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# **Research Article**

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Author for correspondence: Joice Sifuentes dos Santos, Email: Joice.sifuentes@gmail.com

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Joyce Valle Borges, José Augusto de Souza, Rafael Fagnani, Giselle Nobre Costa and Joice Sifuentes dos Santos

Mestrado em Ciência e Tecnologia de Leite e Derivados, Universidade Pitágoras Unopar (UNOPAR), Londrina, Brazil

### Abstract

This research paper aimed to evaluate the role of inulin as a fat replacer on the quality of Frescal sheep milk cheese. Sheep milk and its derivatives are a promising niche in the dairy industry, mainly due to increasing interest of consumers in diversified products. Three Frescal sheep milk cheese formulations, namely whole milk cheese (WMC), semi-skimmed cheese (SSC) and semi-skimmed cheese with 5 g/100 g inulin (SSCI) were prepared. Their composition was evaluated and the feasibility of using inulin as a fat substitute was investigated. SSC and SSCI were considered 'reduced fat' or 'reduced calorie' products. The addition of inulin to SSCI cheeses yielded textural parameters (firmness, adhesiveness, cohesiveness, and gumminess) with intermediate characteristics between SSC and WMC. All the formulations presented scores higher than 7.6 in sensory analysis. In conclusion, the use of inulin in semi-skimmed sheep cheese allowed the production of cheese with texturizing properties similar to whole milk sheep cheese, enabling the development of a foodstuff with lower caloric content and beneficial characteristics valued by consumers.

The use of fat replacements in cheese while maintaining the original functional and sensory properties is a challenge that has attracted great attention and interest from consumers and the food industry. Removal of fat from cheese causes rheological, textural, functional, and sensory defects such as rubbery texture, lack of flavor, bitterness, off-flavor, poor meltability and undesirable color (Mistry, 2001; O'Connor and O'Brien, 2011). It is difficult to make fat-free or low-fat cheeses with desirable properties (Fadaei *et al.*, 2012).

Recently, research has increasingly focused on sheep milk production and composition. Sheep milk is a high quality raw material, presenting a reduced allergenic potential compared with that of cow milk. The cow milk hyperallergenic properties are mainly due to the  $\alpha$  S-1 portion of casein. In sheep and goat milk, this sequence differs markedly from that of cow milk (Masoodi and Shafi, 2010). Sheep milk is rich in fat (7.9% on average) and protein (6.2% on average), composed of more total solids (around 20.0%), and has an improved nutrient content compared to cow's milk (3.6, 3.2, around 13.0%, respectively; Park *et al.*, 2007). The fat globule size is smaller in sheep milk (65% of globules are less than 3  $\mu$ m). This yields better digestibility and a more efficient lipid metabolism than cow milk fat (Park *et al.*, 2007). Sheep milk cheese has a better yield since its solids ratio is higher than that in milk from other ruminants.

Minas Frescal cheese is a dairy product mostly consumed in Brazil, with its origin in Minas Gerais state, influenced by Portuguese colonization. It is a fresh and soft white cheese, slightly salty and with a mild taste of lactic acid (Cunha *et al.*, 2006). Minas Frescal cheese is produced by enzymatic coagulation of pasteurized milk with rennet (Buriti *et al.*, 2005).

One major focus of the food industry has been the creation of new products catering to consumers with certain health needs, including products lower in fat, cholesterol, or sugar content, as well as lower-calorie foods. Even though consumers are advised to reduce their dietary fat consumption, they are not willing to compromise for taste and functionality of the foods (Verbeke, 2006). Strategies for changing the nutritional profile of cheese include reducing fat (reduced fat or low-fat cheese), replacing fat with proteins or carbohydrates (fat replacers), and enriching cheese with nutrients.

Among the products used as fat substitutes is inulin, which is a carbohydrate polymer of fructose units (2–60 fructose units) built up from  $\beta(2,1)$ -linked fructosyl residues mostly ending with a glucose residue (Modzelewska-KapituŁa and KŁębukowska, 2009). Inulin has a variety of uses, including as a replacement for fat and sugar (Kocer *et al.*, 2007; Rodríguez-García *et al.*, 2014), a low-calorie bulking agent, and a texturizing agent. It is also used for its physiological features as a soluble dietary fiber and for its prebiotic properties (Tungland and Meyer,

2002). The average daily consumption of inulin and oligofructose has been estimated to be 1-4 g in some countries. It is generally recognized as safe (GRAS status) or as 'natural food ingredient'. There is no official Recommended Daily Allowance (RDA) for inulin intake, although the recommended range of dietary fiber intake is 25-38 g/d (Giri et al., 2017). Different studies have included inulin in cheese formulation. Koca and Metin (2004) replaced fat in fresh kashar cheeses by 5% inulin, while Salvatore et al. (2014) used 2, 3 and 7% of inulin in caprine milk fresh cheese. Although some studies have reported the use of inulin in sheep milk derivatives (Balthazar et al., 2009, 2016, 2017), to the best of our knowledge, this is the first report about the use of inulin in any fresh sheep milk cheese. Therefore, the goal of the present study was to evaluate the texture as well as the sensory characteristics of reduced-fat Frescal sheep milk cheese using inulin as a fat substitute.

### **Materials and methods**

#### **Materials**

Pasteurized whole (6.0% fat) and semi-skimmed (1.5% fat) sheep milk (Chapecó, Santa Catarina, Brazil), calcium chloride (Vetec, Rio de Janeiro, Brazil), RSF-736 culture (mesophilic/thermophilic, Christian Hansen, Valinhos, Brazil), commercial rennet with chymosin produced by *Aspergillus niger* var. awamori (strength of 1 : 3000, Estrella, Christian Hansen, Valinhos, Brazil), and inulin with degree of polymerization  $\geq 10$  (Beneo, Orafti<sup>®</sup> ST-Gel, Belgium) were used.

#### Cheese making

Three formulations of Frescal sheep milk cheeses were produced: semi-skimmed cheese with 5 g/100 g inulin (SSCI), semiskimmed cheese (SSC), and whole milk cheese (WMC). Frozen sheep milk was thawed, defatted in milk defatting centrifugal creamer (SSCI and SSC) (Casa da Desnatadeira, model 360, Goiânia, Brazil), and pasteurized at  $65 \pm 3$  °C for 30 min by direct heating. Sheep milk was cooled to  $37 \pm 1$  °C, and commercial rennet (0.9 ml/l of milk), calcium chloride (0.5 ml/l of a 50 g/100 ml calcium chloride solution), mesophilic/thermophilic culture (1 ml/l of a 0.28 g/100 ml starter culture), and inulin (only in SSCI, 50 g/l of milk) were added while gently stirring. The formulations were incubated at  $37 \pm 1$  °C for 30 min. The resulting gel was cut into cubes (2 cm<sup>3</sup>), stirred, allowed to drain, and placed in cylindrical perforated containers, each with a 500-g capacity. Every 15 min, for a total of 1 h, formulations were turned and then dry salted (sodium chloride, 1.5 g/100 g of cheese). Then, the formulations were placed into plastic Cryovac® bags (BN 200, Sao Paulo, Brazil). Finally, the cheeses were cold stored  $(8 \pm 1 \text{ °C})$  for 21 d prior to analysis.

### Chemical analysis

The total solids (TS) content was determined by drying the samples at 105 °C until a constant weight was obtained. The fat content of cheese samples was determined by the Gerber method, and the protein content was determined by measuring total nitrogen using the Kjeldahl method (AOAC, 2011) and converting it into the protein content by multiplying by 6.38. Ash was determined gravimetrically by heating the samples at 550 °C until fully ashed. Carbohydrates were calculated by difference. Caloric

values were calculated using the formula: Calories (kcal/g) =  $(C \times 4) + (P \times 4) + (F \times 9)$ , in which: C = carbohydrate content (g/100 g), P = protein content (g/100 g), F = fat content (g/100 g). The titratable acidity was determined by the Dornic method. The pH of cheese samples was measured with a digital pH meter (Tecnal, Piracicaba, Brazil). All chemical measurements were done in triplicate. Cheese samples were chemically analyzed on day 1. The titratable acidity and pH were evaluated on days 1, 7, 14, and 21 of cold storage (8 ± 1 °C).

# Instrumental texture profile analysis

The textural properties of the cheeses were evaluated with a CT3 Texture Analyzer (Middleboro, MA, USA) using two-bite compression of cylindrical samples of 5 cm diameter and 2 cm height. The acrylic cylindrical probe (35 mm in diameter) was employed, with a distance of 10 mm and a velocity of compression of 1 mm/s (Buriti *et al.*, 2008). The parameters measured were firmness, adhesiveness, cohesiveness, elasticity, and gumminess, obtained by using the Texture Expert for Windows software version 1.20 (Stable Micro Systems, Godalming, United Kingdom). Samples were kept in their packages at 8 °C until analysis. Five measurements were carried out for each of the three replicates at days 1 and 21 of storage.

#### Sensory analysis

The sensory analysis was carried out in two different sessions. The first session was conducted at 7 d after sheep milk cheese production and the second session at 21 d after cheese production. The products were analyzed for the presence of the thermotolerant coliforms, coagulase positive staphylococci, salmonella and *Listeria monocytogenes*, as required by legislation (Brasil, 2001) and were in accordance with this regulation.

The first session included 99 untrained panelists recruited from the staff and students of the Unopar University, and the second session involved 95 panelists. Sensory sessions were performed in individual testing booths under white light. Portions of approximately 10 g of the three sheep milk cheese formulations were served in disposable transparent polyethylene plates coded with randomized 3-digit numbers in a random order. The test was carried out under controlled conditions, with mineral water and cream crackers available to the panelists. The panelists were asked about the consumption of sheep milk and cheese, and they rated appearance, flavor, texture, and overall acceptance. Each panelist used a nine-point hedonic scale (1 = extremely disliked, 5 = neither liked nor disliked, 9 = extremely liked). The acceptability index (AI) was calculated using the ratio of the average score obtained for the product and the maximum score given to the product, multiplied by 100, and expressed as a percentage. The Ethics Committee of the University North of Paraná approved the study (Register 1.928.180/2016), and consent forms indicating voluntary and fully informed participation were signed by all volunteers.

#### Statistical analysis

Cheeses were produced in three different batches, and each measurement was repeated in triplicate. Data were expressed as a mean. The data analysis was carried out using Statistica 13.0 software (StatSoft Inc., Tulsa, OK, USA). The normality of data was tested by Lilliefors test. When this assumption was not verified, non-parametric tests were applied to determine significant differences (P < 0.05). Differences of chemical composition and texture parameters in the three formulations were evaluated by Kruskal– Wallis test. Differences in day 1 and 21 of cold storage in texture parameters were evaluated by Wilcoxon test. A principal component analysis (PCA) was conducted using textural parameters to reduce the large set of variables to a small set as well as to determine how the cheeses were differentiated. Data from sensory analysis were evaluated by parametric tests (P < 0.05). Differences in sensory parameters among the three formulations were evaluated by analysis of variance and Tukey's test. Differences in sensory parameters between 7 and 21 d of cold storage were evaluated by Student's *t* test.

# **Results and discussion**

When fats are reduced in food formulations, other ingredients are often required to fulfill their functional role in maintaining sensorial qualities. The use of fat substitutes is one strategy proposed for improving the flavor and texture of low-fat cheese. The chemical compositions of the three formulations of Frescal sheep milk cheese are presented in Table 1. The variations in chemical composition observed for the formulations are directly related to the differences in milk fat content. The total solids in the Frescal sheep milk cheese ranged between 39.17 g/100 g (SSCI) to 41.36 g/100 g (WMC), with the WMC formulation containing significantly more total solids (P < 0.05). In addition, WMC contained significantly more fat in the total solids (50.70 g/100 g) and ash (1.90 g/100 g) than the formulations prepared with semiskimmed milk (P < 0.05). Higher ash values in fat cheese can be attributed to the presence of some minerals in the membrane of the fat globule (Fox and McSweeney, 1998). The composition of SSCI was similar to that of SSC. The Joint FAO/WHO Expert Committee indicates a maximum limit of 50% reduction in fat from a referenced variety for a cheese to be labeled as reduced-fat (FDA, 2008). Indeed, the SSCI and SSC presented a fat reduction by 36% fat in relation to the WMC, thus adapting to this indication.

According to Broadbent *et al.* (2001), one of the most important strategies for improving the properties of lower fat cheese is to increase its moisture content sufficiently to provide a ratio of moisture to protein in the lower fat cheese equal to or higher than its full fat counterpart. Indeed, the SSC and SSCI met this guideline and were moister than the WMC (P < 0.05). In addition, inulin has the capacity of holding water, with expected higher

Table 1. Chemical composition of Frescal sheep milk semi-skimmed cheese with 5 g/100 g inulin (SSCI), semi-skimmed cheese (SSC), and whole milk cheese (WMC)

	SSCI	SSC	WMC
Total solids (g/100 g)	39.17 <sup>b</sup>	39.41 <sup>b</sup>	41.36 <sup>a</sup>
Fat in total solids (g/100 g)	33.93 <sup>b</sup>	33.22 <sup>b</sup>	50.70 <sup>a</sup>
Protein (g/100 g)	19.26	18.35	19.14
Ash (g/100 g)	1.64 <sup>b</sup>	1.63 <sup>b</sup>	1.90 <sup>a</sup>
Carbohydrates	4.98	6.34	0
Caloric value (kcal/100 g)	216.5 <sup>b</sup>	216.6 <sup>b</sup>	265.5 <sup>a</sup>

 $^{a,b}$ Different superscript letters in the same row represent significant differences by Kruskal– Wallis test (P < 0.05). moisture content in cheeses with medium moisture, like mozzarella (Mcmahon *et al.*, 1996). However, in the present work this feature was not observed, which was also the case in the work of Fadaei *et al.* (2012), who developed a reduced fat cheese with inulin as a fat replacer. Monteiro *et al.* (2013) produced a Frescal sheep milk cheese with a similar moisture content. The proximal composition was 59.7 g/100 g moisture, 10.3 g/100 g protein, 16.7 g/100 g fat, and 10.8 g/100 g carbohydrates. Most of the literature consulted refers to ripened sheep cheese, which differs greatly form Frescal sheep milk cheese composition.

Regarding the caloric value, the WMC contained more calories (265.5 kcal/100 g) than the SSC (216.5 kcal/100 g) and SSCI (216.6 kcal/100 g) (P < 0.05). According to Brazilian (Brasil, 1998) and American legislation (FDA, 2013), reduced-fat products require at least a 25% reduction in the fat level or calories or at least a 3 g fat per 100 g (solid food) or 40 kcal/100 g (solid food) reduction from the traditional level of the referenced variety. In the present work, cheese made from semi-skimmed milk (SSCI and SSC) demonstrated reduction in the fat level, representing an 8 g/100 g fat reduction and a 44 kcal/100 g calorie reduction. Thus, the claim 'reduced fat' or 'reduced calories' can be used for the formulations SSCI and SSC.

Along the storage, all formulations showed a decrease in pH (P < 0.05) and, inversely, an expected increase in titratable acidity (P < 0.05) (Fig. 1). A significant difference was observed between the pH of WMC and SSC at day 1 (P < 0.05). The same pattern was not observed for titratable acidity (P > 0.05). When titratable acidity at the final of cold storage was evaluated, SSC presented higher levels of lactic acid than SSCI and WMC (P < 0.05). Balthazar et al. (2016) observed that inulin contributed to a lower lactic acid content in sheep milk yogurt, similar to the observed in the present study. At day 21, pH presented the same value for the three formulations, probably due a blocking effect of caseins (Whittier, 1929). A reduction in pH values along storage, common in cheeses and fermented dairy products, is naturally observed due to the continuous production of lactic acid and other organic acids from lactose fermentation by the starter cultures and may cause sensory and technological changes in the product (Cardarelli et al., 2008). Cheese pH is of great importance to its final quality, as it influences the texture, appearance, and flavor of the product.

The effects of inulin on the sheep milk cheese texture profile are presented in Table 2. The firmness of sheep milk cheese



Fig. 1. pH and titratable acidity (% lactic acid) of Frescal sheep milk semi-skimmed cheese with 5 g/100 g inulin (SSCI), semi-skimmed cheese (SSC), and whole milk cheese (WMC) during cold storage.

**Table 2.** Texture profile analysis (TPA) of Frescal sheep milk semi-skimmed cheese with 5 g/100 g inulin (SSCI), semi-skimmed cheese (SSC), and whole milk cheese (WMC) during cold storage

Texture parameter	Days of storage	SSCI	SSC	WMC
Firmness (N)	1	8.17 <sup>a,b</sup>	11.99 <sup>a</sup>	5.38 <sup>b</sup>
	21	6.06 <sup>a,b</sup>	11.47 <sup>a</sup>	4.54 <sup>b</sup>
Adhesiveness (mJ)	1	0.59 <sup>B</sup>	0.38	0.37 <sup>B</sup>
	21	2.02 <sup>a,A</sup>	0.75 <sup>b</sup>	0.90 <sup>a,b,A</sup>
Cohesiveness	1	0.55 <sup>b</sup>	0.75 <sup>a</sup>	0.62 <sup>a,b</sup>
	21	0.55	0.68	0.67
Elasticity (mm)	1	5.77	6.16	6.31
	21	6.72	6.15	6.09
Gumminess (N)	1	3.93 <sup>b</sup>	8.93 <sup>a</sup>	3.28 <sup>b</sup>
	21	3.55	8.06	3.07

<sup>a,b</sup>Different superscript lowercase letters in the same row represent significant differences in cheese formulations by Kruskal-Wallis test (P < 0.05).

<sup>A,B</sup>Different superscript capital letters in the same column represent significant differences in texture parameter along storage by Wilcoxon test (P < 0.05).

with inulin was intermediate between that of whole and semiskimmed cheese. This pattern was observed on the day of production and after 21 d of cold storage. During storage, firmness of each formulation was kept at the same levels (P > 0.05). Koca and Metin (2004) observed a softening effect following the addition of 5 g/100 g inulin to low-fat cheese. This softening effect could be attributed to both the higher ratio of moisture to protein and the increase in filler volume, which decreases the amount of protein matrix. The adhesiveness of SSCI was higher than that of SSC after 21 d of cold storage (P < 0.05). Increasing adhesiveness was observed during storage of SSCI, and the same was observed for WMC (P < 0.05). Adhesiveness is also a parameter influenced by fat present in food. Higher the fat level, higher the adhesiveness (Kealy, 2006). The cohesiveness of SSCI was lower than that of SSC on the day of production (P < 0.05). Protein is fundamental to gelation and to cohesiveness observed in cheeses. When lower levels of fat and moisture are observed, a higher content of casein is expected, favoring protein-protein interaction and promoting a stiffening of the protein matrix, letting cheese more cohesive. Finally, the gumminess of SSCI resembled that of WMC, with both measuring lower than SSC (P < 0.05). Overall, the presence of inulin in cheese made with semiskimmed milk provided the same texture properties as full fat cheese. To consumers, this is important, because fat plays a crucial role in cheese texture. Inulin molecules and fat globules have similar properties, and are dispersed within the casein micelles. This behavior make an interference in the protein matrix formation. As a result, there is the formation of a softer gel (Paseephol et al., 2008), observed by the similar firmness between SSCI and WMC. Skimmed products, in turn, have a compact protein matrix with fewer spaces, and are therefore firmer (Brennan and Tudorica, 2008). Inulin has the capacity to form microcrystals, which interact with each other and absorb a large amount of water, producing a fine texture that imitate milk fat properties.

The principal component analysis (PCA) indicated that two factors explained 99% of the textural variation among the cheeses (Fig. 2). It appears that there were no marked differences in the textural properties between SSCI and WMC. However,



Fig. 2. Principal component analysis of the textural parameters of Frescal sheep milk semi-skimmed cheese with 5 g/100 g inulin (SSCI), semi-skimmed cheese (SSC), and whole milk cheese (WMC) during cold storage.

while no significant differences were observed in consumer preferences, the SSC showed different textural parameters when compared with cheeses made with whole sheep milk and/or with semi skimmed sheep milk with inulin. Inulin has been identified as a suitable fat substitute in low-fat cheeses, especially because it contributes to a better mouthfeel (Meyer *et al.*, 2011). The fat-substituting property of inulin is based on the stabilization of the aqueous phase structure, which improves the creaminess.

Among the participants who completed the sensorial analysis, 32.9% were younger than 21 years of age, 47.6% were between 21 and 30 years of age, and 19.5% were more than 31 years of age. The majority of respondents were female (73.3%). Participants were asked if they had previously consumed sheep milk, and most indicated that they had not (88.2%). The same pattern was observed when they were asked about the consumption of sheep milk cheese (89.3%). Surprisingly, when they were asked if they had consumed Roquefort cheese, the total of negative responses differed from that obtained for sheep milk cheese (70%). This response suggests that many people consume Roquefort cheese and are not aware that this cheese is made from sheep milk. Finally, when they were asked about the consumption of Minas Frescal cheese, most of the respondents had already consumed this product (86.6%). Based on this information, we observed that panelists are accustomed to consuming Frescal cheese but are not used to consuming sheep dairy products.

The results from the sensory analyses are presented in Table 3. No difference was observed for the parameters evaluated for the three cheese formulations after 7 d of storage (P > 0.05). This indicates that the addition of inulin did not contribute to the increase of acceptance of semi-skimmed formulation. Nonetheless, a better appearance was reported for SSCI when compared with SSC at 21 d of storage (P < 0.05). The acceptability index (AI) for the overall impression of the cheeses at 7 or 21 d of storage was above 85% for the three formulations evaluated, considered highly acceptable. These results demonstrate that the addition of inulin did not contribute to the increase of acceptance of semi-skimmed formulation. Notwithstanding that a few differences were observed in cheese composition (Table 1) and texture parameters (Table 2), consumers were not able to differentiate the formulations.

**Table 3.** Sensory analysis of Frescal sheep milk semi-skimmed cheese with 5 g/ 100 g inulin (SSCI), semi-skimmed cheese (SSC), and whole milk cheese (WMC) at 7 and 21 d of cold storage

Cold storage	Parameter	SSCI	SSC	WMC
7 d	Appearance	$8.1 \pm 1.1$	$8.1^{A} \pm 1.1$	$7.7 \pm 1.4$
	Flavor	$7.6 \pm 1.6$	$7.7 \pm 1.3$	$7.8 \pm 1.5$
	Texture	$7.9 \pm 1.4$	$7.9 \pm 1.1$	$7.8 \pm 1.5$
	Overall acceptance	7.8±1.5	7.9±1.3	$7.8 \pm 1.5$
21 d	Appearance	$8.1^{a} \pm 1.1$	$7.6^{b,B} \pm 1.4$	$7.8^{a,b} \pm 1.4$
	Flavor	$7.8 \pm 1.2$	7.7 ± 1.3	$8.0 \pm 1.1$
	Texture	$7.9 \pm 1.2$	7.7 ± 1.3	$7.8 \pm 1.2$
	Overall acceptance	8.0±1.0	7.8±1.2	7.9±1.1

Data are presented as mean ± standard deviation.

 $^{\rm a,b}$  Different superscript lowercase letters in the same row represent significant differences by Tukey's test (P < 0.05).

 $^{A,B}$ Different superscript capital letters in the same column represent significant differences by Student's t test (P < 0.05).

Inulin addition in low concentrations does not strongly affect product sensory quality, because inulin has a neutral or slightly sweet taste (Nair *et al.*, 2010). No statistical difference was observed for ratings of sheep cheese formulations based on age groups, gender, or whether the respondent has already consumed sheep milk (P > 0.05). Inulin could be used without compromising the general acceptance of the product. In addition, inulin added to the product may contribute to nutritional positive effects.

In conclusion, the use of inulin in semi-skimmed sheep milk cheese yielded a product with similar textural properties to whole milk sheep cheese. The use of semi-skimmed milk in cheese manufacturing provides a less caloric product, meeting requirements for 'reduced fat' or 'reduced calorie' products.

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