

ORIGINAL
RESEARCH

Development of reduced-fat cheeses with the addition of Agave fructans

DIANA R PALATNIK,¹ PAMELA ALDRETE HERRERA,²
ANA N RINALDONI,¹ ROSA I ORTIZ BASURTO² and
MERCEDES E CAMPDERRÓS^{1*}

¹Instituto de Investigaciones en Tecnología Química (INTEQUI-CCT-CONICET), Facultad de Química, Bioquímica y Farmacia (UNSL), Ejército de los Andes, 950-5700 San Luis, Argentina, and ²Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Av. Tecnológico 2595, Col. Lagos del Country C.P. 63175 Tepic, Nayarit, Mexico

Cheeses containing Agave fructans were compared with both full-fat and reduced-fat samples without fructans. Microstructure results showed that the cheeses obtained were very similar to the control samples (without fructans), even the full-fat control sample, demonstrating the texturing role of the carbohydrates. Regarding the composition, a moisture content of 47.96 ± 1.45 and a good protein retention with a low-fat content were found. Therefore, the cheese yield was not adversely affected, and no significant differences were observed in sensory aspects. Considering the health benefits of fructans and their abundance, this development could represent an innovation for dairy industry.

Keywords Agave fructans, Low-fat cheese, Cheese microstructure, Functional cheese.

INTRODUCTION

Cheese is the generic name for a group of fermented milk-based food products produced throughout the world in a great diversity of flavours, textures and forms (Fox *et al.* 2000). The nutritional value of this food stands out above other products because cheese contains a high content of biologically valuable protein with good digestibility. Cheese is an important dietary source of several minerals, in particular calcium, phosphorus and magnesium (O'Brien and O'Connor 2004). It is an appropriate food for growth in children and adolescents. An adult, with no medical contraindications, can consume 125 g of cheese per day. However, cheese consumption is sometimes restricted due to its high calorie content (300–400 cal/100 g), high saturated fats (20 to 30% w/w) and high cholesterol levels (0.09–0.1% w/w), added to the sodium content (about 1% w/w, almost the extreme daily dose) (Panjkota Krbavcic and Colic Baric 2004; Lucas *et al.* 2008).

In addition, during the last decade, consumers have become increasingly aware of the

importance of upholding adequate nutrition, and there is an increasing demand for alternative foodstuffs formulated to lower health risks (Cardarelli *et al.* 2008; Juan *et al.* 2013). Moreover, low-fat cheese production has significantly increased throughout the world (Mistry 2001). Besides the reduction in the fat content, it is interesting to add an ingredient to provide specific functional properties. In this respect, probiotics and prebiotics can be incorporated in cheese production, introducing beneficial changes in the composition and/or activity of the gastrointestinal microbiota (Gomes da Cruz *et al.* 2009).

Fructans, as nondigestible fermentable carbohydrates, are amongst the most studied and well-established prebiotics (Allsopp *et al.* 2013; Gibson *et al.* 2004; Henelly *et al.* 2006; Niness 1999; Pascoal *et al.* 2013; Juan *et al.* 2013; Sáyago-Ayerdi *et al.* 2014). Furthermore, it has been demonstrated that the consumption of fructans in humans increases calcium absorption, improving bone mineral content and density (Bosscher *et al.* 2006). Fructans are fructose polymers and a rich natural source of these

*Author for
correspondence: E-mail:
mcamp@unsl.edu.ar,
mcampd@gmail.com

compounds, are the *Agave plants*, abundant in arid regions of Latin America, which belong to the family of *Agavaceae*. México has been considered the centre of origin and biodiversity of *Agave* species. From the wide variety of species available, numerous fermented and distilled beverages can be obtained by hydrolysis (García-Aguirre *et al.* 2009; Ávila-Fernández *et al.* 2011). Given the abundance of the plant and the degree of industrialisation reached, new applications arise, and recently, the fructans had been assessed as lyoprotector agents for bovine proteins (Rodríguez Furlán *et al.* 2014). The *Agave* plant contains between 13% and 17% (w/w) fructan in mature plants, which is similar to the amount found in chicory (15.2–20.5% (w/w) on a fresh weight basis), the current source of inulin (Van Loo *et al.* 1995; Ávila-Fernández *et al.* 2011; Mellado-Mojica and López 2012). Fructans present in *Agave*, particularly in *Agave tequilana*, have a polymerisation degree (PD) between 3 and 29 with several β (2–6) bonds in branching fructose molecules. The higher PD or branched fructans are degraded more slowly in the colon (Roberfroid 2000; Allsopp *et al.* 2013; Salvatore *et al.* 2014). Due to their rheological properties, higher PD fructans are more suitable as fat replacers than other carbohydrates (Verraest *et al.* 1996).

The possibility of producing a cheese capable of presenting a potentially synbiotic effect, due to the incorporation of fructans from *Agave*, is indeed promising. These products, rich in dietary fibre and bioactive compounds, are a challenge for food processors, especially as consumers prefer natural supplements, fearing that synthetic ingredients may be a source of toxicity (Elleuch *et al.* 2011). Keeping in mind the importance of developing a new popular food from this abundant raw material, the influence of the incorporation of *Agave* native fructans (NF) and of two different

fractions – high-performance fructans (HPF) and high degree of polymerisation fructans (HDPF) – on a cheese matrix was assessed.

MATERIAL AND METHODS

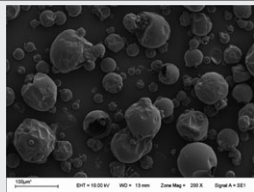
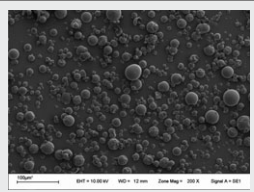
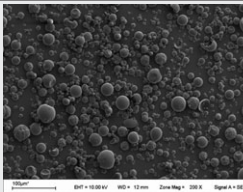
Raw materials

Full-fat milk (3% w/w fat, 3.5% w/w protein, 4.8% w/w lactose) and partially skimmed milk (1.5% w/w fat, 3.5% w/w protein, 4.8% w/w lactose) provided by Milkaut (San Luis, Argentina) were used. The milk was standardised, homogenised and pasteurised in the factory.

Commercial products of *Agave tequilana* native fructans (NF) in powder and 72°Brix were donated by the company Agaviótica (Monterrey, Mexico). Two fractions of fructans concentrated by tangential ultrafiltration process were obtained from the Instituto Tecnológico de Tepic, Nayarit, Mexico. To obtain the high-performance fructans (HPF) and high degree of polymerisation fructans (HDPF), a solution of native fructans in 20°Brix was ultrafiltered in commercial membrane modules (Pellicon-2 from Millipore, MA, USA), with a nominal molecular cut-off (MWCO) of 1 and 10 kDa, respectively (20 °C, 3 bar and 4 L/min). After separation, the fructan fractions retained on each membrane were spray-dried using a model LPG5 drier (CIMA Industries Inc., China) (inlet/outlet temperature: 100/80°C, atomiser speed: 30000 rpm and feed flow: 17.5 mL/min) (Aldrete-Herrera 2013; Espinosa-Castrejón 2012). Physico-chemical characteristics and morphological aspects of the three fructans from *agave* used as additives in cheesemaking are shown in Table 1.

Starter cultures (homofermentative mesophilic lyophilised for direct inoculation) containing *Lactococcus lactis*

Table 1 Composition and morphology of *Agave tequilana* fructans

Characteristic (g·100/g)	NF ^a	HPF ^a	HDPF ^a
Morphology SEM magnification: 200X			
Moisture	4.62 ± 0.2	3.01 ± 0.03	2.5 ± 0.2
Total Carbohydrate	97.5 ± 1.7	98.2 ± 0.7	98.6 ± 0.5
Reducing sugars	13.6 ± 0.5	0.8 ± 0.06	0.4 ± 0.4
Fructans	83.9 ± 0.4	97.4 ± 0.5	98.2 ± 0.3
Ash	0.11 ± 0.02	0.15 ± 0.05	0.10 ± 0.05
Enriched DP	–	4–80	24–80
a _w	0.41 ± 0.02	0.33 ± 0.01	0.30 ± 0.00

^aHPF, high-performance fructans; HDPF, high degree of polymerisation fructans; and NF, native fructans.

subspecies *cremoris* and *Lactococcus lactis* subspecies *lactis* (CHR Hansen, Argentina) were used.

Enzymic coagulant powder (Chy-Max extra CHR Hansen, Argentina) was employed.

Cheese manufacture and yield evaluation

Partially skimmed milk was divided into seven portions of 2.5L for each batch. One of the portions was reserved to produce a low-fat cheese as a control sample (LFC) and to the rest of the portions, different fructans such as HPF, HDPF and NF (in concentrations of 0.5% and 5% (w/v)) were added. Full-fat control cheeses, without fructans, were also produced for comparison.

Production was carried out in a batch process. Each formulation was replicated at least twice and analysed independently, and also each set of samples were made on the same day. Manufacturing stages for cheese-like product production are shown in Figure 1.

The initial pH was measured (6.89 ± 0.10). After the addition of fructans, the samples were mixed. Then, the temperature was brought to $34.5 \pm 2^\circ\text{C}$ and 1 g of CaCl_2 (BDH Chemicals Laboratory Reagents, United Kingdom) was added which contributes to obtain a proper floc. Then, the starter (0.1 g) was added to the batches. When the pH was at 6.5 ± 0.2 , the temperature was raised to $37.5 \pm 0.5^\circ\text{C}$ and 0.1 g of coagulant (2080 IMCU/g powder) was added. The curd was cut 35 ± 5 min later with a curd knife into 2-cm^3 cubes. The cut curd was allowed to settle for 10 min. then the temperature was increased by $2 \pm 0.5^\circ\text{C}$ while gently mixing so as not to break the curd granules. After that, the samples were poured onto a sieve covered with a cheese cloth for the drainage of whey, which was collected in a graduated cylinder.

The cheese curds were put into cylindrical plastic moulds of 250 g capacity (Vigna S.A., Argentina) and pressed for one hour and half. After this time, the pH was monitored up to a value of 5.3 ± 0.2 and placed in saturated brine, calculating the dwell time in the brine according to the weight of the cheeses. The samples were held at $6 \pm 2^\circ\text{C}$ in a refrigerator and were packaged in a plastic film after 24 h.

The yield (Y) reached in the cheese production was calculated by equation (1):

$$Y = \frac{\text{weight of product}}{\text{weight of milk}} \times 100 \quad (1)$$

Analysis

Raw materials and cheeses samples were analysed in duplicate according to AOAC (Association of Official Agricultural Chemists, 1995) methods. Analyses were performed 48 h after cheese manufacture.

pH was measured using a digital pH meter (Testo 206-pH2, Germany).

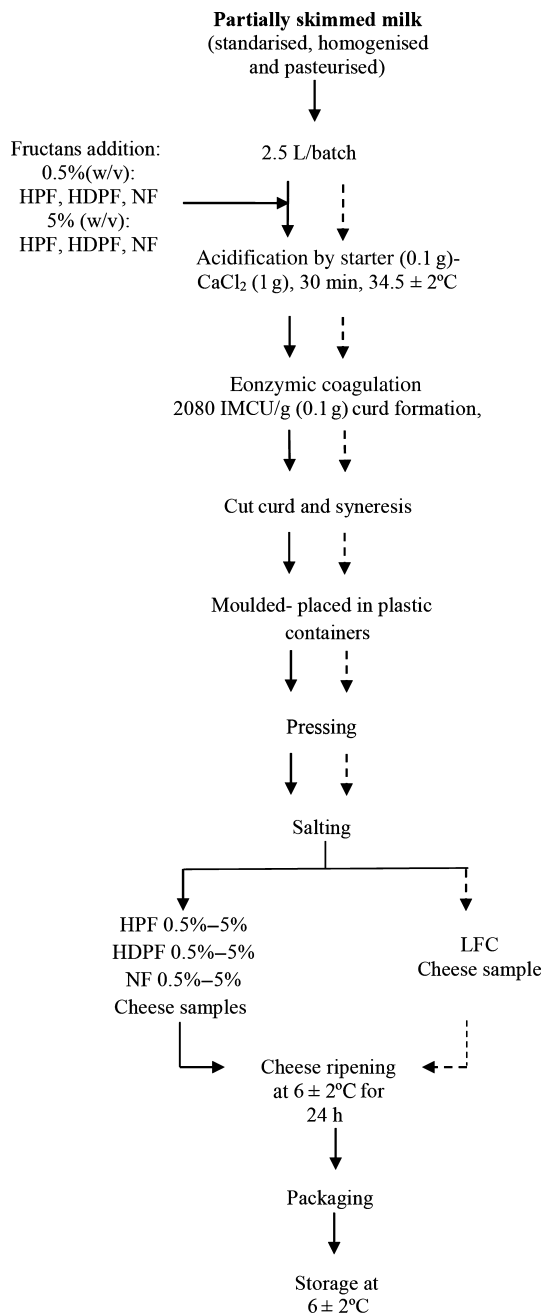


Figure 1 Manufacturing procedure for fresh soft cheese with fructans.

The protein content was calculated by the determination of total nitrogen by the Kjeldahl method using Digestion Blocks and a semiautomatic Kjeldahl Distiller (Selecta, Spain); the conversion factor used to express the results was 6.38 (AOAC 991.22). The fat content was measured by the Rosse–Gottlieb method (AOAC 933.05). Total solids were determined by weight difference after oven drying at $70 \pm 1^\circ\text{C}$ for 24 h (AOAC 925.23). The moisture content was determined by gravimetric method (IDF 1982). 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 total carbohydrates (lactose plus fructan) was carried out in whey, the byproduct of cheese manufacture, using a refractometer (Arcano, China, range 0–32°Brix) in which the soluble compounds are expressed as °Brix. The measurement takes only few seconds and has a good correlation with dissolved solids (Van Waes *et al.* 1998). The determination of fructan was carried out by difference between the amount of total carbohydrates measured and the lactose recorded in the control sample. The fructan value retained in cheeses was obtained by difference between the amount of fructan added and the amount found in the whey.

Scanning electron microscopy

The microstructure of cheese-like products was analysed by scanning electron microscopy (SEM) using an LEO1450VP equipment (Zeiss, Germany). The samples were mounted on double-sided adhesive carbon on aluminium sample holder. The micrographics were determined under VP mode (variable pressure) (Sammons and Marquis 1997). The low vacuum mode of SEM is a special form, where the column is opened and fixed between the SEM chamber, the chamber can be maintained (where the samples are placed) at low vacuum (70 Pa), while the column remains at high vacuum levels. In this way, it is possible to observe sensitive samples, biological or with water content, without dehydrating and metal coating the sample. The images were analysed by Image-Pro Plus 6.0 (Media Cybernetics Inc., Bethesda, USA) software.

Analysis of surface colour

The surface colour was measured by a MiniScan EZ digital spectrophotometer and software (HunterLab, Virginia, USA). The chromometer was calibrated with the standard white and black colour. 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, while a^* and b^* values are the chromaticity values, +redness/-greenness and +yellowness/-blueness, respectively (Morales and Van Boekel 1999).

Sensory evaluation

The samples were tested in a uniformly illuminated room, by a 45-member panel selected from a pool of students and staff members of our Department. Prior to assessment, each model cheese sample was divided into various portions and equilibrated at room temperature ($22 \pm 2^\circ\text{C}$). A discrimination test was employed in which the evaluator had to establish the difference between a control sample and one or more problem samples, using a scale from 0 (no

difference) to 6 (very much different). The samples were coded with three-digit random numbers and were shown in pairs: control vs. sample, and control vs. control (as blind witness). The attributes evaluated comparatively were as follows: flavour, colour, texture, sweetness and acidity. Panelists were exposed to each sample on an individual Petri plastic dish and were asked to assess a number of specific attributes. Water was provided for rinsing between samples, to clean the palate (Meilgaard *et al.* 2006). The average for each attribute of each sample was calculated, and the differences were analysed with the analysis of variance using the statistic software 'GraphPad InStat'.

Statistical analysis

Data from the cheesemaking trials were statistically analysed using the pc program 'GraphPadInStat'. The data were statistically evaluated by the Tukey–Kramer multiple comparison test in the cases where 2 or more comparisons were considered. Otherwise, the t-test was used, assuming that a $P < 0.05$ was statistically significant (SAS 1989).

RESULTS AND DISCUSSION

Cheese composition

The results of the physicochemical characterisation of the products are shown in Table 2. As expected, the highest difference was observed in the fat composition, between the whole cheese control and the rest of samples ($P < 0.05$). In effect, as established by the codex general standard for cheese (Kosikowski and Mistry 1997), the full-fat cheese, FFC, corresponds to a medium-fat cheese (25–45% w/w fat on dry basis), and the rest of the samples, to a low-fat cheese (10–25% w/w fat on dry basis). As the fat content of cheese is lowered, moisture content increases and protein plays a greater role in texture development (Mistry 2001). So, according to the Argentinean legislation (Código Alimentario Argentino 2014), the samples correspond to cheeses of high moisture (46–54.9% w/w of moisture content) and reduced fat (10.0–24.9% w/w of fat content). Regarding the protein content, the results showed a high protein content for the type of cheese under study, compared with other reports (Milesi *et al.* 2007; Francolino *et al.* 2010; Salvatore *et al.* 2014). Furthermore, the amount of fructans remaining in the developed cheeses contributed to the compositional and textural balance of the samples. It was observed that by increasing the amount of fructans added to the formulation, the amount retained in the cheese also increased, with an average retention of 1% and 4.12% for samples with 0.5% and 5% fructan addition, respectively. Salvatore *et al.* (2014) reported a high retention of long-chain inulin fructans in fresh caprine milk cheese with 22% of total solids. The pH was similar for all samples, in the range of 5.07–5.52, which was optimal for flavour

Table 2 Physicochemical characterisation and mean yield of cheese-like products with different concentrations of fructans (means \pm SD)

Sample*	Moisture (%w/w)	Fat (%w/w)	Protein (%w/w)	Fructans (%w/w)	Lactose (%w/w)	Ash (%w/w)	Yield (Y)
FFC	47.25 \pm 1.98 ^a	24.07 \pm 0.51 ^a	23.60 \pm 0.06 ^a	–	1.58 \pm 0.69	3.50 \pm 0.24 ^a	8.76 \pm 1.51 ^a
LFC	47.52 \pm 4.35 ^a	15.83 \pm 0.16 ^b	26.97 \pm 1.01 ^b	–	5.63 \pm 1.47	4.05 \pm 0.35 ^a	8.68 \pm 1.91 ^a
HPF 0.5	47.45 \pm 1.36 ^a	15.84 \pm 4.54 ^b	26.59 \pm 0.91 ^b	2.31 \pm 0.49 ^a	3.76 \pm 1.47	4.06 \pm 0.06 ^a	8.20 \pm 2.30 ^a
HPF 5	48.55 \pm 2.43 ^a	13.08 \pm 3.48 ^c	27.09 \pm 0.34 ^b	3.90 \pm 0.14 ^b	3.48 \pm 1.32	3.90 \pm 0.21 ^a	8.28 \pm 1.71 ^a
HDPF 0.5	49.43 \pm 2.25 ^a	13.24 \pm 4.56 ^c	26.43 \pm 0.56 ^b	1.40 \pm 0.49 ^a	5.51 \pm 1.62	3.97 \pm 0.25 ^a	8.12 \pm 2.52 ^a
HDPF 5	47.89 \pm 1.88 ^a	12.96 \pm 1.52 ^c	27.72 \pm 0.81 ^b	3.55 \pm 0.61 ^b	3.99 \pm 0.99	3.89 \pm 0.16 ^a	8.36 \pm 2.31 ^a
NF 0.5	48.14 \pm 1.67 ^a	12.98 \pm 0.55 ^c	28.45 \pm 0.76 ^b	0.80 \pm 0.05 ^a	5.58 \pm 1.92	4.05 \pm 0.19 ^a	8.22 \pm 2.70 ^a
NF 5	47.46 \pm 1.90 ^a	12.52 \pm 0.14 ^c	30.58 \pm 0.54 ^c	4.12 \pm 0.76 ^b	1.65 \pm 0.72	3.67 \pm 0.13 ^a	8.26 \pm 2.00 ^a

(a–c) Means with different subscript in the same row are significantly different ($P < 0.05$). *FFC, full-fat cheese; LFC, low-fat cheese; HPF 0.5; 5, cheese with high-performance fructans at 0.5 and 5% (w/v); HDPF 0.5; 5, cheese with high degree of polymerisation fructans at 0.5, and 5% (w/v); NF 0.5; 5, cheese with native fructans at 0.5 and 5% (w/v). Number of replicates: 2 (two).

considerations (Bachmann 2001). As a result of this microenvironment performance, cheeses added with fructans showed practically the same yield as the control cheeses (Table 2).

Microstructure study

The electron micrographs were taken to establish the relationships between the microstructure of cheeses and their composition. Figure 2 shows the images obtained for the control samples and cheese-like products with fructans.

In all samples, protein matrixes with small numbers of dispersed fat globules were observed, similar to previous reports (Karaman and Akalin 2013). This type of structure is common for cheeses made from pasteurised milk. Indeed, according to Morales-Celaya *et al.* (2012), these cheeses have a more stratified structure than the casein strand arrangement obtained from raw milk products. Comparing both control samples, full-fat control cheese and low-fat control cheese, it can be observed that the full-fat sample showed a higher screening due to its higher fat content, with smaller holes in the cheese structure. The smaller size of the milk-fat globules seems to be related to less coalescence. In this regard, Michalski *et al.* (2003) obtained similar images for Camembert cheeses with small fat globules. They demonstrated that the small fat globules were better entrapped in the casein matrix and had a greater surface area and thinner casein strands due to the smaller interglobular distance. This result was consistent with cheese composition having high protein and moisture contents.

The presence of fructans appears to determine a more intricate protein matrix, particularly for formulations with a higher oligosaccharide concentration, that is HPF, HDPF and NF at 5% w/v, in which the structures tend to be like the structure of the full-fat cheese. This result suggests that the presence of fructans effectively acts as a texturing agent, replacing fat cheese. Additionally, it was observed that the control samples showed a ‘honeycombing’ structure in the

protein matrix. This structure was observed in samples HDPF 5 and more clear in NF 5. Electron micrographs published by Henelly *et al.* (2006) showed similar results for inulin-containing cheeses which were smoother and more uniform respect to the control, suggesting that the added oligosaccharide had bound any loose water, thus preventing the formation of honeycomb structures during sample preparation. Moreover, Picone *et al.* (2011) reported that the presence of inulin in casein gels at concentrations of 0–6% (w/w) resulted in a more closed-pore network. Gel deformability increased as sodium caseinate concentration was enhanced, reaching a maximum of 0.60 at 5.58% (w/w) of sodium caseinate, but it was not affected by inulin concentration.

Regarding the structure or morphology of the different fructans (Table 1) added into the cheese matrix, an evaluation of the particle sizes is shown in Figure 3. It was found that NF, HDPF and HPF showed mean diameters of: 19.55 \pm 2.74 μ m, 11.01 \pm 0.73 μ m and 10.00 \pm 0.74 μ m, respectively. Furthermore, while HDPF and HPF showed less variability, the NF had particles with diameters upto 120 μ m. This suggests that the increased heterogeneity achieves a better integration in the cheese matrix and therefore retains more protein, as is shown in Table 2. They also act better as fat replacements although with less fat, they better mimic control cheese while retaining a larger amount of a component with prebiotic characteristics, the native fructans.

Cheese colour

The results of colour measurements on cheese-like products are shown in Table 3. The control samples and cheeses enriched with fructans showed high L^* values (around 85) which reflects the degree of lightness, and this parameter has a major impact on the perceived appearance of the product. The values were similar to others obtained for white cheese milk (Juric *et al.* 2003). The a^* values were

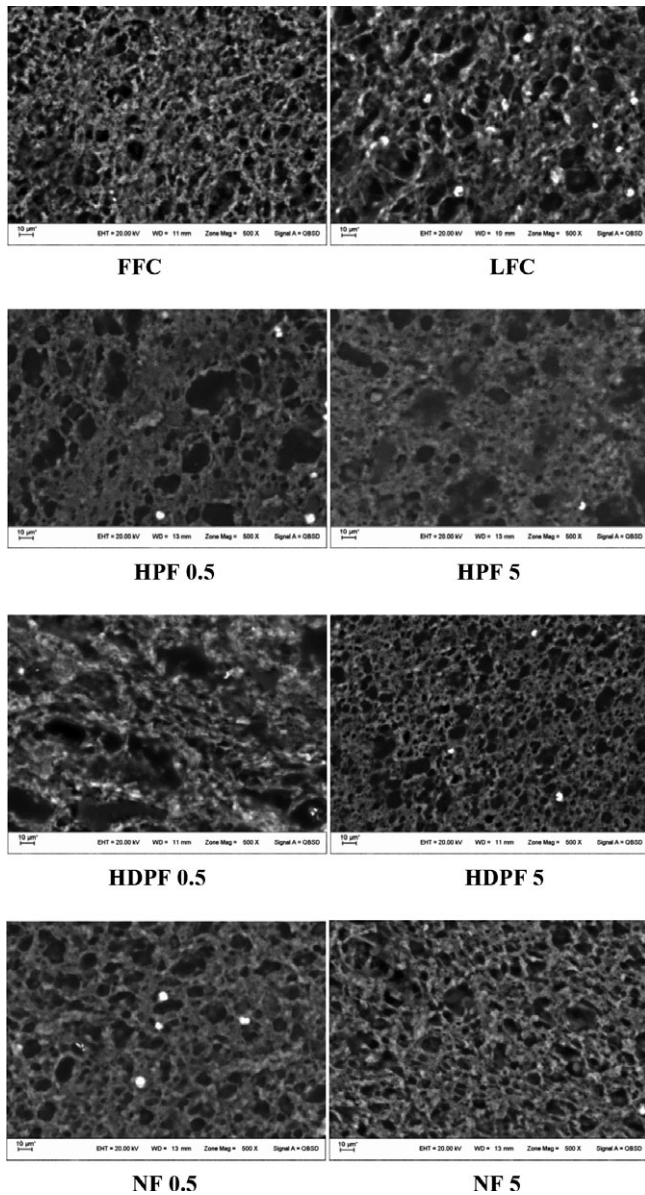


Figure 2 Scanning electron micrographs of the different samples, with and without fructans. FFC: Full-fat cheese; LFC: low-fat cheese; HPF 0.5; 5: cheese with a concentration 0.5, and 5% of high-performance fructans; HDPF 0.5; 5: cheese with a concentration 0.5, and 5% of high degree of polymerisation fructans; NF 0.5; 5: cheese with a concentration 0.5 and 5% of native fructans. Magnification 500 \times .

positives and close to zero reflecting the low degree of redness, without significant differences between samples ($P > 0.05$), with the exception of the full-fat control sample, which has the higher a^* value ($P < 0.05$). The b^* positive value indicated the degree of yellowness. These values were suitable taking into account that in low-fat cheese, the removal of fat imparts a translucent appearance. In effect, the colorimetric parameters obtained were in the same order to those reported by Wadhvani and McMahon (2012), for

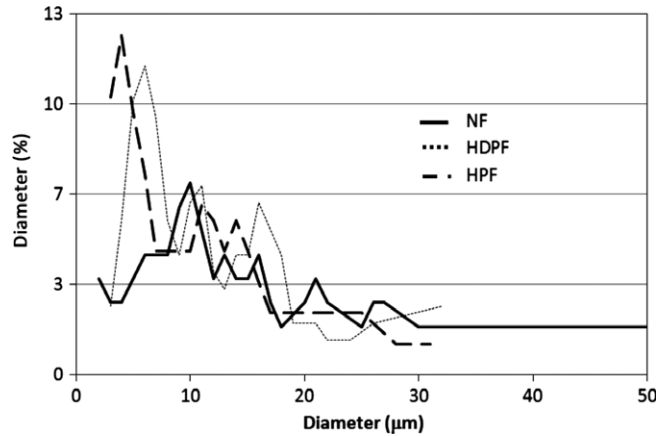


Figure 3 Fructans' size distribution evaluated from micrographs of Table 1.

Table 3 Surface cheese-like products colour

Sample*	L^*	a^*	b^*
FFC	85.30 \pm 2.14 ^{a,b}	1.20 \pm 0.11 ^a	18.37 \pm 2.43 ^a
LFC	86.11 \pm 3.41 ^{a,b}	0.19 \pm 0.16 ^b	14.89 \pm 2.57 ^a
HPF 0.5	85.78 \pm 1.64 ^{a,b}	0.18 \pm 0.16 ^b	14.92 \pm 2.24 ^a
HPF 5	84.72 \pm 0.23 ^{a,b}	0.41 \pm 0.16 ^b	15.14 \pm 1.64 ^a
HDPF 0.5	85.92 \pm 1.70 ^{a,b}	0.22 \pm 0.15 ^b	14.00 \pm 1.55 ^a
HDPF 5	86.59 \pm 1.29 ^a	0.32 \pm 0.18 ^b	13.38 \pm 1.05 ^a
NF 0.5	81.50 \pm 1.01 ^b	0.48 \pm 0.18 ^b	16.32 \pm 1.13 ^a
NF 5	89.60 \pm 1.14 ^a	0.34 \pm 0.09 ^b	16.66 \pm 0.45 ^a

(a–b) means with different subscript in the same row are significantly different ($P < 0.05$). *FFC: Full-fat cheese; LFC, low-fat cheese; HPF 0.5; 5, cheese with high-performance fructans at 0.5, 1.5 and 5% (w/v); HDPF 0.5; 5, cheese with high degree of polymerisation fructans at 0.5 and 5% (w/v); NF 0.5; 5, cheese with native fructans at 0.5 and 5% (w/v).

low-fat cheese without annatto colorant. Also, Michalski *et al.* (2003) found that Camembert cheeses exhibited less yellow colour for the samples with small fat globules.

Thus the presence of fructans did not significantly affect the colour of samples with respect to the control cheese.

Sensory analysis

Meeting consumers' needs is a priority in market-oriented firms and in this sense, acceptability of a food product is considered a trigger for subsequent purchases and, accordingly, a contributing factor to the success of the product to be developed in the long run.

For sensory analysis, the method used was peer discrimination. It was selected to compare cheeses with fructans with control reduced-fat cheese, as these two groups of samples were the most similar between them. The determinations were carried out on all samples with fructans comparing with the control samples; however, as no

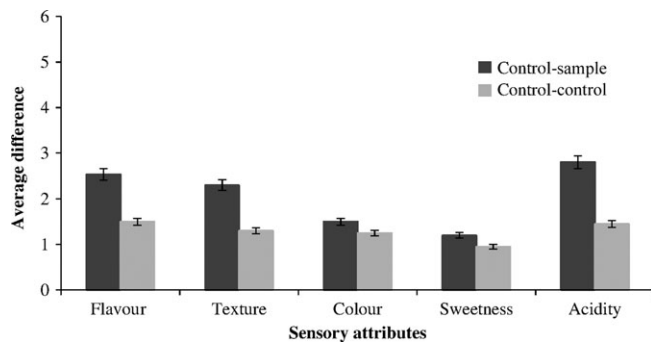


Figure 4 Sensory evaluation by discrimination test for two samples groups: cheese control (LFC) and cheeses enriched with fructans (HPF, HDPF and NF) (average results, $P > 0.05$). Number of replicates: 3 (three).

statistically significant difference between cheeses with HPF, HDPF and NF ($P > 0.05$) was observed, so these values were averaged. The results are shown in Figure 4.

The discrimination test applied to control cheese and the samples with fructans did not show marked differences with respect to the attributes considered. Similar conclusion was found by Juan *et al.* (2013), employing inulin as fat replacer in reduced-fat fresh cheese.

CONCLUSIONS

Reduced-fat cheeses were developed from partially skimmed bovine milk with the addition of Agave fructans. The samples with fructans showed an appropriate moisture and protein retention, especially with the higher fructan concentration added, being the formulation with native fructans which retained the higher amount of fructans. The sensorial aspects, including colour determinations, did not show significant difference with regard to the control samples, indicating that the fructans did not affect these parameters. Even though it would be difficult to mimic entirely a full-fat cheese after fat has been removed, the presence of fructans in reduced-fat formulations suggests an acceptable likeness in relation to structure and general characteristics of the full-fat control cheese. This fact constitutes a technological gain.

The role of the Agave fructans in the cheese matrix is significant, taking into account that they are considered as soluble fibre from natural and abundant source, categorised as prebiotics. Thus, they become a valuable alternative as a functional ingredient to obtain functional foods.

ACKNOWLEDGEMENTS

Financial support provided by the SCyT, UNSL (Project 22Q/011) and PICT 2012-0155 (ANPCyT), also fellowships of Diana Palatnik of the CONICET (Argentina) and Pamela Aldrete Herrera of the CONACYT (México) are gratefully acknowledged.

REFERENCES

- Aldrete-Herrera P I (2013) Separación de una fracción de fructanos de Agave tequilana Weber variedad azul, con alto GP mediante filtración tangencial, master's thesis, Instituto Tecnológico de Tepic. Tepic, Nayarit, México.
- Allsopp P, Possemiers S, Campbell D, Saldaña Oyarzábal I, Gill C and Rowland I (2013) An exploratory study into the putative prebiotic activity of fructans isolated from *Agave angustifolia* and the associated anticancer activity. *Anaerobe* **22** 38–44.
- AOAC (1995) *Official Methods of Analysis*, 16th edn. Vol. II. Arlington: AOAC International.
- Ávila-Fernández A, Galicia-Lagunas N, Rodríguez-Alegría M E, Olvera C and López-Munguía A (2011) Production of functional oligosaccharides through limited acid hydrolysis of agave fructans. *Food Chemistry* **129** 380–386.
- Bachmann H P (2001) Cheese analogues: a review. *International Dairy Journal* **11** 505–515.
- Boscher D, Van Loo J and Franck A (2006) Inulin and oligofructose as functional ingredients to improve bone mineralization. *International Dairy Journal* **16** 1092–1097.
- Cardarelli H R, Buriti F C A, Castro I A and Saad S M I (2008) Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially symbiotic *petit-suisse* cheese. *LWT – Food Science and Technology* **41** 1037–1046.
- Código Alimentario Argentino (2014). Capítulo VIII (Alimentos Lácteos) Art. 553–642.
- Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C and Attia H (2011) Dietary fibre and fibre-rich by-products of food processing: characterization, technological functionality and commercial applications: a review. *Food Chemistry* **124** 411–421.
- Espinosa-Castrejón R (2012) Separación y Caracterización Parcial de Fructanos de Alto Rendimiento de Agave tequilana Weber var. azul. Master tesis, Instituto Tecnológico de Tepic. Tepic, Nayarit, México.
- Fox P F, Guinee T P, Cogan T M and McSweeney P L H (2000) *Fundamentals of Cheese Science*, 1a edn. Gaithersburg, MD, Estados Unidos: Aspen Publishers Inc.
- Francolino S, Locci F, Ghiiglietti R, Iezzi R and 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.
- García-Aguirre M, Sáenz-Álvaro V A, Rodríguez-Soto M A, Vicente-Maguey F J, Botello-Álvarez E, Jiménez-Islas H, Cárdenas-Marríquez M, Rico-Martínez R and Navarrete-Bolaños J L (2009) Strategy for biotechnological process design applied to the enzymatic hydrolysis of agave fructo-oligosaccharides to obtain fructose-rich syrups. *Journal of Agricultural and Food Chemistry* **57** 10205–10210.
- Gibson G R, Probert H M, Van Loo J, Rastall R A and Robertfroid M B (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Review* **17** 259–275.
- Gomes da Cruz A, Buriti F C A, Souza C H B, Faria J A F and Saad S M I (2009) Probiotic cheese: health benefits, technological and stability aspects. *Trends in Food Science & Technology* **20** 344–354.
- Henelly P J, Dunne P G, O'Sullivan M and O'Riordan E D (2006) Textural, rheological and microstructural properties of imitation cheese containing inulin. *Journal of Food Engineering* **75** 388–395.

- IDF (1982) std. 4A; 1986 std. 20A; 1988 std. 88A; 1997 std. 152A. Brussels: International Dairy Federation
- Juan B, Zamora A, Quintana F, Guamis B and Trujilla A J (2013) Effect of inulin addition on the sensorial properties of reduced-fat fresh cheese. *International Journal of Dairy Technology* **66** 478–483.
- Juric M, Bertelsen G, Mortensen G and 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 and 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.
- Kosikowski F V and Mistry V V (1997) Cheese and fermented milk foods, Volume 1. Origins and principles. Westport, CT: F.V. Kosikowski L L C.
- Lucas A, Coulon J B, Agabriel C, Chilliard Y and Rock E (2008) Relationships between the conditions of goat's milk production and the contents of some components of nutritional interest in Rocamadour cheese. *Small Ruminant Research* **74** 91–106.
- Meilgaard M C, Carr T and Civille G V (2006) *Sensory Evaluation Techniques*, 4th edn. USA: CRC Press.
- Mellado-Mojica E and López M G (2012) Fructan metabolism in A. Tequilana weber blue variety along its developmental cycle in the field. *Journal Agriculture Food Chemistry* **28** 11704–11713.
- Michalski M C, Gassi J Y, Famelart M H, Leconte N, Camier B, Michel F and Briard V (2003) The size of native milk fat globules affects physico-chemical and sensory properties of camembert cheese. *Lait* **83** 131–143.
- Milesi M M, Candioti M and 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.
- Morales F J and Van Boekel M A J S (1999) A study on advanced Maillard reaction in heated casein/sugar solutions: colour formation. *International Dairy Journal* **8** 907–915.
- Morales-Celaya M F, Lobatto-Calleros C, Alvarez-Ramirez J and Vernon-Carter E J (2012) Effect of milk pasteurization and acidification method on the chemical composition and microstructure of a Mexican pasta filata cheese. *LWT – Food Science and Technology* **45** 132–141.
- Niness K R (1999) Inulin and Oligofructose: What Are They? *Journal of Nutrition* **129**(7) 1402–1406.
- O'Brien N M and O'Connor T P (2004) Nutritional aspects of cheese. In: *Cheese: chemistry, physics and microbiology, volume 1*. London: Elsevier Academic Press.
- Panjkota Krbavcic I and Colic Baric I (2004) Influence of deep fat frying on some nutritional parameters of novel food based on mushrooms and fresh soft cheese. *Food Chemistry* **84** 3417–3419.
- Pascoal G B, Filisetti T M, Alvares E P, Lajolo F M and Menezes E W (2013) Impact of onion (*Allium cepa* L) fructans fermentation on the cecum of rats and the use of *in vitro* biomarkers to assess *in vivo* effects. *Bioactive Carbohydrates and Dietary Fibre* **1** 89–97.
- Picone C S, Maximo G J, Kuhn K R, Ros-Polski V and Cunha R L (2011) An assessment of the texture of acidified sodium caseinate gels with added inulin using response surface methodology. *International Journal of Dairy Technology* **64** 353–359.
- Roberfroid M B (2000) Chicory Fructooligosaccharides and the gastrointestinal tract. *Nutrition* **16** 677–679.
- Rodríguez Furlán L, Aldrete Herrera P, Pérez Padilla A, Ortiz Basurto R and Campderrós M (2014) Assessment of agave fructans as lyoprotectants of bovine plasma proteins concentrated by ultrafiltration. *Food Research International* **56** 146–158.
- Salvatore E, Pes M, Mazzarello V and Pirisi A (2014) Replacement of fat with long-chain inulin in a fresh cheese made from caprine milk. *International Dairy Journal* **34** 1–5.
- Sammons R and 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 Guide: Statistic. Versión (1989) SAS Inst. Inc., Cary, NC, USA.
- Sáyago-Ayerdi S G, Mateos R, Ortiz-Basurto R I, Largo C, Serrano J, Granada-Serrano A B, Sarriá B, Bravo L and Taberero M (2014) Effects of consuming diets containing Agave tequilana dietary fibre and jamaica calyces on body weight gain and redox status in hypercholesterolemic rats. *Food Chemistry* **148** 54–59.
- Van Loo J, Coussement P, de Leenheer L, Hoebregs H and Smits G (1995) On the presence of inulin and oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science and Nutrition* **35** 525–552.
- Van Waes C, Baert J, Carlier L and Van Bockstaele E (1998) A rapid determination of the total sugar content and the average inulin change length in roots of chicory (*Cichorium intybus* L). *Journal of the Science of Food and Agriculture* **76** 107–110.
- Verraest D L, Peters J, Van Bekkum H and Van Rosmalen G M (1996) Carboxymethyl Inulin: a new inhibitor for calcium carbonate precipitation. *Journal of the American Oil Chemists' Society* **73** 55–62.
- Wadhvani R and McMahon D J (2012) Color of low-fat cheese influences flavor perception and consumer liking. *Journal of Dairy Science* **95** 2336–2346.