Free Amino Acid Profiles During Ripening of Port Salut Argentino Cheese After Frozen Storage

R.A. VERDINI, S.E. ZORRILLA, AND A.C. RUBIOLO

ABSTRACT: Free amino acid profiles of Port Salut Argentino cheeses at different storage conditions (ripening with and without previous frozen storage), ripening times (1, 6, 13, 27, and 56 d), and cheese zones (central and external) were studied. Derivatization with o-phtaldialdehyde and a C_{18} column were used for chromatographic separations. Principal component analysis reduced data to 2 dimensions where unripened cheeses (1 to 13 d) were grouped independently of ripening time, sampling zone, and storage condition. Ripened cheeses (27 and 56 d) showed scattering according to ripening time. The contribution of free amino acid profiles to quality in cheeses preserved by frozen storage may be considered similar to that in traditionally ripened cheeses.

Keywords:free amino acids in cheese, principal component analysis, HPLC, cheese proteolysis, cheese frozen storage

Introduction

DURING CHEESE RIPENING, CASEIN IS DEGRADED TO BREAKDOWN products such as peptides and free amino acids. The extent of this degradation process plays an important role in determining cheese flavor and texture (Law 1987). Significant concentrations of amino acids occur in most of the cheeses that have been investigated (Polo and others 1985; Barcina and others 1995; Fox and McSweeney 1996; Frau and others 1997; Katsiari and others 2000). Every type of cheese has its own characteristic free amino acid pattern, resulting from the enzymatic degradation of peptides by various enzymes, and also from amino acid interconversion, and degradation (Polo and others 1985). Although none of the amino acids has a cheese-like flavor, those compounds contribute to the savory taste of mature cheese (Fox and McSweeney 1996). Catabolism of free aminoacids can result in a number of compounds, including ammonia, amines, aldehydes, phenols, indole and alcohols, which may also contribute to cheese flavor. Moreover, many studies have been undertaken to particularly determine biogenic amine content in cheeses, which are useful indicators of hygienic quality and cheese manufacturing conditions (Pinho and others 2001).

Freezing of dairy products is an alternative to extend stability or shelf-life. Freezing of cheese was normally avoided because of the tendency towards physical breakdown in body and structural characteristics caused by ice crystal formation (Webb and Arbuckle 1977). However, several authors found that frozen storage did not significantly affect cheese quality for some freezing conditions. Lück (1977) reviewed cheese preservation and discussed several results about the effect of frozen storage on cheese flavor and texture. Camembert cheeses frozen immediately after manufacture and ripened under regular conditions for up to 3 wk were acceptable. The quality of full-cream Gouda cheese was ranked satisfactory to good after being stored at –20 °C for 6 mo. Tempered Mozzarella cheeses frozen at different rates exhibited the same textural quality as refrigerated cheeses with no freezing rate effect (Bertola and others 1996). Moreover, Chaves and others (1999) showed that the extent of proteolysis of slowly frozen Mozzarella cheeses after thawing and tempering was similar to that obtained for control cheeses. However, there is scarce information related to changes in amino acid profiles of cheeses preserved by frozen storage.

On the other hand, salt content also influences the rate of casein hydrolysis (de Jong 1978; Law 1987). In cheeses salted by immersion in brine, there is a large salt gradient from the surface to the center. Salt distribution during ripening is a slow process and, therefore, salt concentration changes with ripening time and position in the cheese (Zorrilla and Rubiolo 1994). Moreover, the different NaCl concentration history for central and external zones can affect the primary casein degradation (Zorrilla and Rubiolo 1997). Therefore, it is also of great interest to analyze the effect of salt content on amino acid profiles at different positions in the cheese.

Considering that amino acid profiles generally lead to a large amount of data to be processed, principal component analysis (PCA) can be a useful tool to reduce dimensionality and to examine data variation. Pripp and others (2000a, 2000b) used PCA to evaluate the contribution of rennet and starter enzymes to proteolysis and also found that multivariate analysis of proteolytic profiles was a powerful method to differentiate between cheese varieties, cheese qualities, and starter strains.

In particular, the major cheese production in Argentina is based on soft cheeses, Port Salut Argentino cheese being one of the most popular varieties (Zalazar and others 1999). The Food Code of Argentina (CAA 1981) describes Port Salut Argentino as a semi-cooked cheese which is produced from pasteurized milk, acidified by lactic bacteria, coagulated by rennet, and ripened for a short period. Expanding commercialization of Port Salut Argentino cheeses has increased interest in preserving its characteristics for a longer storage period. Verdini and Rubiolo (2002a) applied PCA to study the ripening of Port Salut Argentino cheeses matured before freezing and reported that there was no effect of frozen storage time on cheese proteolysis. However, other promising alternatives such as frozen storage of unripened Port Salut Argentino cheese have not been studied yet. Further research using PCA of free amino acid profiles may be very useful for a better understanding of casein proteolysis during cheese ripening and for relating it to the preservation process.

The objective of our work was to evaluate the effect of frozen storage on the ripening of Port Salut Argentino cheeses analyzing free amino acid profiles at different ripening times and positions in the cheese.

Materials and Methods

Cheese samples

Commercial Port Salut Argentino cheeses (3.55 ± 0.11 kg wt, 23.2 ± 0.3 cm dia, 7.7 ± 0.3 cm ht, 28.7 ± 0.7 % w/w fat, 20.4 ± 0.9 % w/w total protein, and 48.8 ± 2.6 % w/w moisture) were manufactured at a local factory. Cheeses were salted in a brine solution for 3 h at 3 °C, stored for 20 h, and packed in heat-shrinkable plastic.

Thirty cheeses were transported in insulated boxes with ice from the factory to our laboratory and randomly separated into 2 groups. Fifteen cheeses were held at 5 °C for ripening (cheeses R). Fifteen cheeses were frozen in a Tabai Comstar PR 4GM chamber (Tabai Espec Corp., Osaka, Japan) at –30 °C until the center reached –22 °C. Cheeses were held in frozen storage at –22 °C for 30 d, then they were thawed at 5 °C. The freezing and thawing rates were approximately 4.5 °C/h and 1.5 °C/h, respectively. The temperature of the samples during the freeze-thaw cycle was monitored until the center reached the desired temperature using a Tabai Comstar THP–18 temperature recorder. After thawing, cheeses were held at 5 °C for ripening (cheeses F).

Three cheeses R and 3 cheeses F were sampled at different ripening times: 1, 6, 13, 27, and 56 d. Cubic pieces of 25 mm were cut as described by Creamer and Olson (1982) from 2 different cheese zones, central zone (C) and external zone (E), as shown in Figure 1. Sample identification was related to storage condition and sampling zone. Thus, a sample FC indicates a sample obtained from central zone and frozen before ripening.

Water-soluble fraction extraction

Grated cheese (10 g) mixed with 3 times the sample weight of water was homogenized using an Ultra-Turrax® T25 (IKA®Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany) homogenizer for 2 min (Kuchroo and Fox 1982). The homogenate was held at 40 °C for 1 h, pH was adjusted to 4.4 to 4.6, and the suspension was centrifuged for 30 min at 5 °C and 4800 rpm (Biofuge 28RS; Heraeus Sepatech, Osterode, Germany). After centrifugation 3 layers were obtained, the upper layer of fat was removed. The supernatant was filtered through Whatman nr 42 paper and diluted to 100 mL, constituting the water-soluble fraction.

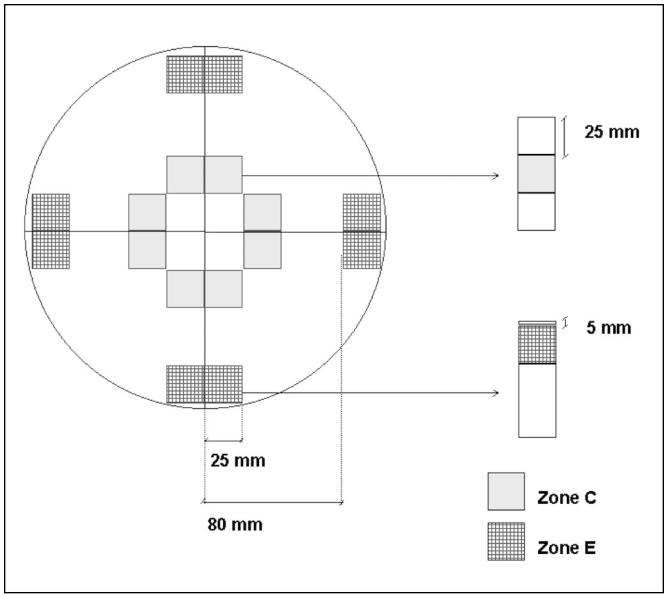

Figure 1—Schematic view of sampling: top-down view on the left and side-on view on the right

Total cheese nitrogen (TN) and water-soluble nitrogen (WSN) were determined using the micro-Kjeldahl method as described by Verdini and Rubiolo (2002b). Indexes of maturation were expressed as a percentage of the water-soluble nitrogen of the total cheese nitrogen content (IM = WSN × 100 / TN). All determinations were carried out in duplicate.

Free amino acid extraction and analysis

Sulfosalicylic acid (SSA) is one of the most commonly used precipitating agents for cheese samples prior to amino acid analysis (Reiter and others 1969; Polo and others 1985); therefore, it was used for the cleanup of the samples before derivatization and HPLC analysis. Precolumn derivatization was performed with o-phtaldialdehyde (OPA) as proposed by Jones and others (1981).

Two mL of 15% w/v SSA was added to 10 mL of the water–soluble fraction to reach a final concentration of 2.5% w/v SSA (Reiter and others 1969). The suspension was centrifuged for 30 min at 20 °C and 4800 rpm (Biofuge 28RS; Heraeus Sepatech). The supernatant solution was adjusted to pH 4.0 and stored in a freezer at –22 °C for chromatographic analysis.

Precolumn derivatization was as follows: 200 mL of standards or samples were mixed with 200 mL of 2% sodium dodecyl sulfate in 0.4 M sodium borate (pH 9.5) and then 200 mL of derivatizing solution was added. The resulting solution was mixed thoroughly and, after 1 min, 400 mL of 0.1 M potassium phosphate (pH 4.6) was added. Derivatizing solution was prepared as described by Jones and others (1981). The solution was mixed and filtered through a disposable 0.2-mm filter (Alltech Associates, Inc., Deerfield, Ill., U.S.A.) before 10 mL was injected. Samples were diluted before derivatization to fit the range of the calibration curves.

A chromatograph with a gradient programmer model 2360 (Isco, Inc., Lincoln, Nebr., U.S.A.), a fluorescence detector model FL-2(Isco, Inc.) was used. Chromatographic separation was accomplished with an Ultrasphere ODS (250 × 4.6 mm, particle size 5μ) C_{18} column (Beckman Instruments, Inc., Fullerton, Calif., U.S.A.) at 30 °C. The following fluorometer settings were used: 9 μL flowcell, excitation filter in the 305 to 375 nm range, emission filter in the 430 to 470 nm range, time constant of 0.5 s, and a sensitivity of 0.02 units.

Gradient elution was used for OPA derivative separation with solvent A: tetrahydrofuran:methanol:0.05 M sodium acetate, pH 5.9 (1:19:80), and solvent B: methanol:0.05 M sodium acetate, pH 5.9 (80:20). The gradient program was: initial composition 0% B, isocratic step at 0% B for 1 min, linear step to 14% B in 5 min, isocratic step at 14% B for 5 min, linear step to 50% B in 5 min, isocratic step at 50% B for 4 min, linear step to 100% B in 12 min, isocratic step at 100% B for 8 min. The flow rate was 1.7 mL/min. All the reactants used were of HPLC grade. Data were processed with the Chem Research Data System Program version 3.0.2. 1994 (Isco, Inc.).

Validation of the RP-HPLC method for free amino acids

Linear response for each of the 16 amino acids studied was evaluated and coefficient of determination (R^2) was obtained. Analytical sensitivity (γ) was determined as proposed by Arancibia and Escandar (1999):

$$\gamma = b/S_R \quad (1)$$

where b is the slope of the calibration curve and S_R is the standard deviation of the residuals. Repeatability was expressed as an aver-

Table 1—Validation of the simultaneous determination of 16 o-phtaldialdehyde derivatives of amino acids by RP-HPLC

Amino acid	Linear range mgL^{-1}	$R^{2(1)}$	$\gamma^{(2)}$mg^{-1}L	LD$^{(3)}$mg L^{-1}	LQ$^{(4)}$mg L^{-1}	Repeatability(%)
Asp	2.02 - 18.43	0.99	1.39	0.67	2.02	5.54
Asn	2.23 - 22.33	0.99	1.37	0.74	2.23	4.81
Ser	3.40 - 31.33	0.99	0.79	1.12	3.40	6.92
Gln	1.52 - 17.95	0.98	1.21	0.50	1.52	5.61
Gly	1.57 - 8.43	0.99	3.23	0.52	1.57	8.48
Thr	0.83 - 11.24	0.99	2.69	0.27	0.83	4.56
Arg	3.30 - 23.76	0.99	1.03	1.09	3.30	7.37
Ala	1.68 - 13.67	0.98	1.76	0.56	1.68	5.13
Tyr	2.50 - 21.43	0.99	1.17	0.82	2.50	4.94
Trp	4.05 - 13.40	0.97	0.68	1.34	4.05	7.89
Met	1.96 - 22.52	0.99	1.14	0.65	1.96	4.47
Val	0.77 - 13.19	0.99	2.52	0.25	0.77	3.98
Phe	1.45 - 23.67	0.98	0.80	0.48	1.45	4.85
Ile	1.45 - 17.95	0.99	2.21	0.48	1.45	2.99
Leu	1.23 - 17.62	0.98	1.16	0.41	1.23	4.08
Lys	4.36 - 36.86	0.96	0.41	1.44	4.36	13.43

(1)Coefficient of determination
(2)$\gamma = b/S_R$
(3)LD = 3.3 S_R^* /b
(4)LQ = 10 S_R^* /b

age percentage of the coefficients of variation of 4 concentrations 3 replicates each as suggested by the U.S. Food and Drug Administration (FDA 1996).

Limit of detection (LD) and limit of quantification (LQ) were calculated as suggested by the U.S. Food and Drug Administration (FDA 1996) with Eq. 2 and 3, respectively:

$$LD = 3.3\, S_R^*/b \quad (2)$$

$$LQ = 10\, S_R^*/b \quad (3)$$

where S_R^* is the standard deviation of the residuals corresponding to a calibration curve containing the analyte in the low-concentration zone.

Statistical analysis

Data were analyzed using ANOVA. When differences between treatment effects were significant ($p < 0.05$), a multiple comparison of means was performed using Tukey's test. Principal component analysis was used to reduce the dimensionality of the data obtained from free amino acid profiles. Principal component analysis was applied to the mean centered data matrix as suggested by Verdini and Rubiolo (2002a). Quadratic discriminant analysis was applied using principal components' scores as predictors (Girard and Nakai 1994) in order to classify cheeses according to groups outlined from the principal component analysis. Minitab 13.20 (Minitab, Inc., State College, Pa., U.S.A.) was used to perform statistical analysis.

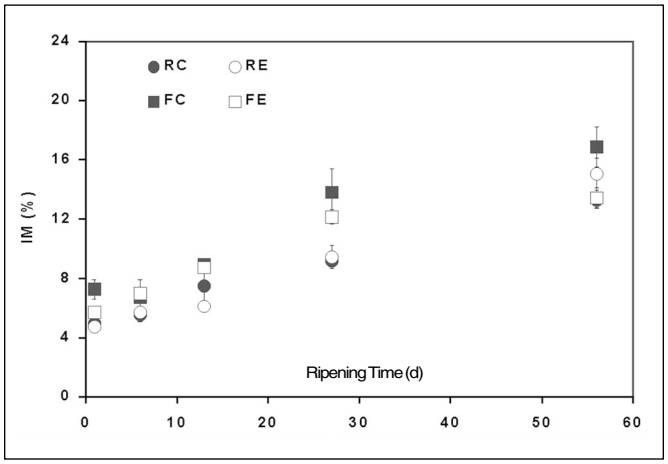

Figure 2—Index of maturation changing with ripening time. IM = WSN x 100 / TN, IM: index of maturation, WSN: water soluble nitrogen, TN: total nitrogen, R: cheeses ripened at 5 °C, F: cheeses held in frozen storage at –22 °C for 30 d, thawed at 5 °C, and ripened at 5 °C.

Results and Discussion

Water-soluble fraction

Figure 2 shows that maturation index (IM) increased throughout ripening. Storage condition significantly affected IM but a definite trend was not observed. There was no effect of sampling zone in IM of cheeses R; however, there was a significant difference between IM for zones C and E of cheeses F at 56 d. Similar results were obtained by Laborda (2000). That author determined IM for Fynbo cheeses at different ripening temperatures and sampling zones and found significant differences between zones when ripening was carried out at 12 °C and 16 °C, but no effect was found when ripening was conducted at 5 °C.

Validation of the RP-HPLC method for free amino acids

Sixteen amino acids out of the 18 injected were resolved in 40 min. Glutamic acid and histidine coeluted; consequently, those amino acids could not be analyzed. Increasing amounts of amino acid standards, ranging from 2 mg/L to 37 mg/L, were injected to test the linear relationship between the amount of the standards and the sample peak areas. Linear range, coefficient of determination, repeatability, and the analytical method validation parameters, including analytical sensitivity, limit of detection, and limit of quantification obtained from Eq. 1 to 3, are shown in Table 1. In general, results of validation parameters were reliable and satisfactory.

Free amino acids

A typical chromatogram of a cheese sample is shown in Figure 3. Amino acids were identified according to their retention times by comparison with a standard solution chromatogram. Free amino acid

Table 2—Free amino acid contents of cheeses R at different sampling zones and ripening times.

Cheese Zone	Ripening Time (days)	Amino acids (mg amino acid/100 g of cheese)[1]															
		Asp	Asn	Ser	Gln	Gly	Thr	Arg	Ala	Tyr	Trp	Met	Val	Phe	Ile	Leu	Lys
C	1	ND[2]	2.00 (1.74)	NQ[3]	2.91 (0.28)	ND	2.81 (0.78)	NQ	NQ	4.32 (0.04)	ND	NQ	1.20 (0.00)	3.93 (0.86)	NQ	4.75 (1.50)	13.30 (2.14)
	6	ND	3.11 (0.30)	NQ	2.91 (0.21)	NQ	2.84 (0.60)	NQ	NQ	4.85 (1.01)	ND	NQ	1.45 (0.19)	4.65 (1.33)	NQ	5.44	13.16 (1.93)
	13	NQ	4.90 (0.03)	2.85 (2.47)	3.59 (0.21)	NQ	4.32 (0.24)	NQ	2.45 (0.51)	7.67 (0.51)	ND	NQ	1.86 (0.35)	7.83 (0.93)	NQ	10.24 (1.37)	16.07 (1.91)
	27	NQ	19.66 (1.94)	NQ	12.16 (0.32)	NQ	13.27 (0.54)	NQ	NQ	14.28 (1.20)	ND	NQ	5.90 (0.57)	20.88 (2.44)	NQ	28.16 (3.46)	42.97 (8.28)
	56	NQ	42.89 (10.57)	8.30 (7.20)	16.99 (1.31)	6.91 (0.67)	27.10 (2.47)	8.83 (7.67)	4.75 (4.11)	18.34 (3.09)	ND	4.81 (4.17)	16.61 (5.86)	38.60 (8.49)	8.36 (2.52)	79.22 (14.35)	53.61 (7.52)
E	1	ND	0.92 (1.60)	NQ	2.59 (0.09)	NQ	2.04 (0.23)	NQ	NQ	1.15 (1.99)	ND	NQ	NQ	3.17 (0.43)	NQ	3.76 (0.29)	11.67 (1.62)
	6	NQ	3.46 (0.25)	NQ	2.76 (0.10)	NQ	3.23 (0.38)	NQ	2.78 (0.37)	5.65 (0.72)	ND	NQ	1.12 (0.03)	6.04 (0.99)	NQ	6.69 (1.06)	13.02 (1.05)
	13	NQ	4.40 (0.17)	4.85 (0.38)	0.92 (1.59)	NQ	4.27 (0.43)	NQ	2.94 (0.20)	5.79 (1.70)	ND	NQ	1.64 (0.32)	8.49 (0.77)	NQ	8.19 (1.71)	13.63 (0.99)
	27	NQ	17.95 (0.90)	NQ	11.01 (0.15)	NQ	11.44 (0.43)	NQ	NQ	15.62 (0.60)	ND	NQ	6.42 (0.73)	19.86 (0.82)	NQ	30.56 (1.77)	60.27 (12.05)
	56	NQ	25.50 (3.33)	NQ	14.59 (1.00)	NQ	21.34 (2.85)	NQ	2.37 (4.11)	16.08 (2.80)	ND	NQ	10.18 (2.04)	28.41 (5.94)	3.84 (3.33)	55.65 (10.90)	36.57 (4.50)

(1)upper line, mean value of concentrations determined for 3 cheeses; lower line, standard deviation
(2)not detected
(3)not quantified

contents are shown in Tables 2 and 3. Most of the studied amino acids were detected in cheese samples and their concentrations increased at different rates during the studied ripening period. Aspartic acid was not detected in cheese R, while tryptophan was detected in neither cheese R nor cheese F. Tryptophan was not detected in artisanal blue cheese by González de Llano and others (1991) because it could have been involved in catabolic reactions. However, the absence of triptophan in Port Salut Argentino, a short ripened cheese, may be related to its low proportion in α-casein. Arginine, responsible for unpleasant or bitter taste (Polo and others 1985) was present in small quantities in Port Salut Argentino cheese.

Total free amino acid content in Port Salut Argentino cheese was determined as the sum of the detected amino acid concentrations. Figure 4 shows total free amino acid contents for cheeses R and F, respectively. Although total free amino acid contents strongly depends on cheese characteristics, those contents determined in Port Salut Argentino cheese were in agreement with values reported by other authors (Polo and others, 1985; Frau and others, 1997). There was a significant increment of the total free amino acid contents during ripening for different storage conditions and sampling zones. Cheeses stored frozen showed higher total free amino acid contents during ripening. Total free amino acid content was lower in zone E than in zone C for both cheeses R and F, however, sampling zone effect was more notorious for cheeses F. Several authors

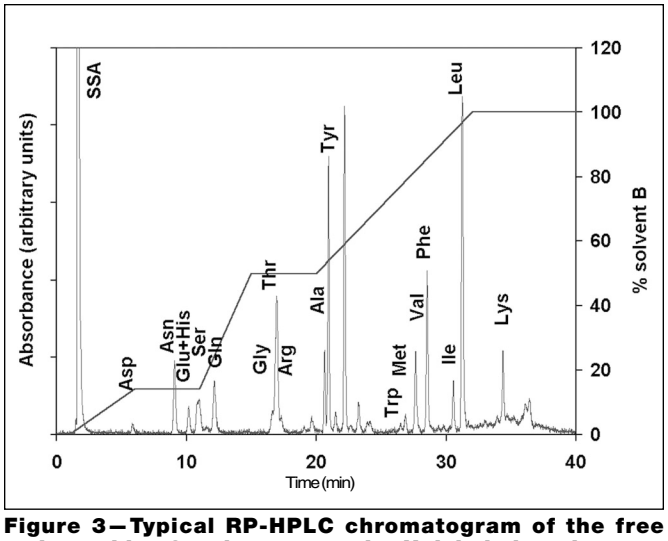

Figure 3—Typical RP-HPLC chromatogram of the free amino acids of a cheese sample. Unlabeled peaks were not identified.

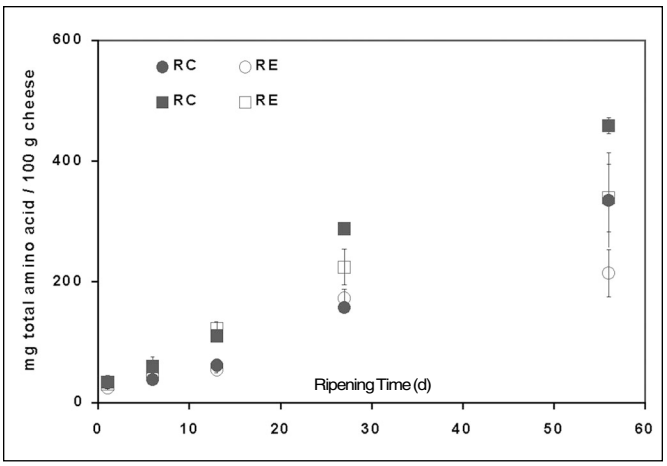

Figure 4—Total free amino acid contents changing with ripening time. R: cheeses ripened at 5 °C, F: cheeses held in frozen storage at –22 °C for 30 d, slowly thawed at 5 °C, and ripened at 5 °C.

Table 3—Free amino acid contents of cheeses F at different sampling zones and ripening times.

Cheese Zone	Ripening Time (days)	Amino acids (mg amino acid/100 g of cheese)[1]															
		Asp	Asn	Ser	Gln	Gly	Thr	Arg	Ala	Tyr	Trp	Met	Val	Phe	Ile	Leu	Lys
C	1	ND[2]	2.76 (0.13)	NQ[3]	3.05 (0.21)	NC	2.99 (0.50)	NQ	NQ	3.46 (0.22)	ND	NQ	1.28 (0.11)	3.67 (0.11)	NQ	5.34 (0.43)	11.50 (1.00)
	6	ND	5.02 (0.88)	NQ	3.67 (0.42)	0.69 (1.19)	4.76 (0.82)	NQ	2.74 (0.55)	5.78 (1.11)	ND	NQ	2.20 (0.82)	8.07 (2.51)	0.59 (1.01)	11.31 (3.25)	15.14 (3.62)
	13	0.96 (1.66)	11.70 (1.57)	1.68 (2.90)	5.00 (1.00)	0.77 (1.34)	9.22 (1.17)	3.50 (3.05)	3.36 (0.41)	7.67 (0.51)	ND	NQ	4.49 (0.35)	13.41 (1.87)	2.61 (0.43)	22.98 (3.31)	22.41 (1.53)
	27	NQ	35.17 (1.54)	NQ	16.00 (1.35)	2.15 (3.72)	23.70 (1.77)	8.79 (7.63)	7.06 (0.50)	14.28 (1.20)	ND	NQ	15.68 (0.47)	36.24 (1.39)	8.76 (0.98)	68.56 (0.71)	44.25 (4.40)
	56	2.75 (4.77)	55.20 (3.36)	15.41 (1.92)	22.12 (0.06)	3.10 (5.37)	34.55 (2.79)	19.22 (0.53)	9.34 (0.66)	18.34 (3.09)	ND	10.58 (0.27)	31.30 (2.26)	50.06 (8.49)	18.46 (1.58)	95.21 (3.20)	64.04 (1.75)
E	1	ND	1.00 (1.74)	NQ	2.94 (0.08)	NQ	2.78 (0.31)	NQ	0.75 (1.30)	2.50 (2.24)	ND	NQ	1.26 (0.30)	3.93 (0.84)	NQ	5.22 (1.19)	10.68 (0.59)
	6	NQ	5.00 (0.41)	NQ	3.01 (0.18)	NQ	3.87 (0.57)	NQ	0.76 (1.72)	5.98 (1.54)	ND	NQ	2.18 (0.40)	6.88 (1.23)	NQ	9.93 (1.67)	15.04 (2.53)
	13	2.00 (1.74)	11.97 (0.69)	1.47 (2.54)	3.51 (1.24)	1.70 (1.47)	8.79 (0.78)	3.32 (2.87)	3.85 (0.39)	10.60 (0.91)	ND	1.76 (1.53)	6.43 (1.25)	15.37 (0.62)	3.37 (0.68)	26.73 (2.11)	21.66 (1.42)
	27	NQ	26.01 (2.90)	NQ	14.31 (0.89)	NQ	18.15 (0.92)	NQ	NQ	16.56 (2.20)	ND	NQ	12.46 (2.27)	28.08 (4.82)	6.70 (4.82)	52.37 (9.37)	43.42 (6.29)
	56	NQ	37.52 (5.83)	NQ	18.90 (2.13)	3.30 (5.71)	29.32 (1.87)	13.46 (0.55)	13.46 (0.55)	21.56 (3.32)	ND	7.28 (0.27)	19.19 (3.54)	36.74 (6.74)	11.15 (1.82)	81.24 (12.07)	45.31 (5.42)

(1)upper line, mean value of concentrations determined for 3 cheeses; lower line, standard deviation
(2)not detected
(3)not quantified

found an increase of total free amino acids during ripening. Puchades and others (1989) found that the free amino acid content increased during Cheedar cheese ripening depending on the starter used. Frau and others (1997) reported that total free amino acid content was strongly different between batches of Mahon cheese. However, there was an increment of total amino acids during ripening of all batches.

Principal component analysis (PCA) was applied to data obtained for cheeses R (control cheeses) in order to analyze the amino acids that characterize the ripening of Port Salut Argentino cheese and to visualize the distribution of cheese samples at different ripening times and sampling zones. The 30 samples and 14 mean centered variables yielded 2 principal components (PC) that explained 97.8% of the data set variation (PC1 90.2% and PC2 7.6%). PC1 and PC2 scores (Figure 5) outlined 2 major groups, 1 group corresponded to unripened cheeses (from 1 to 13 d) and the other corresponded to ripened cheeses (27 and 56 d). Unripened cheeses formed a compact group on the left of Figure 5, while ripened cheeses formed an apparently disperse group that can be subdivided into 2 groups corresponding to 27 and 56 d.

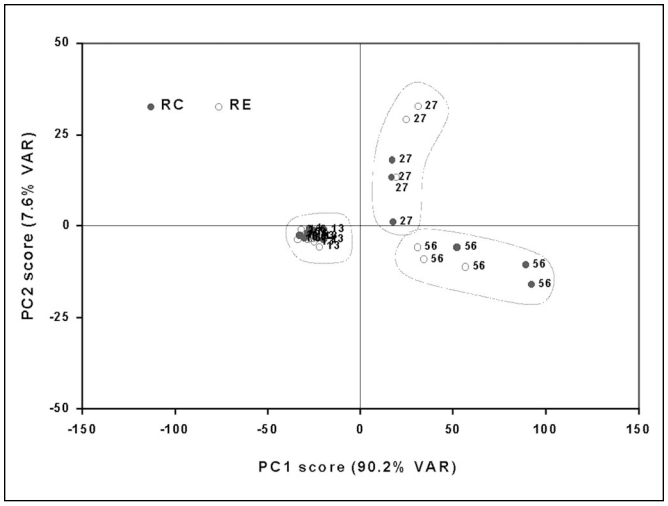

Figure 5—Plot of the scores of the 2 first-principal components when data for control cheeses were analyzed. Dotted lines show sample grouping when QDA of the 2 first-principal components was applied.

Principal component analysis becomes a useful tool when there is a need for classification without preliminary grouping (Vodovotz and others 1993). However, further classification methods are needed to validate the trends outlined in PC plots (Furtula and others 1994). Quadratic discriminant analysis (QDA) was applied using PC1 and PC2 scores as predictors (Girard and Nakai 1994) to classify cheeses according to the 3 groups outlined from PCA. Proportion of correct classification was 100% for cheeses R in 3 groups: I (1 to 13 d), II (27 d), and III (56 d) as shown in Figure 5 with dotted lines.

Amino acids with higher PC loadings that characterized the ripening of Port Salut Argentino cheese were leucine, lysine, asparagine, phenylalanine, threonine, tyrosine, glutamine, and valine (Table 4). Some authors detected the same amino acids during early ripening of cheeses. Frau and others (1997) found that samples of fresh Mahon cheese (10 d of ripening) had high values of leucine, phenylalanine, and valine. Katsiari and others (2000) also reported that the major free amino acids found in young Feta cheese were leucine and valine. Casein hydrolysis by rennet occurs during the 1st stage of cheese ripening. Then, amino acids situated in terminal position or next to the points of hydrolysis are more exposed to bacterial peptidase activity. Results obtained from model cheeses in which the production of amino acids from limited rennet-digested casein showed that initially produced amino acids were: leucine, arginine, and phenylalanine followed by glutamic acid, valine, lysine, tyrosine, and isoleucine (Exterkate and others 1997).

Table 4—Loadings of the 2 first-principal components when data for cheeses R and F were analyzed separately

	Cheese R		Cheese F	
	PC1 loading (90.2% variance)	PC2 loading (7.6% variance)	PC1 loading (97.0% variance)	PC2 loading (1.2% variance)
Leu	0.67	–0.41	0.67	0.17
Lys	0.44	0.85	0.36	–0.17
Asn	0.36	–0.11	0.36	0.20
Phe	0.32	–0.08	0.32	0.10
Thr	0.23	–0.10	0.23	0.01
Tyr	0.15	0.10	0.19	–0.27
Gln	0.15	0.04	0.17	0.11
Val	0.14	–0.07	0.15	0.10
Ile	0.06	–0.12	0.12	–0.50
Arg	0.06	–0.10	0.12	–0.19
Ser	0.04	–0.12	0.07	–0.60
Gly	0.04	–0.07	0.07	0.07
Met	0.03	–0.06	0.06	–0.34
Ala	0.03	–0.10	0.02	–0.10
Asp	—	—	0.01	–0.14

Principal component information can explain the chemical information contained in the variables (Gardnier 1997). Several authors applied PCA to cheese samples and related the 1st PC to ripening time. González de Llano (1991) related the 1st PC to ripening time and the 2nd PC to batch effect on blue cheeses. Frau and others (1997) found a relationship between the 1st PC and ripening time; however, the 2nd PC distinguished between cheeses made from pasteurized milk and raw milk. Table 4 shows that PC1 loadings of the analyzed data for Port Salut Argentino cheeses were all positive. Therefore, the 1st PC (90.2% of the total variation could be related to the increase of amino acid contents along ripening, which is the most important transformation observed in Port Salut Argentino cheeses (Table 2 and Figure 5). Most PC2 loadings were negative except for lysine, tyrosine, and glutamine. Table 2 shows that amino acids increase along ripening at different rates. Amino acids with positive PC2 loadings are responsible for the scattering of cheeses corresponding to 27 d towards the upper hemisphere in Figure 5. Lysine, tyrosine, and glutamine increase more slowly than the other amino acids between 27 and 56 d. Consequently, the 2nd PC may be related to the different rates of amino acid formation that may depend on the different enzyme activities.

Principal component analysis was then applied to cheeses F in order to ascertain whether the amino acids that characterize the ripening of cheeses F differed from those that characterize the ripening of cheeses R. The 30 samples and 15 mean centered variables yielded 2 principal components that accounted for 98.2% of the total variation. Loadings of the 2 first PC of cheeses F are shown in Table 4. Loadings of PC1 of cheeses F were quite similar to those of cheeses R. However, some differences were found when PC2 was analyzed. Considering the contribution of each PC to the total variation (PC1 97.0% and PC2 1.2%), no differences between free amino acid profiles of cheeses R and F was considered.

In order to evaluate frozen storage as a preservation technique for Port Salut Argentino cheese, cheeses R and F were examined together. When PCA was applied to the 60 samples and 15 mean centered variables, the variance explained by the 2 1st-principal components was 97.5% (PC1 93.7% and PC2 3.8%). Figures 6 and 7 show PC scores and PC loadings, respectively. It can be observed that the distribution of cheeses R and F in the 2-dimensional PC space was related to the increment of free amino acid content in the same fashion as discussed for cheeses R. Unripened cheeses (from 1 to 13 d) formed a compact group on the left of Figure 6, while ripened cheeses (27 and 56 d) showed scattering. QDA was applied using PC1 and PC2 scores as predictors according to the 3 groups determined for control cheeses. The proportion of correct classification was 93.3% for the 3 groups. Misclassifications corresponded to 1 cheese RC (d 56) and 3 cheeses FC (d 27) and showed that the mature cheese grouping according to ripening time was overlapped without differences because of storage condition, as can be seen in Figure 6.

Unripened cheeses (1 to 13 d) were grouped independently of ripening time, sampling zone, and storage condition. Ripened cheeses (27 and 56 d) showed scattering according to ripening time.

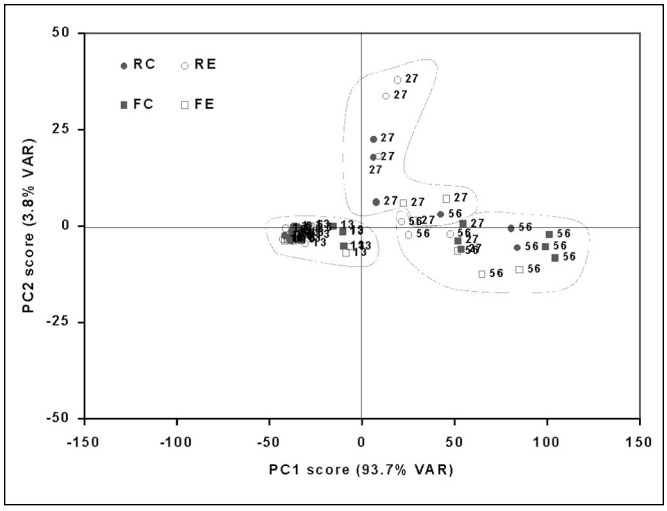

Figure 6—Plot of the scores of the 2 first-principal components when data for all cheeses were analyzed. Dotted lines show sample grouping when QDA of the 2 first-principal components was applied

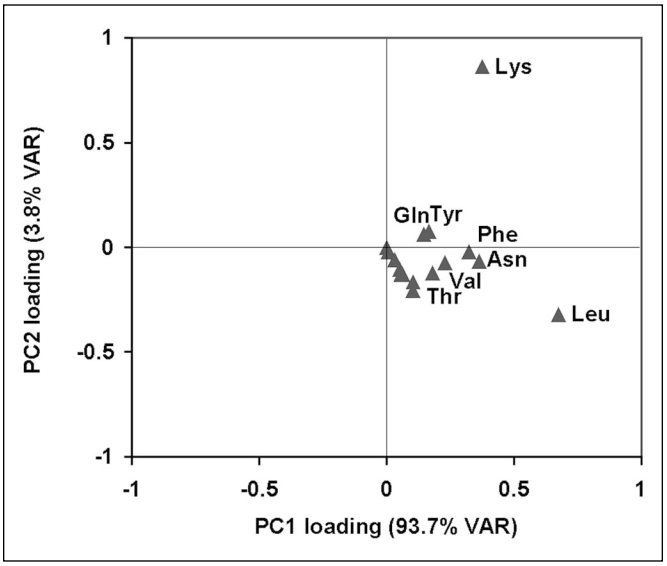

Figure 7—Plot of the loadings of the 2 first-principal components when data for all cheeses were analyzed

Therefore, free amino acid profiles of cheeses preserved by frozen storage were similar to those of traditionally ripened cheeses.

Conclusions

FREE AMINO ACID PROFILES OF PORT SALUT ARGENTINO CHEESES, ripened with and without previous frozen storage, and sampled at 2 different zones, allowed us to evaluate the effect of frozen storage. The major amino acids present in Port Salut Argentino cheese were leucine, lysine, asparagine, phenylalanine, threonine, tyrosine, glutamine, and valine. Total free amino acid contents increased during ripening, being higher in the central cheese zone. Moreover, cheeses stored frozen showed higher total free amino acid contents. Principal component analysis successfully reduced data to 2 dimensions, while quadratic discriminant analysis applied to the 2 first-principal component scores facilitated cheese classification. Unripened cheeses (1 to 13 d) were grouped independently of ripening time, sampling zone, and storage condition. Ripened cheeses (27 and 56 d) showed scattering according to ripening time. Therefore, free amino acid profiles of cheeses preserved by frozen storage were similar to those of traditionally ripened cheeses.

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MS 20020106 Submitted 2/19/02, Revised 4/23/02, Accepted 6/4/02, Received 6/4/02

We thank SanCor Cooperativas Unidas Ltd. for the supply of cheese. This work was done with the financial support of Univ. Nacional del Litoral (Santa Fe, Argentina), Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina), and Agencia Nacional de Promoción Científica y Tecnológica (Argentina).

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