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Changes of organic acids, volatile aroma compounds and sensory characteristics of Halloumi cheese kept in brine

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Abstract

This study examines the changes in organic acids, volatile aroma compounds and sensory characteristics of ovine Halloumi cheese kept in brine (~10% NaCl) at 4 °C for 45 days. The highest total score of the organoleptic assessment was observed at day 1, before storage in brine. During cheese storage, a large reduction of lactose was observed, especially in the first stages of storage while, concurrently, a significantly progressive increase in lactic and acetic acids was observed. Among the volatile aroma compounds determined were alcohols, aldehydes, ketones, acids, esters, hydrocarbons, sulphur compounds, as well as a variety of other compounds in very small quantities. Ethanol and acetic acid were the dominant volatile aromatic compounds and their concentrations increased during storage. The lipolysis of Halloumi cheese during storage was not excessive. The dominant free fatty acids were palmitic, oleic and acetic.

Keywords: Halloumi cheese; Organic acids; Lactose; Volatile aromatic compounds; Lipolysis; HPLC

1. Introduction

Halloumi cheese is a traditional and distinctive cheese of Cyprus. It is widely popular in Cyprus and other countries of the eastern Mediterranean and, more recently, the product has gained international acceptance and recognition. Total exports of Halloumi cheese from Cyprus have risen to ≈2500 metric tonnes (Papademas & Robinson, 2000). The committee for standards of the Cyprus Ministry of Commerce and Industry (1985) established the definition and standards for Halloumi cheese. Large quantities are sold immediately after production and this fresh product has a characteristic aroma, its texture is elastic and compact with no holes, and it is easily sliced. A proportion of the total Halloumi cheese production in Cyprus is preserved in brine. During storage, Halloumi changes markedly in taste and texture and the cheese becomes salty and moderately acidic. However, the fermentation of lactose, the production of organic acids, volatile aromatic compounds, free fatty acids and other compounds during the storage of Halloumi cheese have not been studied up to now. These components, which determine the sensory characteristics of ovine Halloumi cheese, were studied in this work so as to assist the determination of the identity and features of this cheese.

2. Materials and methods

2.1. Milk

Ovine milk from the flock of the Agricultural University of Athens was used.

2.2. Cheese making and sampling

Halloumi cheese was manufactured at the pilot plant of the Dairy Laboratory of the Agricultural University of Athens, following the cheese making procedure described

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by Anifantakis and Kaminarides (1983). Five cheese making trials were conducted. Cheese was subjected to chemical analysis 1 day after preparation and following 15, 30, and 45 days of brine preservation (~10% NaCl) at 4 °C.

2.3. Physicochemical analyses

Lactose and organic acids were analyzed by high-performance liquid chromatography (HPLC). A Varian HPLC (Varian Associates Inc. 2010, CA, USA) equipped with an RI detector (GBC Scientific Equipment Pty Ltd., LC 1240, Vic., Australia) was used with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm, Hercules, CA, USA). Before HPLC analysis, proteins and fat of samples were removed according to the procedure of Standard 28A described by the International Dairy Federation (IDF, 1974). One microlitre of the filtrate was mixed with 50 μl 70% perchloric acid, for the precipitation of very small peptides, and centrifuged at 14,000 rpm and 4 °C for 30 min (Heraeus Sepatech, Biofuge 22R). The upper phase was passed through a filter (0.45 μm) and the filtrate was injected into the 20 μl loop for HPLC analysis. Analysis was performed isocratically at 35 °C with 5 mM H_2SO_4 and at a flow rate of 0.5 ml/min. Analytical grade organic acids were used as standards. Quantification was based on peak area measurements, using a Waters recorder and data module (model 746, Waters Corporation, Milford, USA).

The volatile compounds present in the headspace fraction of samples were isolated and identified using a balance pressure (static) Perkin–Elmer HS40 headspace system (Perkin–Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 (Shimadzu Co., Kyoto, Japan). Five grammes of each milk and Halloumi cheese sample were weighed and introduced into 22 ml vials, which were then sealed with aluminium–rubber septa. The vials containing the samples were held at 80 °C for 30 min, purged and pressurized with helium at a flow rate of 35 ml/min. The volatile compounds were driven through the transfer line that was held at 100 °C to the injector of the GC. The volatile compounds were separated on an HP Innowax capillary column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) under the following conditions: injector temperature, 200 °C; carrier gas helium 0.6 ml/min; temperature programme: 35°C for 3 min, increasing to 80 °C at a rate of 4 °C/min, held for 12 min and then raised to 200 °C at a rate of 7 °C/min and held for 6 min. The GC column was directly connected without splitting to the ion source of a QP 5050 quadrupole mass spectrometric detector, operating in the scan mode within a mass range of m/z 35–300 at 2 scans/s. The interface line to the MS was set at 250 °C. The MS operated in an electron impact mode at electron energy of 70 eV and was calibrated by auto-tuning. Identification of the compounds was carried out by computer-matching of mass spectral data with those in the Shimadzu NIST62 mass spectral database and by comparing their retention times and mass spectra with those of some standard compounds (when available) using the same conditions The quantification of the volatile compounds was performed using the Shimadzu Class 500 software by integrating the peak areas of total ion chromatograms (TIC).

Free fatty acids (FFA) were extracted from cheese according to the method of Nieuwenhof and Hup (1971) and determined by gas chromatography. A Shimadzu model GC-17A gas chromatograph, equipped with an on-column injector and a flame ionization detector (FID) was used with a Nucol fused silica capillary column (length 30 m, internal diameter 0.25 mm) coated with free fatty acid (FFA) phase OV-351 (bonded polyglycol- nitroterephthalic, film thickness 0.25 μm). Direct on-column injection took place at 110 °C. The injector temperature was raised from 110 to 220 °C at a rate of 8 °C/min and then held at 220 °C for 55 min. Oven temperature was initially held at 60 °C for 2 min. Afterwards, it was raised to 70 °C at a rate of 1 °C/min and then immediately raised to 220 °C at a rate of 10 °C/min and held at 220 °C for 18 min. The FID temperature was 230 °C. The carrier gas (helium) flow rate was 2.5 ml/min. The identification of the individual fatty acids of the cheese samples was based on a comparison of the retention times of the unknown FFA with those obtained from known FFA standards (Sigma, Steinheim, Germany) under identical conditions. The quantification of the FFA of cheese samples was performed using the internal standardization technique with C7:0 and C17:0 as internal standards and processing the chromatograms with the Chromeleon™ version 6.2 Software System (Shimadzu Scientific Instruments Inc., Dionex Corp., P.D., Sunnyvale).

2.4. Sensory evaluation

Following storage for 1, 15, 30 and 45 days, cheese was subjected to sensory evaluation by a 10-member panel of the Dairy Laboratory of the Agricultural University of Athens. Panel members, who were familiar with Halloumi cheese, evaluated each cheese for appearance, texture and flavour (odour and taste) using a 10-point scale, scoring 1 for the worst and 10 for the best quality. The attributes of flavour and texture were given priority over appearance, as advised by the IDF (1987), by multiplying their scores by 5 and 4, respectively. The total score was obtained by adding the scores for the three attributes. An excellent cheese would receive a total score of 100. The results are expressed as a mean score by the whole panel for each cheese.

2.5. Statistical analysis

The results for cheese at each sampling age, were subjected to analysis of variance (ANOVA) using the software Statgraphics (Statistical Graphics Corp., Rockville, MD, USA). A randomized complete block design was used and paired comparisons of means were made using the Duncan test ($P \leqslant 0.05$).

3. Results and discussion

3.1. Sugars and organic acids

The main sugars and organic acids that were determined qualitatively and quantitatively in the samples of milk and Halloumi cheese at 1, 15, 30 and 45 days were lactose, citric acid, lactic acid, acetic acid and pyruvic acid.

3.1.1. Lactose

The concentration of lactose in ovine milk was found on average to be 46 mg/ml. The concentration of lactose was significantly higher in ovine milk than in Halloumi cheese (Table 1). Statistical differences ($P < 0.05$) were observed in the lactose content of Halloumi cheese following storage in brine. The initial rapid reduction in lactose during storage resulted mainly from diffusion of lactose into the brine that was used for cheese storage. Subsequent decrease in the concentration of lactose resulted from fermentation by microorganisms. Other researchers have also observed a reduction of lactose during cheese ripening. According to Bouzas, Kantt, Bodyfelt, and Torres (1991), the concentration of lactose in Cheddar cheese decreased from 7.25 to 3.85 mg/g of cheese after 8 days at 12 °C, while it was not detected in samples at 48 days. Also, in commercial Cottage cheese, the lactose was found to vary between 0.00 and 4.31 mg/g of cheese. In Ossolano cheese, the concentration of lactose amounted to 0.87 mg/g of cheese (Zeppa, Conterno, & Gerbi, 2001).

3.1.2. Citric acid

Citric acid, present in ovine milk at 2.63 mg/ml on average, was not detected in Halloumi cheese during ripening. It is likely that most citric acid was lost in the whey because 94% of the citric acid is in the soluble phase of the milk. Nevertheless, the lack of citric acid in Halloumi cheese may also, be due to the metabolism of citric acid to volatile flavour compounds (acetic acid, diacetyl, acetoin and 2,3-butanediol) by some lactic acid bacteria (McSweeney & Sousa, 2000). Panari (1986) reported that no citric acid was detected in Parmigiano–Reggiano (maturation 20 months), Swiss Emmental (maturation 4–5 months), French Emmental (maturation 5 months), Fontina (maturation 3 months), Sbrinz (maturation 12 months) or Beaufort (maturation 4 months) cheeses because, at the low retention volume, where it elutes, other peaks appear.

3.1.3. Lactic acid

The concentration of lactic acid in ovine milk was found to be on average 1.96 mg/ml, which was less than in Halloumi cheese. Moreover, during the ripening of Halloumi cheese, the concentration of lactic acid increased continuously (Table 1), mainly as a result of the fermentation of lactose by thermotolerant bacteria. The production of lactic acid is essential for the consistent ripening (Califano & Bevilacqua, 2000). The percentage of the lactic acid produced is influenced by the age of the cheese. Its concentration increases during the ripening of many cheeses, such as Ossolano, Gouda and Cheddar (Bouzas et al., 1991; Mullin & Emmons, 1997; Zeppa et al., 2001). Lactic acid dominates in aged cheeses and its concentration in different cheeses ranged from 1.94 to 17.4 mg/g cheese (Bevilacqua & Califano, 1989).

3.1.4. Acetic acid

Acetic acid is the second most abundant acid that was detected in Halloumi cheese and increased after 30 days storage (Table 1). Acetic acid is produced either by the metabolism of lactose by lactic acid bacteria, or the metabolism of citric and lactic acid, or the catabolism of amino acids (McSweeney & Sousa, 2000). Many authors (Bevilacqua & Califano, 1989; Marsili, Ostapenko, Simmons, & Green, 1981; Mullin & Emmons, 1997) have reported that the concentration of acetic acid in different cheeses, such as Cheddar, Provolone, Blue cheeses, Emmental, Fontina, Sbrinz, Ossolano and Beaufort ranged from 0.13 to 2.96 mg/g cheese.

3.1.5. Pyruvic acid

Pyruvic acid was detected in small amounts in Halloumi cheese (Table 1). Pyruvic acid is produced by lactic acid bacteria as an intermediate of both glucose and citrate metabolism (McSweeney & Sousa, 2000). Also, it acts as a substrate in various metabolic pathways in which formic acid, ethanol, diacetyl and acetoin are produced. According to Bevilacqua and Califano (1989), Bouzas et al. (1991), Marsili et al. (1981), Mullin and Emmons (1997) and Zeppa et al. (2001), the concentration of pyruvic acid in different cheeses such as Cheddar, Cottage, Port Salut, Quartirolo, Provolone, Blue cheeses, Emmental, Fontina, Sbrinz, Ossolano and Beaufort ranged from 0.004 to 0.20 mg/g cheese.

Table 1
Lactose and organic acids (mg/g) of Halloumi cheese during ripening (x = means of five trials and S_x = standard error of mean)

Age of cheese (days)	Lactose		Lactic acid		Acetic acid		Pyruvic acid	
	x	S_x	x	S_x	x	S_x	x	S_x
1	23.1[d]	2.07	0.25[a]	0.25	1×10^{-6}[a]	3×10^{-7}	0.02[a]	0.01
15	9.22[c]	0.64	0.59[b]	0.37	0.42[b]	0.17	0.04[a]	0.03
30	6.05[b]	1.26	2.86[c]	1.09	0.19[b]	0.11	2×10^{-7}[a]	4×10^{-8}
45	2.95[a]	1.16	4.07[d]	0.45	0.49[c]	0.14	2×10^{-7}[a]	6×10^{-8}

[a,b,c,d] Means in the same column with the same superscript do not differ significantly ($P > 0.05$).

3.2. Volatile compounds

3.2.1. General

Twenty-five volatile compounds were identified in Halloumi cheese during ripening. These belonged to the following chemical groups: alcohols, aldehydes, ketones, volatile acids, esters, hydrocarbons, sulphur compounds and other substances (Table 2).

3.2.2. Alcohols

Ethanol, 3-methyl-1-butanol and 2-methyl-1-propanol were identified during the storage of Halloumi cheese in brine. Ethanol was the principal volatile aromatic compound produced during the storage of Halloumi cheese, a fact that was also observed in other brine cheeses (Kondyli, Katsiari, Masouras, & Voutsinas, 2002, 2003). The high concentration of ethanol in Halloumi cheese is probably due to the native microflora initially present in the raw milk, the thermotolerant bacteria and the yeast growth in the cheese after production, resulting in the formation of large amounts of ethanol by lactose fermentation (Contarini & Toppino, 1995; Ortigosa, Torre, & Izco, 2001). 2-Methyl-1-propanol was detected in Halloumi cheese, only on day 1, while the concentration of 3-methyl-1-butanol increased during the early stage of storage. These alcohols can be formed by the reduction of the aldehydes, formed during the metabolism of fatty acids and amino acids (Barbieri et al., 1994; Bellesia et al., 2003).

Table 2
Volatile aroma compounds of Halloumi cheese at 1, 15, 30 and 45 days (peak area X10^3 TIC in arbitrary units)

Volatile aroma compounds	Age of cheese (days)			
	1	15	30	45
Alcohols				
Ethanol	6686	4105	10107	19445
3-methyl-1-butanol	1385	2596	580	1132
2-methyl-1-propanol	257	–	–	–
Aldehydes				
Acetaldehyde	15	–	2037	16284
3-methyl-butanal	455	31667	487	303
3-hydroxy-butanal	2054	1137	1745	–
Pentanal	10998	–	–	–
Heptanal	–	1193	–	–
Ketones				
Acetone	3674	2586	810	2267
3-methyl-2-butanone	195	–	–	333
Diacetyl	473	638	483	827
3-hydroxy-2-butanone (acetoin)	–	435	417	261
Volatile acids				
Acetic acid	433	590	671	1105
2-methyl-propanoic acid	–	–	154	150
Butanoic acid	228	–	177	1726
3-methyl-butanoic acid	405	159	–	163
Hexanoic acid	202	–	357	1939
Esters				
Butanoic acid, methyl ester	–	–	–	3129
Hydrocarbons				
1-chloro-3-methyl-butane	–	73	–	–
1-methoxy-4[1-propenyl]-benzene	2038	222	835	3906
Sulphur compounds				
1-propanethiol	–	1284	–	–
2-methyl-2-propanethiol	612	–	–	–
2-methyl-2-butanethiol	–	–	–	172
1-pentanethiol	2445	3277	2485	3141
Other compounds				
Acetonitrile	1816	883	281	997

3.2.3. Aldehydes

In Halloumi cheese, acetaldehyde, 3-methyl-butanal, 3-hydroxy-butanal, pentanal and heptanal were detected. Aldehydes may be produced mainly as a result of amino acid catabolism, or by decarboxylation of ketoacids. Acetaldehyde was found in all cheeses apart from that at 15 days. It is the most common aldehyde found in fermented dairy products (Kondyli et al., 2002, Kondyli, Massouras, Katsiari, & Voutsinas, 2003), and characterized by its sweet, pungent smell (Barbieri et al., 1994; Kondyli et al., 2002, 2003; Milo & Reineccius, 1997). The levels of acetaldehyde increased after 15 days of storage. 3-Methyl-butanal was found at all stages, the level increasing up to 15 days of storage and then decreasing. However, it contributes considerably to the aroma because of its synergistic effect with alcohols (Seuvre, Diaz, & Voilley, 2000). 3-Hydroxy-butanal was found in Halloumi cheese at 1, 15 and 30 days. Pentanal was identified in Halloumi cheese only on day 1. Heptanal was identified in Halloumi cheese of 15 days and gives a woody flavour to the product (Kondyli et al., 2003).

3.2.4. Ketones

Acetone, diacetyl (2,3-butanedione), 3-hydroxy-2-butanone (acetoin) and 3-methyl-2-butanone, were detected in Halloumi cheese during storage. Ketones are common constituents of most dairy products (Engels, Dekker, de Jong, Neeter, & Visser, 1997; Kondyli et al., 2003). Acetone was the main ketone found during storage and is a normal constituent of cheese (Kondyli et al., 2002, 2003; Urbach, 1993), formed by degradation of diacetyl. During storage of Halloumi cheese, the concentration of acetone decreased up to 30 days of storage and then increased. Diacetyl was detected at all stages. It is produced mainly through citrate metabolism (Kondyli et al., 2003; McSweeney & Sousa, 2000; Munoz, Ortigosa, Torre, & Izco, 2003; Ortigosa et al., 2001). It has a buttery, nut-like flavour (Engels et al., 1997; Kondyli et al., 2003) and it is more volatile than acetoin (3-hydroxy-2-butanone). Acetoin was found at all stages after day 1. It is produced by the reduction of diacetyl or by decarboxylation of α-keto lactic acid (Izco & Torre, 2000; McSweeney & Sousa, 2000; Munoz et al., 2003; Ortigosa et al., 2001). It has been found to contribute a buttery character to Cheddar cheese (Chin & Rosenberg, 1997; Kondyli et al., 2002, 2003). Also, 3-methyl-2-butanone was detected in cheeses aged 1 and 45 days.

3.2.5. Volatile acids

Acetic acid, 2-methyl-propanoic acid (isobutyric acid), butanoic acid (butyric acid), 3-methyl-butanoic acid (isovaleric acid) and hexanoic acid (caproic acid) were identified in ovine Halloumi cheese. Acetic acid increased during cheese ripening. This is in accordance with the results of Kondyli et al. (2002) for Feta cheese. It is produced by the fermentation of lactose or citrate and lactate (Barbieri et al., 1994; Izco & Torre, 2000; Molimard & Spinnler, 1996; McSweeney & Sousa, 2000; Munoz et al., 2003; Ortigosa et al., 2001). Moreover, it is derived from the degradation of amino acids (alanine and serine) by bacteria (Kondyli et al., 2003; Molimard & Spinnler, 1996) and characterized by a sour flavour (Kondyli et al., 2003; Milo & Reineccius, 1997). Butyric acid and caproic acid, initially detected on day 1, were not found on day 15, but then increased. Butyric acid, produced mainly by the fermentation of lactose and lactic acid (Barbieri et al., 1994; Molimard & Spinnler, 1996; Munoz et al., 2003; Ortigosa et al., 2001), has been found to be a potent odorant in cheese (Rychlic & Bosset, 2001). 2-Methyl-propanoic acid (isobutyric acid) and 3-methyl-butanoic acid (isovaleric acid) were present in low amounts that could be attributed to the metabolism of the amino acids valine and leucine (Munoz et al., 2003; Ortigosa et al., 2001).

3.2.6. Esters

Butanoic acid methyl ester (methyl butanoate) was detected in Halloumi cheese after 45 days of storage. Esters are formed by the esterification of free fatty acids and alcohols (Bellesia et al., 2003; Izco & Torre, 2000; McSweeney & Sousa, 2000). They make an important contribution to the aroma of the cheeses, because they have synergistic effects with alcohols (Izco & Torre, 2000; Ortigosa et al., 2001).

3.2.7. Hydrocarbons

Hydrocarbons detected in Halloumi cheese were 1-chloro-3-methyl-butane at 15 days and 1-methoxy-4 [1-propenyl]-benzene at all stages of storage. The latter decreased up to the 15th day of storage, but then increased. These components may serve as precursors for the formation of other aromatic compounds (Munoz et al., 2003; Ortigosa et al., 2001).

3.2.8. Sulphur compounds

In Halloumi cheese, 1-propanethiol, 2-methyl-2-propanethiol, 2-methyl-2-butanethiol and 1-pentanethiol were detected. 1-Propanethiol, 2-methyl-2-propanethiol and 2-methyl-2-butanethiol were detected only on days 15, 1 and 45, respectively. By contrast, 1-pentanethiol was detected at all stages and was higher in Halloumi cheese stored for 15 days. Sulphur compounds usually confer a garlic cheese aroma (Izco & Torre, 2000). They originate principally from methionine (McSweeney & Sousa, 2000; Munoz et al., 2003).

3.2.9. Other compounds

Acetonitrile, detected in Halloumi cheese throughout storage, is not a metabolic product, but has been found in various other types of cheese (Izco & Torre, 2000; Munoz et al., 2003; Ortigosa et al., 2001). Apart from acetonitrile, β-myrcene and 3,7-dimethyl [*Z*]-1,3,6-octatriene, derived from the mint (*Mentha viridis*) that was used in cheese making were detected in very small quantities. These aromatic compounds were not detected in all samples because, after the addition of mint to the surface of the cheese, it was rinsed with water and most of the mint was washed away.

3.3. Concentration of free fatty acids

The free fatty acids (FFA) that were detected in Halloumi cheese during storage in brine are shown in Table 3. Acetic acid was found at a high concentration, which increased during cheese ripening. It probably results from the metabolism of lactose, citric and lactic acid by thermotolerant microorganisms in the cheese (Papademas & Robinson, 2000) or the catabolism of amino acids (McSweeney & Sousa, 2000). Acetic acid is known to be the main volatile acid extracted with FFAs, which generally characterizes pickled cheeses (e.g. Feta and Teleme) and contributes greatly to the final flavour of these products (Efthymiou, 1967; Kondyli et al., 2002; Mallatou, Pappa, & Massouras, 2003). The other volatile (C4:0 to C8:0) fatty acids were found in lower concentrations than that of acetic acid. This finding is in accordance with the results of Mallatou et al. (2003) and Kondyli et al. (2002) for ovine Teleme and Feta cheese, respectively. Medium-chain free fatty acids (capric acid, lauric acid and myristic acid) were determined at low concentrations in Halloumi cheese. Myristic acid is the major acid of this group. The long-chain FFAs found in Halloumi cheese included palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Palmitic and oleic acid dominated among the saturated and unsaturated long-chain FFAs of Halloumi cheese. However, they do

Table 3
Free fatty acids (mg/kg) of Halloumi cheese at different stages of storage

Fatty acids	Storage time (days)			
	1	15	30	45
Acetic (C2:0)	72	132	240	360
Butyric (C4:0)	54	24	36	154
Caproic (C6:0)	60	12	90	402
Caprylic (C8:0)	66	294	72	12
Capric (C10:0)	36	90	30	24
Lauric (C12:0)	72	54	24	24
Myristic (C14:0)	84	174	108	126
Palmitic (C16:0)	420	474	276	372
Stearic (C18:0)	60	90	42	78
Oleic (C18:1)	114	160	160	178
Linoleic (C18:2)	72	2,4	6	2,4
Linolenic (C18:3)	0	1,2	2,4	1,2

Table 4
Sensory evaluation of Halloumi cheese at different stages of storage (x = means of five trials and S_x = standard error of mean)

Age of cheese (days)	Flavour (1–50)		Texture (1–40)		Appearance (1–10)		Total (1–100)	
	x	S_x	x	S_x	x	S_x	x	S_x
1	43.9^{b}	2.73	35.0^{a}	1.77	8.9^{a}	0.18	87.8^{b}	4.65
15	40.4^{b}	2.40	34.3^{a}	1.76	9.0^{b}	0.14	83.6^{a}	4.15
30	39.2^{b}	1.32	34.1^{a}	1.18	8.5^{a}	0.19	81.8^{a}	2.63
45	35.6^{a}	2.83	32.4^{a}	1.57	8.6^{a}	0.16	76.6^{a}	4.38

[a,b] Means in the same column with the same superscript do not differ significantly ($P > 0.05$).

not intrinsically contribute to cheese flavour quite as much as short-chain FFAs do, since they have higher perception thresholds (Barbieri et al., 1994; Molimard & Spinnler, 1996; Papademas & Robinson, 2000; Pavia, Trujillo, Sendra, Guamis, & Ferragut, 2000). By contrast, linolenic acid was detected at only very low concentrations in relation to the above FFAs. The total free fatty acid content of Halloumi cheese was markedly lower than that of many other cheese varieties. The low degree of lipolysis in Halloumi cheese may be due to high thermal kneading of the curd after pressing, which inactivates the indigenous lipoprotein lipase, the complete inactivation of which, requires heating at ⩾78 °C for 10 s (Mallatou et al., 2003; McSweeney & Sousa, 2000). Moreover, some researchers have observed an inhibitory effect of NaCl on lipolytic activity (Azarnia, Ehsani, & Mirhadi, 1997; Freitas, Pintado, Pintado, & Malcata, 1999; Pavia et al., 2000; Vlaemynck, 1992), and in this study Halloumi cheese was salted in a brine solution of 10% NaCl.

3.4. Sensory evaluations

The results of the sensory evaluation of Halloumi cheese at various stages of storage showed that the fresh cheese (1 day-old), received the highest total scores and the cheese on day 45 the lowest flavour score (Table 4). During storage, the scores decreased, the flavour of mature cheese became more salty or acidic and the body of cheese was more friable than that of fresh cheese. So, the lowest total, texture and flavour scores were received after 45 days of storage.

4. Conclusions

During the storage of Halloumi cheese in brine, a significant reduction in lactose was observed, followed by a progressive increase in lactic acid, acetic acid and ethanol as a result of the activity of thermotolerant microorganisms in cheese. In contrast, the lipolysis of the cheese was not intense as a result of cooking and salting of the curd.

The volatile aromatic compounds that contribute to the flavour of Halloumi cheese were alcohols, aldehydes, ketones, acids, esters, hydrocarbons and sulphur compounds.

During storage a slight decrease in the total score of the organoleptic assessment occurred and the mature cheese became more salty, acidic and friable than the fresh one.

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