LWT - Food Science and Technology 55 (2014) 139–147

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Soft cheese-like product development enriched with soy protein concentrates

Ana N. Rinaldoni [a], Diana R. Palatnik [a], Noemi Zaritzky [b], Mercedes E. Campderrós [a, *]

[a] Instituto de Investigaciones en Tecnología Química (INTEQUI) – CCT-CONICET, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina

[b] Centro de Investigación y Desarrollo en Criotecnología de Alimentos CIDCA (UNLP-CONICET La Plata), Facultad de Ingeniería, UNLP, La Plata, Bs As, Argentina

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form
2 September 2013
Accepted 3 September 2013

Keywords:
Soy protein
Functional cheese
Cheese-like products characterization
Shelf-life

ABSTRACT

The objective of this work was to develop spreadable cheese-like product from skimmed cow's milk, supplemented with soybean proteins. The proteins were concentrated by ultrafiltration, and then freeze-dried. The cheese-like products were prepared using the acid coagulation method, without maturation, obtaining smooth products with creamy texture. The samples were identified as low-fat cheese, being the fat content between 10 and 13 (g/100 g), with moisture in the range of 68.07–70.75 (g/100 g). Compared to a control cheese, the samples containing soy protein concentrate, showed an increase of 6.8–17 in proteins and 22–32 (g/100 g) in fats; also the yield increased. The viscoelastic behavior of the samples was analyzed using oscillatory dynamic tests; in all the samples G' was higher than G''. Texture, surface color and microstructure of the cheese samples were determined. Microbial analysis showed that the incorporation of potassium sorbate increased more than twice the shelf-life of the products (up to 60 days) in comparison to the samples without preservative. Furthermore, the products were accepted by a sensory panel. Considering that both, proteins and fats, are of vegetable origin with high biological value and unsaturated fats, the developed cheese-like products were considered as functional foods.

1. Introduction

Protein foods are essential to ensure adequate nutrition. The development of new sources of proteins and the optimization of the existing ones are issues of great interest and study (Cheftel, Cuq, & Lorient, 1989). Moreover, the use of ingredients beneficial to health has been identified as a steadily growing trend in the food industry. In this way, functional foods refer to foods or food ingredients that provide specific physiological beneficial effects and/or reduce the risk of chronic disease beyond basic nutritional functions (Gomes da Cruz, Buriti, Souza, Faria, & Saad, 2009; Mazza, 1998).

Cheese is a food with high protein content, with a widespread consumption. Besides containing proteins of good quality, contributes to calcium binding and generally has fewer digestive problems that other dairy product. However, it has a high content of saturated fats, an important contribution of calories and in general, is an expensive product, due to the low yield (Karaman & Akalin, 2013; Krbavčić & Barić, 2004; Mistry, 2001).

Therefore, it is a challenge for the food industry the formulation of foods with ingredients that help to lower health risks, as in the case of substituting animal fats by vegetable fats and oils, obtaining foodstuffs low in cholesterol and saturated fats. In this sense, the cheese seems to be a good matrix to incorporate vegetable proteins. Thus, cheese analogs, imitation cheese or processed cheese food, are gaining increasing acceptance with food processors and consumers because many potential benefits (Bachmann, 2001; El-Neshawy, Farahat, & Wahbah, 1988; Farahmandfar, Mazaheri Tehrani, Razavi, & Habibi Najafi, 2010; Mounsey & O'Riordan, 2001).

Between vegetable proteins, soybean is a highly nutritious food material that contains well balanced amino acids and desirable fatty acids and it plays an important role as a protein source for many people around the word. Furthermore it must be considered that the cost of producing cheese analogs can be less than that products obtained only from animal proteins. There are various food formulations that incorporate soy proteins for various

* Corresponding author. Tel.: +54 266 4424689; fax: +54 2652 426711.
E-mail addresses: mcamp@unsl.edu.ar, mcampd@gmail.com (M.E. Campderrós).

0023-6438/$ – see front matter
http://dx.doi.org/10.1016/j.lwt.2013.09.003

purposes, usually associated health benefits or employed soy to fortification milk products for ameliorates the problem of milk availability (Canabady-Rochelle & Mellema, 2010; Che Man & Yee, 1996; El-Neshawy et al., 1988; Farahmandfar et al., 2010; Kim, Park, & Rhee, 1992; Rani & Verma, 1995; Rinaldoni, Campderrós, & Pérez Padilla, 2012). Moreover there are a lot of dairy products that should be assessed in order to obtain novel products that can meet market needs, in terms of adequate protein content, nutritional benefits, production costs, higher availability and stability over time. In this regard, the content of sorbic acid/sorbate as preservative in cheeses with high moisture has been studied (Brocklehurst & Lund, 1985).

The objectives of this study were to develop a cheese-like product enriched with soy protein concentrate and to determine the effect of the incorporation of vegetable proteins in the matrix of a spread product with respect to physico-chemical and viscoelastic behavior, texture, microstructure, sensory and preservation during storage.

The shelf life of the products was studied with and without the use of sorbate as preservative and through pH, acidity, sensorial and microbiological assessments during storage.

2. Materials and methods

2.1. Raw materials

Partially skim-milk was provided by MILKAUT S.A (Argentina). The milk was pasteurized, homogenized and fortified with A and D vitamins in the factory. Commercial soy milk (ADES, Argentina) was used as source of soy protein. The soy milk was concentrated employing membrane technology. A combination of microfiltration with a frontal filter polyethylene with a pore size of 5–10 μm (Pall Corporation, USA) and ultrafiltration with a Pellicon cassette module (Millipore, USA) containing a modified polysulfone membranes with a cut-off of 10 kDa was employed (Rinaldoni et al., 2012). The process was carried out in batch mode, by continuously removing the permeate stream, at 25 ± 2 °C and a transmembrane pressure of 325 kPa. Temperature, recirculation rate, transmembrane pressure, pH and permeate flux rate were continuously recorded. The solids content was measured in the concentrate stream and the process was stopped when the desired concentration was achieved. The volume concentration ratio (VCR) was 2.3, determined as reported by Cheryan (1986) as the ratio between the initial feed volume and the obtained concentrate volume. After each filtration the membrane was cleaned in-line according to instructions provided by the manufacturer. The membrane hydraulic permeability recuperation was always tested to verify that the cleaning procedure was correctly done.

Then, the soy protein concentrates (SPC) were placed on stainless steel trays and frozen in a freezer at −40 ± 2 °C and freeze-dried using a lyophilizer (Rificor S.A., Argentina) at 1 bar of pressure for 48 h. The samples temperature was controlled by a temperature sensor. This procedure simplifies aseptic handling and enhances stability of dry powder, without excessive heating of the product (Fellows, 1994).

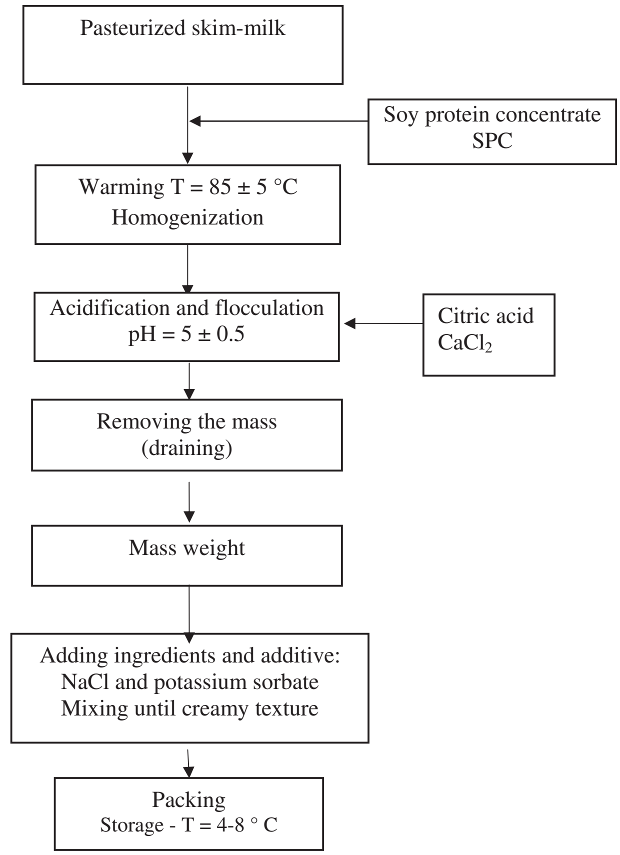

Fig. 1. Flow diagram for manufacturing control cheese and cheese-like products with SPC.

The composition of the raw materials is presented in Table 1.

2.2. Preparation of cheese-like product samples

Cheese-like product was prepared in batch according to the method shown in Fig. 1. Each sample was made in duplicate in a 5 L vat, and the volume of milk used each time was 2.5 L. One sample was reserved as witness without the incorporation of soy proteins concentrate. Different amount of freeze-dried concentrate were added to the remaining samples.The powder addition was carried out slowly, so that the protein aggregation stage is slower respect to denaturation, thus the partially unfolded protein chains can oriented more easily. This favors the formation of a homogeneous ordered gel, with smooth consistency, strongly expanded, very elastic, transparent, and more stable to syneresis and bleeding (Cheftel et al., 1989). The amounts of soy protein concentrate incorporated to milk were: 5, 10 and 15 g/L. The mixture was heated to 85 ± 5 °C and citric acid (40 g/100 mL) (Parafarm) were added until reach a pH = 5 ± 0.5 (near the isoelectric point of both, milk and soy proteins) to produce acidification and proteins aggregation. Moreover,

Table 1
Chemical compositions and pH of raw materials (means ± SD).

Material	pH	Protein (g/100 g)	Fat (g/100 g)	Sugar (g/L)	Total solid (g/100 g)	Ash (g/100 g)
Cow Milk	6.88 ± 0.02	3.5 ± 0.08	2 ± 0.18	50 ± 0.3	10.3 ± 0.10	0.7 ± 0.05
Soy Milk	7.02 ± 0.05	3.2 ± 0.05	1 ± 0.12	30 ± 0.7	8.9 ± 0.10	0.6 ± 0.08
SPC[a]	8.82 ± 0.03	43 ± 0.10	17.5 ± 0.2	10 ± 0.9	97.78±0.15	7.1 ± 0.10

[a] soy protein concentrate, freeze-dried by liophilization.

1 g of $CaCl_2$ (BDH Chemicals Laboratory Reagents) was added which contributes to obtain a proper floc and the samples were allowed to stand at 65–75 °C, approximately 60 min. At this point, the appearance of white clouds on a yellow serum was observed. The preparation was poured onto a sieve covered with cheesecloth for the drainage of whey, which was collected in a graduated cylinder. The curd was weighed and NaCl was added directly to the mass. At this stage, the sample was fractionated and a portion was set aside without addition of preservatives, while in the other fractions, potassium sorbate was added as preservative (210 ± 20 mg, depending of sample weight, being always < 1000 mg/kg of product). The samples were then homogenized with a mixer until creamy. Next, the samples were transferred into sanitized plastic containers of 100 g, weighed and stored in a frigorific chamber (Stesa, Argentina) with temperature and humidity controlled, until further analysis.

2.3. Physico-chemical analysis and yield evaluation

Raw materials, soy protein concentrate and cheese-like products samples were analyzed in duplicate according to standard replication AOAC (Association of Official Agricultural Chemists, 1995) methods.

pH was measured using a digital pH-meter (Orion, USA) and a digital pH-meter (Testo 206-pH2) for viscoplastic substances. Titratable acidity was determined with 0.1 mol/L NaOH, expressed as lactic acid (AOAC 15004). The total protein content was calculated by determination of total nitrogen by the Kjeldahl method using a Digestion Blocks and a semiautomatic Kjeldahl Distiller (Selecta, Spain), the conversion factor used to express the results was 6.38 (AOAC 15017). The fat content was measured by the Rossee-Gottlieb method (AOAC 15029). Total solids were determined by weight difference, drying in an oven at 70 ± 1 °C during 24 h (AOAC 15016). For ash determination, samples were weighted into porcelain crucibles and incinerated in a muffle furnace (Indef, Argentina) with a temperature programmer to reach 520 °C (AOAC 945.46).

The determination of syneresis was carried out, after 24 h of cold storage. The gels were stirred for 60 s on a platform and centrifuged for 20 min at 5000 rpm in an ultracentrifuge (Beckman USA) at 4 °C (Aichinger et al., 2003). Syneresis, S (g/100 g) was calculated as mass of serum *m (serum)* that had separated from the gel due to centrifugation, related to the total mass of gel *m (gel)* that was centrifuged:

$$S = \frac{m(\text{serum})}{m(\text{gel})} \times 100 \tag{1}$$

The actual yield (*Ya*) reached in the cheese-like product production was calculated from the Equation (2):

$$Ya = \frac{\text{weight of product}}{\text{weight of milk}} \times 100 \tag{2}$$

To eliminate differences associated with milk composition (fat, protein) or cheese moisture content, yield was also calculated as *Yafpam* and *Ymfpam* using the formulas proposed by Guinee, O'Kennedy, and Kelly (2006).

Yafpam is the actual yield per 100 kg of cheese normalized to reference or control level of fat and protein and it was calculated as follows:

$$Yafpam = \frac{Ya\,x\,(Frm + \mathrm{Prm})}{(Fcm + Pcm)} \tag{3}$$

where *Frm* and *Prm* correspond to the percentages of fat and protein in the reference or control cheese respectively; *Fcm* and *Pcm* are the actual fat and protein contents of the cheese-like products.

Ymafpam is the moisture adjusted cheese yield per 100 kg of cheese milk adjusted to reference levels of fat and protein and it represents the ratio between the weight of cheese with a reference moisture value (68.07 ± 0.2%) and 100 kg of normalized cheese milk. It was calculated as follows:

$$Ymafpam = \frac{Yafpam\,x\,(100 - MA)}{(100 - MR)} \tag{4}$$

where *MA* and *MR* correspond to actual and reference moisture, respectively.

2.4. Determination of rheological parameters

The visco-elastic behavior of the samples was determined by oscillatory tests evaluating the elastic modulus G' and the viscous modulus G'' (Kealy, 2006). The tangent of phase shift or phase angle ($\tan\delta = G''/G'$) was also evaluated. All measurements were made in duplicate at 20 °C in a Haake RS 800 oscillatory rheometer equipped with a temperature control sensor DC50 using parallel plates 35 mm (PP 35/S), with a gap between plates of 1.5 mm. The surfaces of both upper and lower plates were serrated to remove the possibility of slip at the boundary.

First, stress sweep tests (0.01–1000 Pa) were performed to determine the linear viscoelastic range at a constant frequency (1 Hz-6 28 rad s^{-1}); these tests were carried out using the control cheese samples and the cheese containing the highest concentration of SPC added to select the maximum stress corresponding to the linear viscoelastic range. The obtained value of 20 Pa was maintained in the frequency sweeps carried out in the range of 0.1–600 rad s^{-1} for all the tested samples.

In addition the apparent viscosity was measured with a rotational viscometer Haake (with a rotor 2) which has a shear rate of 3750 s^{-1} at 15 ± 0.5 °C.

2.5. Texture profile analysis

Instrumental texture profile analysis (TPA) of cheeses, control and with SPC, was performed on a TAXT2i Texture Analyzer (Stable Micro Systems) using a load cell of 25 kg and a disc-shaped probe (45 mm of diameter). Testing was carried out after the samples had been equilibrated to a standard temperature of 12 ± 1 °C. Two consecutive compressions performed automatically at a test speed of 0.5 mm s^{-1} and compression ratio of 30%. The waiting time between one cycle and another was set at 5 s. From the force curve versus time, the following mechanical properties were determined (Kealy, 2006): hardness (*N*), adhesiveness (*N*), springiness, cohesiveness and resilience.

2.6. Sensory analysis

The samples were tested at room temperature (22 ± 2 °C), in a uniformly illuminated room, by a 45-member panel selected from a pool of students and staff members of our Department. One hour prior to the evaluations, top slice samples from randomly chosen packages were transferred to closed plastic beakers. The samples were presented in a randomized order in plastic containers. The aim was to compare the choice between more than two samples therefore the method of ordering by preference was applied. The samples were coded with three-digit random numbers. Cookies were provided for rinsing between samples, to cleanse the palate. The sensory attributes evaluated were color, flavor and spreadability. Then statistical analysis was performed using the Friedman test, which determines whether the sums of the total orders for

Table 2
Physico-chemical characterization and mean yield of cheeses control and cheese-like products with different concentrations of SPC* (means ± SD).

SPC	Moisture (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Actual yield (Ya)	Normalized yield (Yafpam)	Moisture normalized yield (Ymafpam)
0 g/L	68.07 ± 0.2	1.80 ± 0.90	10.00 ± 1.20	16.38 ± 0.08[a]	19.0 ± 1.9	19.00 ± 2.5	19.00 ± 3.2
5 g/L	69.54 ± 0.10	1.70 ± 0.27	12.25 ± 1.96	17.50 ± 0.05[b]	19.5 ± 2.3	21.99 ± 2.7	20.93 ± 2.9
10 g/L	69.77 ± 0.20	1.50 ± 0.18	12.91 ± 1.71	18.75 ± 0.10[c]	20.1 ± 2.5	24.12 ± 2.2	22.78 ± 3.0
15 g/L	70.75 ± 0.70	1.45 ± 0.68	13.26 ± 0.53	19.16±0.12[d]	22.5 ± 2.0	27.65 ± 2.1	25.27 ± 3.5

*SPC (Soy protein concentrate).
[a–d] means with different subscript in the same row are significantly different ($P < 0.05$).

each sample differ significantly (Meilgaard, Arbor, Carr, & Civille, 2006).

For the shelf-life studies, the sensory attributes evaluated were odor, color and flavor, using a five-point hedonic scale (from 1 = I disliked very much to 5 = I liked very much), following the same proceeding described below.

2.7. Analysis of surface color

Color perception is based on personal references; thus it is difficult to objectively communicate color without a rule allowing a comparison with accuracy. It is therefore necessary to use measuring instruments to identify color and assigned a numerical value. The surface color was measured by a digital spectrophotometer (Mini Scan EZ) provided with the software. The chromometer was calibrated with the standard white and black color. The results reported are averages of measurements of three slices (five measurements per slice), using CIELAB L^*, a^*, b^* values. L^* value is the lightness variable from 100 for perfect white to zero for black, whilst a^* and b^* values are the chromaticity values, +redness/−greenness and +yellowness/−blueness, respectively (Morales & Van Boeckel, 1999). Because combining a^* and b^* gives a better indication of color than their individual values, the hue angle, $h°$ (arctan (b^*/a^*)) and the saturation index (chroma: $C = (a^{*2} + b^{*2})^{½}$) was calculated (Wadhwani & McMahon, 2012).

2.8. Microbiological analysis

Microbiological counts were performed in the cheese-like sample with 5 g/L of SPC, with and without preservative (potassium sorbate), during storage. Populations of Coliforms were enumerated on Bile Red Violet Agar (BRVA); the plates were incubated for 24 h at 32 °C (Christen, Davidson, McAllister, & Roth, 1993). Moulds and yeasts were developed on yeast extrac-dextrose (glucose)-cloramphenicol agar (YGCA) at 25 °C for 5 days (Frank, Christen, & Bullerman, 1993).

2.9. Scanning electron microscopy

The microstructure of cheese-like products was analyzed by scanning electron microscopy (SEM) using an LEO1450VP equipment (Zeiss, Germany). The samples were mounted on double sided adhesive carbon on aluminum sample holder. The micrographics were determined under VP mode (variable pressure). The low vacuum mode of SEM is a special form, where an opening through the column and fixed between the SEM chamber, the chamber can be maintained (where the samples are placed) at low vacuum at 70 Pa, while that the column remains at high vacuum levels. In this way it is possible to see sensitive samples, biological or water content, without dehydrating the sample and metalize (Sammons & Marquis, 1997).

2.10. Cheese-like products shelf-life study

The shelf-life of a foodstuff comprising the elapsed time between preparation and the moment when significant changes occur in it, which can generate the final consumer rejection. The shelf-life may vary by the production process, the nature of the product and the storage time, causing changes at microbiological, sensory and/or physical-chemical properties. For this study, basic design was used, where cheese-like products enriched with soy proteins concentrate (5 g/L), were stored in a cold chamber (Stesa, Argentina) at 4–8 °C and 86% of humidity. Sampling was performed every 15 days and measurements of sensory, physicochemical and microbiology analysis as indicators of stability of the product were performed (Giménez, Ares, & Ares, 2012).

2.11. Statistical analysis

The obtained data were statistically evaluated by test of variance (ANOVA) on the three groups and their corresponding responses, assuming that a $P < 0.05$ was statistically significant (SAS, 1989).

3. Results and discussion

3.1. Cheese-like product characterization

The results of physico-chemical analysis of the control and the samples with the incorporation of soy proteins concentrate are shown in Table 2. According to the Argentinean legislation (Código Alimentario Argentino), the "white soft cheese reduced in fat" must present a maximum moisture content of 77 (g/100 g) and fats between 10 and 19.9 (g/100 g). The results showed in Table 2 indicated that all samples fall into this category.

The moisture values showed a slight increase ($P < 0.05$) with increasing soy protein concentrate content; this may be due to the hydrophilic nature of these proteins (Rani & Verma, 1995).

The protein content of the samples increased ($P < 0.05$) with the soy protein concentrate increased in the formulation. There was an increase in the range of 6.8%–17% of protein in the cheese-like products. These values are in agreement with the expected taking into account the actual yield obtained, which are presented in Table 2 (Ya). In this sense, the yield for this type of cheese like product is normally higher than other cheeses due to the high moisture content (Nair, Mistry, & Oommen, 2000). Furthermore it was found that the performance also increased with increasing soy protein concentrate content. Similar result was obtained by Francolino, Locci, Ghiglietti, Iezzi, and Mucchetti (2010), employing a milk protein concentrate to standardize milk composition in a Mozzarella cheese and by El-Neshawy et al. (1988), using different sources of proteins for production of processed cheese. Furthermore, the higher performance could be related with water retention capacity of soy proteins (Cheftel et al., 1989; Rodriguez Furlán, Rinaldoni, Pérez Padilla, & Campderrós, 2011).

With respect to fats, a tendency to increase with the incorporation of SPC was observed, although the difference was no statistically different. This result suggests that, fats incorporated in the preparation are indeed retained in the floc matrix. Here, it should be consider that the membrane treatment applied for soymilk concentration, allowed to concentrate proteins and fats by removing in the permeate stream the soybeans anti-nutrient compounds (Ali, Ippersiel, Lamarche, & Mondor, 2010; Rinaldoni et al., 2012). These results demonstrated that the cheese-like products obtained can be identified as functional foods, since both proteins and fats incorporated, are of vegetable origin and present health benefits (Moon, Balasubramanian, & Rimal, 2011). Indeed, the soybeans proteins have a high biological value similar to those of meat, fish or eggs, and also are capable of lowering triglyceride and cholesterol (total cholesterol, LDL-cholesterol and VLDL-cholesterol), (Bachmann, 2001; Genovese & Lajolo, 2002; Mattos et al., 2009). With respect to fats, soymilk basically contains essential polyunsaturated fatty acids, primarily linoleic, linolenic and arachidonic (Messina, Messina, & Setchel, 2002). Furthermore, the protein content in the cheese-like products elaborated were higher than the obtained by Che Man and Yee (1996) for a soybean spread product but similar to the elaborated by Milesi, Candioti, and Hynes (2007) for a mini soft cheese milk, while with less fat content.

The ash content (Table 2) presented a slight decrease being not statistically significant ($P > 0.5$). The values of pH and acidity were in average for the four samples 5.77 ± 0.08 and 0.99 ± 0.16 respectively, showing no statistically significant difference.

3.2. Viscoelastic measurements

The data obtained through this type of dynamic (or oscillatory) measurements are the contributions to the internal structure of the sample from the elastic and viscous portions of flow, G' and G'' (Pa), respectively. The measurements in the linear viscoelastic region involve probing the structure of the sample in a non-destructive manner (Kealy, 2006). The results are showed in Fig. 2. For all samples tested was found that the value of G' was above G'' ($P < 0.01$). This means that in the response of the samples, the elastic component predominated. Also, the significant difference between both moduli and the slight variation with the frequency across the whole range of frequencies studied, suggested a strong gel structure (González-Tomás, Bayarri, Taylor, & Costell, 2008). This result can be related with the low syneresis of the samples, even during storage (Table 5). The tan (δ) values were: 0.27 for 0 g/L SPC, 0.26 for 5 g/L SPC, 0.26 for 10 g/L SPC and 0.27 for 15 g/L SPC. These values did not show significant differences ($P < 0.01$) and were lower than 1.0, thus indicating that the elastic nature of the samples matrix dominated over its viscous counterpart. Similar results were reported by Pereira, Franco, Gomes, and Malcata (2011) for cheeses manufactured from several milk sources.

The apparent viscosity values decreased significantly from 130,000 ± 5 mPas for the control cheese to 20,000 ± 5 mPas (at 15 ± 0.5 °C) for the higher amount of SPC added (15 g/L), this can be explained considering the high water retention capacity of soy proteins (Cheftel et al.., 1989).

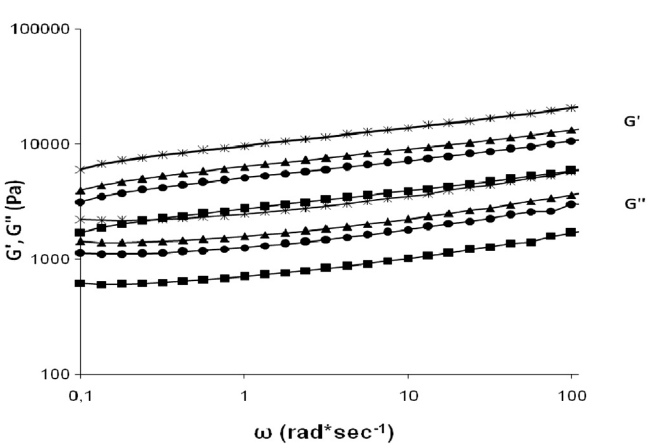

Fig. 2. Viscoelastic moduli for the control cheese and cheese-like products, measured at 12 ± 1 °C. (✳) 0 g/L, (▲) 5 g/L, (●) 10 g/L, (■) 15 g/L.

Table 3
Results of TPA of the samples.

SPC	Hardness (N)	Cohesiviness	Springiness	Adhesivness (N)	Resilience
0 g/L	18.83 ± 1.05^{a}	0.48 ± 0.02^{a}	0.92 ± 0.10^{a}	−7.14 ± 1.12^{a}	0.10 ± 0.01^{a}
5 g/L	12.55 ± 1,09^{b}	0.52 ± 0,06^{b,a}	0.80 ± 0.12^{a}	−5.02 ± 1.30^{b}	0.10 ± 0.02^{a}
10 g/L	8.48 ± 1.22^{c}	0.57 ± 0.05^{b,c}	0.91 ± 0.09^{a}	−4.11 ± 0.24^{b,c}	0.09 ± 0.01^{a}
15 g/L	5.21 ± 0.30^{d}	0.63 ± 0.04^{c}	0.88 ± 0.01^{a}	−2.40 ± 0.18^{c}	0.08 ± 0.01^{a}

$^{a-d}$ means with different subscript in the same row are significantly different ($P < 0.05$).

3.3. Texture

The data measured by the texture analyzer are presented in Table 3. The application of analysis of variance revealed no significant differences among the tested samples in the parameters springiness and resilience. However, there were statistically significant differences ($P < 0.05$) for cohesiveness, adhesiveness and hardness being this last parameter the one that showed the largest differences among the samples. Hardness of the samples increased with the decreasing concentration of SPC. This result should be related with the elastic modulus G' (Fig. 2), since the higher G', greater is the energy required to deform it, suggesting that harder is the material. Thus, the control cheese (SPC: 0 g/L) which had the higher hardness, was also the one with higher G'. Regarding the cohesiveness and adhesiveness, the major differences were found between the control sample and samples with SPC. With respect to the elasticity (the ability of the samples had to regain its original configuration after being removed off the force responsible of the deformation), Table 3 shows that the four samples studied have similar elasticity values. These results correspond well with springiness values which do not present statically difference between the samples as for tan (δ) values ($P < 0.05$).

3.4. Sensory assessment

The assessment by untrained judges on samples enriched with soy protein concentrate, considering the attributes of color, flavor, spreadability and overall acceptance, are shown in Fig. 3. Cheese-like products containing SPC and control products were almost

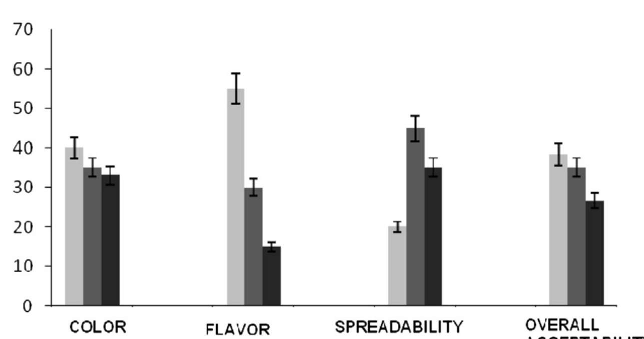

Fig. 3. Sensory analysis of cheese-like product. (▪) 5 g/L; (▪) 10 g/L; (■) 15 g/L.

Table 4
Color measurement of cheeses control and cheese-like products with different concentrations of SPC*(means ± SD).

SPC	L^*	a^*	b^*
0 g/L	84.99 ± 0.28^a	-0.17 ± 0.23	12.90 ± 0.14
5 g/L	83.17 ± 0.53^a	-0.22 ± 0.19	13.05 ± 0.20
10 g/L	80.80 ± 0.25^b	-0.15 ± 0.04	13.53 ± 0.23
15 g/L	81.15 ± 0.38^b	-0.08 ± 0.06	13.41 ± 0.16

* SPC (Soy protein concentrate).
[a–b] means with different subscript in the same row are significantly different ($P < 0.05$).

similar in color and overall acceptability ($P > 0.5$). However, the sample with 5 (g/L) of SPC incorporated became more acceptable in terms of flavor ($P < 0.05$). The flavor is a very important attribute since it determines consumer preference. The results showed that the soy protein content was seen as an off-flavor due to lack of habit in consumption, especially in our country, although in recent years many soy products have been incorporated in the markets because of the legume nutritional and functional properties (Moon et al., 2011). Further studies will determine which ingredients may allow improve the flavor attribute, such as the use of certain herbs. With regard to spreadability, a very important attribute for this type of product, it was found that the order of acceptance was: 10 > 15 > 5 (g/L) ($P < 0.05$), indicating an optimal concentration of soy protein concentrate in the matrix with reference to this parameter.

3.5. Instrumental analysis of color

The results of color measurements on soft cheese-like products are shown in Table 4. The control cheese and samples enriched with soy protein concentrate, showed high L^* value (around 82.5) which reflects the degree of lightness. The control and the sample with 5 g/L of SPC showed the lightest color ($P < 0.05$). As expected, the values were closer to white cheese milk, than products processed only with soybeans (Che Man & Yee, 1996; Juric, Bertelsen, Mortensen, & Petersen, 2003). The a^* value showed a slight degree of greenness, without significant differences between samples ($P > 0.5$). The b^* positive value indicated the degree of yellowness. These values were adequate considering that in low-fat cheese, the removal of fat impart a translucent appearance. In effect, the values of b^* and a^* obtained, were similar to those reported by Wadhwani and McMahon (2012), for low-fat cheese without annatto colorant. The results obtained with Hue and Chroma revealed that the tonality (h°) was in average 90.67 ± 0.28 without significant differences between the samples, indicating a yellow tonality, the value of the hue angle is within the expected values of 40° to 90° transition from orange to yellow (Wadhwani & McMahon, 2012). The saturation index (C) was 12.90 ± 0.14 for the control sample, while for the cheese-like products was in average: 13.33 ± 0.2. From the results it was verified that the presence of SPC did not significantly affect the color of cheese-like products respect to the control sample.

3.6. Microbiological analysis during storage time

The microbiological tests performed for the cheese-like product formulation with soy protein concentrate (5 g/L), with and without preservative are shown in Fig. 4. As cheeses were obtained under controlled microbiological conditions, coliform bacteria, moulds and yeasts counts for samples with sorbate were acceptable, that is below the maximum admissible level in the legislation: coliforms/g < 10 cfu/g ($n = 5$; $c = 2$; $m = 500$; $M = 5000$) and moulds and yeasts < 10 cfu/g ($n = 5$; $c = 2$; $m = 500$; $M = 5000$), where

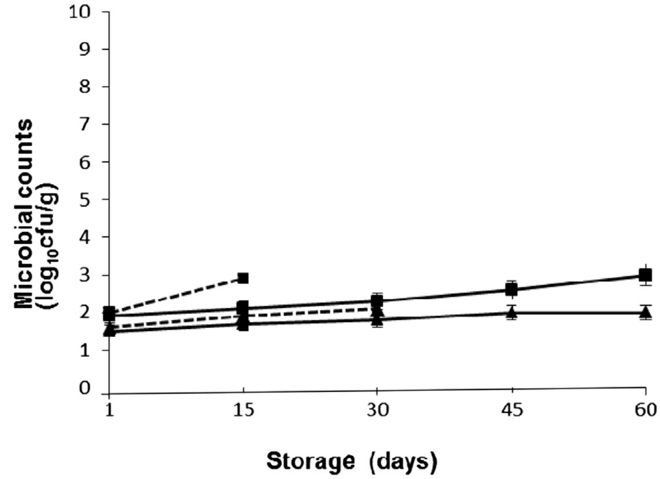

Fig. 4. Evolution of microbial populations during storage days of sample enriched with 5 g/L of SPC. (■) moulds and yeast, (▲) coliform bacteria, (—) with preservative, (– – –) without preservative.

n = number of sample units analyzed; c = maximum number of sample units where m = maximum level of the microorganisms in the food for an acceptable quality; M = maximum level of the microorganisms in the food provisionally acceptable quality (Código Alimentario Argentino). In the sample without preservative an evident development of moulds ($M > 5000$ cfu/g) was detected after 15 days of storage.

3.7. Analysis of the microstructure

Electron micrographs (SEM) were made in order to establish relationships between the microstructure of samples and its composition. Fig. 5 shows the images obtained for the control cheeses and cheese-like products enriched with SPC. Compact protein matrixes with small numbers of unevenly dispersed fat globules were observed, similar to that informed by Karaman and Akalin (2013) for his control cheeses. This type of structure is common for cheeses made from pasteurized milk and acidified. Indeed, according to Morales-Celaya, Lobatto-Calleros, Alvarez-Ramirez, and Vernon-Carter (2012), these cheeses have a more stratified structure than the casein strands arrangement obtained from raw milk products. The smaller size of the milk-fat globules seems to be related with a lower occurrence of coalescence. The presence of soy protein concentrate appears to determine a more intricate protein matrix, coating the fat globule. This result can be explained considering soy proteins capacity as thickener (Cheftel et al., 1989; Kumar, Choudhary, Mishra, Varma, & Mattiason, 2002). Also the moisture originally present within the fat-serum channels should be absorbed into the protein matrix, resulting in a well hydrated protein (Morales-Celaya et al., 2012). This result was consistent with the cheese-like product composition with high protein and moisture contents. For the sample with 10 g/L of SPC, more uniformity can be observed; this fact should be associated with the better spreadability found in the sensory analysis.

3.8. Shelf life time study

As a general rule, food stability is the parameter that defines the product commercial value. The results showed that the shelf life of the samples stored at 4 ± 1 °C without preservative addition was less than 30 days (Table 5), when evident spoilage was observed. In contrast, samples with sorbate as preservative showed a longer shelf-life (more than 30 days), taking into account the high

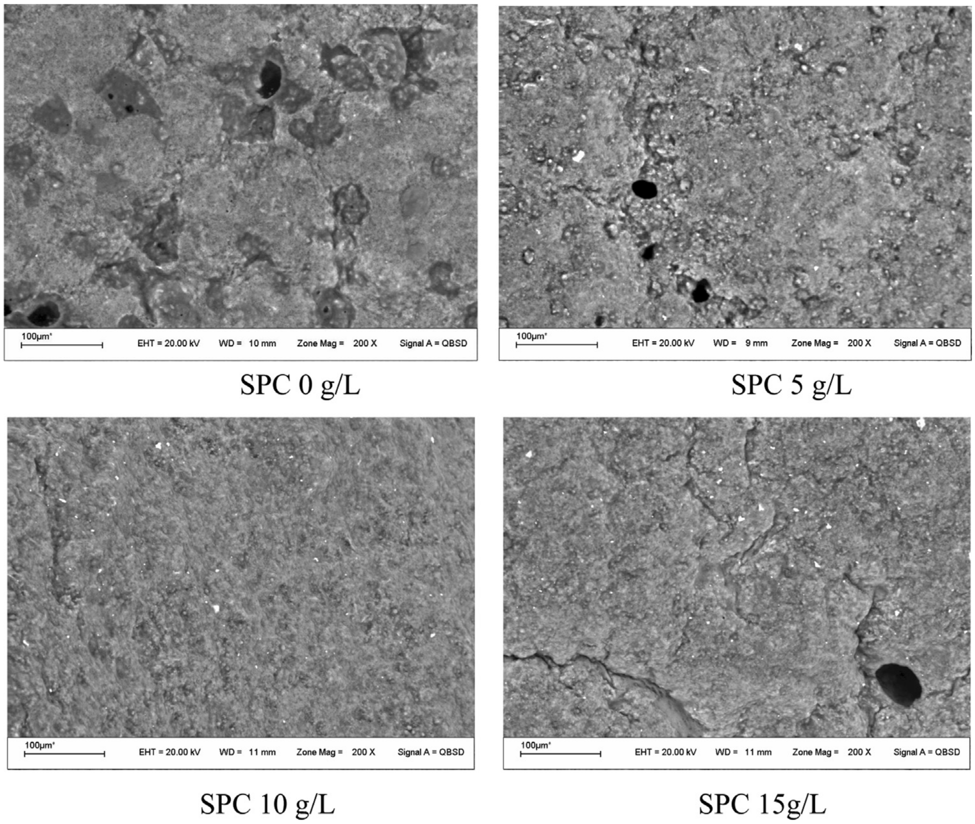

Fig. 5. SEM images of cheese control and cheese-like products. Magnification 200×.

humidity content of these products. So, this additive acted as was expected, being an effective preservative against moulds and yeasts, with some effectiveness against bacteria in agreement with reports of Brocklehurst and Lund (1985). Besides, the preservative activity is directly linked to changes in pH, and it was found that this parameter showed slight changes during storage. In effect, minor variations in pH and acidity, may be related to amino acids and free fatty acids being produced during proteolysis and lipolysis, respectively (Dermiki, Ntzimani, Badeka, Savvaidis, & Kontominas, 2008). The fat content increased during the first 45 days and then decreased ($P < 0.05$), indicating physical changes. In this sense, El-Neshawy et al. (1988) reported slight increase in fat content of cheese from the different blends during storage.

The syneresis increased during storage ($P < 0.05$), however even the highest value obtained (11.3 ± 0.5 g/100 g), was acceptable for these products. The low syneresis values obtained could be related to the cheese making process and the composition of the product. In effect, the brief thermal treatment, the pH of the products (≈5.7) with minor changes during storage and the high protein content, make a firmer gel with low syneresis production (Fox & Cogan, 2004). This result was consistent with the rheological characterization and SEM images, which showed a compact structure. Furthermore, the reduced fat samples also favors the stability of gel (Mateo et al., 2009).

There were non-significant difference in terms of odor and color during the storage, this means that the chemical changes produced, cannot be perceived by the panelists. However it was found that the flavor softens during storage, resulting the products more agreeable, as can be seen in the flavor score increase with the storage time (Table 5). Similar results were reported in processed cheese food enriched with vegetable and whey proteins (El-Neshawy et al., 1988).

Table 5
Effects of incorporation of a preservative on physico-chemical and sensorial parameters of cheese-like products (SPC: 5 g/L) during storage ($T = 4 \pm 1$ °C), (means ± SD).

Storage (days)	1		15		30		45	60
Sample	Without preservative	With preservative	Without preservative	With preservative	Without preservative	With preservative	With preservative	With preservative
pH	5.7 ± 0.03	5.7 ± 0.01	5.67 ± 0.02	5.68 ± 0.03	Development of molds[a]	5.61 ± 0.01	5.55 ± 0.05	5.55 ± 0.05
Acidity (g/100 g)	0.94 ± 0.3	0.92 ± 0.1	0.98 ± 0.1	0.97 ± 0.2		1.01 ± 0.2	1.15 ± 0.2	1.15 ± 0.2
Syneresis (g/100 g)	4.64 ± 0.3	4.70 ± 0.5	5.69 ± 0.2	4.70 ± 0.3		5.7 ± 0.3	11.30 ± 0.5	11.30 ± 0.5
Fat (g/100 g)	12.33 ± 0.03	12.27 ± 0.05	12.42 ± 0.03	12.44 ± 0.02		14.7 ± 0.07	11.5 ± 0.06	11.5 ± 0.06
Odor	4.02 ± 0.23	4.01 ± 0.18	4.02 ± 0.19	4.00 ± 0.12		4.10 ± 0.62	4.03 ± 0.59	4.00 ± 1.10
Flavor	3.01 ± 0.52	3.00 ± 0.29	3.00 ± 0.37	3.73 ± 0.21		4.01 ± 0.28	4.05 ± 0.15	4.00 ± 1.52
Color	3.12 ± 0.39	3.20 ± 1.26	3.42 ± 1.42	3.30 ± 0.90		3.25 ± 1.05	3.12 ± 0.75	3.15 ± 1.21

[a] $M > 5000$ cfu/g.

4. Conclusions

Spreadeable reduced fat cheese-like products were developed from partially skimmed cow's milk with the addition of soy protein concentrate (SPC). It was established that cheese-like product formulations had higher protein content with the increase of SPC content ($P < 0.05$) confirming that part of the soy proteins remained into the casein matrix, also the fat content increase to 22%, respect to control cheese. However the products are classified as reduced in fat (CAA).

The values of normalized yields, that eliminated differences associated with milk composition (fat, protein) and cheese moisture, were significantly higher ($P < 0.01$) for cheese-like products with SPC than for control ones, and this confirmed that the addition of soy protein concentrate improved the cheese yield. The rheological characteristics of the samples revealed that $G' > G''$ indicating that the elastic properties dominated over the viscous ones, in a typical solid like behavior. The texture analysis showed the highest significant differences in the values of hardness; the increment in the SPC content showed a decrease in hardness, in agreement with the results of the apparent viscosity. These results should be associated with the higher moisture content, in samples containing soy proteins.

The SEM micrographs showed compact proteins matrixes with dispersed fat globules. The greater homogeneity of the sample with SPC 10 g/L, was correlated with a better spreadability ($P < 0.05$), found in the sensory analysis.

From measurements of microbial counts, pH, syneresis, fat and sensory evaluation, it was observed that the storage life of the cheese-like product without the addition of potassium sorbate in the formulation, was less than 30 days. In contrast, the formulated samples containing the preservative (in amounts below the limits established by legislation) exhibited a storage life higher than 60 days.

The products were considered as functional, since the added protein source was of vegetable origin with high biological value, because of the high content of amino acids and essential fatty acids. Other physicochemical parameters such as pH, ash content, and especially, sensory attributes did not show significantly differences between the soy formulated samples and the control ones, indicating that the amount of added soy protein concentrate is sufficient to enrich the cheese matrix, without adversely affecting physicochemical properties, sensory quality or product stability.

Acknowledgments

Financial support provided by the SCyT, UNSL (Project 22Q/011) and fellowships of Eng. Palatnik Diana of the CONICET are gratefully acknowledged. The assistance of Dr. Gabriel Lorenzo of the CIDCA Institute, (University of La Plata-CONICET) in the rheological characterization and texture analysis of the samples is gratefully acknowledged.

References*

AOAC (Association of Official Agricultural Chemists). (1995) (16th ed.).. *Official methods of analysis* (Vol. II) Arlington: AOAC International.
Aichinger, P. A., Servais, M. M., Dillmann, C., Rouveet, M. L., D`Amico, M., & Zink, N. (2003). Fermentation of a skim concentrate with *Streptococcus thermophilus* and chymosin: structure, viscoelasticity and syneresis of gels. *Colloids and Surface B Biointerfaces, 31*, 243–255.
Ali, F., Ippersiel, D., Lamarche, F., & Mondor, M. (2010). Characterization of low-phytate soy protein isolates produced by membrane technologies. *Innovative Food Science and Emerging Technologies, 11*, 162–168.
*Bachmann, H. P. (2001). Cheese analogues: a review. *International Dairy Journal, 11*, 505–515.
Brocklehurst, T. F., & Lund, B. M. (1985). Microbiological changes in cottage cheese varieties during storage at +7°C. *Food Microbiology, 2*, 207–233.
Canabady-Rochelle, L. S., & Mellema, M. (2010). Physical-chemical comparison of cow's milk proteins versus soy proteins in their calcium-binding capacities. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 366*, 110–112.
*Che Man, Y. B., & Yee, Y. N. (1996). Development and properties of soybean spreads. *Journal of Food Processing and Preservation, 20*, 347–358.
*Cheftel, J. C., Cuq, J. L., & Lorient, D. (1989). In Acribia (Ed.), *Proteínas Alimentarias*. Zaragoza, España.
Cheryan, M. (1986). *Ultrafiltration handbook*. USA: Technomic Publishing Co.
Christen, G. L., Davidson, P. M., McAllister, J. S., & Roth, L. A. (1993). Coliform and other indicator bacteria. In R. T. Marshal (Ed.), *Standard methods for the examination of dairy products* (pp. 247–269). Washington, DC: American Public Health Association Inc.
Código Alimentario Argentino, Administración Nacional de Alimentos, Medicamentos y Tecnología Médica Capítulo VIII (Alimentos Lácteos). http://www.anmat.gov.ar/alimentos/codigoa/CAPITULO_VIII.pdf.
Dermiki, M., Ntzimani, A., Badeka, A., Savvaidis, I. N., & Kontominas, M. G. (2008). Shelf-life extension and quality attributes of the whey cheese "Myzithra Kalathaki" using modified atmosphere packaging. *LWT – Food Science and Technology, 41*, 284–294.
El-Neshawy, A. A., Farahat, S. M., & Wahbah, H. A. (1988). Production of processed cheese food enriched with vegetable and whey proteins. *Food Chemistry, 28*, 245–255.
Farahmandfar, R., Mazaheri Tehrani, M., Razavi, S. M. A., & Habibi Najafi, M. B. (2010). Effect of soy cheese and trisodium citrate on pizza cheese. *International Journal of Food Engineering, 6*. http://dx.doi.org/10.2202/1556–3758.1777. Article 2.
Fellows, P. (1994). Zaragoza. España. In S. A. Acribia (Ed.), *Tecnología del procesado de alimentos: Principios y Prácticas*.
Fox, P. F., & Cogan, T. M. (2004). Factors that affect the quality of cheese. In P. F. Fox, P. L. H. Mc. Sweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese: Chemistry, physics and microbiology* (pp. 595). London: Elsevier Academic Press.
Francolino, S., Locci, F., Ghiglietti, R., Iezzi, R., & Mucchetti, G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT- Food Science and Technology, 43*, 310–314.
Frank, J., Christen, G., & Bullerman, L. (1993). Tests for groups of microorganisms. In R. T. Marshal (Ed.), *Standard methods for the examination of dairy products* (pp. 271–283). Washington, DC: American Public Health Association Inc.
Genovese, M., & Lajolo, F. (2002). Isoflavones in soy-based foods consumed in Brazil: levels, distribution, and estimated intake. *Journal of Agricultural and Food Chemistry, 50*, 5987–5993.
Giménez, A., Ares, F., & Ares, G. (2012). Sensory shelf-life estimation: a review of current methodological approaches. *Food Research International, 49*, 311–325.
Gomes da Cruz, A., Buriti, F. C. A., Souza, C. H. B., Faria, J. A. F., & Saad, S. M. I. (2009). Probiotic Cheese: health benefits, technological and stability aspects. *Trends in Food Science &Technology, 20*, 344–354.
González-Tomás, L., Bayarri, S., Taylor, A. J., & Costell, E. (2008). Rheology, flavour release and perception of low-fat dairy desserts. *International Dairy Journal, 18*, 858–866.
Guinee, T. P., O'Kennedy, B. T., & Kelly, P. M. (2006). Effect of milk protein standardization using different methods on the composition and yields of Cheddar cheese. *Journal of Dairy Science, 89*, 468–482.
Juric, M., Bertelsen, G., Mortensen, G., & Petersen, M. A. (2003). Light-induced colour and aroma changes in sliced, modified atmosphere packaged semi-hard cheeses. *International Dairy Journal, 13*, 239–249.
Karaman, A. D., & Akalin, A. S. (2013). Improving quality characteristics of reduced and low fat Turkish white cheeses using homogenized cream. *LWT – Food Science and Technology, 50*, 503–510.
Kealy, T. (2006). Application of liquid and solid rheological technologies to the textural characterization of semi-solid foods. *Food Research International, 39*, 265–276.
Kim, S. Y., Park, P. S. W., & Rhee, K. C. (1992). Textural properties of cheese analogs containing proteolytic enzyme- modified soy protein isolates. *Journal of the American Oil Chemists' Society, 69*, 755–759.
Krbavcić, I. P., & Barić, I. C. (2004). Influence of deep fat frying on some nutritional parameters of novel food based on mushrooms and fresh soft cheese. *Food Chemistry, 84*, 417–419.
Kumar, R., Choudhary, V., Mishra, S., Varma, I. K., & Mattiason, B. (2002). Adhesives and plastics based on soy protein products. *Industrial Crops and Products, 16*, 155–172.
Mateo, M. J., Everard, C. D., Fagan, C. C., O'Donnell, C. P., Castillo, M., Payne, F. A., et al. (2009). Effect of milk fat concentration and gel firmness on syneresis during curd stirring in cheese-making. *International Dairy Journal, 19*, 264–268.
Mattos, A. P., Ribeiro, T. C. M., Mendes, P. S. A., Valois, S. S., Mendes, C. M. C., & Ribeiro, , Jr.. (2009). Comparison of yogurt, soybean, casein, and amino acid-based diets in children with persistent diarrhea. *Nutrition Research, 29*, 462–469.

* References indicate previous works showing that underlie the different topics covered in this paper: – a clarifier review of cheese analogs; – food proteins, particularly soy proteins properties; – properties and behavior of a soybean spreads; – soft cheese processing, development and characterization; – use of membrane technology for protein concentrates.

Mazza, G. (1998). In S. A. Acribia (Ed.), *Alimentos funcionales. Aspectos bioquímicos y de procesado*. Zaragoza España.
Meilgaard, M. C., Arbor, A., Carr, T., & Civille, G. V. (2006). *Sensory evaluation techniques* (4th ed.). New Jersey, USA: CRC Press.
*Messina, M., Messina, V., & Setchel, K..,D. R. (2002). In Amalevi (Ed.), *Beneficios de la soja para la salud. ASGA: Asociación Argentina de Grasas y Aceites*. Mza, Argentina.
Milesi, M. M., Candioti, M., & Hynes, E. (2007). Mini soft cheese as a simple model for biochemical studies on cheese-making and ripening. *LWT – Food Science and Technology, 40*, 1427–1433.
Mistry, V. V. (2001). Low fat cheese technology. *International Dairy Journal, 11*, 413–422.
Moon, W., Balasubramanian, S. K., & Rimal, A. (2011). Health claims and consumers' behavioral intentions: the case of soy-based food. *Food Policy, 36*, 480–489.
Morales-Celaya, M. F., Lobatto-Calleros, C., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2012). Effect of milk pasteurization and acidification method on the chemical composition and microestructure of a Mexican pasta filata cheese. *LWT – Food Science and Technology, 45*, 132–141.
Morales, F. J., & Van Boeckel, M. A. J. S. (1999). A study on advanced Maillard reaction in heated casein/sugar solutions: colour formation. *International Dairy Journal, 8*, 907–915.
Mounsey, J. S., & O'Riordan, E. D. (2001). Characteristic of imitation cheese containing native starches. *Journal of Food Science, 66*(4), 586–591.
Nair, M. G., Mistry, V. V., & Oommen, B. S. (2000). Yield and functionality of Cheddar cheese as influenced by homogenization of cream. *International Dairy Journal, 10*, 647–657.
Pereira, C. I., Franco, M. I., Gomes, A. M. I., & Malcata, F. X. (2011). Microbiological, rheological and sensory characterization of Portuguese model cheeses manufactured from several milk sources. *LWT – Food Science and Technology, 44*, 2244–2252.
Rani, M., & Verma, N. S. (1995). Changes in organoleptic quality during ripening of cheese made from cows and soya milk blends, using microbial rennet. *Food Chemistry, 54*(4), 369–375.
*Rinaldoni, A. N., Campderrós, M. E., & Pérez Padilla, A. (2012). Physico-chemical and sensory properties of yogurt from ultrafiltreted soy milk concentrate added with inulin. *LWT – Food Science and Technology, 45*, 142–147.
Rodriguez Furlán, L. T., Rinaldoni, A. N., Pérez Padilla, A., & Campderrós, M. E. (2011). Assessment of functional properties of bovine plasma proteins compared with other protein concentrates, application in a Hamburger formulation. *American Journal of Food Technology, 6*(9), 717–729.
Sammons, R., & Marquis, P. (1997). Application of the low vacuum scanning electron microscope to the study of biomaterials and mammalian cells. *Biomaterials, 18*, 81–86.
SAS USER. (1989). *GUIDE: Statistic version*. Cary, NC, USA: SAS Inst. Inc.
Wadhwani, R., & McMahon, D. J. (2012). Color of low-fat cheese influences flavor perception and consumer liking. *Journal of Dairy Science, 95*, 2336–2346.