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Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening

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Abstract

Proteolysis is one of the major biochemical events which takes place during cheese ripening and its products, amino acids and peptides, have a considerable influence on the sensory characteristics of the cheese. This paper deals with the free amino acid profile of Manchego cheese made with a defined starter culture, the same defined strain starter culture and an adjunct culture *(Lactobacillus plantarum)* or a commercial starter. Manchego cheeses made with the defined strain starter cultures reached higher concentrations of total free amino acids at the end of the ripening period than did cheeses made with the commercial starter culture. Principal components analysis (PCA) applied to the three types of Manchego cheeses at different ripening times divided the samples into two general groups: cheeses with a short ripening period (15 and 45 days), with low concentrations of Phe, Val, Leu, Cys and Tyr, and cheeses with higher ripening times (90 and 150 days), with higher contents of these free amino acids. The variable ripening time seemed to be more important than the variable starter culture for differentiating the samples on the basis of their free amino acid concentrations.

Keywords: Manchego cheese; Amino acids; Defined strain starter; Commercial starter; Ripening time

1. Introduction

Manchego cheese is a high-fat pressed cheese, made in the La Mancha region (Spain) from locally produced ewe's milk. It is manufactured under the methodology described by the designation of origin for Manchego (BOE, 1995) but this regulation does not specify the type of starter culture that can be used in its manufacture. Nevertheless, the milk is generally inoculated with commercial mixed-strain starter cultures, which comprise strains of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*.

It is known that proteolysis is one of the major biochemical events which takes place during cheese ripening (González de Llano, Polo, Ramos, & Martín-Alvarez, 1991) and that its degradation products, amino acids and peptides, have a considerable influence on the sensory characteristics of the cheese (González de Llano, Polo, & Ramos, 1991; Urbach, 1993). Due to the action of rennet, proteinases and peptidases from starter bacteria and the secondary microflora and indigenous milk enzymes, peptides and amino acids are liberated (Barcina, Ibáñez, & Ordóñez, 1995; Langsrud, Reinbold, & Hammond, 1977).

Free amino acid composition has been evaluated to serve as a typicality and quality index of several cheese varieties (Bütikofer & Fuchs, 1997; Resmini, Hogenboom, Pazzaglia, & Pellegrino, 1993; Resmini & Pellegrino, 1986).

The concentrations of the different amino acids in a cheese are related to the manufacturing technology (type of curd, addition of proteinases, starters, ripening conditions), duration of ripening and the extent and type of proteolysis (Christensen, Johnson, & Steele, 1995; Mariani et al., 1993).

Lactobacilli constitute the majority of non-starter lactic acid bacteria (NSLAB) in cheese. In recent years, there have been many studies focussed on the effect, on ripening, of adding lactobacilli as adjunct cultures to pasteurised cheesemilk (Gómez, Gaya, Núñez, & Medina,

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1996; Lane & Fox, 1996; Lynch, McSweeney, Fox, Cogan, & Drinan, 1996; Peterson & Marshall, 1990).

The apparent importance of NSLAB in flavour development has prompted much interest in the deliberate addition of selected lactobacilli to cheesemilk (McSweeney et al., 1995).

In a previous paper (Poveda, Sousa, Cabezas, & McSweeney, 2003) we studied the proteolysis in terms of peptide profile in Manchego cheese made with different starter cultures. In that study, principal component analysis (PCA) of the RP-HPLC chromatograms of the ethanol (70%)-insoluble and soluble fractions divided the samples into three groups, according to the different starter culture used in their manufacture.

This paper deals with the free amino acid profile of Manchego cheese manufactured with three different starter cultures (defined strain starter culture with and without an adjunct culture or a commercial starter). The aim of this work was to study the free amino acid profile in the three types of Manchego cheeses and the evolution of the individual free amino acids during ripening.

2. Material and methods

2.1. Cultures

Lactococcus lactis ssp. *lactis, Leuconostoc mesenteroides* ssp. *dextranicum* and *Lactobacillus plantarum,* which had been isolated from high quality raw milk Manchego cheeses and selected on the basis of their technological properties (García, Palop, & Cabezas, 1997) were used as the defined-strain starter cultures for the manufacture of Manchego cheeses.

A commercial starter (Rhodia, Dangé-Saint Romain, France), composed of two strains of *Lactococcus lactis*, was used as the commercial culture.

2.2. Cheese manufacture and sampling

Manchego cheeses were made at a cheesemaking plant located in the La Mancha region. Six hundred litres of pasteurised (65 °C, 30 min) ewe's milk (pH 6.35) set at 35 °C were used to manufacture three different batches, using three different starter cultures:

Batch 1: *Lactococcus lactis* ssp. *lactis* (80%) and *Leuconostoc mesenteroides* ssp. *dextranicum* (20%).
Batch 2: *Lactococcus lactis* ssp. *lactis* (80%), *Leuconostoc mesenteroides* ssp. *dextranicum* (10%) and *Lactobacillus plantarum* as starter adjunct (10%).
Batch 3: commercial starter.

Batch 1 and Batch 2 were manufactured using a defined strain starter culture of lactic acid bacteria isolated from good quality raw milk Manchego cheese, added at a rate of 1% v/v. Batch 3 was inoculated using a commercial starter, in a proportion comparable to those of the defined strain starters (1% w/v). After 30 min, 7.5 g of bovine rennet (strength 1/150,000; 94% chymosin, 6% pepsin) was added to each batch of milk, and coagulation took place at 35 °C in 45 min. The curds were cut into 5 mm cubes, scalded at 38 °C for 10–15 min and filled into cheese moulds. Cheeses were pressed for 5–6 h at 15° C, salted for 22 h in brine (20% NaCl) at the same temperature, and ripened for 150 days at 12 °C and a relative humidity of 85%.

For each experiment, three cheeses (1 kg each) were manufactured and analysed at 15, 90 and 150 days of ripening.

2.3. Free amino acid analysis

Concentrations of individual free amino acids were determined using a Beckman model 6300 amino acid analyser equipped with a Beckman model P-N 338052 Na^+ cation-exchange column (12×0.4 cm). Large peptides were precipitated using 12% TCA and supernatants were diluted with 0.2 M sodium citrate buffer, pH 2.2, to give approx. 250 nmol of each amino acid residue. The samples were diluted (1:2) with internal standard (norleucine) to give 125 nmol of each amino acid residue per injection volume, and filtered through a 0.2 μm filter. Filtrate (50 μl) was loaded onto the analyser. The amino acids were separated using ion exchange chromatography with post-column derivatisation and visible colorimetric detection; amino acids were detected at 570 nm with the exception of proline, which was detected at 400 nm. Results were recorded using a Minichrom© data handling system.

2.4. Statistical analysis

Principal component analysis (PCA) was applied to the data of the free amino acid concentration using the correlation matrix and Varimax rotation. The Pearson correlation coefficient test was applied to estimate the relationship between the amino acid concentrations and the ripening time. These statistical analyses were performed using the SPSS statistical package (version 9.0, SPSS, Inc., Chicago, Illinois, USA).

3. Results and discussion

Table 1 presents the evolution of total free amino acids (TFAA) during ripening from Manchego cheeses made with two different defined strain starter cultures and with a commercial starter. The concentrations of

Table 1
Total free amino acid (TFAA) concentrations in Manchego cheeses made with three different starters (Batch 1, Batch 2 and Batch 3) at various ripening times

Total FAA (mg/kg cheese)	Ripening time (days)			
	15	45	90	150
Batch 1	4284	7592	12028	23939
Batch 2	10902	6109	14551	20356
Batch 3	9195	6716	15113	16657

TFAA generally increased during ripening. At the end of the ripening period (150 days), cheeses from batches 1 and 2 (both made with a defined strain starter) contained higher amounts of TFAA than cheeses in Batch 3 (made with a commercial starter). The TFAA concentrations found in this study were larger than those reported by Barcina et al. (1995) in Idiazábal cheese (ewes' milk cheese) ripened for 360 days.

The concentrations of individual free amino acids for the three batches of Manchego cheeses during the ripening period are shown in Fig. 1. The major free amino acids found throughout the whole ripening period and in the three batches of cheeses were Glu, Val, Leu, Phe and Lys. These amino acids were also the major amino acids found in other cheese varieties during ripening, although with different concentrations, e.g., in Cheddar cheese (Dilanian, 1980; Hickey, Van Leeuwen, Hilier, & Jago, 1983; Law, Castanon, & Sharpe, 1976; Puchades, Lemieux, & Simard, 1989; Wilkinson, 1993), Gruyère and Sbrinz (Lavanchy & Sieber, 1993) and Mahón cheese (Barcina et al., 1995), and also in ewes' milk cheeses such as Idiazábal cheese (García-Palmer, Serra, Palou, & Gianotti, 1997) and in Manchego cheese ripened in olive oil (Ordóñez & Burgos, 1980).

In the Manchego cheeses made in this study, concentrations of almost all free amino acids showed a clear tendency to increase with ripening time, as was expected, since, during proteolysis, these compounds are released by the proteolytic agents, mainly by the microbial enzymes, through the biochemical reactions that take place during cheese ripening. The proteolysis of α_{s1}-casein, which has a high content of the amino acids Leu, Phe and Val, occurs during the first days of ripening, which predominates over the proteolysis of β-casein (Frau, Massanet, Rosselló, Simal, & Cañellas, 1997). The high content of Glu in Manchego cheese samples could be due to the contribution of different factors, such as the high percentage of Glu in the casein and the formation of Glu from Gln, Val, Leu and Ile. In Manchego cheese ripened in olive oil for 11 months, Glu was the most abundant amino acid only until 3–4 months of ripening, after which the level was constant (Ordóñez & Burgos, 1980). Although proline is one of the major amino acids in β-casein, free proline concentrations found in the samples were not very high, which is indicative of the low degree of proteolysis undergone by β-casein.

It can be observed from Fig. 1 that, at the end of the ripening period, the amino acids Glu, Leu, Val, His and Lys reached higher concentrations in cheeses from batches 1 and 2 (made with the defined strain starters) than those in cheeses from Batch 3.

In order to establish the relationships between the different variables and to detect the most important causes of variability, PCA was applied to the free amino acids data for the three batches of Manchego cheeses ripened for 15, 45, 90 and 150 days. Two principal components (PC) were obtained, accounting for 88.8% of the total variance (TV). Free amino acids were represented as a function of PC 1 and PC 2 (Fig. 2). PC 1 explained 75.6% of the TV and the free amino acids that correlated best with this PC and their factor loadings were Phe (0.980), Val (0.961), Leu (0.957), Cys (0.934) and Tyr (0.902). The free amino acids best correlated with PC 2 (that accounted for 13.2% of TV) and their factor loadings were: Asp, 0.894 and Arg, 0.849. Fig. 3 shows the distribution of the samples in the plane defined by PC 1 and PC 2. It can be observed that samples appear separated according to their ripening times: cheeses with the lowest ripening times (15 and 45 day-old cheeses), located in the negative side of PC 1, had lower contents of the free amino acids Phe, Val, Leu, Cys and Tyr than samples of 90 and 150 days of ripening, located in the positive side of this PC. Taking into account that PC 1 explained a high percentage of the total variance (75.6%), it can be assumed that PC1 could be associated with the ripening time effect. However, PCA did not seem to distribute the cheese samples according to the starter employed in their manufacture. In this study, cheeses were only ripened for a period of 150 days, and perhaps the starter effect would be more evident at later stages of maturation.

Finally, correlation analysis between free amino acid concentration and ripening time was applied (Table 2). For cheeses in Batch 1, all the free amino acids presented a correlation coefficient higher than 0.9, with the exception of Arg. For cheeses in Batch 2, the correlation coefficients were above 0.9 for the amino acids Pro, Gly, Val and Leu and, in cheeses of Batch 3, correlation coefficients were >0.9 for Pro, Gly, Leu and Tyr. Other authors obtained good correlation coefficients for free amino acids with ripening time. In Idiazábal cheese, the correlation coefficients were above 0.9 for the amino acids Glu, Val, Leu, Phe, and Lys (Barcina et al., 1995). Also, García-Palmer et al. (1997) and Bütikofer and Fuchs (1997) found strong correlation between levels of almost all free amino acids with the ripening times of Mahón and Emmental cheeses, respectively.

The low correlation of Arg with ripening time in the cheeses is in agreement with the results reported by other authors in different cheese varieties, including

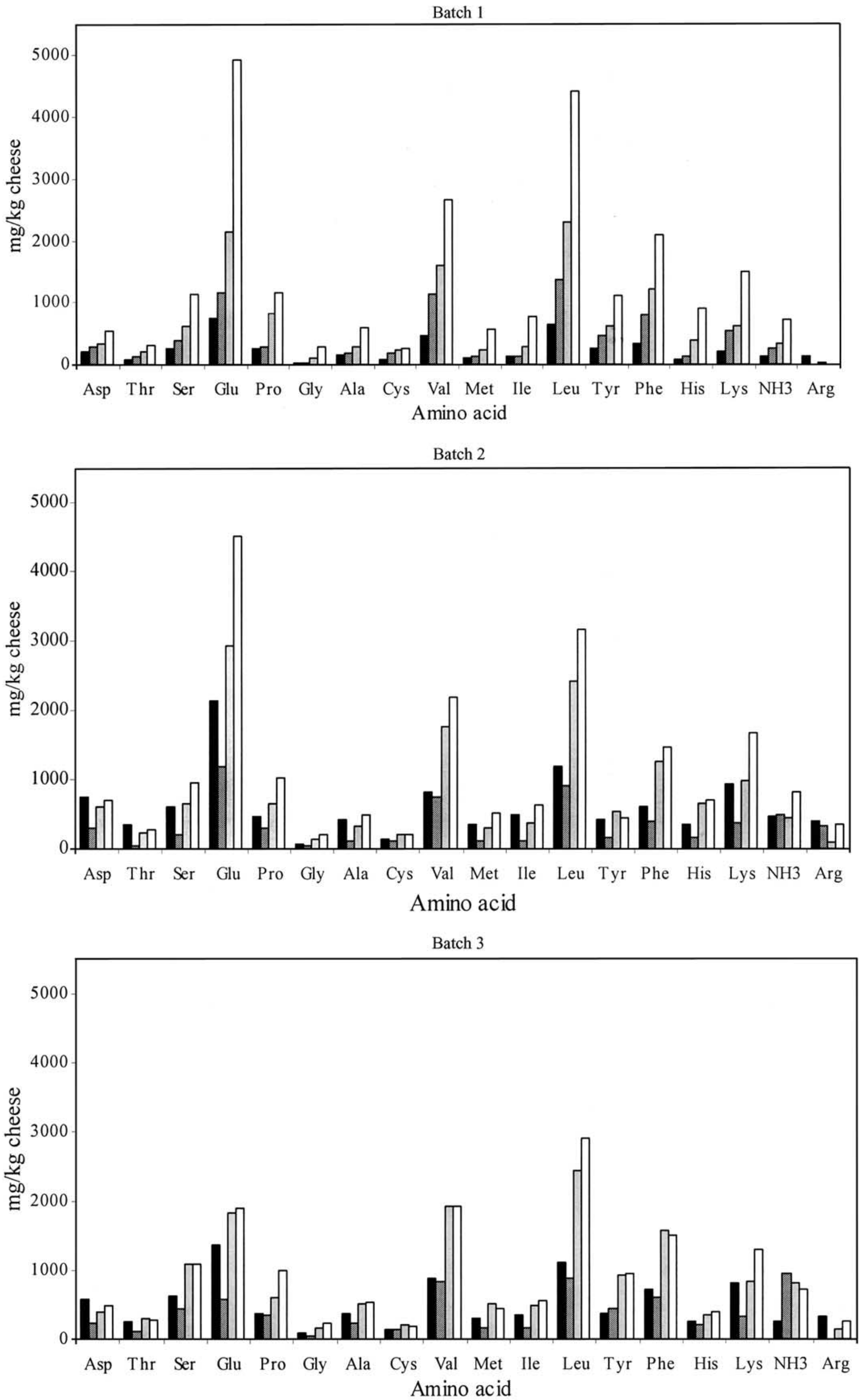

Fig. 1. Evolution of the individual free amino acids during ripening of Manchego cheeses made with a defined strain-starter (Batch 1), a defined strain-starter and adjunct starter (Batch 2) or a commercial starter (Batch 3): ■ 15 days; ▨ 45 days; ■ 90 days; □ 150 days.

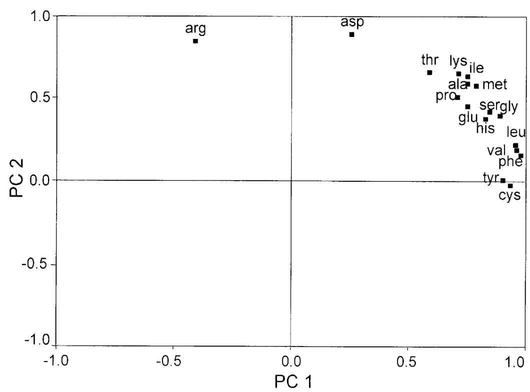

Fig. 2. Loadings of the free amino acid concentrations for the two first principal components.

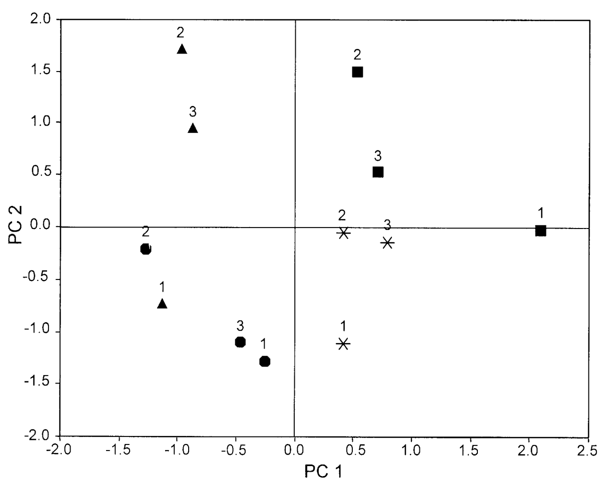

Fig. 3. Projection on the plane defined by the two first principal components of Manchego cheeses made with three different starter cultures (1, 2 and 3) at different ripening times: ▲ 15 days; ● 45 days; ✳ 90 days; ■ 150 days.

Appenzeller, Emmental, Gruyère, Raclette, Sbrinz and Tilsit (Bütikofer & Fuchs, 1997) and it is important that this amino acid concentration does not increase with ripening time, since it may be responsible for bitter off-flavours (Kirimura, Shimizu, Kimizuka, Ninomiya, & Katsuya, 1969; Lemieux & Simard, 1992).

4. Conclusions

Manchego cheeses in Batches 1 and 2 (made with the defined strain starter cultures) contained, at the end of the ripening period studied, higher concentrations of total free amino acids than cheeses made with the commercial starter culture.

PCA of the individual free amino acid concentrations showed that the free amino acids that most contributed to separate the cheeses according to the ripening time (samples with a short ripening time and samples with a longer ripening period) were Phe, Val, Leu, Cys and Tyr.

Table 2
Pearson correlation coefficients (r) between the free amino acid concentrations and ripening times of 15, 45, 90 and 150 day-old Manchego cheeses made with three different starters (Batch 1, Batch 2 and Batch 3)

Amino acid	Batch 1	Batch 2	Batch 3
	r		
Asp	0.980	0.244	0.011
Thr	0.996	0.089	0.449
Ser	0.988	0.710	0.811
Glu	0.971	0.882	0.661
Pro	0.939	0.909	0.964
Gly	0.963	0.919	0.905
Ala	0.954	0.472	0.748
Cys	0.923	0.817	0.805
Val	0.994	0.954	0.880
Met	0.965	0.665	0.680
Ile	0.938	0.496	0.760
Leu	0.992	0.939	0.921
Tyr	0.988	0.413	0.911
Phe	0.997	0.897	0.833
His	0.977	0.814	0.880
Lys	0.954	0.779	0.748
NH_3	0.974	0.819	0.419
Arg	−0.678	−0.245	0.051

On the other hand, the amino acids that best correlated with the ripening time in the Manchego cheeses manufactured with three different starter cultures were Phe, Val, Leu, Cys, Pro, Gly, and His.

We can conclude that the variable ripening time seemed to be more important than the variable starter culture for differentiating the samples on the basis of their free amino acid concentrations. Nevertheless, further work could be done, by ripening the cheeses for a longer ripening time (>150 days) to study the effect of the different starters in the free amino acid production.

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