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Effect of Freezing on Soluble Nitrogen Fraction of Cabrales Cheese

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ABSTRACT

The present study deals with changes in the free amino acid content (by HPLC) and soluble nitrogen fraction (PAGE) in artisanal Cabrales cheese and in samples from these kept in frozen storage for four and eight months before ripening.

The amino acids in highest concentration at the end of ripening in all batches (control and frozen) were glutamic acid, leucine and lysine, which together accounted for 35·1–39·3% of the total amino acids.

The free amino acid content was similar to that in the control batch in cheeses that underwent frozen storage for eight months, and somewhat lower than in the control batch for cheeses frozen for four months.

Proteolysis of whey proteins was low. α-Lactalbumin and β-lactoglobulin remained at the end of the ripening period. Similar results were obtained for the batches of frozen cheese.

INTRODUCTION

Cabrales cheese is typical of the mould-ripened cheese varieties manufactured in Spain. It is an artisanal blue cheese made primarily of cows' milk with 20–30% of goats' and ewes' milk. Some studies on the microbiological and physicochemical aspects of this cheese have been published (Nuñez, 1978; Juarez *et al.*, 1983).

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In view of the seasonal nature of ewes' and goats' milk production, additional research has been directed at freezing curds in an effort to make the manufacture of cheeses that use the milk of these species as raw material, independent of fluctuations in availability (Dalles *et al.*, 1984; Filchacova *et al.*, 1983).

The present study was undertaken to discover the effects of the freezing process and frozen storage (four and eight months) on the soluble nitrogen fraction of Cabrales cheese.

MATERIALS AND METHODS

Cheese samples

Two batches of Cabrales cheese were prepared according to traditional methods (Nuñez, 1978). No lactic starter or mould spore powder was inoculated into the milk or curd. After drying at room temperature for ten days the cheeses were ripened in natural mountain caves, where a temperature of 9–10°C and a relative humidity of 90–95% favour the growth of natural microflora, in particular *Penicillium roqueforti* (Nuñez, 1978). One-third of the sample cheeses were allowed to ripen under these conditions, and the remaining two-thirds were flash frozen in a Sabroe-Aathus (Denmark) plate freezer designed to attain temperatures of −40°C with a freezing velocity of 1·33 cm/h. After freezing, samples were stored at −20°C for four or eight months. Following this storage period the cheeses were thawed and ripened in caves by traditional methods under conventional conditions for four months.

Physicochemical analysis

The pH, fat, total solids, total nitrogen, ash and NaCl were analyzed according to the standards of the International Dairy Federation (IDF).

Water-soluble nitrogen-gel electrophoresis

Soluble extracts of the cheese were obtained using Skir's method (Stadhouders, 1960) and then freeze-dried. Samples containing 20–50 μg of protein were analyzed by vertical slab gel electrophoresis using Hillier's method (Hillier, 1976), T = 9·4%, C = 4·25% at pH 8·9. The slab was stained with Coomassie Blue without discoloration (Blakesley & Boezi, 1977).

Whey proteins of cows', goats' and ewes' milk, and proteose peptone obtained in our laboratory (Ramos *et al.*, 1986) were used as standards.

High performance liquid chromatography of free amino acids

The amounts of individual free amino acids in the sulphosalicylic acid (SSA)-soluble fraction, were determined by high performance liquid chromatography of the *o*-phthaldialdehyde derivatives of the amino acids. All separations were performed using a Waters Associate device with two Model 6000 A pumps, a 720 system controller, a RCM-100 radial compression module, and a U6K injector. Fluorescence was detected using a 420 AC fluorimeter with standard flow cell and standard filters (340 ± 6 nm excitation filter and 425 nm emission (long pass) filter). A reverse phase column (10 cm × 8 mm ID), Radial Pak C-18 (10 μm) and a Bondapak C-18/Corasil (37–50 μm) guard column were used.

An aliquot of a sample containing 14 μg of amino nitrogen was allowed to react with 0·5 ml of *o*-phthaldialdehyde/mercaptoethanol solution (prepared according to Cooper *et al.*, 1984) for exactly 1 min and then injected. Separation was performed at room temperature using a polarity gradient similar to that used by Jones *et al.* (1981).

RESULTS AND DISCUSSION

Composition

Table 1 shows the mean values of global composition from the two batches at the end of ripening, for the control (F–0) and those stored in the frozen state for four and eight months (F–4) and (F–8).

The results indicate that there are no noticeable differences, for the variables mentioned, between the control batch and frozen cheeses.

Free amino acids

Table 2 presents the mean values of the free amino acid contents of the control batch (F–0) and those stored in the frozen state for four and eight

TABLE 1
Global Composition of Four-Month-Old Cabrales Cheese. F–0 Control Batch, F–4 and F–8 Cheeses from Curds Frozen for Four and Eight Months Respectively

	pH	*Fat* (%)	*Protein* (%)	*Total solids* (%)	*Ash* (%)	*NaCl* (%)
F–0	6·77	30·6	24·5	61·28	5·52	3·73
F–4	6·37	32·3	22·6	60·97	4·89	4·34
F–8	6·39	30·0	22·4	59·10	4·83	3·54

TABLE 2
Free Amino Acid Contents (mg/100 g DM) of Four-Month-Ripened Cabrales Cheese. F–0 Control Batch, F–4 and F–8 Cheeses from Curds Frozen for Four Months and Eight Months Respectively

	F–0		*F–4*		*F–8*	
	mg/100 g DM	(%)	*mg/100 g DM*	(%)	*mg/100 g DM*	(%)
Aspartic acid	470	5·0	318	5·5	357	3·6
Glutamic acid	1 241	13·1	751	13·0	1 365	13·9
Asparagine	91	1·0	76	1·3	85	0·9
Serine	129	1·4	202	3·5	240	2·4
Glutamine	279	2·9	299	5·2	658	6·7
Histidine	229	2·4	229	4·0	441	4·5
Glycine	170	1·8	58	1·0	174	1·8
Threonine	496	5·2	198	3·4	210	2·1
Alanine	309	3·3	344	6·0	568	5·8
γ-aminobutyric acid	59	0·6	150	2·6	41	0·4
Tyrosine	545	5·8	258	4·5	767	7·8
α-aminobutyric acid	43	0·4	42	0·7	68	0·7
Arginine	46	0·5	29	0·5	39	0·4
Methionine	440	9·6	185	3·2	428	4·4
Valine	735	7·8	454	7·9	588	6·0
Tryptophan	107	1·1	66	1·1	198	2·0
Phenyl alanine	719	7·6	401	6·9	676	6·9
Isoleucine	673	7·1	359	6·2	500	5·1
Leucine	1 228	13·0	773	13·4	1 351	13·8
Ornithine	193	2·0	74	1·3	142	1·4
Lysine	1 246	13·2	504	8·7	904	9·2
Total	9 448		5 770		9 800	
Tyramine	314		129		70	
Histamine	40		15		5	

months (F–4 and F–8) after four months of ripening. The coefficient of variation obtained in the four replications performed on samples from a single cheese was less than 6% in each case, and was highest for the less prevalent amino acids (asparagine, glutamine and ornithine). Similar results were obtained in a previous study about free amino acids in Mahon cheese (Polo *et al.*, 1985) in which dansyl chloride was used as the derivatization agent.

In the control batch and in the frozen batches at the end of ripening the amino acid contents were very high. This was in good agreement with Sala-Trepat & Burgos (1972) who studied Cabrales cheese for seven weeks. Ismail & Hansen (1972), in a study of different types of cheese, found a higher

content of free amino acids in Danablue cheeses. This is probably due to the proteolytic activity of *Penicillium roqueforti* which is responsible for cheeses ripening.

The amino acids in highest concentration at the end of ripening in all batches (control and frozen) were glutamic acid, leucine and lysine, which together accounted for 35·1–39·3% of the total amino acids. The percentage of each amino acid present was roughly constant.

The total amino acid content in the cheeses frozen for four months, was lower than in the control cheeses. During frozen storage, the microbial flora suffered partial destruction, which prevented normal proteolysis (Peláez, 1983). In spite of this, the aminogram profile was similar; the percentage of each amino acid was roughly the same as in the controls.

The total amino acid content in the cheeses frozen for eight months was similar to the control batches. In experiments on the freezing of Camembert-type curds, Hote-Baudart (1969) recorded enzyme activity even at temperatures of $-15°$ and $-20°C$. This might explain why the curds that underwent eight months of frozen storage had a higher amino acid content that those stored for four months at $-20°C$. Both these freezing phenomena, ongoing enzyme activity and partial destruction of microbial flora, essentially compensated each other, yielding final cheeses similar to the controls. The relative proportions of the various amino acids were also similar. Alonso (1985) found that the index of ripening (soluble nitrogen/total nitrogen × 100), for cheeses of this type frozen for four months, was lower than in control cheeses (77·5 against 88%). The values for that index for cheeses stored frozen for eight months (81·3%) were also lower than in the control cheese; they were higher than the values for cheeses frozen four months. Studies carried out by electrophoresis of caseins also indicated a lower level of proteolysis in frozen cheeses. From this result it is possible to conclude that the proteolysis is somewhat less intense in the frozen Cabrales cheeses when compared with the control cheese.

With this method it is possible to evaluate the concentration of histamine and tyramine. The content is lower in frozen cheeses than in the control batch.

Electrophoretic analysis of the soluble nitrogen fraction

Figure 1 shows the results of vertical slab electrophoresis on polyacrylamide gel of the soluble nitrogen fraction from Cabrales cheese at the four months of ripening of control and frozen batches for four and eight months.

A series of bands was visible for the soluble nitrogen fraction in all the samples. The mobility of these bands corresponded to that of the whey proteins serumalbumin, α-lactalbumin, and β-lactoglobulin A and B. Two

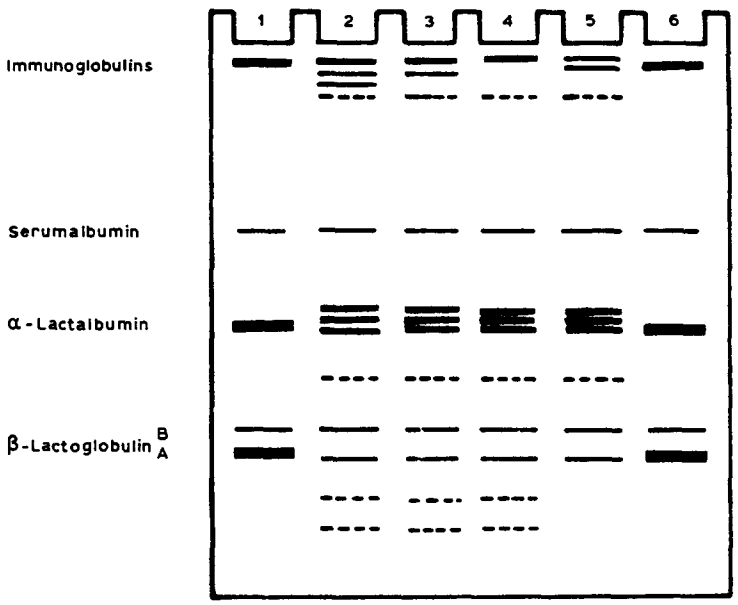

Fig. 1. Diagramatic representation of polyacrylamide gel electrophoresis patterns of soluble nitrogen in Cabrales cheeses. (1) and (6) whey proteins; (2) and (3) control cheeses after four months of ripening; (4) and (5) frozen cheeses (four and eight months) after four months of ripening.

bands migrated between serumalbumin and α-lactalbumin and these coincided with the β-lactoglobulins and the α-lactalbumin of goats' and ewes' milk, whose mobility is lower than that of the cows' milk proteins. The band between α-lactalbumin and β-lactoglobulin could correspond to that of proteose-peptone component 5. Component 5 was recently shown to be a product of the degradation of β-casein by plasmin and to correspond to the N terminus fragment of β-casein (Eigel *et al.*, 1984). Three or four bands of greater mobility than the β-lactoglobulins were also found to be present. The band with the highest mobility might be the fast proteose-peptone component consisting of residues 1–28 of the N terminus portion of β-casein (Eigel *et al.*, 1984). While studying the action of rennet and fermentation bacteria on Cheddar cheese, O'Keefe *et al.* (1978) also found a series of bands for the fraction soluble at pH 4·6 whose mobility coincided with that of proteose-peptone.

The results of electrophoretic analysis of the water-soluble nitrogen fraction in frozen cheeses were similar to those obtained for the controls. The whey proteins α-lactalbumin and β-lactoglobulins A and B were also found to be present at the end of the ripening period of both the control and frozen cheeses. This has also been reported for other types of cheese (O'Keefe *et al.*, 1978; Polo *et al.*, 1985). The soluble nitrogen/nonprotein

nitrogen fraction did not undergo any significant variation during ripening (Alonso, 1985).

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