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ORIGINAL RESEARCH

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

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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 ($ZnSO_4$ or $ZnCl_2$) 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 years, the 'functional foods' concept has achieved a great importance for consumers and dairy products are the most sought after. It has been reported that blood cholesterol 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 a reduction in dietary cholesterol consumption (Dias *et al.* 2010). Reduced cholesterol dairy products is a way to avoid the risk of these diseases whilst keeping the rest of the milk fat, since this 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 removal of fat from dairy products affect their sensory characteristics in a negative way (Kim *et al.* 2009).

The methods to reduce cholesterol from dairy products include physical, chemical or biological strategies; among them, the extraction with high-methoxyl pectins, plant sterols, adsorption with saponin and solvent could be mentioned. However, most of these methods are not suitable, as 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 remainder of the milk fat. This method is selective and avoids the removal of flavours and nutritional components during cholesterol extraction. β-CD has a central cavity, which allows complex formation with the cholesterol molecule (Kwak *et al.* 2001, 2002). In addition, β-CD is nontoxic, edible, nonhygroscopic, chemically stable and easy to

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separate. Furthermore, in 1998 it was introduced into the Generally Recognized As Safe (GRAS) list of compounds, because it is not absorbed in the upper gastrointestinal tract, and it is completely metabolised 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 positive 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 it has a recognised 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, deficiency of this essential nutrient has 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 for females and 4.2 mg/day for males, assuming a bioavailability of dietary zinc of 50% (diet high in meat), and 9.8 mg/day for females and 14 mg/day 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 dietary intake and the total daily absorption of this trace element (Hess and Brown 2009). In addition, it 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+} solubilisation increasing its bioavailability (Aquilanti *et al.* 2012). Zinc sulphate 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 Alimetario Argentino as having 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) to cholesterol removal and Zn^{2+} fortification may alter the characteristics of dairy products. 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 obtain the cream base (CB) were purchased from Manfrey (Santa Fe, Argentina). Commercial β-CD (Kleptose®, Roquette, France) was the extracting agent used to remove cholesterol from the CB. The fortification agents, $ZnSO_4.7H_2O$ and $ZnCl_2$ (anhydrous), were purchased from Novalquim (Santa Fe, Argentina) and Cicarelli (Santa Fe, Argentina), 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%) and skim milk (0.05%) required to obtain a cream base (CB: 10% w/w fat content) were mixed and homogenised using a two-stage homogeniser ST2 (Simes S.A., Santa Fe, Argentina) with a total pressure of 100 atm at 70 °C 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 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).

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

Each type of cheese was made from CB treated with β-CD and diluted with skim milk (Milkaut S.A., Santa Fe, Argentina) to reduce the fat content to 3% w/w (treated milk, TM), which was pasteurised at 65 °C for 20 min. This TM was then cooled to 40 ± 1 °C, followed by the addition of $CaCl_2$ (200 mg/Kg; Cicarelli, Santa Fe, Argentina) and the Zn^{2+} salts (16 mg Zn^{2+}/kg) in the case of fortified cheese samples (Aquilanti *et al.* 2012; Kahraman and Ustunol 2012). The lactic starter culture for Cuartirolo soft cheese (STD, Diagramma S.A., Santa Fe, Argentina) was added at a ratio of 0.01 g/kg of TM. After that, bacterial rennet (0.94 UR/mL; Chy-Max®; Chr.Hansen, Hoersholm, Denmark) was added at a ratio of 0.5 mL/kg of TM. A coagulum was formed after 30 min, and cubes of approximately 2 cm^3 were cut and allowed to heal for 15 min.

Whey and curd particles were gently stirred for 5 min. This operation was 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 moulded cheese was maintained in a cold brine solution (20% w/w NaCl) for a period of 1 h/kg 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: (i) a cholesterol-reduced cheese (ChC-R: made of TM obtained from cholesterol-reduced CB and without Zn^{2+} fortification), (ii) a $ZnCl_2$-fortified cheese ($ChZnCl_2$: made of TM obtained from cholesterol-reduced CB and fortified with $ZnCl_2$ salt), (iii) $ZnSO_4$-fortified cheese ($ChZnSO_4$: made of TM obtained from cholesterol-reduced CB and fortified with $ZnSO_4$ salt) and (iv) a cheese without any treatment (ChWT: made without cholesterol extraction treatment and without fortification). Each cheese sample was made in duplicate.

Quantification of cholesterol

The 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, Argentina) according to the technique published by Pavón *et al.* (2014). Cholesterol quantification was made by an enzymatic method using a commercial kit (Wiener Lab., Rosario, Argentina). The 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 (which belongs to the commercial kit). Cholesterol extraction percentage (%ExtChol) was calculated as follows:

$$\%ExtChol = 100 - [(\text{amount of cholesterol in } \beta\text{-CD treated CB} \times 100) / (\text{amount of cholesterol in untreated CB})] \quad (1)$$

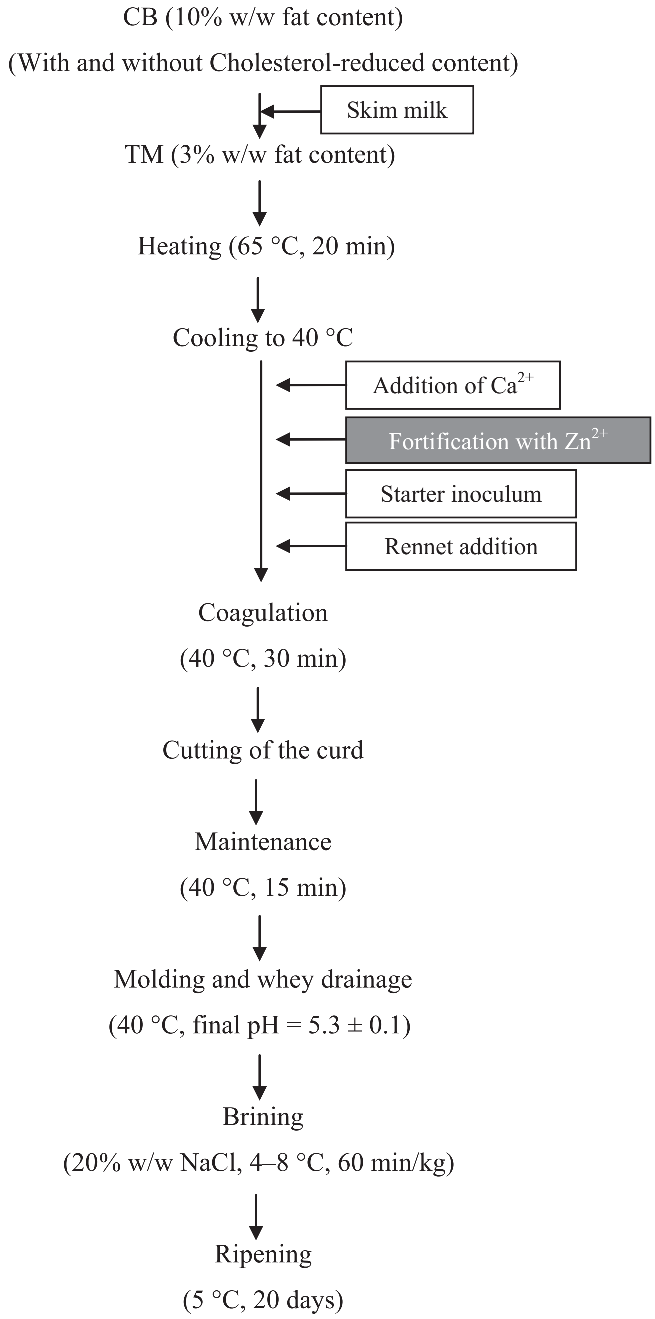

Figure 1 Flow diagrams for Cuartirolo cheese manufacture.

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 (2005). The values corresponding to the percentage of Zn^{2+} fortification (%Zn^{2+} Fortification) are calculated as follows:

$$\%Zn^{2+}\ \text{Fortification} = [([Zn^{2+}]_{ChF} - [Zn^{2+}]_{ChC-R})/[Zn^{2+}]_{max}] \times 100 \quad (2)$$

where $[Zn^{2+}]_{ChF}$ and $[Zn^{2+}]_{ChC\text{-}R}$ are the Zn^{2+} concentration in the fortified cheese and the ChC-R respectively. $[Zn^{2+}]_{max}$ 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 cheesemaking, and the yield percentage (%Yield) according to the following formula:

$$\%\text{Yield} = [(\text{wt.cheese} \times 100)/(\text{wt.milk})] \quad (3)$$

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

Moisture content. For cheese moisture content determinations, 2 g of shredded cheese samples were weighed onto porcelain dishes and dried at 105 ± 1 °C until constant weight was reached, according to the Association of Official Analytical Chemists' official method (2005).

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 motorised 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, 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, Nikon, CABA, Argentina) 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., San José, California, USA) according to the method proposed by Soazo *et al.* (2014) 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 × 5 × 2 mm, were cut with a scalpel placed in a microscope slide and one drop (50 μL) of 0.01 mg/mL Rhodamine B (Sigma-Aldrich, St. Louis, MO, USA) was added to the cut surface. Rhodamine B binds to the protein 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, Nikon, Tokyo, Japan), with an objective magnification of 60× (oil immersion lens) and a numerical aperture of 1.4.

The digital images were acquired with a pixel resolution of 1024 × 1024 and stored in a TIFF format in order to be analysed.

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. To determine cheese pore diameter from the CSLM images, a plug-in of the ImageJ (version 1.485, Biotechnology and Biological Science Research Council, Swindon, United Kingdom) 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 to quantify the fusion capability of each cheese sample.

Sensory analysis

Cheese samples were evaluated by a trained sensory panel composed of ten members (six females and four males from 25 to 60 years old), all of whom had used quantitative descriptive analysis (ISO-8589 1988) 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 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 as follows: 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 randomised 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, 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 analysed 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 and 0.7 ± 0.1 mg/mL before and after β-CD treatment, respectively. Therefore, the average %ExtChol was $93 \pm 1\%$. 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 as 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

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

As expected, notably higher Zn^{2+} levels ranging from 129 ± 8 to 131 ± 8 mg/kg were found in fortified cheese samples ($ChZnCl_2$ and $ChZnSO_4$). In addition, Zn^{2+} content values found in fortified cheese and in nonfortified ChWT and ChC-R are comparable with those reported by Aquilanti *et al.* (2012) for Zn^{2+}-fortified squacquerone cheese and caciotta cheese (Italian cheeses that are similar in manufacture and texture to Cuartirolo soft cheese).

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

Assuming a low bioavailability (15%) of Zn^{2+}, a 30-g portion of cheese with the % Zn^{2+} fortification levels ($ChZnCl_2$ and $ChZnSO_4$) represents 40% and 28% of the RDA for women and men, respectively. On the other hand, assuming a high bioavailability (50%) of Zn^{2+}, it represents about 100% and 90% of the RDA for women and men, respectively. No significant differences were observed between the fortifying agent used and the % fortification value obtained.

The slightly higher yield observed for the cheese obtained from cholesterol-reduced TM compared with 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 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 Zn^{2+} salts might promote water retention. Previous reports showed that moisture values of other soft cheese increase with the homogenisation process (Kwak *et al.* 2001, 2002).

Firmness determinations

The firmness of cheese samples is shown in Figure 2, in which ChWT firmness was statistically higher compared with the other types of cheese analysed. These results agree

Table 1 Chemical composition and yield of samples (data shows mean and standard deviation)*

Sample	*[Zn^{2+}] (mg/kg)*	*Yield of cheese (% w/w)*	*Zn^{2+} fortification (% w/w)*	*Moisture (% w/w)*
ChWT	$(33 \pm 2)^a$	$(12.2 \pm 0.5)^a$	–	$(56.4 \pm 0.5)^a$
ChC-R	$(26 \pm 2)^b$	$(13.1 \pm 0.5)^{ab}$	–	$(53.9 \pm 0.4)^b$
$ChZnCl_2$	$(129 \pm 8)^c$	$(13.5 \pm 0.1)^b$	$(87 \pm 7)^a$	$(55.8 \pm 0.9)^a$
$ChZnS0_4$	$(131 \pm 8)^c$	$(14 \pm 1)^b$	$(94 \pm 7)^a$	$(55.8 \pm 0.1)^a$

*The same letters in the same column indicate that there was no significant difference among the samples (P>0.05).

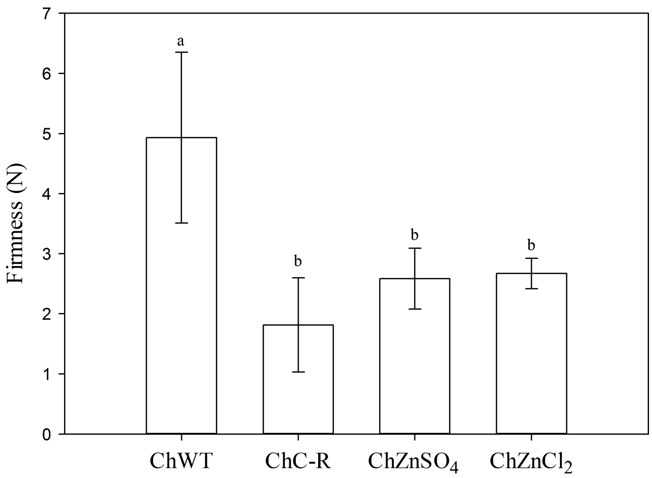

Figure 2 Firmness estimated at 25 °C by penetrometry on Cuartirolo cheese samples. The same letters indicate that there were no significant differences among the samples (P>0.05),

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 the CB homogenisation process causing greater fat globule dispersion in the curd and a reduction in the amount of free casein available to form the casein network, resulting in improper curd matting.

On the other hand, no statistical differences in firmness value were observed between cheese obtained from cholesterol-reduced TM (ChC-R; $ChZnSO_4$; $ChZnCl_2$), indicating that Zn^{2+} salt fortification does not have an effect on firmness value. Therefore, the Zn^{2+} concentrations seem not to affect the establishment of rearrangements that increase the coagula firmness.

Digital colorimetric measurement

Figure 3(a,b) shows the images of the different cheese samples under study and the colour parameters for each, respectively. In Figure 3(b), 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 observed in the values for all analysed cheese samples for the L* parameter. These results agree with previous findings by Rudan *et al.* (1998) and Kwak *et al.* (2001). They reported a change in the cheese structure as a consequence of the reduction in 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 in intensity values of pixels, which might contain information of geometric structure of objects as a great change in 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

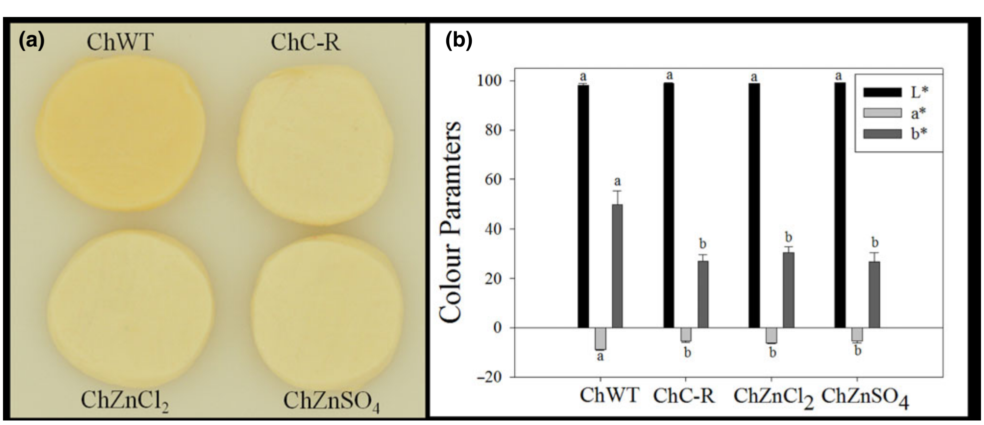

Figure 3 (a) Cheese sample images. (b) Parameters of colour of cheese samples. The same letters indicate that there was no significant difference among the samples (P>0.05).L*=Lightness (0=black; 100=white); a*= red-green component; b*= yellow-blue component.

Table 2 Mean and standard deviation of textural parameters obtained from digital images of different cheese samples: Shannon entropy (S), smoothness (K), uniformity (U) and mean normalised grey-level variance (σ^2 (N)).* All texture parameters are dimensionless

Texture parameters	*ChWT*	*ChC-R*	*ChZnSO$_4$*	*ChZnCl$_2$*
S	5.8 ± 0.2^a	5.4 ± 0.1^b	5.24 ± 0.02^b	5.28 ± 0.08^b
K	0.043 ± 0.005^a	0.017 ± 0.002^b	0.014 ± 0.001^b	0.016 ± 0.001^b
σ^2(N)	2900 ± 300^a	1100 ± 100^b	930 ± 60^b	1050 ± 80^b
U	0.023 ± 0.004^a	0.031 ± 0.003^b	0.031 ± 0.001^b	0.030 ± 0.002^b

*The same letters indicate that there was no significant difference among the samples (*P*>0.05).

(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 pore 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+} 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 summarised in Table 3.

Appearance

Cheese appearance was homogeneous with no significant differences among all analysed cheese samples. Colour was more intense in ChWT than in the other types, which is in agreement 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 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).

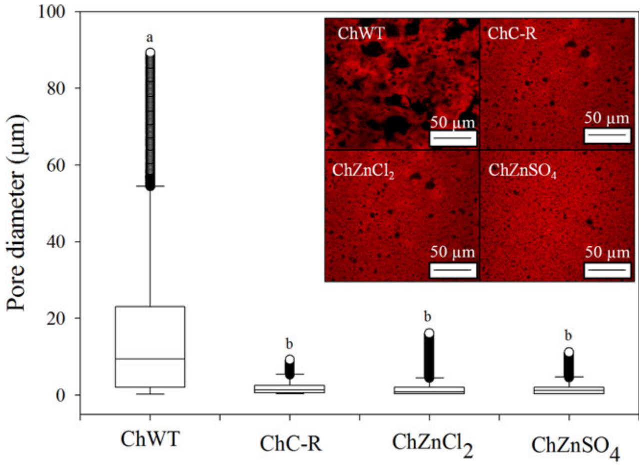

Figure 4 Box plots of the cheese samples pore size distribution. The same letters indicate that there was no significant difference among the samples (*P*>0.05).

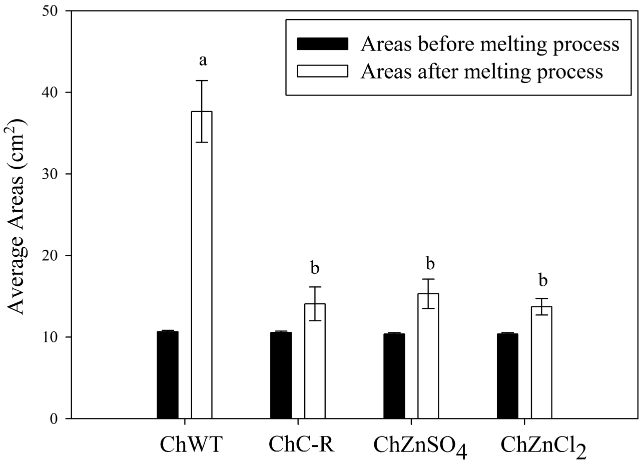

Figure 5 Areas before and after melting test. The same letters indicate that there was no significant difference among the samples (*P*>0.05).

Table 3 Mean and standard deviation of sensory characteristics of different cheese samples storage at 4 °C for 20 days*

Sample	*Odour*	*Colour*	*Cheese appearance*	*Elasticity*	*Adherence*	*Cohesiveness*
ChWT	4.15 ± 1.70[c]	4.71 ± 1.75[b]	8.39 ± 0.55[a]	4.83 ± 2.12[b]	2.77 ± 1.37[a]	5.54 ± 1.77[ab]
ChC-R	2.40 ± 1.96[ab]	2.98 ± 1.35[a]	7.98 ± 0.98[a]	5.70 ± 1.47[b]	3.88 ± 1.58[ab]	6.91 ± 1.19[b]
$ChZnCl_2$	3.54 ± 2.10[cd]	2.71 ± 1.20[a]	7.23 ± 2.03[a]	5.17 ± 2.57[b]	5.01 ± 2.44[b]	5.65 ± 1.83[b]
ChZnSO4	1.69 ± 1.88[a]	1.87 ± 1.07[a]	7.32 ± 2.24[a]	2.76 ± 2.28[a]	5.58 ± 3.44[b]	4.09 ± 1.39[a]
P-value	0.0190	0.0012	0.4103	0.0243	0.0413	0.0075

Sample	*Chewiness*	*Mouthfeel*	*Sweet taste*	*Acid*	*Salty*	*Biter*
ChWT	3.34 ± 1.69[a]	5.43 ± 2.10[a]	1.39 ± 1.03[a]	3.37 ± 1.69[a]	4.29 ± 1.61[a]	1.98 ± 1.47[a]
ChC-R	4.29 ± 1.46[a]	6.39 ± 1.20[a]	1.51 ± 1.04[a]	3.10 ± 1.05[a]	3.67 ± 1.69[a]	1.98 ± 0.66[a]
$ChZnCl_2$	3.79 ± 0.87[a]	5.61 ± 1.55[a]	1.59 ± 1.18[a]	3.61 ± 2.20[a]	3.81 ± 1.11[a]	1.70 ± 1.48[a]
$ChZnSO_4$	2.77 ± 1.01[a]	6.14 ± 1.72[a]	1.58 ± 1.11[a]	3.53 ± 1.62[a]	3.97 ± 1.94[a]	2.64 ± 1.40[a]
P-value	0.1128	0.6060	0.9797	0.9244	0.8717	0.2703

Sample	*Cream flavour*	*Metallic flavour*	*Astringency*	*Residual flavour*
ChWT	3.44 ± 1.84[a]	2.07 ± 1.67[a]	1.84 ± 1.93[a]	4.29 ± 2.87[a]
ChC-R	4.38 ± 1.43[a]	1.34 ± 1.43[a]	2.30 ± 1.15[a]	3.49 ± 1.63[a]
$ChZnCl_2$	4.10 ± 1.92[a]	1.63 ± 0.88[a]	2.68 ± 2.16[a]	4.00 ± 1.70[a]
$ChZnSO_4$	2.80 ± 1.75[a]	1.89 ± 1.07[a]	3.53 ± 1.88[a]	4.42 ± 2.03[a]
P-value	0.1821	0.6292	0.1042	0.8001

*The same letters indicate that there was no significant difference among the samples (P>0.05).

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 than 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 the 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 $ChZnCl_2$ seems 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 maximise 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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