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Influence of the Breed of Sheep on the Characteristics of Zamorano Cheese

Domingo Fernández , Patricia Combarros-Fuertes , Erica Renes , Daniel Abarquero, José María Fresno and María Eugenia Tornadijo *

Department of Food Hygiene and Technology, Faculty of Veterinary Science, University of Leon, 24071 León, Spain; domin000@gmail.com (D.F.); pcomf@unileon.es (P.C.-F.); erenb@unileon.es (E.R.); dabac@unileon.es (D.A.); jmfreb@unileon.es (J.M.F.)
* Correspondence: metorr@unileon.es; Tel.: +34-987-293253

Abstract: This work aimed to study the effects of using ewe's milk from Churra, Assaf, or both breeds on the physicochemical and sensory characteristics of Zamorano cheese at the end of ripening. Zamorano cheese is a hard variety with protected designation of origin (PDO) produced in the province of Zamora (Spain) with raw or pasteurized ewe's milk. Five batches of Zamorano cheese were produced with pasteurized ewe's milk. One batch was elaborated using milk from the Churra breed, the other using milk from the Assaf breed, and the remaining three employed milk mixtures of Churra and Assaf breeds in the proportions 75:25, 50:50 and, 25:75, respectively. Cheeses made with a higher proportion of Churra milk showed a predominance of hydrophilic peptides, while hydrophobic peptides predominated in cheeses with a greater percentage of milk from the Assaf breed. The largest content of most free amino acids was found in cheeses produced with the highest percentage of Churra milk. These cheeses presented the highest values for fat acidity index and free fatty acids content and showed greater elasticity and adhesiveness, as well as lower granularity and hardness. In the sensory evaluation, aftertaste and persistence were higher in these cheeses, being scored with the best overall values.

Keywords: ewe's milk; sheep breed; cheese; ripening; texture; sensory characteristics

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1. Introduction

Zamorano cheese is an uncooked pressed-curd variety produced in the Province of Zamora in Castilla y León, Spain, using raw or pasteurized ewe's milk from Churra and Castellana breeds. The traditional manufacturing procedure for this cheese involves curdling the milk with animal rennet at 28–32 °C for 30–45 min, cutting the curds until they reach rice grain size. Then, they were stirred and heated up to a temperature of 36–38 °C. After the whey is strained off, the curds are transferred to molds and pressed, finally reaching a pH value of 5.4–5.5. Thereafter, the cheeses are salted in brine and ripened for a minimum period of 60–100 days, depending on the weight of the cheeses, although in general such cheeses are marketed with at least six months of ripening. The core of the cheese is firm and the crust varies in color from yellow to darkish brown. Traditional Zamorano cheese is characterized by presenting an aroma of ewe's milk, which intensifies with ripening, as well as a certain spicy sensation.

Zamorano cheese is a Spanish protected designation of origin (PDO) hard cheese [1]. According to a regulation made by the Spanish Regulation this name refers to a hard cheese made with ewe's milk from Spanish Churra and Castellana breeds, and ripened for a minimum period of 60 days for those weighing 1.5 kg or less, and of 100 days for those over 1.5 kg [2]. This regulation was later modified to permit also the use of milk from first crosses (F1) of sheep of these breeds of the Spanish Assaf breed, although in this latter case it was not permissible to restock a farm with the descendants of such crossbreeds [3]. It is included in the Official Cattle Breeds Catalog of Spain under the heading “breeds from third parties” [4].

There are currently farms and cheese-makers who are pressing for the inclusion of new breeds in the PDO regulations for Zamorano cheese so as to adjust to changes that are taking place in flocks in Zamora, also claiming that there ought not to be any appreciable differences between cheeses made with ewe’s milk from local or outsider breeds. However, other producers which belong to the PDO point out the contrary. They stated that the characteristics of a cheese produced with milk from breeds other than native breeds may present major differences in the cheese’s final quality. This takes into account the fact that milk from the local Churra and Castellana breeds has a higher average fat and protein content than in another ewe’s milk from elsewhere in Spain, even if the overall milk yield of the native types may be lower. The Assaf milk yield is more than double that of the Churra, with 278 and 127 L respectively, in lactations at 150 days, although Assaf milk has lower fat and protein content (6.82% and 5.43%, respectively) than Churra milk (7.24% and 5.62%, respectively) [5,6].

Several studies have addressed the question of the effects breeds have on properties such as total cheese yield, curdling of milk, the firmness of curds and syneresis, all of these being aspects connected to the final quality of cheeses [7,8]. However, little work has been reported about sheep influence on the ripening process. In fact, ripening constitutes a fundamental stage in cheese-making. This is a process governed by a complex enzymatic system, involving enzymes naturally present in the milk, the curdling enzyme and microbial enzymes. Moreover, the activity of these agents on the main components of milk may be influenced by many factors. Some are intrinsic, such as pH, water activity and salt-to-humidity ratios. Others are extrinsic, for instance ambient temperature and relative humidity, chamber air velocity or ripening time. During the ripening period, a number of processes affect the product characteristics from a physical, chemical and sensory point of view. Among the principal biochemical events contributing to the ripening of cheese, proteolysis is the most complex of all, and it exercises a marked influence upon the texture, aroma and flavor of cheeses. On the other hand, sheep and goat milk ripened cheeses can develop an intense lipolysis that affects the flavor due to the greater presence of FFA but also due to the formation of other compounds derived from the catabolism of FFA [9,10].

There are several studies on the quality of PDO Zamorano cheese [11–15]. In these pressed-curd cheeses, proteolysis exercises a major impact upon the texture and sensory properties acquired by cheeses during ripening. Nevertheless, little information is available on the influence that ewe’s milk from different breeds may have on the characteristics and quality of the cheese. Consequently, the aim of this work was to evaluate the effects of using ewe’s milk from the Churra and Assaf breeds, and of their mixtures, on the final characteristics of Zamorano cheese.

2. Materials and Methods

2.1. Cheese Making and Sampling

Five batches of Zamorano cheese were produced, in duplicate, with pasteurized ewe’s milk: (a) 100% Churra, (b) 75% Churra and 25% Assaf mix, (c) 50% Churra and 50% Assaf mix, (d) 25% Churra and 75% Assaf mix, and (e) 100% Assaf. The fat and protein content in Churra milk was 7.71% and 5.53%, respectively, while in Assaf milk was 6.78% and 5.20%, respectively.

The cheeses were made in accordance with the procedure approved by the Regulatory Council for the PDO Zamorano Cheese. Once the milk was pasteurized, it was kept in the vat at 30–32 °C. Thereafter, the starter culture (1%) was added, this being made up of *Lactococcus lactis* subsp. *lactis* and *Lactococcus. lactis* subsp. *cremoris* (Choozit^{TM} MA 11 LYO 50DCU; Danisco, France) with calcium chloride at 0.2 g L^{-1} and sodium nitrate at 0.25 g L^{-1}. After thirty minutes, 25 mL of liquid sheep rennet (80% chymosin and 20% pepsin, coagulant power of 175–180 international units (IMCU) (Cuajos Caporal, Valladolid, Spain)) was added for each 100 L of milk. Then, 40–45 min were allowed for coagulation, after which the curds were cut and stirred for 50 min until they were of the size of a grain of rice. The temperature of the curds was increased by 1 °C every 5 min in this phase, until it reached 36 °C. At that point, the whey was drained off and the curds were placed in cylindrical molds 21 cm in diameter and 15 cm high. The next step was to press the curds for 5–6 h under pressure which ranged from 1.5 kg cm^{-2} up to 3.5–4 kg cm^{-2}. The pressed curds were then removed from the molds and submerged in a 20% brine solution at 8–10 °C for 20–24 h. Finally, the cheeses were ripened in chambers at 10 °C and a relative humidity of 80–85% for 240 days. In every batch, six cheeses weighing approximately 3 kg each were obtained.

By the end of the ripening period, the rind of the cheeses was removed, and they were cut up and placed into packets with an approximate weight of 50 g each. These were stored in a deep freeze at −30 °C until required for analysis.

2.2. Peptide Profile Analysis

Peptide profile indicates considerable qualitative and quantitative differences between the cheeses during ripening. Moreover, the hydrophobic/hydrophilic ratio has been suggested as an indicator of bitterness in cheese [16,17].

The water-soluble extracts (WSE) from the cheese for peptide analysis were obtained using the procedure described by Andersen et al. [18]. The peptide profile analysis was carried out as indicated in Parra et al. [19], by means of reversed-phase high performance liquid chromatography (RP-HPLC). This used a Waters Symmetry300 C18 column, 5 µm pore (4.6 mm × 250 mm; 300 Å) and Waters Symmetry300 pre-column (4.6 mm × 20 mm) with a thermostatically controlled temperature of 50 °C. Detection was at 214 nm. Elution of the samples involved a gradient of two solvents: (A) 0.1% (*v*/*v*) of trifluoro-acetic acid (TFA), sequencing grade (Sigma, Saint Louis, MO, USA) in de-ionized HPLC grade water and (B) 0.1% TFA (*v*/*v*) in a 1:1 mixture of acetonitrile and water. Flow speed was 1 mL min^{-1}. On each chromatogram, three zones were established as a function of the retention time: Zone 1, hydrophilicity (13–22 min), Zone 2, intermediate hydrophobicity (22–30 min) and Zone 3, extreme hydrophobicity (30–45 min). Peptides were also grouped as hydrophobic (the sum of the areas in Zones 2 and 3) relative to hydrophilic (the sum of the areas in Zone 1). The results were determined as the relative areas obtained by dividing the sum of the areas within the peaks of a given zone in the chromatogram by the total area of all the peaks.

2.3. Free Amino Acid Content

Separation, identification and quantification of free amino acids were performed by means of reversed-phase high performance liquid chromatography (RP-HPLC). The procedure followed was as described by Alonso et al. [20], with some modifications. Sample derivatization was undertaken using phenyl isothiocyanate. Separation of free amino acids was carried out in a model 2695 chromatograph (Waters, Milford, MA, USA) fitted with a C18 Brisa LC2 reverse phase column (Teknokroma, Barcelona, Spain) with 5 µm pore size (4.6 mm × 250 mm) at a thermostatically controlled temperature of 50 °C. Detection was performed at 254 nm. To quantify the free amino acids, twenty-four standard solutions were prepared at a concentration of 25 mM for each amino acid, except tyrosine, for which the concentration was 12.5 mM. Using these standard solutions of each amino acid, a stock solution was made up representing the profile of amino acids present in Zamorano cheese. From this stock solution, eight dilutions were produced that underwent derivatization under the same conditions used for the cheese samples. They were injected into the chromatograph, and the relevant calibration curves were found for each amino acid. These standard curves permitted quantification of the free amino acids.

2.4. Analysis of Lipolysis and Quantification of Free Fatty Acids

The degree of lipolysis was established by using the fat acidity index determined in accordance with ISO 1740:2004 [21].

Extraction of the fat from the cheese and the subsequent separation of the free fatty acids from the triglycerides were achieved by following the procedure described by De Jong and Badings [22], with slight modifications. Identification and quantification of free fatty acids was by means of gas chromatography, using a 6890 Series GC System chromatograph (Hewlett Packard, Wilmington, NC, USA), fitted with a 7683 automatic sample injector (Hewlett Packard, NC, USA) and a 5973 mass selective detector (Hewlett Packard, NC, USA) as described in Arenas et al. [23].

2.5. Texture Profile Analysis and Sensory Analysis

The texture profile analysis (TPA) allowed various parameters to be determined: crumbliness or fracturability, hardness, adhesiveness or stickiness, springiness, cohesiveness, chewiness and gumminess. This was done with a Stable Micro Systems TA-XT2i texturometer (Aname, Madrid, Spain), as described by Bourne [24]. Samples were extracted with a punch and compressed to 80% of their original depth, with two compression cycles at constant velocity. Each sample was analyzed in quintuplicate.

A descriptive sensory analysis of the cheeses was performed by panels of six to ten tasters trained by the Regulatory Council (RC) of the PDO "Zamorano cheese", following the ISO 8586:2012 standard [25]. Twenty-six descriptors for attributes grouped under four headings—appearance, aroma, texture and taste—on the basis of the criteria of the RC of the PDO "Zamorano cheese".

In addition, a public tasting session was run, with one hundred and two participants, in order to know the opinion of the consumers. They completed a simplified tasting sheet giving a score running from 1 (I dislike this a lot) to 10 (I like this a lot) for the attributes listed above, as well as an overall rating for the cheeses.

2.6. Statistical Analysis

Analyses were carried out at least in duplicate. Significant differences were evaluated through an analysis of variance (ANOVA) using Fisher's least significant difference test, while data grouping checks used principal component analysis. The Statistica 7.0 software package (TIBCO Software, Tulsa, OK, USA) was used. Sensory variables were analyzed using the Kruskal-Wallis test at 5% significance level.

3. Results and Discussion

3.1. Peptide Profile

Table 1 shows the peptide contents in three groupings: hydrophilic, mildly hydrophobic and hydrophobic. It also indicates the ratios of hydrophobic and hydrophilic peptides in Zamorano cheese made with different mixtures of Churra and Assaf milk after 240 days of ripening. The peptide content revealed significant differences ($p \leq 0.05$) between the samples of cheese. Cheeses produced with a higher proportion of milk from the Churra breed showed a predominance of the hydrophilic peptides, unlike what was observed in cheeses made with a greater percentage of milk from the Assaf breed. This feature is of importance because the presence of hydrophobic peptides of small size could be responsible for bitter taste in cheeses [26]. These tendencies were corroborated by the ratio between hydrophobic and hydrophilic peptides; then the hydrophobic peptides were 1.7 more prevalent in cheeses with a higher percentage of Assaf milk, relative to those made wholly with Churra milk. These results differ from the findings of Hayaloğlu et al. [27], who described very little influence on the peptide profile of cheeses produced with milk from different breeds, although it is true that this study did not look at differences in terms of the hydrophobicity or hydrophilicity of peptides.

Table 1. Relative areas of hydrophilic and hydrophobic zones of chromatograms from permeates of soluble extract in Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
	100% Ch	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
Zone 1	0.34 ± 0.01 [c]	0.27 ± 0.01 [b]	0.25 ± 0.01 [a]	0.24 ± 0.01 [a]	0.24 ± 0.01 [a]
Zone 2	0.38 ± 0.01 [c]	0.36 ± 0.01 [b]	0.35 ± 0.01 [a,b]	0.33 ± 0.01 [a]	0.34 ± 0.01 [a,b]
Zone 3	0.28 ± 0.01 [b]	0.24 ± 0.01 [a]	0.38 ± 0.01 [c]	0.43 ± 0.01 [d]	0.41 ± 0.01 [d]
Hyphob/Hyphil	4.00 ± 0.11 [a]	5.35 ± 0.15 [b]	5.69 ± 0.16 [b,c]	5.86 ± 0.16 [c]	6.69 ± 0.19 [d]

Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). Zone 1 = hydrophilic peptides; Zone 2 = intermediate hydrophobic peptides; Zone 3 = hydrophobic peptides; Hyphob/Hyphil = relation of hydrophobic peptides respect to hydrophilic peptides.

3.2. Free Amino Acids

Table 2 shows the average free amino acid contents of Zamorano cheese produced with different mixtures of ewe's milk from Churra and Assaf breeds after 240 days of ripening. Significant differences ($p \leq 0.05$) were observed in the total amino acid content between batches of cheese. The cheeses with the greatest amount of free amino acids (3085.50 mg 100 g^{-1} of cheese) were those made with 100% Churra milk. In contrast, the lowest values corresponded to the cheeses produced with 100% Assaf milk, with 1515.69 mg 100 g^{-1} of cheese. The total free amino acid content of the remaining cheeses analyzed lie at intermediate values, although those manufactured predominantly with milk from Churra breed showed levels of free amino acids that were slightly higher. These results agree with the behavior observed for the fraction of nitrogen soluble in 5% phosphotungstic acid [8]. This may be related to the high counts on De Man, Rogosa and Sharpe (MRS) agar in cheeses made with 100% Churra milk. The lower salt-to-humidity ratio of these cheeses [11] allowed the development not only of the lactococci of the starter culture but also of contaminant lactic acid microbiota. At late stages of ripening, the dominant microbiota would probably be lactobacilli, as was demonstrated in a similar study by Ferrazza et al. [14], and would be able to develop an intense aminopeptidase activity.

The free amino acid content of the cheeses in the present study was similar to those described by Poveda [28] in Manchego cheese made with pasteurized milk, where an average total amount of 2394 mg 100 g^{-1} of cheese was recorded. Likewise, the values fall within the range noted by other authors [29–31] for ewe's milk cheeses.

The highest amount of every one of the free amino acids analyzed was found in cheeses with the greatest percentage of milk from Churra breed, with the exception of Cit, GABA and Cys. The predominant amino acids in cheeses made from 100% Churra milk were the combination of Gln+Gly, Leu, Glu, Lys, Val, Asn and Tau, these together representing nearly 73% of the total of amino acids. In contrast, cheeses produced with 100% Assaf milk had as their main amino acids Gln+Gly, Leu, Glu, Lys, Val, Phe and GABA, which together amounted to 66% of the total. These profiles are very similar to those recorded in other studies on Zamorano cheese [30,31], although these also included Pro among the predominant amino acids (Table 2). The profiles for the main free amino acids were practically the same in all the cheeses studied, independently of the breed whose milk was used for cheese-making due to the amino acid composition of caseins in ewe's milk. Nevertheless, the concentration of amino acids does vary from one type of cheese to another.

Principal component (PC) analysis allowed data to be grouped with 91.24% of explained variation with two PCs. Cheeses produced from 100% Churra milk were associated with the negative zone of PC1. Those made with 100% Assaf milk and with 25% Churra and 75% Assaf milk were related to the positive side of PC2. In contrast, cheeses manufactured with the mixtures 75% Churra and 25% Assaf milk, and 50% Churra and 50% Assaf milk were negatively correlated to PC2.

With regard to details of the projection of variables (Figure 1), it was observed that all the free amino acids were strongly related to the negative zone of PC1 (mostly with correlations greater than −0.95), this coinciding with the group of cheeses produced from 100% Churra milk. The amino acids Asp, Pro, Cit and His were associated primarily with cheeses manufactured from mixtures involving 75% or 50% of Churra milk. The amino acid His, together with GABA, was linked to batches made with 75% of 100% Assaf milk.

Table 2. Average free amino acids content (expressed as mg 100 g^{-1} of cheese) in Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
Amino Acids	**100% Ch**	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
Asp	40.55 ± 0.86 ^{c}	42.92 ± 0.93 ^{d}	39.85 ± 0.84 ^{c}	30.35 ± 0.58 ^{b}	25.05 ± 0.43 ^{a}
Glu	357.09 ± 9.82 ^{b}	120.90 ± 3.14 ^{a}	128.15 ± 3.34 ^{a}	119.89 ± 3.11 ^{a}	115.61 ± 2.99 ^{a}
Asn	193.64 ± 5.19 ^{e}	53.87 ± 1.52 ^{c}	66.49 ± 1.74 ^{d}	31.19 ± 0.60 ^{a}	40.21 ± 0.85 ^{b}
Ser	63.04 ± 1.50 ^{d}	46.63 ± 1.04 ^{c}	38.57 ± 0.81 ^{a,b}	39.39 ± 0.97 ^{b}	36.32 ± 1.03 ^{a}
Gln+Gly	709.38 ± 19.78 ^{d}	323.38 ± 9.15 ^{c}	316.09 ± 8.80 ^{c}	217.29 ± 5.58 ^{a}	283.73 ± 7.46 ^{b}
His	32.41 ± 0.63 ^{b}	29.12 ± 0.68 ^{a}	29.29 ± 0.55 ^{a}	36.23 ± 0.74 ^{c}	33.32 ± 0.66 ^{b}
Cit	7.89 ± 0.34 ^{a}	38.24 ± 1.08 ^{c}	43.67 ± 0.95 ^{d}	20.30 ± 0.29 ^{b}	39.47 ± 0.83 ^{c}
Tau	121.44 ± 3.15 ^{d}	52.30 ± 1.20 ^{c}	41.48 ± 0.89 ^{b}	11.53 ± 0.04 ^{a}	12.31 ± 0.07 ^{a}
Gaba	26.08 ± 1.87 ^{a}	41.22 ± 0.88 ^{b}	47.92 ± 1.07 ^{c}	49.41 ± 0.41 ^{c}	78.76 ± 1.94 ^{d}
Arg+Thr	101.56 ± 2.59 ^{c}	67.61 ± 1.91 ^{a,b}	71.54 ± 1.74 ^{b}	64.98 ± 1.56 ^{a}	64.70 ± 1.55 ^{a}
Ala	67.92 ± 1.64 ^{c}	39.63 ± 1.12 ^{b}	42.33 ± 0.91 ^{b}	34.55 ± 0.84 ^{a}	34.25 ± 0.69 ^{a}
Pro	26.63 ± 0.47 ^{d}	36.35 ± 1.03 ^{e}	23.70 ± 0.39 ^{c}	20.85 ± 0.31 ^{b}	18.37 ± 0.24 ^{a}
Tyr	68.02 ± 1.64 ^{d}	20.53 ± 0.58 ^{c}	21.87 ± 0.34 ^{c}	17.25 ± 0.35 ^{b}	15.04 ± 0.14 ^{a}
Val	220.99 ± 5.97 ^{d}	108.28 ± 2.78 ^{b,c}	111.68 ± 2.88 ^{c}	91.23 ± 2.44 ^{a}	101.38 ± 2.58 ^{b}
Met	48.56 ± 1.09 ^{d}	19.64 ± 0.27 ^{b,c}	20.55 ± 0.30 ^{c}	18.44 ± 0.38 ^{b}	16.29 ± 0.18 ^{a}
Cys	ND	23.71 ± 0.39 ^{b}	22.95 ± 0.37 ^{b}	18.80 ± 0.39 ^{a}	18.78 ± 0.25 ^{a}
Ile	42.67 ± 0.92 ^{d}	24.11 ± 0.68 ^{b}	29.33 ± 0.55 ^{c}	17.46 ± 0.35 ^{a}	17.64 ± 0.22 ^{a}
Leu	392.25 ± 10.81 ^{d}	274.20 ± 7.47 ^{c}	257.86 ± 7.01 ^{c}	212.19 ± 5.86 ^{a}	237.05 ± 6.42 ^{a}
Phe	95.49 ± 2.42 ^{c}	92.21 ± 2.61 ^{c}	57.98 ± 1.36 ^{a}	61.98 ± 1.47 ^{a}	83.76 ± 2.09 ^{b}
Trp	118.50 ± 3.07 ^{c}	79.77 ± 1.97 ^{b}	80.66 ± 2.00 ^{b}	68.35 ± 1.79 ^{a}	75.78 ± 1.86 ^{b}
Orn	99.33 ± 2.53 ^{c}	67.28 ± 1.62 ^{b}	68.07 ± 1.64 ^{b}	57.86 ± 1.50	63.99 ± 1.53 ^{a}
Lys	252.06 ± 6.85 ^{d}	138.54 ± 3.64 ^{c}	121.83 ± 3.16 ^{b}	108.12 ± 2.92 ^{a}	103.90 ± 2.94 ^{a}

Aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine and glycine (Gln+Gly), histidine (His), citrulline (Cit), taurine (Tau), gamma-aminobutyric acid (GABA), arginine and threonine (Arg+Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cysteine (Cys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), ornithine (Orn), lysine (Lys). Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). ND = not detected.

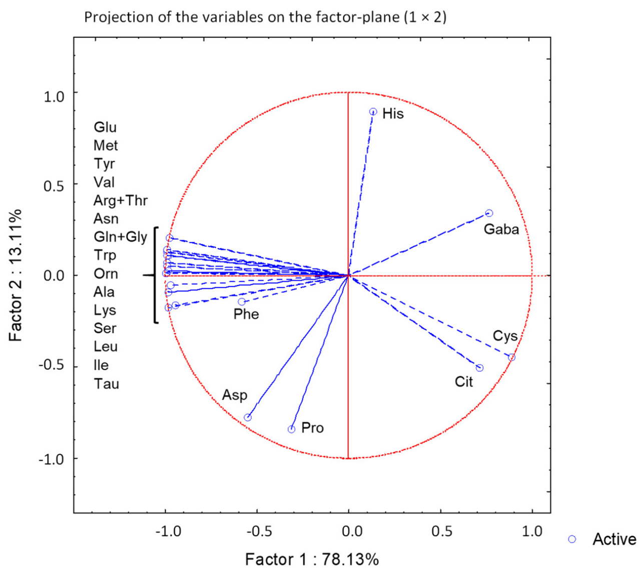

Figure 1. Principal component analysis for free amino acids in Zamorano cheese after 240 days' ripening as a function of breed through the representation of projections.

From the point of view of nutrition, the presence of a large amount of GABA in 100% Assaf milk cheeses should be highlighted—quantities being similar to those observed by Diana et al. [29] in other ewe's milk cheeses, even if they equated to more or less half of what was described by Renes et al. [31]. Finally, it should be noted that ornithine (Orn) was detected in larger amounts in cheeses made with 100% Churra milk, with values more than ten times greater than those recorded by Diana et al. [29]. The bio-active properties ascribed to GABA and Orn may bring added value to these cheeses.

3.3. Lipolytic Changes and Quantification of Free Fatty Acids

The average values for the fat acidity index (FAI) and free fatty acids (FFAs) in the batches of Zamorano cheese made with different proportions of milk from Churra or Assaf after 240 days of maturation are shown in Table 3. Significant differences ($p \leq 0.05$) were observed between the various batches of cheese in the FAI. Values were slightly higher in cheeses produced with a larger proportion of Churra milk relative to those manufactured with a greater proportion of Assaf milk.

Table 3. Free fatty acids (FFA) content in Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
	100% Ch	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
FAI	$2.61 \pm 1.11^{a,b}$	$2.50 \pm 0.19^{a,b}$	3.66 ± 0.08^{b}	$2.48 \pm 1.16^{a,b}$	2.45 ± 0.70^{a}
C2:0	2.71 ± 0.04^{d}	2.61 ± 0.04^{c}	2.58 ± 0.03^{c}	2.45 ± 0.03^{b}	2.31 ± 0.03^{a}
C4:0	$10.50 \pm 0.26^{b,c}$	11.04 ± 0.27^{c}	$10.02 \pm 0.25^{b,c}$	9.59 ± 0.23^{b}	8.52 ± 0.20^{a}
C6:0	28.16 ± 0.76^{d}	$18.78 \pm 0.49^{b,c}$	19.77 ± 0.52^{c}	17.42 ± 0.45^{b}	15.64 ± 0.40^{a}
C8:0	10.23 ± 0.25^{c}	4.85 ± 0.10^{b}	4.89 ± 0.10^{b}	4.08 ± 0.08^{a}	3.75 ± 0.07^{a}
C10:0	33.28 ± 0.90^{d}	18.25 ± 0.48^{c}	16.74 ± 0.44^{b}	15.56 ± 0.40^{b}	13.85 ± 0.35^{a}
C10:1	0.50 ± 0.02^{a}	0.58 ± 0.02^{c}	$0.53 \pm 0.02^{b,c}$	0.97 ± 0.01^{d}	1.39 ± 0.00^{e}
C12:0	20.11 ± 0.53^{d}	10.34 ± 0.25^{c}	8.20 ± 0.19^{b}	7.51 ± 0.17^{b}	5.49 ± 0.12^{a}
C14:0	39.80 ± 1.09^{d}	20.10 ± 0.53^{c}	20.55 ± 0.54^{c}	17.54 ± 0.46^{b}	14.80 ± 0.38^{a}
C14:1	0.77 ± 0.02^{e}	0.62 ± 0.02^{d}	0.53 ± 0.02^{c}	0.39 ± 0.03^{b}	0.00 ± 0.00^{a}
C15:0	2.91 ± 0.04^{d}	1.47 ± 0.00^{b}	1.67 ± 0.01^{c}	1.45 ± 0.00^{b}	0.72 ± 0.02^{a}
C16:0	59.07 ± 1.39^{e}	38.05 ± 1.04^{d}	32.29 ± 0.88^{c}	28.59 ± 0.77^{b}	21.24 ± 0.56^{a}
C16:1	0.65 ± 0.02^{a}	0.89 ± 0.01^{b}	1.14 ± 0.01^{d}	1.05 ± 0.01^{c}	2.69 ± 0.04^{e}
C18:0	25.74 ± 0.69^{d}	19.36 ± 0.51^{c}	16.72 ± 0.43^{b}	18.18 ± 0.48^{c}	11.98 ± 0.30^{a}
C18:1	46.23 ± 1.27^{d}	24.66 ± 0.66^{c}	24.88 ± 0.67^{c}	19.28 ± 0.51^{b}	15.69 ± 0.41^{a}
C18:2	8.22 ± 0.19^{d}	2.95 ± 0.05^{b}	2.57 ± 0.03^{a}	3.23 ± 0.05^{c}	3.33 ± 0.06^{c}
C18:3	0.98 ± 0.01^{d}	$0.31 \pm 0.03^{b,c}$	0.16 ± 0.03^{a}	0.34 ± 0.03^{c}	0.25 ± 0.03^{b}
C18:2 Conjugated	0.82 ± 0.02^{d}	$0.39 \pm 0.03^{a,b}$	0.35 ± 0.03^{a}	0.43 ± 0.03^{b}	0.61 ± 0.02^{c}
SFA	232.50 ± 17.38^{c}	144.86 ± 10.65^{b}	133.42 ± 9.39^{b}	122.38 ± 8.45^{b}	98.29 ± 6.55^{a}
MUFA	48.16 ± 21.11^{b}	26.74 ± 11.10^{a}	27.08 ± 11.18^{a}	21.70 ± 8.56^{a}	19.76 ± 6.51^{a}
PUFA	10.02 ± 3.78^{b}	3.65 ± 1.35^{a}	3.08 ± 1.20^{a}	4.00 ± 1.47^{a}	4.19 ± 1.51^{a}

Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). FAI = fat acidity index (mg KOH 100 g^{-1} fat); FFA (mg 100 g^{-1} of cheese), being acetic [C2:0], butyric [C4:0], caproic [C6:0], caprylic [C8:0], capric [C10:0], decenoic [C10:1], lauric [C12:0], myristic [C14:0], myristoleíc [C14:1], pentadecanoic [C15:0], palmitic [C16:0], palmitoleic [C16:1], stearic [C18:0], oleic [C18:1], linoleic [C18:2], linolenic [C18:3], octodecadienoic conjugated [C18:2 conjugated]; saturated fatty acids [SFA]; monounsaturated fatty acids [MUFA]; polyunsaturated fatty acids [PUFA].

Lipolysis yields FFAs, which contribute to the aroma profile of a wide range of cheeses [17,32], as well as constituting a very useful tool for the cheese industry by acting as an indicator of the ripening of cheeses [33,34]. FFAs with shorter chains (lipid numbers C4:0, C6:0 and C8:0) display a low threshold of perception, so their impact on aroma is greater and it is a positive aspect if the balance is correct [35]. On the other hand, their presence in high concentrations could favor rancidity in cheeses [36].

Significant differences ($p \leq 0.05$) between batches were also noted in the FFA content. Cheeses made with a greater proportion of Churra milk exhibited a higher FFA content, with the exception of C10:1 and C16:1, which were detected in larger quantities in batches produced principally with milk from the Assaf breed. The overall amount of FFAs in the batches manufactured with 100% Churra milk was 2.4 times higher than in those made with 100% Assaf milk, this indicating greater lipolysis. The predominant FFAs were C14:0, C16:0 and C18:1 in cheeses manufactured with percentages of milk from Churra equal to or greater than 50%. In cheeses made with 75% Assaf milk, they were C6:0, C16:0, C18:0 and C18:1, and finally they were C6:0, C16:0 and C18:1 in the batch using 100% Assaf milk. Differences in the profile of fatty acids in goat's milk or ewe's milk from different breeds have been highlighted by various authors [37,38].

In cheeses produced exclusively with milk from the Churra breed, C18:1 was detected in quantities more than twice what was found in cheeses manufactured with milk coming entirely from the Assaf breed. This fact may be related to differences in the expression of genes between the two breeds. In fact, fatty acid binding protein 4 (FABP4), more highly expressed in the Churra breed, codes for a protein transporter with a strong affinity for C18:1 [39]. Differential expression of FABP4 might thus be related to the greater content of this fatty acid in milk from the Churra breed relative to the Assaf breed [15].

When FFAs were grouped as a function of their degree of saturation, it was observed that the average figures for saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) were significantly higher ($p \leq 0.05$) in cheeses made with 100% Churra milk. All the same, the ratios between them were very similar in the various types of cheese, MUFA and PUFA constituting approximately 25% of the total. Similar results were noted in other studies relating to the influence of the breed on the profile of fatty acids in milk and cheese [40,41].

Several authors have found variations in the conjugated linoleic acid (CLA) content of milk initially drawn from different breeds, although in no case did these differences reach significance [37,40]. However, in the present work, the values for CLA detected, comprising the mixture of CLA isomers, were significantly higher in cheeses made from 100% Churra milk relative to those produced from 100% Assaf milk.

The two principal components were able to explain 88% of variance. All cheeses were divided up into three groups as a function of breed. Cheese made from 100% Churra milk was associated with the negative component of PC1, while cheese produced from 100% Assaf milk was related to the positive component of PC1. All the other cheeses were grouped more or less in its central area. If the projection of variables is observed (Figure 2), it can be seen that, in general, most FFAs are strongly correlated to the negative component of PC1, with correlations greater than -0.9, and thus with the cheeses manufactured with milk from the Churra breed. In contrast, C10:1 and C16:1 presented a higher correlation with cheeses made with 100% Assaf milk.

3.4. Texture Profile Analysis

Table 4 gives the average values for the various different texture parameters in Zamorano cheese produced with different mixtures of milk from Churra and Assaf breeds at the end of a period of 240 days of ripening. Significant differences ($p \leq 0.05$) were observed in the parameters fracturability, hardness, chewiness and gumminess, with higher values being shown by cheeses made with a greater percentage of milk from the Assaf breed. These cheeses showed higher total solids, and salt and humidity contents [11], these being chemical parameters with a considerable influence upon texture [42]. Moreover, a high salt and humidity content is associated with the development of a cheese body that is granular, friable, dry, and hard [43]. On the other hand, the stronger proteolysis in cheeses made predominantly with Churra milk, seen because of the figures corresponding to nitrogenous fractions [8], may also have contributed to creating a softer texture in these cheeses.

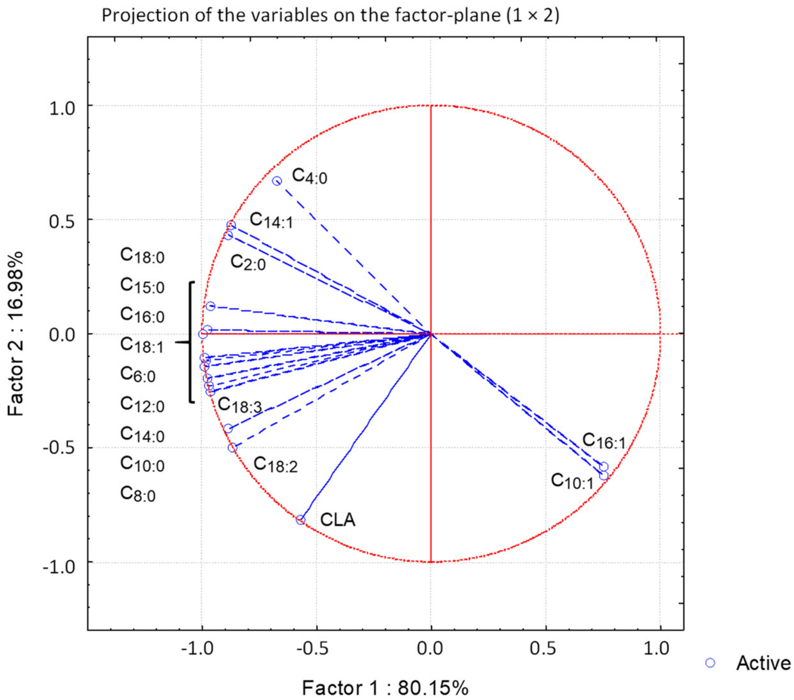

Figure 2. Principal component analysis for free fatty acids in Zamorano cheese after 240 days' ripening as a function of breed through the representation of projections.

Table 4. Average values for texture parameters in Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
	100% Ch	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
Fracturability	53.97 ± 4.91^{a}	68.64 ± 4.68^{b}	71.35 ± 3.04^{b}	76.65 ± 2.05^{b}	103.53 ± 9.21^{c}
Hardness	192.99 ± 6.21^{a}	$205.71 \pm 16.15^{a,b}$	$220.49 \pm 6.52^{b,c}$	239.54 ± 0.23^{c}	246.15 ± 6.17^{c}
Adhesiveness	-6.53 ± 1.34	-6.25 ± 3.62	-3.73 ± 2.66	-4.99 ± 0.21	-4.83 ± 1.15
Cohesiveness	0.14 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.02
Springiness	0.47 ± 0.01	0.42 ± 0.05	0.38 ± 0.07	0.40 ± 0.08	0.47 ± 0.00
Gumminess	$26.10 \pm 0.14^{a,b}$	24.64 ± 1.98^{a}	27.98 ± 0.33^{b}	31.61 ± 1.68^{c}	28.99 ± 0.47^{b}
Chewiness	$12.31 \pm 0.33^{a,b}$	10.39 ± 2.04^{a}	10.69 ± 2.03^{a}	$12.49 \pm 2.01^{a,b}$	13.21 ± 0.32^{b}

Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). Fracturablity (kg m s^{-2}); Hardness (kg m s^{-2}); Adhesiveness (kg m^2 s^{-2}); Gumminess (kg m s^{-2}). Chewiness (kg). Cohesiveness and Springiness are not dimensional parameters.

Several authors have linked limited proteolysis to the development of a harder texture [44,45]. The influence of breed on the texture of hard cheeses was described by several authors [46–48], although in this instance, significant differences were observed only in relation to adhesiveness or stickiness, and not to hardness.

3.5. Sensory Analysis by Tasting Panel

Table 5 shows the average scores awarded by the panel of tasters for various sensory attributes (appearance, aroma, texture and flavor) of Zamorano cheese manufactured with different mixtures of milk from the Churra and Assaf breeds after 240 days of ripening, together with their overall evaluation.

Table 5. Average values assigned by tasting panel for various sensory attributes linked to appearance, aroma, texture and flavor, and an overall evaluation for Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
	100% Ch	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
APPAREANCE					
Color intensity	3.71 ± 0.68	3.38 ± 0.34	3.63 ± 0.65	3.63 ± 0.65	3.88 ± 0.51
Holes size	3.71 ± 0.68	3.25 ± 0.61	3.00 ± 0.40	3.13 ± 0.78	4.13 ± 1.00
Holes number	3.69 ± 0.47	3.47 ± 0.72	3.01 ± 0.57	3.23 ± 0.68	3.97 ± 0.89
ODOR					
Lactic	4.12 ± 0.64	4.36 ± 0.40	4.23 ± 0.37	4.01 ± 0.62	4.24 ± 0.01
Fresh and clean	3.98 ± 0.54	4.23 ± 0.37	4.23 ± 0.37	3.88 ± 0.12	4.13 ± 0.12
Flowers/fruits	4.12 ± 0.35	3.99 ± 0.13	3.87 ± 0.29	4.01 ± 0.62	4.13 ± 0.13
Grassy	3.98 ± 0.54	4.13 ± 0.13	3.73 ± 0.40	3.74 ± 0.04	4.01 ± 0.62
Dry fruits	3.84 ± 0.36	4.01 ± 0.62	3.99 ± 0.13	4.01 ± 0.62	4.01 ± 0.62
Vanilla	4.14 ± 0.09	3.88 ± 0.12	3.99 ± 0.13	3.88 ± 0.12	4.01 ± 0.62
Wax	3.99 ± 0.20	4.01 ± 0.62	3.99 ± 0.13	4.01 ± 0.62	4.01 ± 0.62
TEXTURE					
Elasticity	4.26 ± 0.78	3.62 ± 0.15	3.61 ± 0.40	3.61 ± 0.45	3.48 ± 0.58
Crumbliness	4.27 ± 0.48	3.88 ± 0.12	3.11 ± 0.43	3.23 ± 0.49	3.36 ± 0.59
Adhesiveness	4.25 ± 1.19^{b}	4.12 ± 0.02^{b}	3.23 ± 0.02^{a}	3.37 ± 0.32^{a}	3.23 ± 0.42^{a}
Grainy	2.85 ± 0.74^{a}	2.87 ± 0.18^{a}	$3.71 \pm 0.07^{a,b}$	$3.48 \pm 0.41^{a,b}$	3.99 ± 0.28^{b}
Solubility	4.27 ± 0.44^{c}	$3.98 \pm 0.42^{b,c}$	3.23 ± 0.49^{a}	$3.72 \pm 0.69^{a,b}$	3.36 ± 0.49^{a}
Hardness	3.49 ± 0.06^{a}	3.62 ± 0.13^{a}	$4.01 \pm 0.62^{a,b}$	3.88 ± 0.12^{a}	4.13 ± 0.19^{b}
Creaminess	4.13 ± 0.12^{c}	3.88 ± 0.12^{c}	3.49 ± 0.14^{b}	3.50 ± 0.10^{b}	3.13 ± 0.08^{a}
Rugosity	3.99 ± 0.20	3.98 ± 0.42	3.23 ± 0.42	3.60 ± 0.91	3.12 ± 0.08
Dryness	3.41 ± 0.39	3.87 ± 0.12	3.22 ± 0.89	3.48 ± 0.45	3.12 ± 0.18
TASTE					
Salty	3.74 ± 0.14^{a}	3.74 ± 0.13^{a}	3.74 ± 0.13^{a}	4.12 ± 0.11^{b}	$3.99 \pm 0.07^{a,b}$
Fresh milk	4.11 ± 0.71	3.88 ± 0.12	3.37 ± 0.32	3.74 ± 0.04	3.61 ± 0.45
Acid	3.27 ± 0.49	3.37 ± 0.32	2.99 ± 0.08	3.61 ± 0.45	3.48 ± 0.41
Hot spicy	3.97 ± 0.95	4.11 ± 0.45	3.62 ± 0.30	3.99 ± 0.13	3.74 ± 0.30
Sweet	3.54 ± 0.75	3.86 ± 0.43	3.62 ± 0.05	4.11 ± 0.37	3.87 ± 0.13
Bitter	2.98 ± 0.41	2.99 ± 0.34	2.74 ± 0.18	3.37 ± 0.16	3.23 ± 0.49
Aftertaste and persistence	4.35 ± 0.16^{b}	$4.14 \pm 0.43^{a,b}$	$3.74 \pm 0.14^{a,b}$	3.36 ± 0.49^{a}	3.43 ± 0.51^{a}
OVERALL EVALUATION					
Overall score	7.16 ± 0.62	7.25 ± 0.48	7.04 ± 0.05	6.58 ± 0.33	6.50 ± 0.51

Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). All parameters were scored between 1 and 7. Overall evaluation was scored between 1 and 10.

Attributes relating to appearance and aroma presented no significant differences ($p \geq 0.05$) between cheeses. A slightly stronger lactic smell could be detected in cheeses made with 100% Assaf milk, which may be associated with the lower values for pH and higher values for titratable acidity in these cheeses [11]. The attribute of fresh or clean smell in 100% Assaf milk cheeses yielded values slightly higher than those for 100% Churra milk cheeses. This may be related to greater lipolysis in 100% Churra milk cheeses and higher short-chain fatty acid content, associated with a certain taste of animal fat. Martin et al. [45] described an influence from breed upon the texture and aroma of Cantal cheese.

The attributes relating to texture that presented significant differences ($p \leq 0.05$) among cheeses were adhesiveness, grainy, solubility and hardness. The values for adhesiveness, solubility and creaminess were higher in cheeses made with greater percentages of Churra milk.

Cheeses produced from 100% Churra milk showed a larger amount of long-chain SFA and of C18:1, greater proteolysis and a higher humidity content, with the effect of giving these cheeses more adhesiveness, solubility and creaminess, as noted in a study by Soryal et al. [41]. The greater granularity and hardness observed in cheeses made with 100% Assaf milk may have been an outcome of the lower humidity levels in these cheeses, as also a lesser solubilization of caseins, as indicated in similar work carried out by other researchers [45].

With regard to taste attributes, most of the descriptors showed no significant differences ($p > 0.05$) between cheeses. The exceptions were saltiness, aftertaste and persistence. Cheeses produced with a greater percentage of Assaf milk showed higher levels of salt, which would explain the differences noted by the tasters. The scores associated with a stronger aftertaste and persistence, as also the best overall evaluation ($p > 0.05$) was found in batches of cheese made with a greater proportion of Churra milk. This may be related to the greater proteolysis and lipolysis that developed in these cheeses. Indeed, higher scores for aroma and flavor attributes were given to cheeses where nitrogenous fractions and free amino acid contents are more prevalent [49]. Cheeses made with a higher proportion of Assaf milk showed a higher hydrophobic/hydrophilic ratio, and this fact would also explain the greater degree of bitterness in these cheeses, although no significant differences were detected.

The results obtained from the panel of consumers (Table 6) did establish significant differences ($p \leq 0.05$) for appearance and texture parameters, and for sensations affecting the trigeminal nerve, such as hot, cold or tingly feelings. In general, there was confirmation of the trend towards higher scores for aroma and flavor, and for overall assessment, in batches with a higher percentage of Churra milk.

Table 6. Average values assigned by tasting panel of consumers for various sensory attributes linked to appearance, aroma, texture and flavor, and an overall evaluation for Zamorano cheese made with milk from Churra (Ch) and Assaf (A) breed after 240 days' ripening.

	Milk				
	100% Ch	**75% Ch:25% A**	**50% Ch:50% A**	**25% Ch:75% A**	**100% A**
Aspect	$5.45 \pm 0.35^{a,b}$	$5.20 \pm 0.36^{a,b}$	5.58 ± 0.24^{b}	4.95 ± 0.57^{a}	$5.25 \pm 0.24^{a,b}$
Odor	6.03 ± 0.37	5.80 ± 0.16	6.03 ± 0.34	5.85 ± 0.24	5.65 ± 0.34
Taste	7.20 ± 0.23	6.75 ± 0.51	6.85 ± 0.42	6.80 ± 0.48	6.60 ± 0.62
Texture and trigeminal sensations	$5.33 \pm 0.22^{a,b}$	5.00 ± 0.16^{a}	5.53 ± 0.38^{b}	$5.38 \pm 0.15^{a,b}$	$5.40 \pm 0.34^{a,b}$
Overall score	6.33 ± 0.46	6.45 ± 0.62	6.00 ± 0.45	6.15 ± 0.47	6.03 ± 0.50

Values in the same line with different superscript letters differ significantly ($p \leq 0.05$). All parameters were scored between 1 and 10.

Both lipolysis and proteolysis generate volatile compounds that influence the flavor of cheeses. Differences in the volatile profile of Gokceada cheese elaborated with goat's milk from different breeds have been described by Hayaloglou et al. [27]. Ferreira et al. [48,49] observed the same effect in the Castelo Branco PDO sheep cheese. In both studies, these differences also have been detected by the tasting panel.

Over recent years, there has been increasing research intended to shed light on the connections between sensory attributes of cheese and the breed from which milk is obtained. The aim is to provide the basis for improvements in the quality of cheeses [48,49].

4. Conclusions

This research supports the influence of sheep breed on the physicochemical and sensory characteristics of Zamorano cheese.

Cheeses made with higher proportion of Churra milk were characterized by showing better texture properties. They presented higher values for adhesiveness, solubility and creaminess than the other cheeses. Moreover, the aftertaste and persistence, detected by the tasting panel as positive characteristics, were stronger in cheeses made with higher proportion of Churra milk. This fact could be related to the high proteolysis and lipolysis degree developed in these cheeses. The highest values of free amino acids concentrations and fat acidity index were detected in these cheeses. Moreover, cheeses produced with a higher proportion of milk from Churra breed showed a predominance of the hydrophilic peptides, unlike what was observed in cheeses made with a greater percentage of milk from the Assaf breed. The overall evaluation did not show significant differences between the cheeses, although the best scores were obtained by the cheeses made with 100% and 75% Churra milk. In consequence, greater value might be set on native breeds, encouraging their maintenance and genetic biodiversity.

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