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Changes in the volatile fraction during ripening of Mahón cheese

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Abstract

Mahón cheeses, manufactured in the Island of Minorca (Spain), were produced and ripened under controlled conditions. Cheeses were sampled and analyzed at different stages of ripening. Twenty-one major aroma components were identified. It was observed that, during ripening until the most commercially valuable product was obtained, (60–90 days) only 16 compounds varied significantly with time: butanoic, isovaleric, hexanoic, octanoic and decanoic fatty acids ($p < 0.001$), together with heptanoic acid ($p < 0.005$), 2-methyl pentanone, 2-methyl heptanone, 2-methyl nonanone, methyl ketones ($p < 0.001$), ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate, ethyl hexadecanoate and ethyl esters ($p < 0.001$). These changes could be described by means of zero order kinetics. Through a PCA analysis it was found that three factors explained ca. 87% of the total variance. According to the first two components the variables were grouped by their chemical characteristics (fatty acids, methyl ketones, ethyl esters) and the cases by their ripening time (0/18 days, 35 days, 53/67/82 days).

Keywords: Mahón cheese; Ripening; Volatile; Kinetics

1. Introduction

Cheese ripening includes complex microbiological and enzymatic processes contributing to the appearance of compounds which confer unique flavor and textural characteristics (Collin, Berdague, & Dogning-Bergeret, 1987; Muir, Hunter, & Banks, 1997). Knowledge of these changes is of a great technological relevance as it allows standardization of cheese manufacturing and a better control of the process, avoiding, in some cases, sensory alterations. Improved control of the process could lead to reduction of the ripening time and a more uniform and controlled product, this being of particular economic importance.

Studies on the evolution of volatile compounds in cheese are scarce. Only in a few kinds of cheese, among them Swiss Gruyere, have chemical changes been described in depth, for alkaline and neutral compounds (Bosset & Liardon, 1985) and for volatile fatty acids (Bosset, Collomb, & Sieber, 1993). Likewise, in Gruyere Comté the influence of ripening on the volatile content was addressed, analyzing the effect of the temperature during the ripening time, the seasonal influence and the manufacturer (Berdague, Jeunet, & Grappin, 1987; Guichard, Berdague, & Grappin, 1987). In Cheddar cheese, volatile compounds have been studied as indicators of aging time. From the concentrations of 2-pentadecanone and 3-hydroxy 2-butanone, good correlations were obtained for predicting ripening time (Banks, Brechany, & Christie, 1992; Christensen & Reinnecius, 1995).

Mahón, a non-cooked pressed type of cheese, salted in brine, is manufactured in Minorca island (Spain) from cows milk. Cheeses are parallelepipedic in shape with round edges, of approximately $0.2 \times 0.2 \times 0.08$ m and 2.5 kg weight. The product is manufactured under the methodology described by the "Mahón Cheese Appellation of Origin", whose regulation distinguishes between four different kinds of Mahón cheese in relation to their ripening time: fresh (less than 10 days since manufacturing), half-ripened (from 2 to 5 months of ripening), ripened (from 5 to 10 months of ripening) and old-ripened (more than 10 months of ripening). Due to the fact that the Mahón cheese major market demand is for 90–150 ripening days, the process carried out by the commercial firms includes changing of the ripening chamber conditions after 90 days to allow the cheese to last longer in the more marketable span. Several studies

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have been carried out on the characterization of Mahón cheese regarding proteolysis (Addeo, Garro, Intorcia, Pellegrino, Resmini, & Chianese, 1995; Frau, Massanet, Rosello, Simal, & Cañellas, 1997; Lopez-Fandino, Martin-Alvarez, Pueyo, & Ramos, 1994; Martinez-de-Castro & Martin-Hernandez, 1994), fatty fraction (Fuente, Fontecha, & Juarez, 1993), comparison of volatiles with other European cheeses (Bosset & Gauch, 1993) and scanning electron microscopy SEM (Frau, Blanco, & Rosello, 1993; Frau, Mulet, Simal, Massanet, & Rossello, 1997). Nevertheless, no studies on the evolution of volatiles of the Mahón cheese or similarly manufactured cheeses have been reported.

The objective of this work was to study the kinetics of the evolution of volatile compounds identified in the Spanish Mahón cheese during the ripening period to reach its market value.

2. Materials and methods

The manufacturing procedure includes pasteurization of the milk, the addition of rennet, $CaCl_2$ and starters. Once the curd is obtained, the cutting of the curd into rice-sized pieces is carried out with subsequent stirring. Next, the curd is placed into moulds and pressed.

The samples considered in this work were 12 cheeses of the same batch, manufactured with pasteurized milk in the Coinga (Alayor, Minorca, Spain) facilities. Coinga manufactures more than 50% of the cheese belonging to the Mahón-certified origin, thus ensuring the adequacy of the batch according to the cheese characteristics. All the cheeses were surface-treated 7 days after salting with pimaricin for antifungal purposes. Ripening was carried out for 6 months according to the procedure of this company. During the first 3 months the cheeses were stored in a ripening chamber at 12–13°C and air relative humidity of 88–89%. The next 3 months were spent at 4–6°C and 80% moisture for a better mould control. For kinetic purposes, only the first period was considered. The average weights and dimensions of the cheeses were 2.7 ± 0.1 kg, 0.194 ± 0.004 m in width and 0.079 ± 0.003 m high. The protein and fat contents were 39.0 ± 0.5 g/100 g dry matter (DM) and 51.3 ± 0.7 g/100 g DM, respectively. All the samples were ripened by the manufacturer and two cheeses of each were sent, each time, to the laboratory in refrigerated boxes after: 0, 18, 35, 53, 67 and 82 days. At these times, two portions of approximately 0.2 kg, were taken from each sampled cheese, vacuum-packaged and stored at −30°C until proceeding to the volatile extraction. Cheese portions were defrosted at 8°C for 12 h (it was previously checked that this procedure preserved the cheese characteristics), then approximately 0.5 cm of the outer zone of the cheese portion including the rind and the smear was discarded. Each fraction of cheese was finely ground and thoroughly mixed using a mincer in order to obtain the sample, which was analyzed twice.

2.1. Isolation and concentration of volatile aroma components

Volatile compounds were isolated according to the well-known combined procedure of simultaneous distillation-extraction (SDE) similar to that described by Godefroot, Sandra, and Verzele (1981), using pentane as solvent and camphor as internal standard (Frutos, Sanz, & Martinez-Castro, 1988, 1991). In each analysis, 10 g of a sample were introduced into a 500 ml round-bottom flask, to which 100 ml of bidistilled hot water were added together with 50 μg of camphor. The flask remained 15 min in an ultrasonic bath to facilitate the total disintegration of the sample and was next introduced into the oil bath (110°C) of the extraction equipment. The 50 ml heart flask, which contained 3 ml of pentane solvent, was introduced into a water bath at 50°C. The vapors from both flasks were condensed in the common refrigerated "U-tube" of the equipment. After thirty minutes distillation the entire content of the U-shaped tube of the equipment was collected in a airtight closed tube and frozen at –18°C to facilitate the separation of the aromatic fraction (of lesser density and liquid at –18°C), in which all the aromatic compounds were dissolved. This organic phase was concentrated in a nitrogen stream up to a final volume of approximately 100 μl.

All reagents were of analytical grade. Standard compounds for determination of GC retention times and mass spectra (MS) were purchased from Sigma–Aldrich Quimica S.A. (Madrid, Spain).

2.2. Chromatographic analysis

An Autosystem Perkin Elmer Gas Chromatograph (Norwalk, CT) equipped with a flame ionization detector (FID) was used to analyze the concentrated extracts. A fused silica capillary column Cromlab SL 30 m × 0.25 mm of DB-FFAP phase, specific for free acids, methyl ketones and esters was employed. The column temperature was programmed: 60°C for 5 min; from 60 to 200°C at 4°C/min; 200°C for 34 min; from 200 to 230°C at 10°C/min. The carrier gas was nitrogen at 2 ml/min. Injections were made in the split mode with a split ratio of 1:16 into the injector. The injected volume was 1 μl. The injector and detector temperature was set at 250°C. The chromatograms were recorded and the corresponding peak areas integrated by a Model 1020 Personal Integrator of Perkin Elmer (Norwalk, CT).

2.3. Identification and quantification

The identification of volatile compounds was performed by Gas Chromatography and Mass Spectrometry

(GC–MS). The former capillary column used in the gas chromatograph was installed in a Fisons Trio 1000 GC–MS (Manchester, UK), replacing nitrogen by helium as the carrier gas. Fragmentation was performed by electronic impact EI^+ at 70 eV, SCAN mode between 50 and 450 mass units.

Identification has been carried out by comparison of the retention times with those of standard substances and further confirmation by GC–MS. The mass spectra for all the compounds have been compared with several standard mass spectra provided by the data base of the equipment. Quantification was carried out from peak areas of components and internal standard. GC detector response correction for each component was considered as well. The quantitative results were obtained as an average of eight replicates.

Coefficients of variation (CV) were calculated by repeating the SDE extraction and subsequent analysis of a Mahón cheese sample to determine repeatability of analysis. The CVs for most peaks were smaller then 15% and never exceeded 20%, this being considered a satisfactory result for this type of analysis.

Statistical analysis was performed on pooled data by using the BMDP statistical software package (Dixon, 1992). The 7D (ANOVA) and 4M (principal component analysis) programs were employed.

3. Results and discussion

Twenty-one major volatile compounds were identified. Table 1 shows the concentrations measured for the compounds identified in the cheese volatile fractions during the ripening period considered. Results are presented in Table 1 on a dry basis (μg/g cheese DM) to facilitate comparison on the total content. For kinetic purposes, volumetric concentrations were considered, assuming constant cheese volume (Villota & Hawkes, 1992). It was observed that the volatile compounds changed according to different patterns.

3.1. Kinetics

ANOVA was performed on pooled results in order to evaluate influence of the ripening time of Mahón cheese on the volatile aroma components analyzed. It was concluded that there was a significant influence of ripening time on butanoic, isovaleric, hexanoic, octanoic and decanoic fatty acids ($p < 0.001$) together with heptanoic acid ($p < 0.005$), 2-methyl pentanone, 2-methyl-heptanone, 2-methyl nonanone, methyl ketones ($p < 0.001$), ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate and ethyl hexadecanoate ethyl esters ($p < 0.001$). Ripening time showed no significant influence on the other identified compounds.

3.2. Fatty acids

Fig. 1 presents the results for decanoic and hexanoic acids, two of the most abundant fatty acids. The observed increase in concentration was expected due to the fact that the majority of the fatty acids, especially those with more than six carbon atoms, originate from lipolysis, thus changing according to cheese ripening (Bosset & Gauch, 1993; Weber & Ramet, 1990).

In order to establish a kinetic model different reaction orders were investigated (Villota & Hawkes, 1992). Due to the data scattering observed and the lack of knowledge regarding the main mechanisms involved, it is difficult to establish a suitable reaction order. Nevertheless, for modelling purposes and for the sake of simplicity, a zero order kinetic was chosen, since this model was able to account for more variance than any other considered for the ripening period studied.

For the butanoic, hexanoic, octanoic and decanoic acids, their concentrations increased according to a zero order reaction (Table 1) as shown in Eq. (1):

$$Ca - Ca_0 = kt \tag{1}$$

where k is the zero order reaction rate constant, t the reaction time and C the concentration (μg/g). The confidence level of the correlation coefficient was higher than 95%.

The scattering of the points could be understood as linked to the fact that each point corresponds to a different sampled cheese. Differences among cheeses are responsible for these phenomena.

Decanoic acid was the most abundant volatile for fresh and ripened cheese (Table 1). Other acids, such as octanoic and hexanoic, also increased their concentration. The behaviours of tetradecanoic and dodecanoic acids were similar; the observed variations were not significant and their concentrations could be considered constant within the ripening period.

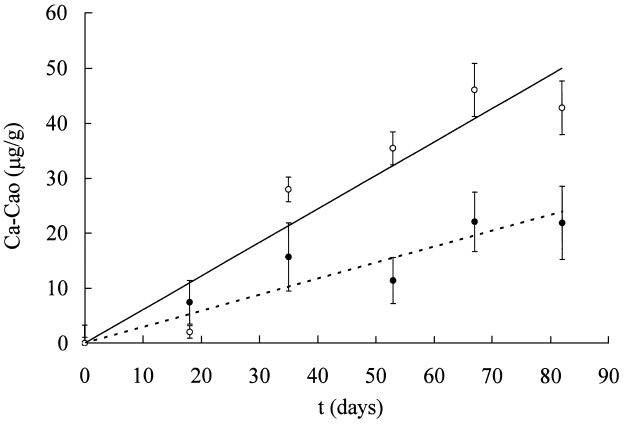

Fig. 1. Zero order reaction fitting for hexanoic (○) and decanoic (●) acids.

Table 1
Moisture and volatile compounds along ripening time for Mahón cheese. Values are given as means with their standard deviation ($n=8$). Zero order reaction rate constant (k) and corresponding explained variances

		Ripening time						Mean	k (μg/g)/day	Explained variance (%)
		0 days	18 days	35 days	53 days	67 days	82 days			
Moisture (g/100 g)		45.5 ± 0.2	43.3 ± 0.1	43.6 ± 0.1	40.3 ± 0.2	39.8 ± 0.1	39.4 ± 0.1			
Major free fatty acids (μg/g dm)	Butanoic	17 ± 3	15 ± 3	19 ± 3	32 ± 5	48 ± 6	43 ± 6	29	0.21	78
	Hexanoic	16 ± 2	19 ± 2	65 ± 4	74 ± 5	91 ± 8	85 ± 8	58	0.61	90
	Octanoic	13 ± 4	29 ± 4	55 ± 5	50 ± 4	53 ± 3	54 ± 6	42	0.38	72
	Decanoic	81 ± 6	91 ± 7	106 ± 11	93 ± 7	110 ± 9	109 ± 11	98	0.29	83
	Dodecanoic	46 ± 8	48 ± 5	53 ± 5	41 ± 7	40 ± 9	38 ± 5	44	−0.037	5
	Tetradecanoic	39 ± 3	39 ± 9	51 ± 5	37 ± 3	33 ± 6	35 ± 2	39	0.015	14
Minor free fatty acids (μg/g dm)	Isovaleric	0.45 ± 0.05	0.53 ± 0.07	1.6 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.6 ± 0.1	1.1	0.01	66
	Pentanoic	0.8 ± 0.09	1.2 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.3	0.006	42
	Heptanoic	1.5 ± 0.2	2.1 ± 0.3	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0.3	2.3 ± 0.4	2.2	0.004	55
	Nonanoic	0.9 ± 0.1	2.1 ± 0.2	2.2 ± 0.3	2.0 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.9	5×10^{-5}	5×10^{-5}
Methyl ketones (μg/g dm)	2-pentanone	0.29 ± 0.03	0.35 ± 0.05	0.35 ± 0.04	0.33 ± 0.1	0.33 ± 0.06	0.40 ± 0.2	0.34	0.0008	48
	2-heptanone	0.7 ± 0.3	0.6 ± 0.1	0.9 ± 0.1	2.1 ± 0.2	2.0 ± 0.3	3.1 ± 0.3	1.6	0.015	81
	2-nonanone	0.6 ± 0.1	1.0 ± 0.2	0.7 ± 0.2	1.2 ± 0.3	1.1 ± 0.1	1.4 ± 0.1	1.0	0.062	74
	2-undecanone	0.7 ± 0.3	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.8	0.4 ± 0.05	0.5	−0.002	41
Ethyl esters (μg/g dm)	Butyrate	0.91 ± 0.09	1.06 ± 0.15	0.7 ± 0.1	0.7 ± 0.1	0.66 ± 0.09	0.6 ± 0.1	0.77	−0.001	51
	Hexanoate	3.5 ± 0.5	2.6 ± 0.3	1.7 ± 0.2	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	2.0	−0.016	64
	Octanoate	5.5 ± 0.7	4.1 ± 0.5	4.0 ± 0.4	3.4 ± 0.3	3.3 ± 0.3	3.2 ± 0.5	3.9	−0.016	67
	Decanoate	9.2 ± 1.2	9 ± 1	8.9 ± 0.9	6.7 ± 0.7	6.6 ± 0.8	6.5 ± 0.5	7.8	−0.014	74
	Tetradecanoate	11 ± 1	8.8 ± 0.9	8.9 ± 1.1	6.6 ± 0.4	6.6 ± 0.9	4.8 ± 0.8	7.8	−0.04	94
	Hexadecanoate	5.5 ± 0.8	5.3 ± 0.7	5 ± 1	3.2 ± 0.5	3.3 ± 0.5	2.6 ± 0.4	4.1	−0.017	83

The concentrations of isovaleric, pentanoic, heptanoic and nonanoic acids were much lower than those mentioned above. Concentrations of pentanoic, heptanoic and nonanoic acids can be considered constant along the whole period, whereas isovaleric acid increases with time.

The increase in concentration of the volatile fatty acids during ripening, has also been reported for other kinds of cheeses (Attaie & Richter, 1996; Contarini & Toppino, 1995; Fernandez-Garcia, 1996; Tuomala & Kallio, 1996). Nevertheless, the main acids found were often of shorter chain lengths than those of Mahón cheese. In Swiss Gruyère cheese (Bosset & Gauch, 1993; Bosset & Liardon, 1985), Greek Gruyère (Zerfiridis, Vafopoulou-Mastrogiannaki, & Lipoulou-Tzanetaki, 1984) and Gruyere de Comté (Berdague, Jeunet, & Grappin, 1987), the main fatty acids found during ripening were ethanoic, propanoic and butanoic, originating mainly from lactose fermentation and amino acids. High levels of those acids are common in cooked paste cheeses and their concentrations have been used by the cheese industry to identify anomalous propionic and butyric fermentations (Berdague, Jeunet, & Grappin, 1987).

3.3. Methyl-ketones

Table 1 shows the change in the methyl-ketones. For both 2-methyl heptanone and 2-methyl nonanone, an increase in concentration, which could be described by a zero order kinetics (95% confidence level), was observed. Nevertheless, the concentrations of 2-methyl pentanone and 2-methyl undecanone could be considered constant due to the low explained variance.

In Mahón cheese, in addition to many other kinds of cheeses, the methyl-ketones of seven and nine carbon atoms were present in larger amounts than any other methyl-ketones (Guichard, Berdague, & Grappin, 1987). This finding has been related to the fact that the β-oxidation mechanism involved in the methyl-ketones formation from the corresponding free fatty acids is common for octanoic and decanoic acids (Frutos, Sanz, & Martinez-Castro, 1991).

The increase in the number of methyl ketones during ripening is characteristic of many kinds of cheeses (Bosset & Liardon, 1985) due to the fact that its occurrence is linked to lipolysis.

3.4. Ethyl-esters

For Mahón cheese there was a significant decrease in the concentration of all the esters identified. Although esters have been frequently identified (Bosset & Liardon, 1984; Frutos, Sanz, & Martinez-Castro, 1991; Guichard, Berdague, & Grappin, 1987; Preininger & Grosch, 1994) there is a lack of studies on their change. Some authors reported that, in different kinds of cheese, esters

appeared as a consequence of enzymic or chemical reactions (Collin, Osman, Delcambre, El-Zayat, & Dufour, 1993); nevertheless other studies show that some esters decrease with time (Yang & Min, 1994).

3.5. Alcohols

The only alcohol identified was heptanol; no neat evolution pattern was observed, the concentration varying between 0.2 and 1.3 μg/g cheese DM, the minimum value being for fresh cheese.

3.6. Clustering: principal component analysis

PCA was applied to pooled measurements in order to establish the relationship between the different chemical variables and to detect the most important factors of variability. The following variables on which ripening time showed the highest influence ($p < 0.005$) were selected to carry out this analysis: butanoic acid, isovaleric acid, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid, 2-methyl pentanone, 2-methyl heptanone, 2-methyl nonanone, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate and ethyl hexadecanoate.

Squared multiple correlation coefficients of each variable with all other variables, and communalities among the selected variables were high (ranging from 0.72 to 0.993 and from 0.68 to 0.95, respectively). Therefore, PCA could be applied to the results without deterioration or loss of information.

PCA of analytical variables resulted in three principal components with eingenvalues greater than 1.0, a common statistical cutoff point (Dixon, 1992). The selected components accounted for 86.3% of the total variance. Thus, the dimensionality of the results was reduced from 15 variables to three uncorrelated principal components with 13.7% loss of variance. The first principal component condensed 64.0% of variance in data space and 74.1% in factor space. Loading coefficients obtained from the application of PCA are shown in Table 2.

Selected chemical variables were represented as a function of both the first (PC1) and second (PC2) principal components in Fig. 2. PC1 was high in ethyl hexadecanoate, ethyl decanoate, ethyl tetradecanoate (positive values) and in butanoic acid and 2-methyl heptanone (negative values). PC2 was high in isovaleric acid, octanoic acid (positive values) and in ethyl butyrate, ethyl hexanoate (negative values). Fig. 2 shows a clustering of acids, ketones and esters, thus suggesting a similar origin and evolution during ripening.

Table 2
Principal components rotated loadings for characteristic volatile compounds

	PC1	PC2	PC3
Decanoic acid	−0.264	0.688	0.374
Butanoic acid	−0.790	0.394	0.153
Hexanoic acid	−0.633	0.687	0.247
Octanoic acid	−0.352	0.703	0.573
Isovaleric acid	−0.240	0.769	0.522
Heptanoic acid	−0.150	0.277	0.877
2-Methyl-pentanone	0.000	0.130	0.876
2-Methyl-heptanone	−0.783	0.190	0.436
2-Methyl-nonanone	−0.693	−0.102	0.627
Ethyl butirate	0.465	−0.802	0.219
Ethyl hexanoate	0.644	−0.638	−0.245
Ethyl octanoate	0.722	−0.502	−0.135
Ethyl decanoate	0.885	−0.337	0.109
Ethyl tetradecanoate	0.820	−0.372	−0.266
Ethyl hexadecanoate	0.897	−0.334	−0.028

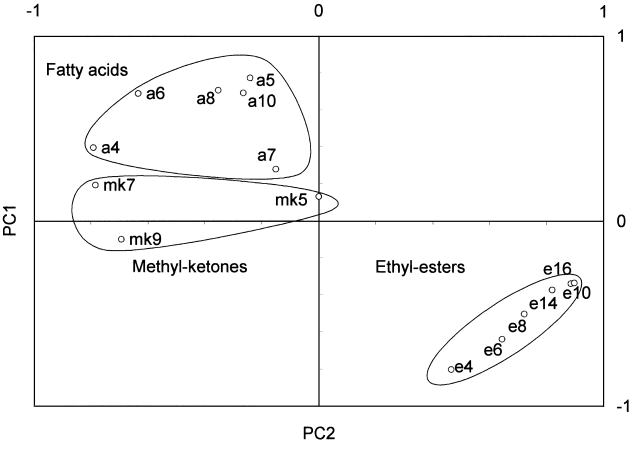

Fig. 2. Principal component analysis. Clustering of volatile compounds according to the first (PC1) and second (PC2) principal components: a4 (butanoic acid), a5 (isovaleric acid), a6 (hexanoic acid), a7 (heptanoic acid), a8 (octanoic acid), a10 (decanoic acid), mk5 (2-methyl pentanone), mk7 (2-methyl heptanone), mk9 (2-methyl nonanone), e4 (ethyl butyrate), e6 (ethyl hexanoate), e8 (ethyl octanoate), e10 (ethyl decanoate), e14 (ethyl tetradecanoate), e16 (ethyl hexadecanoate).

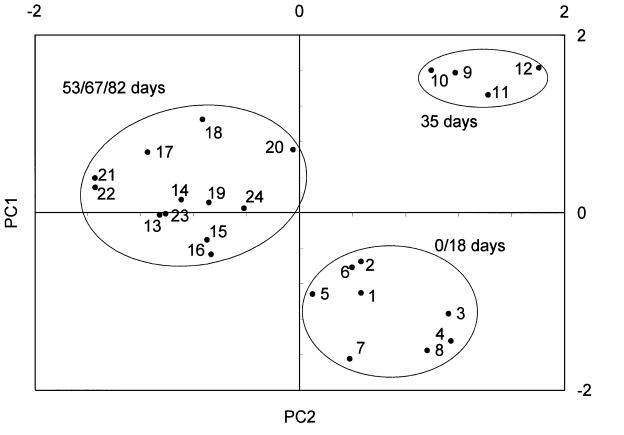

Fig. 3. Principal component analysis. Clustering of Mahón cheese samples (1–24) according to loadings on the first (PC1) and second (PC2) principal components.

To assess the ripening time influence, the loadings of cheese samples for the first two components were plotted as shown in Fig. 3. It is observed that, during a first storage of 18 days, there is a clustering of the samples and another cluster for 53, 67 and 82 days. An intermediate clustering at 35 days was also observed. This result shows the possibility of differentiating cheeses by age range, thus setting up a methodology for the control of the ripening stage.

4. Conclusions

During ripening of Mahón cheese important changes in the volatile fraction takes place. There was an overall increase in fatty acids and methyl ketone contents. This was also observed for other kinds of cheeses.

The main compounds of the volatile fraction were aliphatic non-branched fatty acids. Regardless of ripening time, the main concentration always corresponded to decanoic acid, next in decreasing concentrations being the hexanoic, dodecanoic, octanoic, tetradecanoic and butanoic acids. By the end of the ripening period considered, 82 days, these six acids represented around 95% (weight basis) of the volatiles identified, thus they were present in important quantitative amounts and may contribute significantly to the Mahón cheese aroma development at the different ripening stages.

At the present stage of knowledge, an overall reaction kinetic order for most of the compounds identified could be proposed in order to describe their evolution. For process modelling purposes, the overall zero order kinetics may be adequate over the first 82 days of ripening. Compounds such as pentanoic, heptanoic, nonanoic, dodecanoic and tetradecanoic acids, 2-methyl pentanone, 2-methyl undecanone, ethyl butyrate, and heptanol did not vary significantly during the ripening period considered.

In the clustering analysis of volatiles it was observed that acids, ketones and esters were grouped by using the first two components. This fact suggests a similarity of development during ripening for those series of compounds.

The clustering of cheeses showed that the production of volatiles during the ripening of Mahón cheese produced a grouping starting at 53 days and lasting up to 82 days. This result confirms those previously attained by the experts of the Appellation of Origin who stated 60 days (half-ripened) as a market landmark for all the producers.

Through the statistical treatment (ANOVA, and PCA analyses) of the volatile component parameters measured in different samples of Mahón cheese, it was possible to establish two principal components capable of distinguishing among Mahón cheeses from different ripening times.

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