining a CB with cholesterol-reduced content in the supernatant. The optimal conditions for cholesterol extraction were determined using a response surface methodology, which was performed in an early stage (data not shown).

**Cheese-making process**

The cheese samples were made according to traditional Cuartirolo technology. A bench-top (< 10 L) cheese vat was used. The cheese-making process was made in duplicated for each type of cheese in study.

Each type of cheese was made from CB treated with β-CD and diluted with skim milk (Milkaut S.A., Santa Fe, Argentine) to lower down the fat content to 3 % w/w (treated milk, TM), which was pasteurized at 65 °C for 20 min. This TM was then cooled to 40±1 °C, followed by the addition of CaCl₂ (200 mg Kg⁻¹, Cicarelli, Santa Fe, Argentine) and the Zn²⁺ salts (16 mg Zn²⁺ kg⁻¹) in the case of fortified cheese samples (Aquilanti *et al.* 2012, Kahraman and Ustunol 2012). The starter lactic culture for Cuartirolo soft cheese (STD, Diagramma S.A., Santa Fe, Argentine) was added at a ratio of 0.01 g kg⁻¹ of TM. After that, bacterial rennet (0.94 UR mL⁻¹, Chy-Max®, Chr.Hansen, Denmark) was added at a ratio of 0.5 mL kg⁻¹ of TM. Clot was formed after 30 min, cubes of approximately 2 cm³ were cut and allowed to heal during 15 min.

The mixture whey-curd particles were gently stirred for 5 min. This operation repeated thrice. Whey was drained and the curd was moulded. Moulded cheese (0.5 kg cheese) were stored at 40±1 °C, inverted each every 30 min until the pH of the cheese was near 5.3±0.1. The molded cheese was maintained in a cold brine solution (20 %
w/w NaCl) for a period of 1 h kg\textsuperscript{-1} of cheese. After that, cheese samples were vacuum packed and ripened at 5 °C for 20 days (Figure 1).

Four types of Cuartirolo soft cheese were made: 1) a cholesterol-reduced cheese (ChC-R: made of TM obtained from cholesterol-reduced CB and without Zn\textsuperscript{2+} fortification), 2) a ZnCl\textsubscript{2} fortified cheese (ChZnCl\textsubscript{2}: made of TM obtained from cholesterol-reduced CB and fortified with ZnCl\textsubscript{2} salt), 3) ZnSO\textsubscript{4} fortified cheese (ChZnSO\textsubscript{4}: made of TM obtained from cholesterol-reduced CB and fortified with ZnSO\textsubscript{4} salt), and 4) a cheese without any treatment (ChWT: made without cholesterol extraction treatment and without fortification). Each cheese sample was made in duplicated.

**Quantification of cholesterol**

Cholesterol content of the CB, before and after β-CD treatment, was determined in quadruplicate. Samples of CB were saponified, followed by cholesterol extraction using n-hexane (Cicarelli, Santa Fe, Argentine) according to the technique published by Pavón *et al.* (2014). Cholesterol quantification was made by an enzymatic method using a commercial kit (Wiener Lab., Argentine). Cholesterol content of all samples was determined by absorption at 510 nm using a UV-VIS spectrophotometer (Jasco V550, Japan) and was compared with the cholesterol standard solution (belong to the commercial kit). Cholesterol extraction percentage (\%ExtChol) was calculated as follows:

\[
\%\text{ExtChol} = 100 - \left( \frac{\text{amount of cholesterol in } \beta\text{-CD treated CB} \times 100}{\text{amount of cholesterol in untreated CB}} \right)
\]  

(1)

**Chemical cheese analysis and cheese yield determination**

**Zinc content determination**
Cheese Zn$^{2+}$ content was determined according to the flame atomic absorption spectrophotometric method of the Association of Official Analytical Chemists (1990). The values corresponding to the percentage of Zn$^{2+}$ fortification ($\%$Zn$^{2+}$ Fortification) are calculated as follows:

\[
\%\text{Zn}^{2+} \text{Fortification} = \left( \frac{[\text{Zn}^{2+}]_{\text{ChF}} - [\text{Zn}^{2+}]_{\text{CHC-R}}}{[\text{Zn}^{2+}]_{\text{max}}} \right) \times 100
\]

(2)

where $[\text{Zn}^{2+}]_{\text{ChF}}$ and $[\text{Zn}^{2+}]_{\text{CHC-R}}$ are the Zn$^{2+}$ concentration in the fortified cheese and the CHC-R respectively. $[\text{Zn}^{2+}]_{\text{max}}$ is the Zn$^{2+}$ maximum concentration present in the cheese if all the Zn$^{2+}$ salt added remains in the curd.

*Cheese yield*

Cheese yield was determined as the weight of curd obtained per 100 g of TM used for cheese-making, and the yield percentage ($\%$Yield) according to:

\[
\%\text{Yield} = \left( \frac{\text{wt.cheese} \times 100}{\text{wt.milk}} \right)
\]

(3)

where wt.cheese and wt.milk are the weight of cheese and milk respectively (Kwak et al. 2001, Kwak et al. 2002).

*Moisture content*

For cheese moisture content determinations, 2 g of shredded cheese samples were weighted onto porcelain dishes and dried at 105±1 °C until constant weight was reached, according to the Association of Official Analytical Chemists official method (1990).

*Measurement of cheese firmness*

Cylindrical samples of cheese (height: 20 mm, diameter: 40 mm) were prepared and tempered at room temperature for 1 h before the determinations. The firmness of the cheese was measured using a motorized test frame Mecmesin MultiTest 2.5-d
(Mecmesin, Spain) equipped with a dynamometer of a 100 N load cell. Penetration tests were carried out in the middle of the cylindrical samples, always at the same place, to avoid side effects.

The force in Newton required to push a cylinder probe (diameter 20 mm), at a speed of 10 mm min\(^{-1}\), into the cheese mesh was measured. Cheese firmness was considered as the force required to insert the probe to a depth of 10 mm into the cheese.

**Colorimetric measurement**

A high-resolution digital camera (Nikon, Coolpix P520) was used to measure colour by capturing the colour image of cheese samples under proper lighting. The digital images were processed, using Photoshop software (Adobe Systems Inc., USA) according to the method proposed by Soazo et al. (2014) in order to obtain the L*, a* and b* parameters. Where L* is the luminance or lightness component that goes from 0 (black) to 100 (white), and parameters a* (green to red) and b*(blue to yellow) are both chromatic components, varying from -120 to +120.

**Microstructure analysis**

*Confocal scanning laser microscopy (CSLM)*

CSLM is widely used for the study of food microstructure. Sections of samples, approximately of 5 mm X 5 mm X 2 mm, were cut with a scalpel placed in a microscope slide and one drop (50 μL) of 0.01 mg mL\(^{-1}\) Rhodamine B (Sigma-Aldrich, St. Louis, USA) was added to the cut surface. Rhodamine B binds to proteins fraction in the cheese samples, labelling them in red.

Images of representative areas of each sample were captured using a confocal microscopy (Nikon Eclipse TE-2000-E, Japan), with an objective magnification of 60X (oil immersion lens) and a numerical aperture of 1.4.
The digital images were acquired with a pixel resolution of 1024 X 1024 and stored in a TIFF format in order to be analyzed.

Images textural analysis

Texture parameters: Shannon entropy (S), smoothness (K), uniformity (U) and grey level variance ($\sigma^2(N)$), were determined from the CLSM images according to the method proposed by Ingrassia et al. (2013).

Pore diameter determination

In order to determine cheese pore diameter from the CSLM images a plug-in of the image J (version 1.485) program was employed (Doube et al. 2010). From the CSLM images, the thickness parameter was obtained using the Bone J plug-in (version 1.3.12). This can be interpreted as the width of cavities or as the distance between structures. It can thus be used to estimate parameters such as pore diameter.

Cheese meltability determinations

A modified Schreiber’s test was used to determine the fusion capability of cylindrical samples of cheese (diameter: 37 mm, height: 12 mm) (Muthukumarappan et al. 1999).

All cheese meltability determinations were made after 20 days of ripening. Samples were maintained at 4 °C for 30 min before the determinations and then were placed in a stove at 130 °C for 15 min. Subsequently, both areas (before and after heating) were determined in order to quantify the fusion capability of each cheese sample.

Sensory analysis

Cheese samples were evaluated by a trained sensory panel composed of ten members (6 female and 4 male from 25 to 60 years old), all of whom had used quantitative descriptive analysis (ISO, 1993) on regular basis over the past 2 years. The panel was calibrated in the use of the chosen attributes during five training sessions. During these sessions, panellists discussed and agreed upon the definitions and how to qualify the
attributes on the scale using commercial soft cheese samples and following the recommendations of the International Dairy Federation (IDF Standard, 1997). Different texture, taste and flavour descriptors, on a 10 cm unstructured line scale anchored with appropriate terms at the left and right ends. For texture attributes, the anchor points were: 1= “almost nothing”, 9= “a lot” and for taste and flavour properties: 1=“barely”, 9=“extremely” perceptible. Test samples of 30 g were presented in plates to the panellist in a randomized order, at 10 °C after 20 days of storage. During tasting, each panel member marked in such scale the perceived intensity of every attribute. Afterwards, the intensities of each descriptor were measured in each scale, in order to assign a numeric value for statistical analysis (Pavón et al. 2014).

**Statistical analysis**

All determinations were performed at least in duplicate. ANOVA test was performed in all experimental determinations and the significance of the results was analyzed by the least significant difference (LSD) test. Differences of p<0.05 were considered to be significant.

**RESULTS AND DISCUSSION**

**Quantification of cholesterol**

Cholesterol content of the CB was 10±2 mg mL$^{-1}$ and 0.7±0.1 mg mL$^{-1}$ before and after β-CD treatment, respectively. Therefore, the average %ExtChol was 93±1 %. This value agrees with the one previously reported by Kwak et al. (2001) for milk cholesterol extraction. These authors report the importance of the homogenisation step in the cholesterol extraction protocol since about 80 % of cholesterol exists in the fat globule. Thus the surface area of the fat globule should be increased for an effective adsorption of β-CD to interact with the cholesterol molecule (Kwak et al. 2001).
Chemical cheese analysis and cheese yield determination

Zinc content determination

Zn\textsuperscript{2+} concentrations, \%Yield, \% Zn\textsuperscript{2+} Fortification and moisture found in cheese samples are shown in Table 1.

As expected, notably higher Zn\textsuperscript{2+} levels ranging from 129±8 to 131±8 mg kg\textsuperscript{-1}, were found in fortified cheese samples (ChZnCl\textsubscript{2} and ChZnSO\textsubscript{4}). In addition, Zn\textsuperscript{2+} content values found in fortified cheese and in non-fortified ChWT and ChC-R are comparable with those reported by Aquilanti et al. (2012) for Zn\textsuperscript{2+} fortified Squacquerone cheese and Caciotta cheese (Italian cheeses that are similar in manufacture and texture to Cuartirolo soft cheese).

The high \% Zn\textsuperscript{2+} fortification found, for both salts used, compared to the low yield of the cheese-making process, is in agreement with the existence of an interaction between Zn\textsuperscript{2+} and casein micelles reported by other authors, who studied the Zn\textsuperscript{2+} binding to caseins (Pomastowski et al. 2014). Therefore, most of the added Zn\textsuperscript{2+} remains retained in casein fractions and little is lost in the whey during cheese manufacture.

Assuming a low bioavailability (15 \%) of Zn\textsuperscript{2+}, a 30 g portion of cheese with the \% Zn\textsuperscript{2+} fortification levels (ChZnCl\textsubscript{2} and ChZnSO\textsubscript{4}) represents 40\% and 28\% of the RDA for women and men, respectively. On the other hand, assuming a high bioavailability (50\%) of Zn\textsuperscript{2+} it represents about 100\% and 90\% of the RDA for women and men, respectively. No significant differences were detected between the fortifying agent used and the \% fortification value obtained.
The slightly higher yield observed for the cheese obtained from cholesterol-reduced TM compared to ChWT arise as a result of the homogenisation process that involved the cholesterol extraction protocol. Yield increase was previously reported and could be explained by the fact that protein and fat recovery were higher in cheese made from homogenised milk (Kwak et al. 2001). Homogenisation creates smaller fat globules with a greater total fat-water interfacial surface area that enabled fat globules to interact with the casein matrix (Everett and Auty 2008).

Moreover, Table 1 shows moisture percentages values for each type of cheese. These values slightly varied among samples where the moisture content of the ChC-R was found to be significantly lower than the rest. Additionally, the presence of the Zn²⁺ salts might promote water retention. Previous reports showed that moisture values of other soft cheese increase with the homogenisation process (Kwak et al. 2001, Kwak et al. 2002).

**Firmness determinations**

Cheese samples firmness is shown in Figure 2, in which ChWT firmness was statistically higher compared to the other types of cheese analyzed. These results agree with the observation of a soft and brittle curd during cheese manufacture from β-CD treated CB, also reported by Kwak et al. (2002) for cholesterol-reduced Cheddar cheese manufacture. Therefore, these lower firmness values found in cholesterol-reduced cheese samples could be explained by weak coagulum formation as a consequence of CB homogenisation process that caused greater fat globule dispersion in the curd and a reduction in the amount of free casein available to form casein network, resulting in improper curd matting.

On the other hand, no statistical differences in firmness value were found between cheese obtained from cholesterol-reduced TM (ChC-R; ChZnSO₄; ChZnCl₂).
indicating that Zn\(^{2+}\) salt fortification does not have an effect on firmness value. Therefore, the Zn\(^{2+}\) concentrations added seems not to affect the establishment of rearrangements that lead to increase the coagula firmness.

**Digital colorimetric measurement**

Figure 3A and 3B exhibit the images of the different cheese samples under study and the colour parameters for each, respectively. In Figure 3B, a decrease in the parameter values a* and b* could be observed in cheese samples obtained from the cholesterol-reduced TM in comparison with the values found for ChWT, which reflects a decrease in the intensity of the shades of green (a*) and yellow colours (b*). The visual colour differences in ChWT (Figure 3A) are mainly explained by the variation of b* parameter. Moreover, no differences were found in the values for all analyzed cheese samples for the L* parameter. These results agree with previous findings by Rudan et al. (1998) and Kwak et al. (2001). They had reported a change in the cheese structure as a consequence of the reduction of fat particle size by the homogenisation process, which makes the cheese appearance whiter and less yellow than cheese made from unhomogenised milk (Rudan et al. 1998, Kwak et al. 2001).

**Microstructure analysis**

The concept of texture from a computational point of view refers to the spatial arrangement of the brightness of the pixels in a region of the image. Therefore, texture parameter study through images reflects changes of intensity values of pixels which might contain information of geometric structure of objects since a great change of intensity values may indicate a change in geometric structure (Zheng et al. 2006). CLSM images of cheese samples sections obtained from the TM with cholesterol-reduced content exhibit a diminution in the values of S, K and \(\sigma^2(N)\) and an increment
in the U value, in comparison with the parameter values found for ChWT (Table 2). An increment in the value of U implies a tendency to a uniform distribution of the grey scale of the image, while a diminution of S, K and $\sigma^2(N)$ corresponds to structures in which all the particles are dispersed uniformly over the entire extension of the image (Ingrassia et al. 2013). On the other hand, as an observation of images of cheese samples obtained from the TM with cholesterol-reduced content exhibit smaller pores size and pores homogeneously distributed around the entire image (Figure 4), as a result of the process of homogenisation. Nevertheless, CLSM micrographs for ChWT images showed large, irregular and sectored pores. However, there are no differences in texture parameter values and pore size distributions between the ChC-R, ChZnSO$_4$ and ChZnCl$_2$, reflecting the fact that the addition of Zn$^{2+}$ salts has no effect on cheese microstructure, thus concluding that microstructural differences are due exclusively to the homogenisation process.

**Meltability**

Meltability could be defined as the capability of the cheese to flow or spread upon heating. Figure 5 shows the areas before and after cheese melting process. The results obtained from the difference of areas measured agree with Kwak et al. (2001), who found that meltability decreased with homogenisation. In addition, other authors reported that milk homogenisation breaks down fat globule membranes, making the spread of melted fat more difficult (Lelievre et al. 1990). Therefore, the mechanical homogenisation step over the CB for cholesterol extraction affects in a negative way the meltability of the resulting cheese. On the other hand, Zn$^{2+}$ salt presence would seem not to affect the melting properties of fortified cheese samples.

**Sensory analysis**
The sensory attribute of cheese was studied in appearance, texture and flavour; the average data for all sensory descriptors are summarized in Table 3.

Appearance
Cheese appearance was homogeneous with no significant differences among all analyzed cheese samples. Colour was more intense in ChWT than in the other types, which is in consonance with the results found in the digital colour determinations presented. Appearance is one of the most important attributes for cheese and is closely related to consumer's acceptance. From this point of view, whiteness of cholesterol-reduced cheese would grant a desirable feature for the consumer, avoiding the frequent use of chemical strategies, such as the addition of titanium dioxide to reach such desirable whiteness (Rudan et al. 1998).

Texture
Elasticity for ChZnSO$_4$ showed a significantly lower value than the rest. Adherence to the palate was higher in the case of fortified cheese than for ChC-R and ChWT, indicating that Zn$^{2+}$ salts increase the resistance to remove the product from the palate. Cohesiveness was lower for ChZnSO$_4$ and higher for ChC-R. Chewiness and mouthfeel were similar in all samples.

Flavour
Cream odour was detected with more intensity in ChWT than in the other types of cheese. Sweet flavour was detected in low intensity in all the cheese samples studied, ruling out the possibility that a remainder of β-CD could impart sweetness to the cheese mass. Bitter, salty and acid flavours were barely detected in all samples, presenting no significant differences among them. Cream flavour was detected with the same intensity in all samples, indicating that the process of cholesterol extraction does not alter the
organoleptic properties of the traditional cheese. Metallic flavour was not detected and astringency was low for all samples.

Although negative changes in sensory properties of foods fortified with ZnSO$_4$ have been reported (Salgueiro et al. 2002, Boccio and Monteiro 2004), metallic flavour, acid taste and astringency were detected without statistically significant differences among all samples, indicating that fortification with Zn$^{2+}$ salts does not have a significant negative effect on organoleptic characteristics of cheese. In addition, similar sensorial scores were obtained for the fortified cheese samples with the two salts under study, except for elasticity and cohesiveness sensory attributes, in which ChZnCl$_2$ showed to be more similar to ChWT.

CONCLUSIONS

In the present study, a functional dairy product with a satisfactory level of cholesterol extraction (93%) and a high content of Zn$^{2+}$ level, with adequate physical and sensory characteristics, comparable to those found in traditional Cuartirolo soft cheese, was achieved. However, the differences found between the ChWT and the cholesterol-reduced content cheese samples were due to the mechanical homogenising process used to maximize the cholesterol extraction percentage and were not related with the use of β-CD or with the Zn$^{2+}$ fortification strategy. On the other hand, both Zn$^{2+}$ salts tested showed to be effective and appropriate for Cuartirolo soft cheese fortification. Nevertheless, the use of ZnCl$_2$ showed to be more adequate from a sensorial point of view.

Based on the overall results we can demonstrate the suitability of the manufacture of a cholesterol-reduced and Zn$^{2+}$ fortified Cuartirolo soft cheese that may meet nutritional needs of risk groups.
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**Figure captions**

**Figure 1.** Flow diagrams for Cuartirolo cheese manufacture.

**Figure 2.** Firmness estimated at 25°C by penetrometry on Cuartirolo cheese samples. The same letters indicate that there were no significant differences among the samples (p>0.05)

**Figure 3.** A) Cheese sample images B) Parameters of colour of cheese samples. The same letters indicate that there was no significant difference among the samples (p>0.05). L*=Lightness (0=black; 100=white); a*= red-green component ; b*= yellow-blue component.
Figure 4. Box plots of the cheese samples pore size distribution. The same letters indicate that there was no significant difference among the samples (p>0.05).

Figure 5. Areas before and after melting test. The same letters indicate that there was no significant difference among the samples (p>0.05).

Table 1 Data represent the means and standard deviation of cheese chemical composition and cheese yield measurements per sample.*

Table 2 Data represent the means and standard deviation of textural parameters obtained from digital images of different cheese samples: Shannon entropy (S), smoothness (K), uniformity (U), and mean normalized grey-level variance (\(\sigma^2(N)\)).* All texture parameters are dimensionless.

Table 3 Data represent the means and standard deviation of sensory characteristics of different cheese samples storage at 4°C for 20 days.*
Table 3 Data represent the means and standard deviation of sensory characteristics of different cheese samples storage at 4°C for 20 days. *The same letters indicate that there was no significant difference among the samples (p>0.05)

<table>
<thead>
<tr>
<th>Sample</th>
<th>ODOR</th>
<th>COLOR</th>
<th>CHEESE APPEARANCE</th>
<th>ELASTICITY</th>
<th>ADHERENCE</th>
<th>COHESIVENESS</th>
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<tr>
<td>ChWT</td>
<td>4.15±1.70a</td>
<td>4.71±1.75b</td>
<td>8.39±0.55a</td>
<td>4.83±2.12b</td>
<td>2.77±1.37a</td>
<td>5.54±1.77ab</td>
</tr>
<tr>
<td>ChC-R</td>
<td>2.40±1.96ab</td>
<td>2.98±1.35a</td>
<td>7.98±0.98a</td>
<td>5.70±1.47b</td>
<td>3.88±1.58ab</td>
<td>6.91±1.19b</td>
</tr>
<tr>
<td>ChZnCl₂</td>
<td>3.54±2.10cd</td>
<td>2.71±1.20a</td>
<td>7.23±2.03a</td>
<td>5.17±2.57b</td>
<td>5.01±2.44b</td>
<td>5.65±1.83b</td>
</tr>
<tr>
<td>ChZnSO₄</td>
<td>1.69±1.88a</td>
<td>1.87±1.07a</td>
<td>7.32±2.24a</td>
<td>2.76±2.28a</td>
<td>5.58±3.44b</td>
<td>4.09±1.39a</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0190</td>
<td>0.0012</td>
<td>0.4103</td>
<td>0.0243</td>
<td>0.0413</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>CHEWINESS</th>
<th>MOUTHFEEL</th>
<th>SWEET TASTE</th>
<th>ACID</th>
<th>SALTY</th>
<th>BITER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChWT</td>
<td>3.34±1.69a</td>
<td>5.43±2.10a</td>
<td>1.39±1.03a</td>
<td>3.37±1.69a</td>
<td>4.29±1.61a</td>
<td>1.98±1.47a</td>
</tr>
<tr>
<td>ChC-R</td>
<td>4.29±1.46a</td>
<td>6.39±1.20a</td>
<td>1.51±1.04a</td>
<td>3.10±1.05a</td>
<td>3.67±1.69a</td>
<td>1.98±0.66a</td>
</tr>
<tr>
<td>ChZnCl₂</td>
<td>3.79±0.87a</td>
<td>5.61±1.55a</td>
<td>1.59±1.18a</td>
<td>3.61±2.20a</td>
<td>3.81±1.11a</td>
<td>1.70±1.48a</td>
</tr>
<tr>
<td>ChZnSO₄</td>
<td>2.77±1.01a</td>
<td>6.14±1.72a</td>
<td>1.58±1.11a</td>
<td>3.53±1.62a</td>
<td>3.97±1.94a</td>
<td>2.64±1.40a</td>
</tr>
<tr>
<td>p-value</td>
<td>0.1128</td>
<td>0.6060</td>
<td>0.9797</td>
<td>0.9244</td>
<td>0.8717</td>
<td>0.2703</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>CREAM FLAVOR</th>
<th>METALLIC FLAVOR</th>
<th>ASTRINGENCY</th>
<th>RESIDUAL FLAVOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWT</td>
<td>3.44±1.84a</td>
<td>2.07±1.67a</td>
<td>1.84±1.93a</td>
<td>4.29±2.87a</td>
</tr>
<tr>
<td>ChC-R</td>
<td>4.38±1.43a</td>
<td>1.34±1.43a</td>
<td>2.30±1.15a</td>
<td>3.49±1.63a</td>
</tr>
<tr>
<td>ChZnCl₂</td>
<td>4.10±1.92a</td>
<td>1.63±0.88a</td>
<td>2.68±2.16a</td>
<td>4.00±1.70a</td>
</tr>
<tr>
<td>ChZnSO₄</td>
<td>2.80±1.75a</td>
<td>1.89±1.07a</td>
<td>3.53±1.88a</td>
<td>4.42±2.03a</td>
</tr>
<tr>
<td>p-value</td>
<td>0.1821</td>
<td>0.6292</td>
<td>0.1042</td>
<td>0.8001</td>
</tr>
</tbody>
</table>

p-value: 0.0190, 0.0012, 0.4103, 0.0243, 0.0413, 0.0075, 0.1128, 0.6060, 0.9797, 0.9244, 0.8717, 0.2703
Table 1 Data represent the means and standard deviation of cheese chemical composition and cheese yield measurements per sample.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Zn(^{2+})] (mg kg(^{-1}))</th>
<th>Yield of cheese (% w/w)</th>
<th>Zn(^{2+})Fortification (% w/w)</th>
<th>Moisture (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChWT</td>
<td>(33±2)(^a)</td>
<td>(12.2±0.5)(^a)</td>
<td>-</td>
<td>(56.4±0.5)(^a)</td>
</tr>
<tr>
<td>ChC-R</td>
<td>(26±2)(^b)</td>
<td>(13.1±0.5)(^{ab})</td>
<td>-</td>
<td>(53.9±0.4)(^b)</td>
</tr>
<tr>
<td>ChZnCl(_2)</td>
<td>(129±8)(^c)</td>
<td>(13.5±0.1)(^b)</td>
<td>(87±7)(^a)</td>
<td>(55.8±0.9)(^a)</td>
</tr>
<tr>
<td>ChZnSO(_4)</td>
<td>(131±8)(^c)</td>
<td>(14±1)(^b)</td>
<td>(94±7)(^a)</td>
<td>(55.8±0.1)(^a)</td>
</tr>
</tbody>
</table>

* The same letters in the same column indicate that there was no significant difference among the samples (p>0.05).
Table 2 Data represent the means and standard deviation of textural parameters obtained from digital images of different cheese samples: Shannon entropy (S), smoothness (K), uniformity (U), and mean normalized grey-level variance ($\sigma^2(N)$). All texture parameters are dimensionless.

<table>
<thead>
<tr>
<th>Texture parameters</th>
<th>ChWT</th>
<th>ChC-R</th>
<th>ChZnSO$_4$</th>
<th>ChZnCl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>5.8±0.2$^a$</td>
<td>5.4±0.1$^b$</td>
<td>5.24±0.02$^b$</td>
<td>5.28±0.08$^b$</td>
</tr>
<tr>
<td>K</td>
<td>0.043±0.005$^a$</td>
<td>0.017±0.002$^b$</td>
<td>0.014±0.001$^b$</td>
<td>0.016±0.001$^b$</td>
</tr>
<tr>
<td>$\sigma^2(N)$</td>
<td>2,900±300$^a$</td>
<td>1,100±100$^b$</td>
<td>930±60$^b$</td>
<td>1,050±80$^b$</td>
</tr>
<tr>
<td>U</td>
<td>0.023±0.004$^a$</td>
<td>0.031±0.003$^b$</td>
<td>0.031±0.001$^b$</td>
<td>0.030±0.002$^b$</td>
</tr>
</tbody>
</table>

*The same letters indicate that there was no significant difference among the samples (p>0.05)
CB (10% w/w fat content)
(With and without Cholesterol-reduced content)

TM (3% w/w fat content)

Heating (65 °C, 20min)

Cooling to 40 °C

Coagulation
(40 °C, 30 min)

Cutting of the curd

Maintenance
(40 °C, 15 min)

Molding and whey drainage
(40 °C, final pH=5.3±0.1)

Brining
(20 % w/w NaCl, 4-8 °C, 60min kg⁻¹)

Ripening
(5 °C, 20 days)