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Effects of somatic cells on the protein profile of hard ovine cheese produced from different breeds

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Bulk tank ewe's milks with low (< 500,000 ml^{-1}), medium (1,000,000–1,500,000 ml^{-1}) and high (> 2,500,000 ml^{-1}) somatic cell counts (SCC) from three breeds were used to manufacture hard ewes'-milk cheese. Physico-chemical analysis and capillary electrophoresis of fresh cheeses and cheeses that had been ripened for 1, 2, 3 and 6 months were carried out. The results showed that high SCC levels in milk affected the moisture content of only freshly made cheeses and the pH, fat content and fat acidity of ripened cheeses. Regarding proteolysis, the levels of all β-CNs in freshly made cheeses were significantly lower as the SCC values increased and the Castellana breed was the most affected by SCC levels because a significant decrease in all α-CNs was also observed as SCC levels rose. Analysis of the casein profile by principal component analysis (PCA) revealed that there were no clear differences according the SCC up to the third month. However in the third and sixth months cheeses with low levels of SC were closely grouped and characterised by the highest levels of intact caseins. Regarding the effect of breed, the results point to a more intense proteolytic activity in the Assaf breed, whose more matured cheeses showed the highest content of casein proteolytic fragments.

Keywords: Capillary electrophoresis, cheese, ripening, caseins, proteolysis.

Ewes' milk is used almost exclusively for the production of cheese, and its cheese-making qualities depend on the milk characteristics especially on the concentration of intact casein (Fantuz et al. 2001). Previous studies have shown that high Somatic Cell Counts (SCC) are positively correlated with total nitrogen, non-protein nitrogen and whey proteins (Pirisi et al. 1996, 2000; Leitner et al. 2003; Bianchi et al. 2004; Vivar-Quintana et al. 2006), although the results of such studies are not always in agreement. Indeed, high SCC affords lower levels of β_1-, β_2-, and α_{s1}-I-casein, and α-lactalbumin (Rodríguez-Nogales et al. 2007) because milk from mastitic udders exhibits increased proteolytic activity (Albenzio et al. 2004).

Despite the significance of proteolysis on the quality of ewes' milk hard cheese, the effect of SCC on this process is less well documented. Some authors have reported significantly higher percentages of proteolysis in cheeses made from high-SCC milks, together with some sensory defects (Jaeggi et al. 2003; Revilla et al. 2007), because increasing levels of SCC proteolytic enzymes will contribute to proteolysis in cheese (Marino et al. 2005), and the hydrolysis of caseins directly affects the development of the desired texture and aroma, and the intensity of the background flavour of matured cheese (Fox & McSweeney, 1996). Nevertheless, Pirisi et al. (2000) have reported that milk SCCs have no effects on secondary proteolysis or on sensory characteristics. Much less is known about the effect of SCCs on the different casein fractions, and although in fresh cheese α-casein is more proteolysed than β-casein, in most mature cheeses the percentage of hydrolysis of β-casein is similar to that of α-casein (Revilla et al. 2007).

Taking into account that few studies have investigated proteolytic patterns in ewes' cheeses by means of capillary electrophoresis and that these patterns are probably affected by SCC levels, the aim of the present work was to monitor the evolution of different casein fractions during the ripening process of hard ewes' cheese made from the milk of two Spanish local breeds (Churra and Castellana) and one foreign breed (Assaf) in order to determine ifthat the effect of SCC was independent on the breed. In Spain, the Assaf breed is becoming increasingly important but some works have reported higher incidence of mastitis compared with local breeds (González-Rodríguez et al. 1995; Gonzalo et al. 2005).

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Table 1. Mean values and (SD) for composition parameters of cheeses depending on SCC or breed

		Month	pH	Moisture%	Fat%†	Protein%‡	Ash%§	Fat acidity¶
SCC	$<500{,}000$ ($n=18$)	0	5·07 (0·09)	$38{\cdot}95^{a}$ (0·54)	53·41 (4·65)	40·36 (4·29)	4·96 (1·06)	0·75 (0·44)
		1	4·98 (0·05)	33·93 (4·64)	52·81 (0·68)	37·48 (2·34)	4·73 (0·32)	1·13 (0·76)
		2	5·04 (0·01)	26·24 (1·46)	51·78 (0·93)	38·38 (2·12)	4·89 (0·79)	$1{\cdot}08^{a}$ (0·28)
		3	$4{\cdot}83^{a}$ (0·01)	21·43 (2·77)	51·72 (0·75)	32·64 (2·23)	5·11 (0·42)	$1{\cdot}58^{a}$ (0·45)
		6	$4{\cdot}91^{a}$ (0·15)	16·88 (0·17)	$53{\cdot}66^{b}$ (1·04)	35·13 (0·17)	5·14 (0·90)	$3{\cdot}87^{a}$ (0·71)
	10^{6}–$1{\cdot}5\times10^{6}$ ($n=18$)	0	5·15 (0·05)	$41{\cdot}20^{b}$ (1·86)	51·61 (1·77)	41·60 (1·70)	4·10 (0·42)	0·67 (0·18)
		1	5·02 (0·02)	35·63 (0·72)	51·59 (2·79)	37·48 (3·32)	4·60 (0·29)	1·01 (0·26)
		2	5·12 (0·06)	25·51 (1·87)	52·20 (1·20)	36·30 (1·00)	4·87 (0·30)	$1{\cdot}04^{a}$ (0·29)
		3	$4{\cdot}98^{b}$ (0·06)	23·55 (1·73)	49·92 (2·10)	31·95 (1·12)	4·65 (0·06)	$1{\cdot}70^{a}$ (0·62)
		6	$5{\cdot}51^{b}$ (0·29)	17·55 (1·30)	$50{\cdot}20^{a}$ (0·92)	34·85 (1·30)	5·36 (0·26)	$5{\cdot}23^{b}$ (0·13)
	$>2{\cdot}5\times10^{6}$ ($n=12$)	0	5·10 (0·01)	$40{\cdot}72^{b}$ (3·22)	48·16 (1·56)	42·04 (0·10)	4·08 (0·22)	0·81 (0·08)
		1	5·02 (0·03)	31·86 (1·22)	51·64 (3·93)	36·53 (1·57)	4·73 (0·29)	1·22 (0·30)
		2	5·07 (0·01)	28·13 (1·08)	51·52 (2·75)	41·62 (4·22)	5·02 (0·34)	$1{\cdot}71^{b}$ (0·20)
		3	$4{\cdot}97^{b}$ (0·09)	21·18 (5·20)	50·93 (4·63)	34·86 (4·24)	5·05 (0·41)	$3{\cdot}17^{b}$ (1·16)
		6	$5{\cdot}37^{b}$ (0·10)	18·08 (1·28)	$51{\cdot}32^{a,b}$ (2·58)	35·56 (1·28)	5·12 (0·38)	$6{\cdot}02^{b}$ (0·38)
Breed	Churra	0	5·16 (0·04)	41·86 (2·26)	49·58 (3·54)	42·49 (0·41)	4·21 (0·51)	4·27 (0·41)
		6	5·32 (0·23)	17·60 (0·59)	51·06 (1·93)	35·17 (1·35)	5·65 (0·43)	5·07 (0·32)
	Castellana	0	5·06 (0·05)	39·06 (1·02)	53·41 (3·52)	39·52 (2·99)	3·81 (0·11)	4·90 (0·23)
		6	5·29 (0·76)	17·89 (2·39)	52·02 (4·00)	34·71 (0·32)	4·73 (0·43)	4·38 (1·42)
	Assaf	0	5·09 (0·07)	39·41 (1·05)	51·95 (3·79)	42·49 (0·51)	4·98 (0·48)	5·48 (3·38)
		6	5·16 (0·19)	16·96 (1·18)	52·36 (1·16)	35·38 (0·84)	5·12 (0·41)	4·48 (0·81)

†Fat content on a dry weight basis. ‡Total %N × 6·38, on a dry weight basis. §Ash on a dry weight basis. ¶mg of KOH per gram of fat
[a,b]Different letter in the same column means statistically significant differences between SCC groups within the same month of ripening

Materials and Methods

Milk samples were collected as previously described (Revilla et al. 2009b) and cheeses of each milk type were manufactured in accordance with the procedure reported by Revilla et al. (2009a).

Analytical methods

Cheeses were analysed for pH (potentiometric method, CRISON Basic20), fat (Van Gulik method, ISO, 1975), moisture (IDF, 1982), ash (AOAC, 2000) and fat acidity (IDF, 1969). Total nitrogen (AOAC, 1995) was determined using the Kjeldahl method and the results were expressed as protein equivalents (TN × 6·38) on the basis of dry extract.

Capillary electrophoresis of cheese

Ewes'-milk cheeses that had been ripened for 0, 1, 2, 3 and 6 months were used to obtain isoelectric ovine casein. The caseins were extracted by the method of Ibañez et al. (1995) and analysed by capillary electrophoresis according to the method reported by Revilla et al. (2007). Each sample was analysed three times and the average of the relative area of each peak was calculated.

Statistical analyses

The data for each variable were analysed by one-way analysis of variance (ANOVA). The statistical significance of a factor (sample) was calculated at the $\alpha=0{\cdot}05$ level, using the *F*-test. When significant, the LSD Fisher-test was employed to test for statistically significant differences between means. Among pattern recognition tools, Principal Component Analysis (PCA) was used for data treatment. All statistical analyses were carried out using the Statgraphic Plus for Windows Computer Package (1995 Manugistics, Inc.).

Results and Discussion

Cheese characteristics

Moisture showed higher values as SCC values rose in freshly elaborated cheeses (Table 1), and this is in agreement with previous reports that showed a tendency for the cheese made from the high SCC milk to possess statistically significant higher humidity (Pirisi et al. 2000; Jaeggi et al. 2003).

Previous authors have reported that cheeses made from mastitic milk have higher moisture contents than those made from normal milk (Grandison & Ford, 1986; Mitchell et al. 1986; Barbano et al. 1991), apparently due to an alteration in the milk protein composition and mineral balance (Munro et al. 1984), although some studies have reported that somatic cells themselves may contribute to the increase in the cheese moisture content (Marino et al. 2005). However, in ripened cheeses there were no statistically significant differences in moisture content due to the SCC.

This was the only difference detected in fresh cheese due to the SCC, unlike that which was observed for the composition of the starting milk, in which significantly higher pH values and significantly lower fat contents where

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Table 2. Mean values and (SD) for casein composition of freshly produced cheeses expressed as electropherogram area

	Assaf			Churra			Castellana			SCC			Breed		
SCC ml^{-1}	Low	Medium	High	Low	Medium	High	Low	Medium		Low	Medium	High	Assaf	Churra	Castellana
α_{s2}-CN	141·2^{x} (65·7)	140·9 (65·7)	93·2 (18·8)	162·5^{x} (16·9)	144·2 (37·0)	176·9 (79·2)	299·7^{b,y} (7·2)	98·3^{a} (24·7)		181·4 (74·4)	127·8 (46·8)	135·1 (69·5)	125·1 (53·6)	161·2 (49·8)	165·4 (105·8)
α_{s1}-I-CN	332·2 (164·2)	282·2^{y} (35·8)	210·7 (34·2)	282·9^{b} (13·3)	170·8^{a,x} (37·9)	273·2^{b} (48·9)	317·2^{b} (9·8)	234·5^{a,y} (28·6)		309·4^{b} (100·1)	229·2^{a} (56·9)	241·9^{a} (51·4)	333·3 (202·7)	242·3 (65·1)	262·1 (48·1)
α_{s1}-II-CN	339·6 (195·8)	331·7^{y} (7·6)	254·2^{x} (31·9)	318·8 (46·9)	266·0^{x} (51·1)	321·8^{y} (42·2)	396·5^{b} (8·9)	275·3^{a,x} (15·6)		342·6 (120·1)	291·0 (41·4)	288·0 (49·9)	308·5 (111·2)	302·1 (50·1)	315·6 (63·8)
α_{s1}-III-CN	132·2 (86·6)	121·1 (8·4)	96·8 (19·4)	119·3 (22·0)	134·6 (39·5)	135·6 (28·4)	152·6^{b} (5·0)	110·4^{a} (15·4)		131·1 (53·2)	121·9 (24·8)	116·1 (30·6)	116·6 (49·1)	129·7 (28·9)	124·4 (24·8)
β-CN	202·4 (101·1)	149·8^{y} (16·7)	115·5^{y} (10·2)	165·4^{b} (24·3)	93·6^{a,x} (25·9)	157·9^{b,y} (18·8)	177·9^{b} (3·37)	137·9^{a,y} (16·1)		182·74^{b} (62·6)	127·1^{a} (31·1)	136·7^{a} (26·7)	155·8 (65·5)	138·9 (143·5)	151·2 (24·2)
β_2-CN	946·6^{b} (367·5)	613·5^{a,y} (41·7)	507·6^{a,x} (67·3)	674·1^{b} (55·9)	406·6^{a,x} (83·4)	670·1^{b,y} (53·1)	759·9^{b} (20·6)	560·0^{a,y} (46·4)		800·26^{b} (254·14)	526·7^{a} (106·5)	588·9^{a} (103·4)	689·6 (357·3)	583·6 (143·5)	626·6 (109·7)
β_1-CN	1604·6^{b} (789·0)	960·1^{a} (32·3)	836·8^{a,x} (157·3)	994·0^{a,b} (114·7)	827·5^{a} (198·7)	1079·3^{b,y} (73·1)	1126·3^{b} (62·7)	809·8^{a} (63·6)		1264·7^{b} (501·4)	865·8^{a} (130·6)	958·9^{a} (171·6)	1134·4 (591·2)	966·9 (166·5)	915·3 (173·2)
I-α_{s1}-CN	111·6^{b,y} (15·8)	58·2^{a,x} (15·8)	67·6^{a,b,x} (43·2)	93·4^{a,y} (6·5)	150·4^{b,y} (48·0)	133·7^{a,b,y} (17·2)	63·2^{x} (5·8)	74·1^{x} (13·8)		94·6 (21·2)	94·2 (50·1)	100·6 (46·6)	79·2^{x} (35·14)	125·8^{y} (36·4)	70·4^{x} (12·3)
γ-CN	97·9^{b} (38·3)	66·7^{a,b} (5·1)	46·5^{a,x} (3·5)	80·3^{b} (11·2)	48·0^{a} (29·6)	70·2^{a,b,y} (2·8)	77·2 (7·8)	63·9 (11·0)		86·7^{b} (25·1)	59·4^{a} (18·8)	58·3^{a} (12·9)	70·3 (30·0)	66·1 (21·7)	68·3 (11·4)

^{a,b} Different letter in the same row means statistically significant differences at $p < 0{\cdot}05$ due to the SCC
^{x,y} Different letter in the same row means statistically significant differences at $p < 0{\cdot}05$ due to the breed

observed when SSC increased (Revilla et al. 2009b). This absence of significance may be due to the normalization of pH at 6·5 before the addition of the rennet, as indicated by Pirisi et al. (2000).

Regarding pH, statistically significant differences were observed as SCC increased from low to medium and high SCC at the last stages of ripening considered. This result is due to the higher proteolytic activity, which produces a release of amino acids and leads to higher pH values (Sousa et al. 2001).

The fat content of the cheeses declined as SCC increased and the difference was statistically significant in the 6-month-old cheeses. As SCC increased, higher values of fat acidity were also observed that were statistically significant for high-SCC milk cheese up to the second month of ripening. This is attributable to an increase in lipolytic activity in the highest-SCC milks due to the lipase activity of somatic cells (Delandes, 1998), which produces higher concentrations of free fatty acids (FFAs) (Salih & Anderson, 1979; Bachman et al. 1988).

The ash and protein contents did not show any significant variations due to the change in SC levels at any of the ripening states, in agreement with previous results (Pirisi et al. 2000; Revilla et al. 2007, 2009a).

Regarding the effect of breed, cheeses of the three breeds evolved in a similar fashion and no significant differences were observed in any of the months of ripening for any of the parameters studied. These results agree with those obtained for 12-month old cheeses showing a lack of significant differences in physico-chemical parameters due to breed and statistically significant differences for pH, fat and fat acidity due to SCC levels. Indeed, the tendency for high-SCC cheeses to have higher moisture contents was confirmed by the results for 12-month old cheeses (Revilla et al. 2009a).

Protein profile of freshly produced cheeses

The casein composition of recently manufactured cheeses expressed as peak electropherogram area is shown in Table 2. The caseins were identified according to the electropherogram order of elution, as previously reported by Revilla et al. (2007).

Regarding α_{s1}-CNs, the α_{s1}-I-CN variant showed significantly lower levels with the increase in SCCs that were statistically significant for local breeds. The other two α_{s1}-caseins (α_{s1}-II-CN and α_{s1}-III-CN) and α_{s2}-CN levels were not affected by the SCC, although the Castellana breed showed statistically lower values of these caseins as SCC levels rose. The results also showed that the cheeses made from low SCC milk had the highest levels of β-CN, β_1-CN and β_2-CN.

The results concerning the influence of SCCs on α_s-CN are in agreement with what has been reported for the starting milk (Rodríguez-Nogales et al. 2007). However, for the β-CN in fresh cheese, a stronger influence of SSCs was observed, such that the levels of β-CN and β_1-CN were significantly lower in the medium- and high-SSC cheeses, whereas in the

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milk there was either no effect (β-CN) or it was significant only for high SCC-milk (β_1-CN).

Higher proteolysis of caseins, especially of β-CN, in agreement with the results of Marino et al. (2005), as SCC rise can be attributed to the higher moistures in the medium and high SCC cheeses (Jaeggi et al. 2003). High moisture levels are known to accelerate proteolysis, the hydrolysis of β-CN being more favoured than α-CN hydrolysis (Creamer, 1970). Moreover, the higher β-CN proteolysis may also be due to a higher plasmin activity reported in high SCC milk (Zachos et al. 1992; Gilmore et al. 1995). This higher proteolysis in high-SCC cheeses was specially important in the case of the Assaf breed because high-SCC milk of the Assaf breed had the highest contents of all the individual caseins studied when compared with low and medium-SCC milks (Revilla et al. 2009b), however the corresponding high-SCC cheese had lower values than low and medium-SCC cheeses. In contrast, for the autochthonous breeds the effect of SSCs on fresh cheese coincides with those found in the starting milk (Revilla et al. 2009b).

Regarding the effect of breed, no significant effect of this factor on the cheese casein composition was observed, differing from that observed for the milk (Rodríguez-Nogales, 2007; Revilla et al. 2009b).

With respect to the fragments due to casein hydrolysis, the I-α_{s1}-CN fragment increased with the SCC, the differences being statistically significant for the local breeds, although in the case of the Assaf breed the result was the opposite. Study of the breed factor revealed that the Churra breed showed the highest levels of this fragment while the Castellana breed had lower values; this could be related to the better textural properties of Castellana breed curds (Revilla et al. 2009b).

On the other hand, despite the higher degree of β-CN hydrolysis shown for the cheeses with high SCCs a significant decrease in the levels of the γ-CN fragment, which is the main product of plasmin β-CN hydrolysis, with the SCCs was observed as previously reported by Revilla et al. (2007). The β-CNs can be hydrolyzed to β-I peptides which may undergo further hydrolysis producing β-II and β-III peptides and other minor breakdown products (Mulhivill & Fox, 1978; Trujillo et al. 2000). Finally, no effect of breed factor was observed for the γ-CN fragment.

In general, these results agree with those reported in previous research indicating that when somatic cell counts increase the proteolytic activity increases (DeRham & Andrews, 1982; Verdi et al. 1987), reflecting lower levels of intact caseins and a poorer textural quality (Revilla et al. 2009b).

Study of the effect of SSC on proteolysis

The casein fractions (α_{s2}-CN, α_{s1}-I-CN, α_{s1}-II-CN, α_{s1}-III-CN, β-CN, β_2-CN, β_1-CN) and their fragments (I-α_{s1}-CN and γ-CN) of all the ewe's cheeses were evaluated by principal component analysis (PCA). The results of the statistical evaluation revealed the existence of four significant principal components (eigenvalues greater than unity) explaining

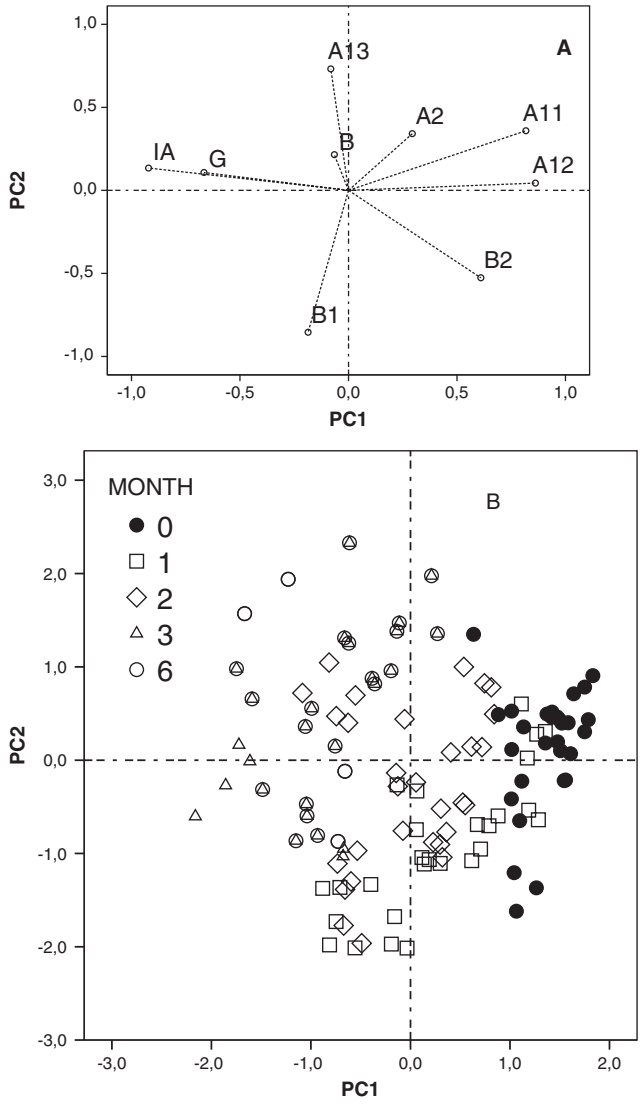

Fig. 1. Loading plot (A) and score plot (B) of first two principal components for classification of samples of cheeses according their ripening time (0–6 months). Codes: A2 = α_{s2}-CN; A11 = α_{s1}-I-CN; A12 = α_{s1}-II-CN; A13 = α_{s1}-III-CN; B = β-CN; B2 = β_2-CN; B1 = β_1-CN; IA = I-α_{s1}-CN; G = γ-CN.

82·1% of the variance contained in the original data. PC1 explained 35·6% of the variance; strongly and positively contributing variables were α_{s1}-I-CN, α_{s1}-II-CN, and β_2-CN (Fig. 1A). PC1 also had a high but negative contribution from the casein fragments I-α_{s1}-CN and γ-CN. PC2 explained 20·7% of the variance and had a high and positive contribution from α_{s1}-III-CN and a negative contribution from β_1-CN.

An analysis of the samples as a function of their ripening time revealed a separation along the PC1 axis (Fig. 1B). The freshly manufactured cheeses were characterized by the highest values of PC1, thus pointing to the highest contents of intact casein (mainly, α_{s1}-I-CN, α_{s1}-II-CN, and β_2-CN), while the samples of hard cheeses (3-, and 6-month-old

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cheeses) were located at negative values of PC1 owing to their higher levels of casein fragments (I-α_{s1}-CN and γ-CN). PC1 was therefore critical in order to classify the samples according to their state of maturity. The values of the loadings showed that the protein fractions α_{s1}-I-CN and α_{s1}-II-CN underwent the strongest degree of hydrolysis. The evolution of the three β-CNs (to a lesser degree for the β_2-CN) shows that this group of proteins was subject to a lower degree of hydrolysis than the alpha caseins, in agreement with previous findings for ewes' cheeses, since their maximum hydrolysis was 40% while in the case of the alpha caseins this value reached 80%. This percentage is similar to or slightly higher than that observed for other ewes' cheeses with the same ripening time (Freitas et al. 1997; Albillos et al. 2006; Revilla et al. 2007). In the case of the β-CN and β_1-CN, these did not undergo an important degree of proteolysis during the ripening time, while the evolution of the β_2-CN variant pointed to a gradual and continuous decrease in intact protein levels throughout maturation.

The samples of cheeses of different SCC levels or from different breeds overlapped in the space defined by the first two PCs. Thus, new PCA was performed for each ripening time because no clear differences were found among the protein profiles of the samples according their SCC or breed.

The results of PCA for 1-month-old cheeses gave three significant components with eigenvalues greater than unity, accounting for 77% of the total variance. From the loadings of the variables, the most influential variables on the positive first principal component (PC1) were the caseins α_{s1}-I-CN and α_{s1}-II-CN, and for the negative PC1 they were β_1-CN and the casein fragments I-α_{s1}-CN and γ-CN (Fig. 2A1). The graphic representation of the 1-month-old cheeses in the space defined by the first two components did not show clear separation among the samples according to their SCC (Fig. 2B1). However, a certain separation of the Assaf breed samples was observed, these being located in the upper left quadrant. This result is in concordance with that observed in freshly made Assaf cheeses, characterized by a higher proteolytic activity that continued until this point of maturation. Similar results were found for the PCA for 2-month-old cheeses, where the score plot shows an overlapping among the samples with low, medium and high levels of SC.

The results obtained applying this statistical analysis to samples with three months of ripening revealed that PCA allowed 85% of the total variance to be explained by the four significant components found. From the loadings of the variables (Fig. 2A2), the most influential variables on the first principal component (PC1) were β_2-CN, β-CN, α_{s1}-II-CN, and α_{s1}-I-CN with positive loading values, and again I-α_{s1}-CN with negative loading values. For the second principal component (PC2), the most important variables were α_{s1}-II-CN, α_{s1}-I-CN, and α_{s2}-CN (positive loadings) and β_1-CN (negative loading). The samples of 3-month-old cheeses with low SCC were grouped and scattered to the right of the origin on the axis of PC1 (Fig. 2B1). This points to a lower degree of proteolysis, as evidenced by the fact that these samples were characterized by high concentrations of intact caseins; mainly, β_2-CN, β-CN, α_{s1}-II-CN, and α_{s1}-I-CN. Unlike the results obtained for 1-month old cheeses, no groups according to breed were observed (Fig. 2C2), revealing a strong influence of SCC on the proteolytic process.

Regarding the results obtained with PCA for the 6-month-old cheeses (Fig. 2A3), four significant components with eigenvalues greater than unity were found, the two first PC accounting for almost 55·3% of the variance. There was positive contribution to PC1 by α_{s2}-CN, α_{s1}-I-CN, and β-CN and negative contribution by I-α_{s1}-CN, γ-CN, and β_1-CN with loading values of 0·77, 0·73, 0·703, −0·73, −0·55, and −0·55, respectively, while PC2 had a positive contribution from α_{s1}-II-CN (loading value 0·63), and a negative contribution from β_2-CN (loading value of −0·89). It can be observed that 6-month-old cheeses with low levels of SC were closely grouped and characterised by positive values of PC1, thus illustrating a more intense effect of SCC on the degree of casein hydrolysis as ripening progressed. The low SCC samples had the highest levels of intact alpha and beta caseins while the samples with medium and high SCC had negative PC1 values (Fig. 2B2). These cheeses showed a higher degree of hydrolysis, as evidenced by their higher levels in I-α_{s1}-CN and γ-CN.

Previous studies have shown that the increase in SCC can increase plasmin activity in ewes' milk, enhancing the hydrolysis of β-CNs and their main proteolytic fragments, γ-CNs (Bianchi et al. 2004), as was observed here. However, the action of plasmin did not account for the increase in I-α_{s1}-CN generated through the degradation of the α_{s1}-CN fraction (Strickland et al. 2001; Gorostiza et al. 2004). Thus, the increase in proteolysis of the α_{s1}-CN fraction observed in high-SCC milk cheeses may be attributed to other endogenous milk enzymes, such as cathepsin D, or to enzymes from somatic cells, such as cathepsin G, elastase or cathepsin B (Considine et al. 2000, 2002, 2004).

Although there was no clear sample separation according to breed (Fig. 2C3), most of the Assaf samples had negative values of PC1 owing to their higher contents in casein fragments. This could be the reason for the lower overall consumer acceptability reported for Assaf cheeses (Revilla et al. 2009a). Higher levels of proteolysis and the increase in the I-α_{s1}-CN fragment are correlated with textural problems (Sousa et al. 2001; Irigoyen et al. 2000). The study of correlations among proteolytic fragments and sensory parameters of these cheeses (Revilla et al. 2009a) revealed a positive correlation between graininess and both I-α_{s1}-CN (0·489) and γ-CN (0·606), but also a positive correlation among these fragments and hardness (0·667 and 0·515, respectively). Indeed, higher levels of γ-CN were correlated with a lower creaminess (−0·478) and consumer preference (−0·538), pointing to the relevance of the proteolytic process on the sensory characteristics of cheeses.

Conclusions

The results obtained point to a significant increase in proteolysis as SCC levels rise. Thus, in freshly made cheeses

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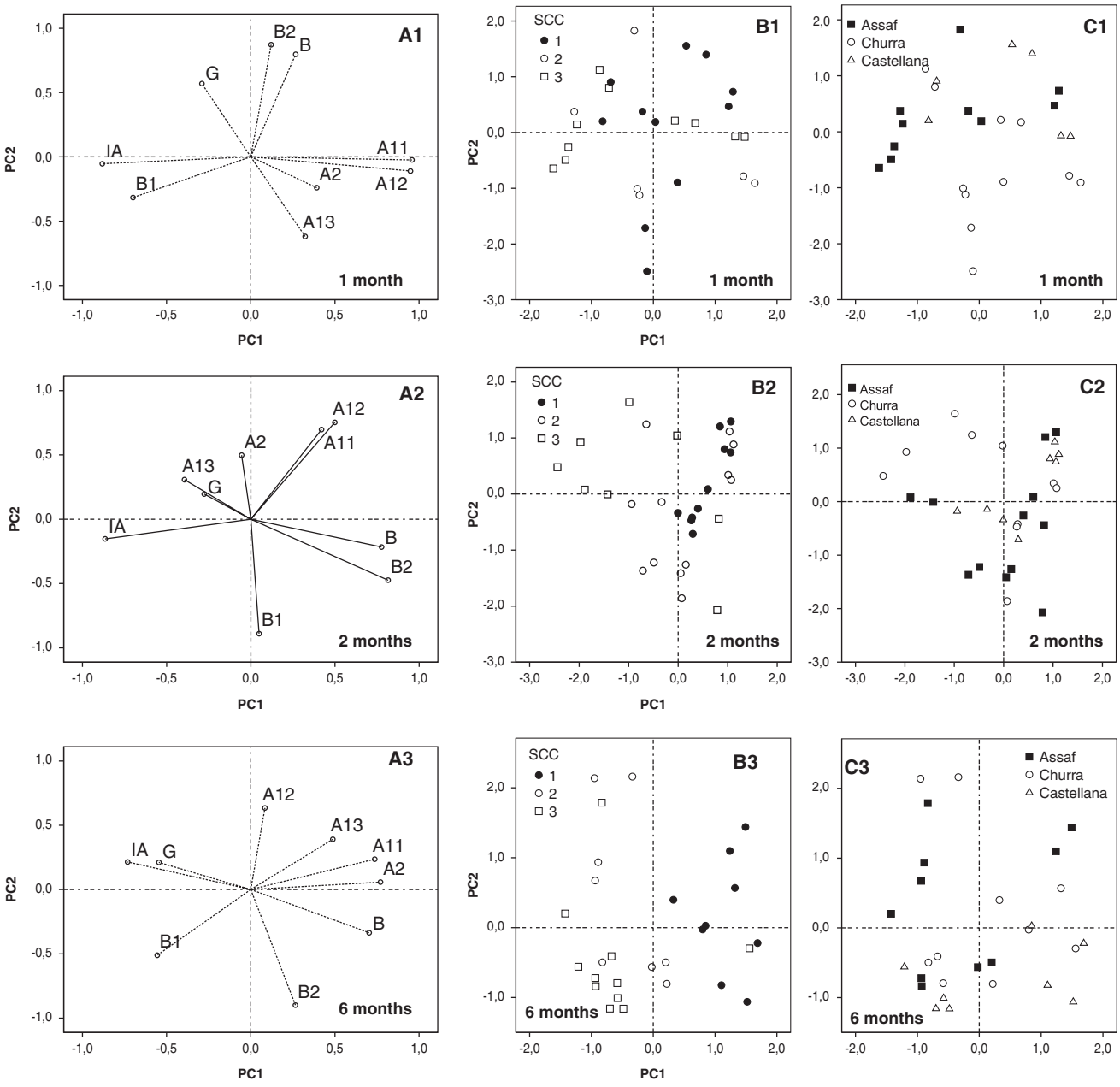

Fig. 2. Loading plot (A) and score plot (B and C) of first two principal components for classification of samples of cheeses according their ripening time (1, 3 or 6 months), somatic cell accounts (B) or breed (C). Codes: 1 = < 500,000 SCC ml^{-1}; 2 = 10^6–$1{\cdot}5 \times 10^6$ SCC ml^{-1}; 3 = $2{\cdot}5 \times 10^6$ SCC ml^{-1}. A2 = α_{s2}-CN; A11 = α_{s1}-I-CN; A12 = α_{s1}-II-CN; A13 = α_{s1}-III-CN; B = β-CN; B2 = β_2-CN; B1 = β_1-CN; IA = I-α_{s1}-CN; G = γ-CN.

the levels of α_{s1}-I-CN and β-CNs were significantly lower as the SCC values increased. An important influence of breed was observed, the Castellana breed being the most affected by SCC. As ripening progressed, a decrease in the fat content and an increase in pH and fat acidity in high-SCC cheeses were observed. Indeed, the analysis by PCA of casein fractions and their fragments at different ripening times revealed that in the third and sixth months low-SCC cheeses were closely grouped and were characterised by the highest levels of intact caseins, while samples with medium and high SCC were characterised by their higher levels in I-α_{s1}-CN and γ-CN.

Regarding breed, the results also point to a more intense proteolytic activity in Assaf breed cheeses, characterized by a strong decrease in intact caseins in freshly made cheeses and by the higher content of casein proteolytic fragments of the more matured cheeses.

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