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Effect of Thistle Ecotype in the Physical-Chemical and Sensorial Properties of Serra da Estrela Cheese

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***Abstract*—**The objective of this study was to evaluate the physical and chemical characteristics of Serra da Estrela cheese and compare these results with those of the sensory analysis. For the study were taken six samples of Serra da Estrela cheese produced with 6 different ecotypes of thistle in a dairy situated in Penalva do Castelo. The chemical properties evaluated were moisture content, protein, fat, ash, chloride and pH; the physical properties studied were color and texture; and finally a sensory evaluation was undertaken. The results showed moisture varying in the range 40-48%, protein in the range 15-20%, fat between 41-45%, ash between 3.9-5.0% and chlorides varying from 1.2 to 3.0%. The pH varied from 4.8 to 5.4. The textural properties revealed that the crust hardness is relatively low (maximum 7.3 N), although greater than flesh firmness (maximum 1.7 N), and also that these cheeses are in fact soft paste type, with measurable stickiness and intense adhesiveness. The color analysis showed that the crust is relatively light (L* over 50), and with a predominant yellow coloration (b* around 20 or over) although with a slight greenish tone (a* negative). The results of the sensory analysis did not show great variability for most of the attributes measured, although some differences were found in attributes such as crust thickness, crust uniformity, and creamy flesh.

***Keywords*—**Chemical composition, color, sensorial analysis, Serra da Estrela cheese, texture.

I. Introduction

THE Serra da Estrela cheese is a cured cheese, with a semi-soft buttery paste, white or slightly yellow, handmade, obtained by slowly draining the curd after coagulation of raw sheep milk achieved by means of thistle flower (*Cynara cardunculus*). This product in Portugal has a Protected Designation of Origin (PDO) and it is a much appreciated product [1].

The characteristics of Serra da Estrela cheese, although varied, are defined by the Portuguese law (Dec. Regul. 42/85 of 05 July 1985). Properties such as moisture content, fat, protein, cheese form, crust characteristics and pulp consistency are typical and result from the type of milk used as well as from the specific manufacture and cure processes [2], [3].

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It has the shape of a low regular cylinder with slight bulging on the sides and on the upper side, and without sharp edges. It possesses a malleable consistency, allowing some fluctuations. It has a buttery texture, shear deformable, well connected and creamy, and the aroma is smooth and slightly acidic. The texture, flavor and aroma of cheese are associated with the chemical composition and pH, as well as with microstructure [4], [5].

The texture of foods, although with a degree of subjectivity, is the principal quality attribute of cheese, and during manufacture several factors may contribute to the final texture. The international standards organization defines texture of food products as "All rheological and structural attributes of the product perceptible by means of mechanical, tactile and, when appropriate, visual and auditory receptors" [6].

Color is very important as the first indicator of food quality, because it function as the most immediate characteristic evaluated by consumers. Color is the first sensation that the consumer perceives and uses as a tool to accept or reject food. In this way, it is pivotal for the acceptance of the product, even before tasting [7].

The profile of volatile compounds has been studied in Serra da Estrela cheese and it evidenced that volatile fatty acids were the major contributors to the overall aroma of this type of cheese, being influenced by the coagulant *Cynara cardunculus* and by the native microflora of raw milk [8], [9].

Sensory analysis in cheese permits assessing its quality, characterize it during product development besides being fundamental for testing its acceptance by the consumer [10].

The objective of this study was to evaluate some physical characteristics, such as texture and color, and also chemical characteristics, such as moisture, chlorides, ash, protein, fat and pH in Serra da Estrela cheeses produced with six different thistle ecotypes, as well as to make a sensory analysis aimed at comparing the different samples tested.

II. Experimental Procedure

A. Samples

The samples evaluated in this study were Serra da Estrela cheeses produced with six different ecotypes of thistle in a dairy situated in Penalva do Castelo, which is within the geographical area of the Protected Designation of Origin.

B. Chemical Analyses

For the chemical analyses the sample collection was made by cutting a shallow portion of approximately 0.3 cm in the crust to access the inner flesh. The samples were homogenized and for each analysis performed three replicates were made.

For the determination of moisture was used the method of weight loss after dehydration to constant weight in a Halogen Moisture Analyser (Mettler Toledo HG53), set at an operating temperature of 120 °C. The mass used each time was 3 grams.

The determination of the ash was performed by the method of Weende, which involves complete calcination by incineration in a muffle furnace at a temperature of 550 °C to obtain a white residue [11]. As it was very difficult to obtain a white residue in the first operation, the residue was treated with concentrated HNO_3 and calcinated again, this time originating the white residue. The sample mass for this analysis was 1 gram each time.

Protein was quantified according to the Kjeldahl method [11] consisting in mineralization of the sample with sulfuric acid, in the presence of a catalyst (a solution of saturated copper sulfate). To a Kjeldahl flask it was added 1 g of sample, 10 mL of concentrated sulfuric acid and 1 mL of saturated solution of copper sulfate, and this was then placed in mineralizer at 400°C for 6 hours. After cooling 50 mL of water was added and then it was placed the distillation apparatus. An Erlenmeyer flask 20 mL boric acid and a drop of mixed indicator were used to collect the distillate. The process proceeded with the addition of 50 mL of a solution NaOH 40%. Finally, the collected distillate (blue) was titrated with 0.1 N HCL until turning to pink. The crude protein content was determined by multiplying the total nitrogen content by the factor 6.38.

Fat was determined by the method of Gerber following the Portuguese Standard NP-2105 [12], by using a butyrometer where an acid hydrolysis is undertaken for protein digestion with sulfuric acid, followed by separation of milk fat by centrifugation and use of amyl alcohol.

In food analysis is very common to determine the chlorine, expressed as sodium chloride ion, by means of silver nitrate such as in the case of Mohr's method [11]. A sample of 1 gram was weighted into a porcelain crucible, which was then carbonized in a Bunsen burner and placed in an oven at 550 ºC to incinerate. After that, three drops of nitric acid 10% were added and also 30 mL of water, following stirring and filtration. Then the crucible and the filter were washed with 50 mL water recovering the filtrate and the washings in an Erlenmeyer flask. It followed the neutralization with sodium bicarbonate to pH 8.2, heating in a thermal water bath until no more release of carbon dioxide was observed. After cooling two drops of indicator potassium chromate 10% were added and the titration was made with a solution 0.1N of silver nitrate.

The pH determination was performed directly on the cheese without dilution. For this was used a potentiometer with a penetration electrode, previously calibrated with standard solutions pH=4 and pH=7.

C. Color Analysis

The color can be evaluated instrumentally using the CIELAB coordinate system defined by the *Commission Internationale de l'éclairage* - "CIE L*a* b* Colour Space". L* respects to brightness, ranging from 0 (black) to 100 (white); a* varies from -60 (green) to +60 (red) and b* varies from -60 (blue) to +60 (yellow) [13].The L*a*b* coordinates were measured in the rind of the cheese using a portable colorimeter Chroma Meter CR-400 from Konica Minolta. Three measurements were made on the upper face and another 3 on the lower face, resulting in 6 measurements performed on each cheese.

D. Texture Analysis

For the analysis of texture it was used a texturometer TA-XT2 from Stable Microsystems, with a 2 mm diameter probe. The test speed was 1 mm/s and the distance was 10 mm. Whole cheeses were used for the tests and five perforations were made in each sample, distributed on the upper surface of the cheese. The parameters analyzed were crust firmness (softness), flesh firmness, adhesiveness and Stickiness, as shown in Fig. 1.

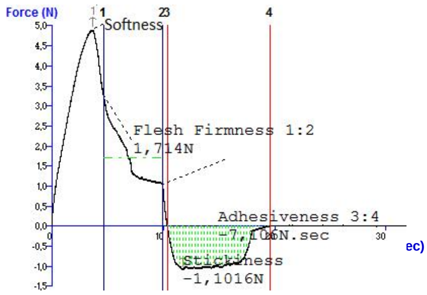

Fig. 1 Example of a texture analysis performed to a cheese

E. Sensorial Analysis

Sensory analysis of the cheeses was made with a panel composed by 9 trained testers aged between 25 and 56 years, in a test room with controlled temperature and relative humidity (21.2°C ± 1°C and 42% ± 1%, respectively). All samples were coded to unable identifying its origin.

The sensory evaluation of the samples was carried out in two stages. Initially, each sample of cheese was presented to each taster, in whole and in halves to allow evaluating the parameters related to aspect of the crust and pulp. Subsequently, a slice of the cheese was presented for tasting to evaluate the flavor and texture.

The register sheet has been adapted from that which is regulated by the Portuguese Standard NP 1922 for tasting Serra da Estrela cheese. All parameters were assessed on a scale from 1 - very little to 7 - very much, except for color where the value 1 corresponded to pure white and the value 7 to strong yellow. The parameters evaluated by the panelists were: thickness, uniformity and color of the crust; uniformity, color and creaminess of the paste; intensity of the aroma; flavor intensity, salt, acidity and bitterness; and lastly an overall assessment.

III. Results and Discussion

A. Chemical Properties

Table I shows the results of the chemical analyses performed to the different cheeses produced with different thistle ecotypes.

The moisture content varied from 40.15% to 48.25%, for ecotypes 2 and 1, respectively. Macedo et al. [5] evaluated the chemical composition of Serra da Estrela Cheese and reported moisture contents ranging from 45.60% to 52.99%, thus being slightly higher than those in the present work. The humidity of most cheeses is conditioned by the rate and duration of syneresis and compaction of the structure of casein. After coagulation, the following processes such as molding, pressing and salting, which differ according to the type of cheese, are accompanied by lowering the pH and cause a considerable loss of moisture by removing the whey [1].

Protein was higher for the cheese made with ecotype 4 (19.88%) and lower for ecotype 1 (14.69%), these values corresponding respectively to 3.12% and 2.30% of total nitrogen. The values found by [5] were considerably higher, varying from 4.20% to 6.33%.

This type of cheese is quite rich in fat, due to the milk used for its manufacture which is sheep milk, a particularly fat rich milk. The amount of fat varied considerably, being 35.00% for ecotype 4 and 45.00% for ecotypes 1 and 2. The values encountered by [5] were higher, varying in the range 51.40% - 58.36%.

The ashes varied from 3.88% to 4.95% whereas the salt content varied from 1.23% to 2.99%. The salt contents were found to be quite similar to those reported by [5], varying from 1.31% to 2.26% for the same type of cheese. Determination of salt is very important for its role as flavor intensifier and also by its bactericidal functions [1].

The pH is of decisive importance in the texture of cheeses due to its effect on the structure of the casein [14]. Furthermore, cheeses made from raw milk develop the characteristic flavor more rapidly than when made from pasteurized milk, due to the lactic acid bacteria present in raw milk. These bacteria are important not only for the acid development, and consequently lowering of the pH, thus hastening the milk coagulation and assisting in expulsion of whey, but also because of their influence on flavor, body and texture of the final cheese [15].

TABLE I
CHEMICAL PROPERTIES

Property	Eco1	Eco2	Eco3	Eco4	Eco5	Eco6
Moisture	48.25	40.15	44.39	47.77	42.96	44.46
(%)	(3.94)	(0.90)	(1.28)	(1.40)	(3.48)	(1.21)
Protein	14.69	16.48	19.35	19.88	17.46	16.95
(%)	(0.99)	(0.53)	(0.74)	(0.32)	(0.47)	(0.54)
Fat	45.00	45.00	40.00	35.00	40.50	44.33
(%)	(0.00)	(1.00)	(0.00)	(1.00)	(0.50)	(0.58)
Ash	4.41	4.39	4.26	4.13	3.88	4.95
(%)	(0.05)	(0.01)	(0.14)	(0.03)	(0.04)	(0.02)
Chlorides	2.99	2.01	1.88	1.34	1.23	2.15
(%)	(0.35)	(0.09)	(0.07)	(0.07)	(0.11)	(0.30)
pH	4.82	5.23	5.24	5.43	5.11	4.91

The results show the mean value plus the standard deviation (in parenthesis)

The values of pH for the cheeses at study stood in the range 4.82 – 5.43, with the minimum pH being registered for ecotype 1 and the maximum value for ecotype 4. Macedo et al. [5] determined values of pH varying from 4.62 to 6.37 for Serra da Estrela Cheeses.

Globally it was found that the cheese made with ecotype 1 presented the highest moisture, fat and salt and the lowest protein and pH. The sample ecotype 2 had the lowest moisture and highest fat as ecotype 1. Ecotype 3 showed for all chemical parameters evaluated values in the middle of the ranges encountered, not standing out for any characteristic. Sample with ecotype 4 had the lowest fat and highest protein and pH. Ecotype 5 showed the lowest ash and salt and finally ecotype 6 stood out for the highest ash content.

B. Texture

Table II resumes the textural properties evaluated for the cheeses at study, produced with thistle form the six different ecotypes. The cheese composition has a great influence on its texture. The texture, flavor and aroma of cheese depend on composition (moisture, protein, fat and mineral content), pH of the curd and ripening conditions, temperature as well as the microbial contamination throughout the mass and the surface of the cheese [1]. The texture of the cheese is initially conditioned by the pH and by the ratio of the intact casein and moisture. The lowering of the pH causes demineralization of the components of the micelles which, in turn, has a great influence on the links between the casein protein network [16]. In the present case it was observed that the cheese made with ecotype 2 had the highest crust hardness and also flesh firmness, followed by ecotype 6. Thus, the harder cheese corresponded to that with the highest pH. The textural properties related to the creaminess of the paste, adhesiveness and stickiness were more intense also in sample ecotype 2 followed by ecotype 6. This creaminess is related to the fat present and hence the highest creaminess was observed for the sample ecotype 2 with the highest value registered for fat content.

TABLE II
TEXTURAL PROPERTIES

Property	Eco1	Eco2	Eco3	Eco4	Eco5	Eco6
Crust hardness	2.75	7.30	2.92	2.83	1.56	4.71
(N)	(0.32)	(0.28)	(0.33)	(0.23)	(0.16)	(0.48)
Flesh firmness	0.69	1.66	0.81	0.81	0.46	1.24
(N)	(0.11)	(0.13)	(0.10)	(0.12)	(0.09)	(0.11)
Stickiness	-0.77	-1.86	-0.76	-0.64	-0.36	-1.34
(N)	(0.10)	(0.30)	(0.05)	(0.09)	(0.06)	(0.14)
Adhesiveness	-5.22	-14.83	-5.36	-3.50	-2.46	-9.69
(N.s)	(0.97)	(2.25)	(0.42)	(0.48)	(0.38)	(1.53)

The results show the mean value plus the standard deviation (in parenthesis)

In the first two weeks of maturation very marked changes are observed in texture due to hydrolysis of αS1-casein. Subsequently, changes in texture are slow and are mainly determined by the rate of proteolysis, by the ratio between salt content and humidity, and by temperature. The decrease of water activity during maturation, due to the release of ionic groups with the capacity to retain water and, in many cases due to evaporation of water, can also be important in the texture development [1]. Although proteolysis plays an important role in the softening of the structure of some cheeses, in other prevails the effect of lowering the water activity and/or water evaporation that causes a hardening of the cheese, by losing the plasticizing properties [15]. In the present case it was observed that sample ecotype 2 had the lowest moisture content and in fact was the hardest either in the crust or in the flesh.

The sample of cheese made with thistle from ecotype 5 showed the lowest value for crus hardness, flesh firmness, adhesiveness and stickiness (these two last in terms of absolute value).

C. Color

Table III shows the CIELab color coordinates for the rind of the cheeses evaluated.

TABLE III
COLOR COORDINATES

Coordinate	Eco1	Eco2	Eco3	Eco4	Eco5	Eco6
L*	59.07	61.36	59.02	56.61	58.37	57.00
	(1.20)	(0.55)	(1.03)	(1.65)	(0.53)	(0.63)
a*	-2.55	-2.37	-2.65	-2.06	-2.16	-3.45
	(0.33)	(0.27)	(0.16)	(0.15)	(0.34)	(0.18)
b*	22.17	21.32	21.11	19.55	19.59	22.04
	(1.07)	(0.37)	(1.30)	(1.22)	(1.85)	(0.67)

The results show the mean value plus the standard deviation (in parenthesis)

The cheese made with the thistle ecotype 2 stood out as the clearer, with highest L* (61.36) whereas the darkest was ecotype 4 (L*=56.61). Regarding greenness (a*) the values are very close to zero, thus indicating just a slight presence of the greenish color. Still, ecotype 6 showed the more intense green coloration among all samples. As to yellowness (b*), the values are positive and high, corresponding to an intense yellow coloration. The cheese chromatic parameters can be influenced by the fat composition and, for example, cheeses with lower fat content result in less yellow cheeses than those with higher fat content [1]. In the present case it was observed that ecotype 4 had the lowest value of b*, and this sample was also that with the lowest fat content.

D. Sensorial Profile

Fig. 2 shows the sensory profiles obtained from the sensorial analysis made to the different samples at study, by a trained panel composed by 9 tasters.

The results show that sample from ecotype 4 had the lowest overall assessment (3.44 points on average). As previously seen, this sample showed the highest hardness (crust and flesh) and highest creaminess. The ample with better overall acceptability was ecotype 3 with 4.67 points on average.

The judges of the panel considered samples of ecotypes 5 and 6 to have higher crust thickness. However, these results are not in total agreement with those obtained by instrumental texture analysis. Relatively to ecotype 6, it was the second hardest as seen on texture analysis and hence this corroborates the judge's evaluation as one of the samples with thicker crust. Conversely, ecotype 5, identified by the panelists as the second thicker crust showed the lowest values for hardness of the crust.

Samples ecotype 1 and 3 had very low scores in relation to crust uniformity, 3.11 and 3.22 points on average, respectively.

The creaminess of the flesh was the property evaluated that allowed obtaining more dissimilar results among all samples at study. Samples ecotype 1 and 5 were considered very creamy, whereas samples ecotype 3 and 4 were evaluated as less creamy.

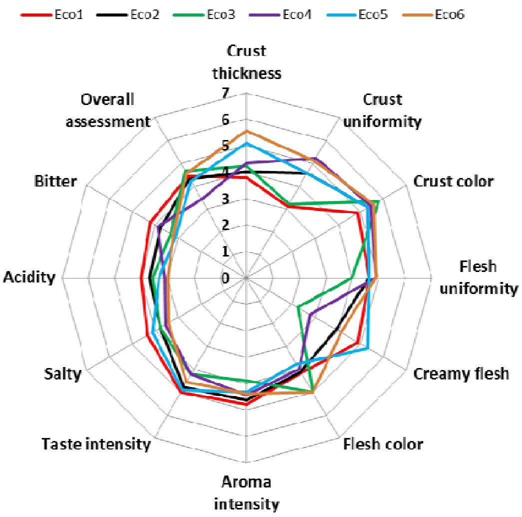

Fig. 2 Sensorial profiles of the cheese samples studied

The aroma and taste intensity were scored in the ranges 3.89-4.78 and 4.22-5.00, respectively. These cheeses were obtained from raw ewe's milk. The cheeses obtained from raw milk develop a more intense flavor and softer texture, which can be explained by the intensity and extent of proteolysis happening in these cheeses [15]. On the other hand, the products resulting from those processes, such as peptides and amino acids, considerably influence the sensory characteristics of the cheese [17]. Also, in cheeses made from raw milk the development of flavor is faster and more complete than in cheeses obtained from pasteurized milk, because the rapid fermentation of lactose, guaranteed by the native bacteria, allows greater efficiency in serum expulsion, thus influencing the flavor and texture of the final product [15].

The type of rennet influences the development of the sensory profile during maturation. When rennet from animal origin is used for the production of cheese, it is observed a lower intensity of flavors, acidity and brightness, besides being firmer and less creamy compared with cheeses coagulated from plant extracts [18]. Lipolysis also influences the development of cheese flavors and, in general, it is believed that this increased intensity of scents can be related to the greater amount of volatile fatty acids present [19].

IV. CONCLUSION

This work focused on the evaluation of some physical, chemical and sensorial properties of Serra da Estrela Cheeses made with six different ecotypes of thistle, in order to evaluate whether this factor would influence the product characteristics.

The results obtained allowed verifying that in fact the type of rennet used, and in particular the thistle ecotype, greatly influenced the cheese properties. There was a great variability in the chemical composition; the texture also diverged importantly among samples and color also revealed noticeable differences.

The sensorial analysis allowed identifying clearly some differences, particularly in terms of creaminess, crust uniformity and crust thickness.

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