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RESEARCH ARTICLE

Production of Serra da Estrela cheese from ultrafiltered sheep's milk

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ABSTRACT

The objective of this study is to elaborate Serra da Estrela cheese with sheep's milk concentrate by ultrafiltration and characterize it in relation to protein, yield, proteolysis, amino acid profile, and acceptability. The cheese elaborated with concentrate milk (F2) presented an increase of 17% yield, and protein content was 2 times higher than cheese without concentrate milk (F1). On the 30th day of storage F2 presented an increase of the amino acids and also of proteolysis extension and depth index in relation to the 1st day. Also presented better sensorial characteristic in relation to F1. Were observed positive correlations (>0.85) between acceptability in relation to protein, depth index, extensionand extension index, valine, methionine, serine, phenylalanine aspartic acid, valine andproline. Cheese elaborated with concentrate milk and coagulated with thistle flower is one alternative to increase cheese yield with differential sensorial characteristics.

Keywords: Cheese; *C. cardunculus L.* thistle; protein concentrate; sheep milk; ultrafiltration.

INTRODUCTION

In Portugal one famous cheese artisanal is Serra da Estrela, and is appreciated worldwide. This cheese is elaborated with raw ewe's milk, the coagulation is made with thistle flower, consumed, with an average maturation of 1 month (Carocho et al., 2016). In this sense, this type of cheese need attention during manufacture and shelf-life storage, because its nutrient contents and adequate conditions of pH and water activity for the growth of microorganisms (Amira et al., 2017). Therefore, the technology of membrane mainly by using ultrafiltration (UF) can benefit this product (Ng et al., 2019).

UF is a membrane process that use mainly pressure to a separation of compousds. In the membrane are retained suspended solids and compounds of high molecular weight, on other side low molecular weight solutes and pass through the membrane. When UF process is used in milk are removed water, lactose, and minerals, and consequently, the proteins are concentrated in the retentate (Farkye and Rehman, 2011; Phil, 2019).

In cheese processing, pre-concentration of milk by UF maximizes the equipment capacity, reduces the amount of rennet and salt needed, resulting in consistent gel structure, with increased in the casein retention and yields (Mistry, 2017; Phil, 2019). In addition, during cheese ripening the proteolysis promotes protein degradation by proteolytic enzymes, especially by the casein, resulting in amino acids and polipeptides that improve to the cheese flavor and taste (Diezhandino et al., 2015, Ribeiro et al., 2018; Chen et al., 2019; Pinho and Minhalma, 2019; Kulozik, 2019).

The UF promotes different characteristics in the cheese due to the milk concentration. This work was to evaluate the influence of concentrated sheep milk by UF on the Serra da Estrela cheese, coagulated with *C. cardunculus* L. thistle flower, evaluating during 30 days the sensorial characteristics, lactic acid bacteria count, proteolysis and physic-chemical characteristics.

MATERIAL AND METHODS

Material

Whole raw sheep milk obtained from a herd of Lacaune sheep located in Chapecó, Brazil containing approximately 13% non-fat solids and 5% (v/v) fat. To cheese manufacture

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the fat content was standard to 0.5% (w/w) using centrifuge (Suck Milk) and pasteurized at 72 to 75°C for 15 s) using pasteurizer plates (BCISMINI, EQUILATI, São Paulo, Brazil), and after was cooled to 4ºC.

The dry flowers (*Cynara cardunculus* L.) were obtained from Serra da Estrela region Viseu- Portugal, located at 40°42'17.5"N, 7°54'45.8"W.

Elaboration of cheese

Serra da Estrela cheese was elaborated using the method of Almeida et al. (2010), with adaptations. In this study, two formulations were investigated: F1 - Sheep milk without concentration and F2 - Concentrated by UF (2 bar and 22°C), in pilot scale, using a module with hollow fiber membrane of polysulfone amide (GE– Osmonics, Sepa MW), molecular weight cut-off (MWCO) mass of 10 kDa, filtration area of 4.4 m², feed pump with flow 1.7 to 5.2 $m^3 h^{-1}$, pressure of 0.5 at 3 bar and power of 3.0 KW (Fig. 1). In a stainless tank (Equilati, São Paulo, Brazil) were added 30 L milk (F1 and F2, 50 mL mesophilic dairy culture *Lactobacilluslactis* ssp. *cremoris* and *L. lactis* ssp. *lactis* (lyophilized culture used was type DVS -direct vat set for direct inoculation of milk in the concentration of 30 mg L^{-1} in order to obtain log 10^8 CFU L^{-1}), 12 mL calcium chloride solution (40%) (Chr Hansen@), and 27 mL vegetable rennet extract. The vegetable extract was obtained with 3.5 g dry flowers from the *C. cardunculus L.* thistle ground and, soaked with 25 g sodium chloride and 1 L sheep milk or concentrated milk for 24 h, and centrifuged (3,000 × g). Coagulation took place at 31°C after 25 min. The cut of the mass was performed in cubes of approximately 0.5 cm with lire and removed around of 75% whey and added 1.2% (w/w) sodium chloride (Swan).

The mass was placed in cylindrical shapes of 400 g and subject to 14 N m^{-2} for 90 min and 20 N m^{-2} for 90 min with inversions the positions. The cheeses were heat shrink in plastic packaging (Supravac VC2 – 55 microns coextruded structures of polyamides - nylon and ethylene vinyl acetate - EVA) with vacuum of - 690 mmHg (Selovac - vacuum systems 200 B) and stored at 6°C for 30 days.

Characterization of milk, concentrate and cheeses

The milk and concentrate milk obtained by UF were characterized in terms of lactose, protein, fat, pH, acidity, total minerals (ash), using methodology of Faion et al. (2019) and AOAC (2005).

Physicochemical analyses of the cheese were performed on the 1st and 30th days of storage. The cheeses were randomly chosen (n=3), grated (Wallita), and homogenized. A quarter was retained for analytical determinations. The cheese were characterized in terms of yield, fat, total nitrogen (TN), non-protein nitrogen, soluble nitrogen (SN), extent of the proteolysis index (EPI), depth of the proteolysis index (DPI), free amino acid profile, and lactic acid bacteria count are described and detailed in previous work of the group (Faion et al., 2019)

Sensory evaluation

For the sensory were used portions of approximately 2 cm² of cheese ripening for 30 days. The samples were distributed in balanced form, in plastic dishes coded with 3 digits random numbers to 50 tasters non-trained. The acceptability, texture, global acceptance and flavor attributes, were evaluated using hedonic scale structured of 9 points where: 1 as the lowest and 9 as the highest values (Faria and Yotsuyanagi, 2002). Before of the sensory evaluations, the research was approved by the Research Ethics Committee of the Regional Integrated University of Upper Uruguai and Missions according to Brazilian National Health Council ethical and scientific requirements (CAAE: 03496312.1.0000.521).

Statistical analysis

Results (n=3) were treated by analysis of variance (ANOVA) followed by comparing the means by t student test, using the *Statistic* 7.0 software with 95% significance level., the Pearson correlation and principal component analysis (PCA) were analyzed using XLSTAT (2019 free) software.

RESULTS AND DISCUSSION

Sheep´s milk and UF fractions

Table 1 present the results of physic-chemical analysis for milk and concentrate samples obtained by UF. The pH of milk differs ($p<0.05$) of the concentrate indicating a slight acidification after UF process. In the UF due milk remains for 30 min in the system, there is greater retention of phosphate, casein and other acidic components of

Fig 1. Pilot scale membrane filtration unit: module with hollow fiber membrane of polysulfone amide (a), concentrate tank (b), heating tank (c), concentrate collection valve (d), and feed pump (e)

the dry matter. The time and temperature promote the growth of lactic acid bacteria that increase the acidity of the concentrate, this development was also observed by Iltchenco et al. (2018) and Faion et al. (2019).

The concentrate milk presents an increase of about 4 times in fat and in protein, and 1.2 times in minerals than milk. In membrane technology, this behavior is expected because the high molecular weight of proteins and fats are retained, that promote a increasing of solids in the concentrate milk (Leite et al., 2006).

The lactose content presented a significant ($p<0.05$) reduction in the concentrate in relation to milk (Table 1). This behavior indicates that the UF process is permeable to lactose (Phil, 2019). The concentrate milk has high protein content (18%) and may provide high yields and nutritional functionality, and promotes improvements in sensory characteristics to the develop products such as cheese.

Characteristics of cheese

It was observed that the F2 cheese formulation presented an increase of 17% in yield when compared to the F1, due to high solids retained in the concentrate milk, as fat and protein, that promotes increase in the yield.

Both formulations (F1 and F2) on the 1st and 30th days of storage presented 56 and 58% and 45 and 52% of moisture, being considered with very high and high moisture cheeses respectively (Brazil, 1996). The moisture values may be related to the size of the curd grains, and because the concentrate milk, which promotes an increase in moisture content due to increased absorption of water by whey proteins. The cheese elaborated with concentrated milk (F2) showed a fat content of 29%, being considered as semi-fat cheese (25.0 to 44.9%) according to Brazil (1996).

The protein content of F2 was approximately 2 times higher than F1, because the concentration milk presented 18% of protein and consequently in the cheese (Table 2).

In other studies with sheep´s milk, Pellegrini et al. (2012) elaborated Pecorino Toscano with 25% protein and 34% moisture on the 1st day of storage, which this value is higher than F1 (Table 2) observed in the present work, because the difference of milk composition characteristics.

The incorporation whey proteins in the concentrate depend on the membrane system and of the milk characteristics. The amount of whey proteins retained depends on the milk and UF operation conditions. Cheeses elaborated with UF milk are considered more soft due to water-binding capacity and high casein, so are less susceptible to dehydration during the ripening when compared with than traditional cheeses (Mistry and Maubois, 2017).

Table 1: Results of pH, acidity, lactose, fat, protein and minerals for the milk and concentrate

Samples	pH	Acidity (%, w/v)	Lactose (%, w/v)	Fat (%, w/v)	Protein (%, (%, w/v)	Minerals (%, w/v)
Milk	6.49^{a} (±0.21)	0.19^{b} (±0.08)	3.80^{a} (±0.21)	0.5^{b} (±0.21)	4.42^{b} (±0.22)	0.95^{a} (±0.32)
Concentrate	6.33^{b} (±0.22)	0.25^{a} (±0.04)	1.12^{b} (±0.33)	2.23^{a} (±0.23)	17.86^{a} (±0.47)	1.15^{a} (±0.23)

*Mean ± standard deviation (n=3) followed by the same lowercase letters in columns do not differ statistically at 5 % level (t student test)

Table 2: Results of protein, depth index (DPI) and extent of proteolysis (EPI), and amino acids for cheeses elaborated with milk and concentrate during the storage (1st and 30th days)

Analysis	Milk (F1)		Concentrate (F2)	
	1st day	30th day	1st day	30th day
Protein (g.100 g^{-1})	18.87^{d} (±0.11)	19.67^{c} (±0.12)	36.02^{b} (±0.31)	37.71^{a} (±0.23)
Depth index - DPI (g SN 100 g^{-1} TN^{-1})	0.71^{c} (±0.05)	6.4^{b} (±0.47)	0.72^{c} (±0.01)	8.9^{a} (±0.17)
Proteolysis Ext. - EPI (g NPN 100g^{-1} TN^{-1})	1.72^{c} (±0.02)	9.9^{b} (±0.19)	1.81^{c} (±0.05)	13.2^{a} (±0.14)
Amino acids (mg 100 g^{-1}) *- Arginine*	1.25^{b} (±0.05)	1.52^{a} (±0.06)	1.34^{b} (±0.03)	1.47^{a} (±0.04)
- Serine	1.41^{d} (±0.08)	3.02^{b} (±0.09)	1.85^{c} (±0.04)	4.33^{a} (±0.13)
- Aspartic acid	4.71^{a} (±0.10)	3.11^{c} (±0.12)	4.28^{b} (±0.08)	3.41^{c} (±0.11)
- Glutamic acid	2.05^{c} (±0.07)	2.87^{b} (±0.08)	1.56^{d} (±0.03)	3.11^{a} (±0.08)
- Threonine	1.03^{a} (±0.04)	0.89^{a} (±0.03)	0.99^{a} (±0.02)	0.79^{a} (±0.03)
- Alanine	3.65^{a} (±0.11)	-	2.85^{b} (±0.05)	-
- Proline	0.91^{c} (±0.03)	2.02^{b} (±0.06)	0.60^{d} (±0.02)	3.11^{a} (±0.07)
- Methionine	2.63^{c} (±0.07)	3.12^{b} (±0.08)	2.01^{d} (±0.02)	4.02^{a} (±0.15)
- Valine	2.01^{b} (±0.06)	3.13^{a} (±0.07)	1.44^{c} (±0.03)	3.17^{a} (±0.14)
- Leucine	-	-	-	-
- Phenylalanine	-	1.07^{b} (±0.02)	-	3.07^{a} (±0.06)
- Lysine	-	-	2.28 (±0.11)	-

*Mean ± standard deviation (n=3) followed by the same lowercase letters in the formulation/lines do not differ statistically at 5 % level (t Student test)

In this study, it was observed that the concentration of milk proteins did not inhibit the action of the proteolytic enzymes of rennet in F2 on the 30th day of storage. This behavior may also be associated with the moisture content of the F2 (53%).

The proteolysis extension index increased ($p<0.05$) during the storage (Table 2). The extent of proteolysis is related to the enzymatic action of milk and of the coagulant agent due to αs1-casein and β-casein that promote primary proteolysis resulting in peptides high molecular weight. During the ripening may factors could influence the proteolysis as milk characteristic, relative humidity of the environment and salt in cheese, temperature, pH and time (De Rensis et al., 2009).

The DPI is associated with proteolytic enzymes from lactic cultures, and of the milk, that hydrolyze peptides with high molecular weight in low molecular weight indicating secondary proteolysis (De Rensis et al., 2009). So, on the 30th day of storage the cheese F2 presenting an increase in proteolysis (13.2 g NPN 100 g^{-1} TN^{-1} of EPI and 8.9 g SN 100 $g^{-1}TN^{-1}$ of DPI) in relation to F1 (9.9 g NPN 100 g^{-1} TN^{-1} of EPI and 6.4 g SN 100 g^{-1} TN^{-1} of DPI). The high proteolysis can be due to the concentrated milk (F2) provide the development of lactic bacteria on the 30th days. Cunha et al. (2006) when elaborated Minas cheese with concentrate milk by UF obtained values of DPI of 6.5 to 8.5 g NPN 100 g^{-1} TN^{-1} on 30th day of storage.

The proteolysis during cheese ripening is one of the most important in the development of the sensory characteristics such as flavor and texture of the product, due to peptides and free amino acids (Soodam et al., 2014; Diezhandino et al., 2015; (Ardö et al., 2017).

For F2 on the 30th day of storage (Table 2) was observed an increase ($p<0.05$) of methionine, serine, glutamic acid, valine, proline and phenylalanine. This is due to the UF process and greater primary proteolysis index on the 30th day, that promote peptides increase (Ramos et al., 2014), due to the action of amine and/or exo-peptides of microbial origin.

F2 diffed ($p<0.05$) of F1 in the attributes general acceptance, texture and flavor (Table 3) with mean >8.2, corresponding on the hedonic scale to the very liked. This may be due to the high rate of proteolysis, proteolytic capacity of the enzymes and the concentration of the proteins, which promotes an increase in the production of amino acids, such as glutamic acid, methionine, proline, serine, valine, and others (Table 2), and contribute to the of cheese flavor. Some amino acids such as proline and serine provide sweetness in the cheese. Proline is one of the main amino acids present in β-casein, where there was an increase in the 30th day for F2. In relation to texture, factors as moisture, protein and proteolysis that may have influenced the high score for F2.

The moisture promotes the dilution and reduction of the bonds in the protein matrix favoring the reduction of the hardness in cheeses. There is a direct relation between proteolysis and creaminess, due to the disintegration of the casein chain in a more homogeneous structure promoting softness in the product (Fox, 2004).

The UF provided the concentration mainly of milk proteins and fats, and thus favoring proteolysis and possibly, also, the lipolysis of the cheese. These characteristics provided detectable sensorial increments (acceptance, taste, and texture) by the tasters. The texture of cheese was influenced by the decomposition of fat, the texture rearranges and secondary junctions between casein networks are created that have beneficial role on the texture perception of cheese and its mouthfeel. In addition, the free fatty acids profile and the secondary junctions between casein networks (Karami, 2017) promoted better sensorial characteristics of F2.

The results obtained in the present work can be better visualized by Pearson correlation analysis and Principal Component Analysis shown in Table 3 and Fig. 2. In PCA (Fig. 2), the variables are represented as vectors which characterize the F1 and F2 cheeses on the 30th day of ripening that are located close to them. The first (PC1) and second (PC2) dimension explained 92.7 % of the total variance, being the principal component 1 (PC1) accounted for 73.60 %, while the principal component 2 (PC2) by 18.47%.

The values obtained from Pearson correlation (Table 4) confirmed the relationship between the parameters observed on PCA, where high positive correlations were observed (>0.85) between acceptability in relation to protein, depth index, extension index, serine, proline,

Table 3: General acceptance, flavor and texture of Serra da Estrela cheese, at the 30th day of storage

Formulations**	Sensorial characteristics*			Acceptability (%)
	General acceptance	Flavor	Texture	
F1	7.30^b (±1.04)	7.17^b (±0.82)	7.73^b (±0.76)	81.1
F2	8.27^a (±1.03)	8.33^a (±1.03)	8.63^a (±1.07)	91.2

*Mean ± standard deviation (n=3) followed by the same lowercase letters in columns do not differ statistically at 5 % level (t student test)

Table 4: Pearson correlation matrix for the variables of acceptability (ACCEP.), flavor, texture, total protein (TP), depth index (DPI), proteolysis Extention (EPI) and amino acids (arginine, serine, acid aspartic, acid glutamic, threonine, alanine, proline, methionine, valine, leucine, phenylamine - Pheny and lysine)

Variable	ACCEP	Flavor	Texture	TP	DPI	EPI	Arginine	Serine	Aspartic	Threonine	Proline	Methionine	Valine	Pheny
ACCEP.	1.000													
Flavor	0.708*	1.000												
Texture	0.554*	0.120	1.000											
TP	0.911*	0.619*	0.504	1.000										
DPI	0.942*	0.743*	0.549	0.977*	1.000									
EPI	0.924*	0.668*	0.508	0.998*	0.989*	1.000								
Arginine	-0.263	0.337	-0.241	-0.502	-0.314	-0.443	1.000							
Serine	0.925*	0.710*	0.465	0.993*	0.990*	0.998*	-0.400	1.000						
Aspartic	0.851*	0.485	0.879*	0.845*	0.881*	0.853*	-0.328	0.829*	1.000					
Threonine	-0.721*	-0.197	-0.522	-0.891*	-0.785*	-0.859*	0.832*	-0.830*	-0.751*	1.000				
Proline	0.924*	0.681*	0.488	0.997*	0.989*	1.000*	-0.432	0.999*	0.842*	-0.851*	1.000			
Methionine	0.922*	0.75*	0.417	0.980*	0.988*	0.990*	-0.342	0.997*	0.800*	-0.788*	0.993*	1.000		
Valine	0.351	0.890*	-0.284	0.232	0.370	0.287	0.616	0.347	0.041	0.213	0.307	0.419	1.000	
Pheny	0.912*	0.633*	0.492	1.000	0.979*	0.998*	-0.490	0.995*	0.839*	-0.883*	0.998*	0.984*	0.251	1.000

*Significant correlation (p<0.05), r>0.55

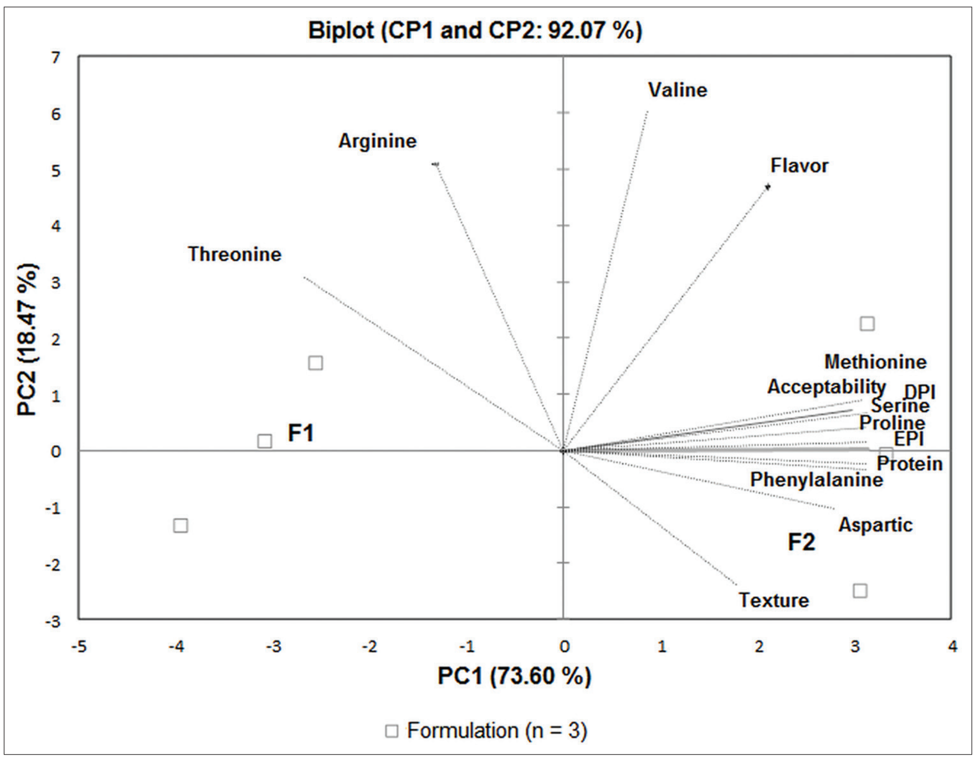

Fig 2. Principal component analysis (PCA) of protein, from 4 independent observations at 30th day of storage for F1 and F2 cheeses

aspartic acid, valine, methionine and phenylalanine. It was also observed a positive correlation of flavor with respect to protein, depth and extension index, serine, valine, methionine, proline, and phenylalanine.

It was observed a good discrimination among the formulations (Fig. 2). The F2 stood out in relation to sensory characteristics (texture, acceptability and flavor), protein and proteolysis (EPI, DPI) and amino acids (proline, aspartic, serine, methionine, and phenylalanine).

On the 1st day of storage the content proline (Table 2) was one of the lowest amino acid fount in the F1 and F2, but on the 30th day of storage was higher (p<0.05), demonstrating to be an indicative of proteolysis by β-casein during the storage. The manufacturing technology of cheese such as the addition of proteases, coagulation type, conditions and ripening period, and type of proteolysis can influence the concentration of some amino acids.

On the proteolysis, occur protein degradation to polypeptides and free amino acids, and small or medium size peptides. These substances are important to the flavor due they act as flavor precursors. The arginine is related to bitterness while proline and serine to sweetness (Fox, 2004; Diezhandino et al., 2015).

CONCLUSION

The Serra da Estrela cheese elaborated with UF sheep's milk presented an increase of 17% on yield, 2 times more protein content and better sensorial characteristics (acceptance, flavor and texture) in relation to the cheese elaborated with sheep´s milk without concentration. Then the elaboration of Serra da Estrela cheese with concentrate milk is one alternative to increase cheese yield with differential sensorial characteristics.

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Authors´ contributions

All the authors contributed equally to the writing of this paper. They were also involved in the overall work of experiments: Andréia Maria Faion and Ilizandra Aparecida Fernandes - Concentration of proteins by ultrafiltration, elaboration of cheese, characterization of milk, concentrate and cheeses and sensory evaluation. Anne Luize Lupatini Menegotto and Clarice Steffens - Assisted in data analysis and drafting the manuscript. Juliana Steffens and Eunice Valduga - Concentration of proteins by ultrafiltration, characterization of milk, concentrate and cheeses, assisted in data analysis and drafting the manuscript.

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