Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 435–441
Food Chemistry
www.elsevier.com/locate/foodchem

A study of the effects of adjunct cultures on the aroma compounds of Feta-type cheese

T. Bintsis [a,*], R.K. Robinson [b]

[a] Laboratory of Food Microbiology and Hygiene, Faculty of Agriculture, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece
[b] School of Food Biosciences, The University of Reading, Whiteknights, P.O. Box 226, Reading, RG6 6AP Berkshire, UK

Received 11 November 2003; received in revised form 16 January 2004; accepted 16 January 2004

Abstract

The influence of adjunct brine cultures on the volatile compounds in Feta-type cheeses made from bovine milk was studied. Four batches of brine were produced: one with no added adjuncts, a second containing *Lactobacillus paracasei* subsp. *paracasei*, a third containing *Lb. paracasei* subsp. *paracasei* plus *Debaryomyces hansenii* and a fourth with *Lb. paracasei* subsp. *paracasei* plus *Yarrowia lipolytica*. All the cultures were isolated from commercial Feta brines.

Aroma compounds were analysed by dynamic headspace analysis, on-line coupled with GC/MS. The most important volatile compounds were quantified in the experimental cheeses; it was concluded that the use of *Lb. paracasei* subsp. *paracasei* and *D. hansenii* as adjuncts in the manufacture of Feta-type cheeses contribute to the formation of a richer pattern of aroma compounds, namely alcohols, aldehydes and esters. The inclusion of *Y. lipolytica* resulted in the production of undesirable aroma compounds that are not part of the usual volatile profile of high quality Feta cheeses.

Keywords: Lactobacilli; Yeasts; Feta-type cheese; Volatile compounds; Aroma compounds

1. Introduction

Feta cheese is the most popular white-brined traditional Greek cheese, which is nowadays globally manufactured on an industrial scale. Traditionally, this cheese was manufactured from raw ovine milk without the addition of starters (artisanal cheese), and development of the typical flavour and texture was based on the natural lactic acid micro-flora (Zygouris, 1952), as well as contaminants from the cheese-making environment and equipment. Good quality milk leads to a cheese with a richer flavour and faster maturation, but this practice may lead to cheeses with a great number of defects, especially during periods of high ambient temperatures. In addition, public health aspects have forced cheesemakers to use pasteurized milk, and nowadays, there is great emphasis on hygienic conditions, production of a safe final product and minimization of the risk of spoilage. These practices, together with the use of phage-resistant starter cultures, selected for their acidification capability, which do not make a significant contribution to the enhancement of the flavour, have resulted in the manufacture of safe and standardized products but, usually, lacking in flavour (Law, 1999).

The microbiology of Feta and related white-brined cheeses is well documented (Bintsis & Papademas, 2002; Bintsis, Litopoulou-Tzanetaki, Davies, & Robinson, 2000; Tzanetakis & Litopoulou-Tzanetaki, 1992; Tzanetakis, Hatzikamari, & Litopoulou-Tzanetaki, 1996) and, typically during the pre-maturation stage for Feta cheese, the population of lactic acid bacteria (LAB) increases significantly, while the pH falls as a result of acid production. Thereafter, microbial counts tend to stabilize for the remaining period of maturation, 60 days at 5–7 °C. If used, mesophilic starter cultures tend to die out early during maturation, as the high salt content (60–80 g/l NaCl) and low pH (pH <5.0) of the brine tend to be highly selective. Consequently, typical Feta brines are colonized by lactobacilli, such as

* Corresponding author. Fax: +30-231-047-3622.
E-mail address: thomasb@panafonet.gr (T. Bintsis).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.057

Lactobacillus paracasei subsp. *paracasei* or *Lactobacillus plantarum* and yeasts, such as *Debaryomyces hansenii* and *Yarrowia lipolytica* (Bintsis et al., 2000).

Cheese aroma is considered to be the result of a balance between various volatile compounds, which individually do not reflect the overall odour (Adda, 1986). Many volatile chemical compounds have been implicated in cheese aroma (Urbach, 1997). These compounds originate from the action of cheese micro-organisms and their enzymes on the lactose, lipids and proteins of cheese curd (Fox, Law, McSweeney, & Wallace, 1993). In a natural biological system, such as a cheese maturing in brine, the varied micro-flora can give rise to a range of volatile metabolites that can contribute to the aroma and flavour of the finished cheese. Available data on the role of lactobacilli and yeasts in the production of volatile aroma compounds in Feta-type cheeses are limited.

Experimental Feta-type cheeses manufactured with starter culture alone (i.e., *Lactococcus lactis* subsp. *lactis*), stored in sterile brine, developed a typical mild balanced flavour; however, the presence of lactobacilli and a selected yeast was associated with a more intense flavour, which achieved higher scores from the sensory panel (Bintsis, Chissimelli, Papadimitriou, & Robinson, 2002).

In the present study, the presence of aroma compounds in experimental Feta-type cheeses was evaluated with dynamic headspace analysis and separated on a GC–MS system, and cheeses were examined for flavour compounds at 2 (fresh cheese) and 60 days (mature cheese) in order to study the impact of the addition of adjunct cultures (i.e., *Lb. paracasei* subsp. *paracasei* alone or in conjunction with *D. hansenii* or *Y. lipolytica*) on the profile of volatile compounds. For comparison, commercial samples of Feta-type cheese were purchased from a local super-market in Reading.

2. Materials and methods

2.1. Manufacture of experimental Feta-type cheeses

The preparation of the inocula and the cheesemaking of two separate batches of Feta-type cheese in the pilot-plant at the University of Reading have been described elsewhere (Bintsis et al., 2002).

In each batch, four lots of 8×500 g blocks were made, and each lot was covered with a different type of brine, namely:

Cheese 1 (S1) – brine (60 g/l NaCl) sterilized at 121 °C for 5 min;

Cheese 2 (S2) – an identical brine inoculated with a culture of *Lb. paracasei* subsp. *paracasei* to give a final concentration of 10×10^3 cfu/ml of brine;

Cheese 3 (S3) – an identical brine inoculated with cultures of *Lb. paracasei* subsp. *paracasei* (10×10^3 cfu/ml of brine) and *D. hansenii* (10×10^2 cfu/ml of brine); and

Cheese 4 (S4) – an identical brine inoculated with cultures of *Lb. paracasei* subsp. *paracasei* (10×10^3 cfu/ml of brine) and *Y. lipolytica* (10×10^2 cfu/ml of brine).

Sample blocks (100 g) were taken from each can at 2 and 60 days for headspace analysis.

2.2. Headspace analysis

2.2.1. Sample preparation

The cheese samples were kept in the freezer (−20 °C) until analysis. The samples were thawed overnight in the refrigerator and cut into small pieces using a sharp knife.

2.2.2. Headspace trapping

Aroma compounds of Feta-type cheese samples were isolated using headspace trapping onto Tenax TA and analysed using thermal desorption combined with GC–MS. Headspace trapping onto an absorbent gives an extract similar to that which is sniffed by a sensory assessor (Elmore & Mottram, 1998).

Chopped cheese samples (10 g) were placed in screw-top conical flasks (250 ml). A Dreschel head was attached to the flask, using an SVL fitting (Bibby Ltd., Stone, UK). The flask was held in a water-bath at 40 °C for 1 h, while nitrogen at 40 ml/min swept the volatiles onto a glass-lined stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg Tenax TA (Scientific Glass Engineering Ltd., Ringwood, Australia). An internal standard (130.6 ng 1,2-dichlorobenzene in hexane) was added to the trap at the end of the collection, and excess solvent and water retained on the trap were removed by purging the trap with nitrogen at 40 ml/min for 5 min.

Headspace collections were performed in duplicate.

2.2.3. Analysis by gas chromatography

Gas chromatography/mass spectrometry (GC/MS) analysis was carried out on a Hewlett Packard (HP) 5972 mass spectrometer (Hewlett Packard Ltd., Bracknell, UK), fitted with an HP 5890 Series II gas chromatograph. A CHIS injection port (Scientific Glass Engineering Ltd., Milton Keynes, UK) was used to thermally desorb the volatiles from the TENAX trap onto the front of a BPX5 fused silica capillary column (50 m × 0.32 mm i.d, 0.5 μm film thickness; Scientific Glass Engineering Ltd.). During the desorption period of 10 min, the oven was held at 0 °C; after desorption, the oven was heated at 40 °C/min to 40 °C and held for 2 min before heating at 4 °C/min to 280 °C. Helium at a pressure of 5.5 N/cm^2 was used as a carrier gas, resulting in a flow of 1.75 ml/min at 40 °C. The mass spectra were determined at an ionisation voltage of 70 eV and anal-

ysed by the HP-Data Analysis System (Hewlett Packard G 1701 AA Version A.03.00).

In order to identify the unknown spectra, the NIST Library of mass spectra and subsets was used. The linear retention indices (LRI) were also calculated for each peak using as a reference the series of hydrocarbons C_6–C_{25} and compared with literature LRI values.

3. Results and discussion

The chemical analyses of the experimental Feta-type cheeses from which the volatiles were extracted were described by Bintsis et al. (2002). These results can be summarized as follows: the use of different adjunct cultures had little impact on the gross chemical composition of the cheeses, while different levels of free fatty acids (FFA) and free amino acids (FAA) were observed in the order S1 < S2 < S3 <S4. S4 was found to have a much softer texture, probably attributable to the high proteolytic activity of *Y. lipolytica* and a harsh flavour, which excluded it from the formal sensory evaluation; the results revealed a significant preference of the panel for the S3 cheese.

The volatile compounds of these experimental cheeses were investigated in the present study, while a commercial Feta-type cheese sample was included for comparison; the flavour compounds comprised 15 alcohols (Table 1), 12 aldehydes (Table 2), 8 ketones (Table 3), 5 esters (Table 4) and 14 miscellaneous compounds (Table 5).

High amounts of ethanol were found to be present in the fresh cheese (Table 1), and these increased in the mature cheeses. Ethanol is the most abundant volatile

Table 1
Amount of alcohols (μg/kg) in the headspace of experimental and commercial Feta-type cheeses; all figures are the means of duplicate determinations of two replicates ± SD

	Commercial	Fresh	S1	S2	S3	S4
Ethanol	154 ± 43	105.5 ± 61	330 ± 130	265 ± 180	180 ± 61	190 ± 44
Propan-1-ol	38 ± 8	–	11 ± 2.5	16 ± 6	11 ± 6.5	33 ± 18
Propan-2-ol	9 ± 7	–	99 ± 48	60 ± 53	23 ± 13	38 ± 18
Butan-1-ol	–	–	–	–	–	7 ± 3
Butan-2-ol	69 ± 26.5	6 ± 1	6 ± 3	7 ± 3	8 ± 4.5	23 ± 13
2-Methyl-propan-1-ol	30 ± 9	–	–	20 ± 11.5	320 ± 37	15 ± 6
2-Methyl-butan-1-ol	–	–	–	18.7 ± 15.1	101 ± 59	–
2-Methyl-2-buten-1-ol	13 ± 0.5	–	7.5 ± 3.2	6.9 ± 5.6	14 ± 9	–
3-Methyl-2-buten-1-ol	–	–	3 ± 1	–	–	17 ± 10
3-Methyl-butan-1-ol	38 ± 15	–	–	311 ± 61	1400 ± 1062	–
Pentan-1-ol	–	–	–	–	29.0 ± 6.5	–
Pentan-2-ol	17 ± 1	–	–	–	–	–
Heptan-2-ol	–	–	–	9 ± 8	–	–
1-Octen-3-ol	–	–	–	–	–	12 ± 2.5
Phenyl ethanol	–	–	–	–	–	43 ± 19

S1, control; S2, *Lb. paracasei* subsp. *paracasei*; S3, *D. hansenii* plus *Lb. paracasei* subsp. *paracasei*; S4, *Y. lipolytica* plus *Lb. paracasei* subsp. *paracasei*.

Table 2
Amount of aldehydes (μg/kg) in the headspace of experimental and commercial Feta-type cheeses; all figures are the means of duplicate determinations of two replicates ± SD

	Commercial	Fresh	S1	S2	S3	S4
Acetaldehyde	–	–	–	310 ± 200	103 ± 110	–
Butanal	–	–	–	–	38 ± 12.5	–
3-Methyl-butanal	17 ± 1	3.5 × 1	27 ± 17	8 ± 1	37 ± 19	–
Pentanal	53 ± 35	–	15 ± 9	26 ± 10	35 ± 15	24 ± 9
Penten-4-al	–	3 ± 1	–	–	–	–
Hexanal	8 ± 4	–	9 ± 4.5	25 ± 17	33 ± 21	9 ± 4
Hexen-3-al	–	6 ± 1	–	–	–	–
Heptanal	–	–	6 ± 4	6 ± 4	12 ± 1.5	5 ± 2
Octanal	–	–	7 ± 3	8.5 ± 3	8 ± 1	–
Nonanal	–	–	–	15 ± 12	9 ± 7	18 ± 9
Decanal	–	–	17 ± 11	–	–	–
Benzaldehyde	–	–	10 ± 7	9 ± 6.5	7.5 ± 2	7 ± 4.5

S1, control; S2, *Lb. paracasei* subsp. *paracasei*; S3, *D. hansenii* plus *Lb. paracasei* subsp. *paracasei*; S4, *Y. lipolytica* plus *Lb. paracasei* subsp. *paracasei*.

Table 3
Amount of ketones (μg/kg) in the headspace of experimental and commercial Feta-type cheeses; all figures are the means of duplicate determinations of two replicates ± SD

	Commercial	Fresh	S1	S2	S3	S4
Acetone	95 ± 90	–	23 ± 11	36 ± 27	–	652 ± 290
2,3-Butanedione	139 ± 127	187 ± 144	37 ± 9.5	83 ± 49	40 ± 29	–
2-Butanone	283.5 ± 215	–	53 ± 35	32 ± 8	80 ± 35	436 ± 314
2-Pentanone	120 ± 67	4 ± 2	22 ± 4.5	30 ± 18	50 ± 34	23 ± 13
3-Hydroxy-2-butanone	–	738 ± 550	21 ± 5	31 ± 23	10 ± 1	136 ± 33
2-Hexanone	5 ± 4	–	–	–	–	–
2-Heptanone	84 ± 35	–	29 ± 25	32 ± 22	9 ± 2	18 ± 3
2-Nonanone	30 ± 19	–	17 ± 14	11 ± 4	4 ± 3	9 ± 3

S1, control; S2, *Lb. paracasei* subsp. *paracasei*; S3, *D. hansenii* plus *Lb. paracasei* subsp. *paracasei*; S4, *Y. lipolytica* plus *Lb. paracasei* subsp. *paracasei*.

Table 4
Amount of esters (μg/kg) in the headspace of experimental and commercial Feta-type cheeses; all figures are the means of duplicate determinations of two replicates ± SD

	Commercial	Fresh	S1	S2	S3	S4
Ethyl acetate	17 ± 4	6.5 ± 1.5	–	30 ± 20	54 ± 22	–
3-Methyl-butyl acetate	–	–	–	–	24 ± 18	–
2-Hydroxy-ethyl propionate	169 ± 90	–	–	–	–	–
Ethyl butyrate	13 ± 10	–	–	–	–	–
3-Methyl-butyl butyrate	–	–	–	–	9 ± 1	–

S1, control; S2, *Lb. paracasei* subsp. *paracasei*; S3, *D. hansenii* plus *Lb. paracasei* subsp. *paracasei*; S4, *Y. lipolytica* plus *Lb. paracasei* subsp. *paracasei*.

Table 5
Amount of miscellaneous compounds (μg/kg) in the headspace of experimental and commercial Feta-type cheeses; all figures are the means of duplicate determinations of two replicates ± SD

	Commercial	Fresh	S1	S2	S3	S4
Toluene	75 ± 52	69 ± 22	87 ± 46	129 ± 77	237 ± 177	113 ± 41
Hexamethyl cyclotrisiloxane	3 ± 0.5	–	7 ± 4	6 ± 4	5 ± 4	16.5 ± 7
Styrene	24 ± 10	12 ± 7	18 ± 4	9 ± 3	20 ± 8	14 ± 6
Limonene	24.5 ± 8	–	9 ± 0.5	14 ± 4	13 ± 6	16 ± 7
Chloroform	18 ± 5.5	–	–	4 ± 0.5	5.6 ± 1	13 ± 7
Benzene	143 ± 89	–	–	–	–	–
Dimethyl disulfide	62 ± 51	–	–	4.5 ± 1	8 ± 2	58 ± 23
Furan	–	4 ± 3	–	–	–	–
2,2,4,6,6-Pentamethyl heptane	–	–	46 ± 20	47 ± 28	25 ± 12	67.5 ± 39.5
Octane	11 ± 0.5	–	3 ± 1.5	–	5 ± 4	–
Nonane	–	–	13 ± 11	6.5 ± 4	8.5 ± 4.5	6.5 ± 3
Decane	–	–	69.5 ± 81	88 ± 50	70 ± 27.5	–
Dodecane	–	–	87 ± 85	–	–	–
Phenol	–	–	13 ± 5	16 ± 12	13 ± 7.5	–

S1, control; S2, *Lb. paracasei* subsp. *paracasei*; S3, *D. hansenii* plus *Lb. paracasei* subsp. *paracasei*; S4, *Y. lipolytica* plus *Lb. paracasei* subsp. *paracasei*.

compound in Feta cheese (Horwood, Lloyd, & Stark, 1981) and Feta-type cheese (Kondyli, Katsiari, Masouras, & Voutsinas, 2002), and is produced by the fermentation of lactose by heterofermentative LAB (Fox & Wallace, 1997) or from amino acid metabolism (Urbach, 1995), or from acetaldehyde reduction (Molimard & Spinnler, 1996). Propan-1-ol and propan-2-ol were detected in all mature experimental and commercial cheeses, and these compounds appeared, also, during maturation of Domiati cheese (Collin, Osman, Delcambre, El-Zayat, & Dufour, 1993).

Butan-1-ol was produced only in S4, probably as a metabolite of *Y. lipolytica*. Butan-2-ol was present in the fresh cheese, and the levels remained constant except for S4. 2-Methyl-propan-1-ol and 2-methyl-butan-1-ol were produced in high levels in S3; these compounds can be derived by reduction of aldehydes formed by Strecker degradation of amino acids (Larsen, 1998). The former

compound was present in Greek Feta cheese (Horwood et al., 1981), while the latter was detected here for the first time in Feta-type cheeses. 3-Methyl-butan-1-ol was detected in S2 and S3, probably produced by the adjunct cultures; the amount in S3 was higher than all other volatiles found in the present study. A high concentration of 3-methyl-butan-1-ol was found in Feta cheese by Kondyli et al. (2002), and this compound is responsible for the pleasant aroma of some soft cheeses, giving an alcoholic floral note (Engels, Dekker, de Jong, Neeter, & Visser, 1997; Molimard & Spinnler, 1996), and is derived from leucine by Strecker degradation (Urbach, 1995).

In addition, pentan-1-ol (amyl alcohol) was detected only in S3 and in the commercial sample made from ovine milk (data not shown) and, according to Horwood et al. (1981), pentan-1-ol is one of the main flavour components of Greek Feta cheese. Since it was found only in S3 of the trial cheeses, it may be that the production of pentan-1-ol can be attributed to the presence of the high population of *D. hansenii*.

One interesting record for S4 was the presence of 1-octen-3-ol, the alcohol responsible for the 'mushroom' note in the typical flavour of Camembert cheese (Gripon, 1993). It could be that the presence of 1-octen-3-ol, together with the presence of phenyl ethanol in S4, were responsible for the 'unclean flavours' observed with this cheese (Bintsis et al., 2002). The latter has been reported to give 'unclean flavours' in Cheddar cheese (Adda, 1986). Phenyl ethanol is one of the major compounds in Camembert cheeses after 7 days of ripening as a result of yeast metabolism (Molimard & Spinnler, 1996).

Alcohols may be rapidly produced from aldehydes under the strong reducing conditions present in cheese (Molimard & Spinnler, 1996), or from other metabolic pathways, namely lactose metabolism and amino acid catabolism. These compounds generate fruity and nutty notes in some cheeses but, when present at high levels, they are responsible for defects in Gouda and Cheddar cheeses (Engels et al., 1997).

High levels of acetaldehyde were found in experimental cheeses S2 and S3 (Table 2). It may be that the high counts of *Lb. paracasei* subsp. *paracasei* were responsible for the formation of acetaldehyde, and it can be formed either by the metabolism of lactate or by the oxidation of ethanol (McSweeney & Sousa, 2000). The formation of butanal in S3, which may be attributed to the presence of high counts of *D. hansenii*, was a notable feature. Additionally, higher amounts of 3-methyl-butanal in S3 were observed, probably due to the high aminopeptidolytic activity of *D. hansenii* (Bintsis, Vafopoulou-Mastrojiannaki, Litopoulou-Tzanetaki, & Robinson, 2003). 3-Methyl-butanal was found to be the most abundant aldehyde in Roncal cheese (Izco & Torre, 2000) and could be formed from leucine through Strecker degradation (Griffith & Hammond, 1989). The complete absence of acetaldehyde and butanal in S4 is surprising, and it may be that *Y. lipolytica* metabolized them to other compounds. Pentanal, hexanal, heptanal and benzaldehyde were detected in all experimental cheeses, while penten-4-al and hexen-3-al were present only in fresh cheese, probably produced by the starter and metabolized throughout the maturation process.

Branched and linear aldehydes are probably derived by the microbial degradation of amino acids (transamination followed by decarboxylation) or via Strecker degradation, while linear aldehydes are additionally formed from lipid oxidation. High levels of branched aldehydes (exceeding 200 μg/kg) caused off-flavours in Cheddar cheese (Dunn & Lindsay, 1985). Some aldehydes have a low perception threshold (Izco, Irigoyen, Torre, & Barcina, 2000) and may play an important role in cheese aroma. They appear in Camembert after the first week, but are quickly transformed into the corresponding alcohol or acid (Dumont & Adda, 1978).

Fresh cheese was found to contain a range of ketones, such as 2,3-butanedione (diacetyl), 2-pentanone and 3-hydroxy-2-butanone (acetoin) (Table 3), probably produced by the starter but, in the control cheese (S1), acetone, 2-butanone, 2-heptanone and 2-nonanone were formed during maturation. S3 was notable for the absence of acetone, while very high amounts of acetone, 2-butanone and 3-hydroxy-2-butanone characterized S4. According to Keen, Walker, and Peberdy (1974), 2-butanone and 2-butanol are formed in Cheddar cheese by the action of non-starter lactic acid bacteria. 2-Butanone is reported to be a major aroma compound in Feta cheese (Horwood et al., 1981). 2-Pentanone and 2-heptanone were the main methyl ketones in Manchego cheese (Martinez-Castro, Sanz, Amigo, Ramos, & Martinez-Alvarez, 1991), while 2-heptanone and 2-nonanone are reported to be the most representative neutral compounds in determining the flavour of mould-ripened cheeses (Sable & Cottenceau, 1999).

It is interesting to note that 2,3-butanedione is produced through metabolism of citrate by LAB and is reduced to 3-hydroxy-2-butanone (Monnet, Condon, Cogan, & Gripon, 1996). The concentrations of these compounds were high in the fresh cheese, probably from the starter, while they markedly declined in the mature cheeses.

Methyl ketones are produced either by microbial oxidation of fatty acids or decarboxylation pathways (Seth & Robinson, 1988), and are commonly found in mould-ripened cheeses, to which they impart a characteristic aroma (Gripon, 1993).

High amounts of ethyl acetate were found in S2 and S3, and 3-methyl-butyl acetate and 3-methyl-butyl butyrate were found in S3 (Table 4). The detection of 3-methyl-butyl butyrate in S3 is worth noting, as this correlates with the high concentration of FFA, and particularly butyric acid, in S3 (Bintsis et al., 2002) and the high scores of S3 in sensory analysis; a possible

explanation could be the high esterase activities of strains of *D. hansenii* (Bintsis et al., 2003). Ethyl-acetate, ethyl propionate, propyl-acetate and ethyl butyrate were found in Feta cheese (Horwood et al., 1981), while Kondyli et al. (2002) found ethyl acetate to be present in Feta cheese but absent in the low-fat Feta-type cheese. It is interesting to note, however, that many types of esters were found in ovine Feta (data not shown), which implies that these compounds may have a special role in the typical flavour of Feta made from ovine milk. It is likely that the high fat content of ovine milk enhances the action of certain lipases and/or esterases.

Esterification reactions occur between short- to medium-chain fatty acids and alcohols (Molimard & Spinnler, 1996). Dahl, Tavaria, and Malcata (2000) reported high amounts of ethyl esters of short- and medium-chain fatty acids in Serra de Estrela cheese, probably as a result of the activity of lipases produced by the yeasts. Most esters encountered in cheeses have very low perception thresholds and are described as having fruity and floral notes (Molimard & Spinnler, 1996); these flavour compounds were the predominant constituents of the volatile fraction of Grana Padano cheese (Moio & Addeo, 1998), Swiss Emmental cheese (Imhof & Bosset, 1994) and Parmesan (Barbieri et al., 1994). Various esters were also recovered from Domiati (Collin et al., 1993). Esters may impart fruity notes to cheeses, and these contribute to the balance of the flavour by minimizing the sharpness imparted by free fatty acids (Collin et al., 1993).

Despite the fact that *Y. lipolytica* is a strongly lipolytic yeast, no esters were detected in S4; probably the enzymic activity of the yeast contributed to the production of methyl ketones from fatty acids rather than esterification reactions.

A number of miscellaneous compounds were detected in the experimental and commercial cheeses. The high levels of toluene and 2,2,4,6,6-pentamethyl-heptane, formed during maturation, were notable in all cheeses, but at different levels. Hydrocarbons are possible secondary products of lipid autoxidation (Barbieri et al., 1994) and do not make a major contribution to cheese aroma, although these components may serve as precursors for the formation of other aromatic compounds (Arora, Cormier, & Lee, 1995). The lack of dimethyl disulfide in S1 is worth noting; low amounts of dimethyl disulfide were found in S2 and S3, but higher amounts in S4 and both commercial samples. Dimethyl disulfide was reported to be a major flavour component of Feta cheese by Horwood et al. (1981) and is formed as an end-product of the Strecker degradation of methionine (Fox & Wallace, 1997); it is an important component of Swiss cheese flavour (Adda, Gripon, & Vassal, 1982), while its presence in Parmesan (Barbieri et al., 1994) and Domiati (Collin et al., 1993) has been reported.

4. Conclusions

All four experimental cheeses exhibited a notable build-up of volatile compounds throughout the maturation.

The results of this study showed that the addition of selected lactobacilli and yeast resulted in a richer pattern of volatiles, in particular alcohols, aldehydes and esters. Some of these compounds, namely, pentan-1-ol, butanal, 3-methyl-butyl acetate and 3-methyl-butyl butyrate were detected only in S3, and have already been identified as essential components of Feta cheese aroma. Additionally, the concentrations of most of the volatile compounds were highest in the cheese containing *Lb. paracasei* subsp. *paracasei* and *D. hansenii* as adjuncts. On the other hand, the inclusion of *Y. lipolytica* resulted in the production of certain volatiles, namely 1-octen-3-ol and phenyl ethanol, which were responsible for 'unclean' flavours.

Acknowledgements

The financial support of the Greek State Scholarship Foundation (IKY) is gratefully acknowledged, as is the technical assistance of Andrew Dodson.

References

Adda, J. (1986). Flavour of dairy products. In G. G. Birch & M. G. Lindley (Eds.), *Developments in food flavours* (pp. 151–172). London, UK: Elsevier Applied Science.

Adda, J., Gripon, J. C., & Vassal, L. (1982). The chemistry of flavour and texture generation in cheese. *Food Chemistry, 9*, 115–129.

Arora, G., Cormier, F., & Lee, B. (1995). Analysis of odour-active volatiles in Cheddar cheese headspace by multidimensional GC/MS/sniffing. *Journal of Agricultural and Food Chemistry, 43*, 748–752.

Barbieri, G., Bolzoni, L., Careri, M., Mangia, A., Parolari, G., Spagnoli, S., & Virgili, R. (1994). Study of the volatile fraction of Parmesan cheese. *Journal of Agricultural Food Chemistry, 42*, 1170–1176.

Bintsis, T., & Papademas, P. (2002). Microbiological quality of white-brined cheeses: A review. *International Journal of Dairy Technology, 55*(3), 113–120.

Bintsis, T., Litopoulou-Tzanetaki, E., Davies, R., & Robinson, R. K. (2000). Microbiology of brines used to mature feta cheese. *International Journal of Dairy Technology, 53*(3), 106–112.

Bintsis, T., Chissimelli, E., Papadimitriou, A., & Robinson, R. K. (2002). Brine microbiology and the chemical and sensory properties of mature feta-type cheese. *Australian Dairy Foods, 8*, 30–32.

Bintsis, T., Vafopoulou-Mastrojiannaki, A., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2003). Protease, peptidase and esterase activities by lactobacilli and yeast isolates from Feta cheese brine. *Journal of Applied Microbiology, 95*, 68–77.

Collin, S., Osman, M., Delcambre, S., El-Zayat, A., & Dufour, J. P. (1993). Investigation of volatile flavour compounds in fresh and ripened Domiati cheeses. *Journal of Agricultural Food Chemistry, 41*, 1659–1663.

Dahl, S., Tavaria, F. K., & Malcata, X. (2000). Relationships between flavour and microbiological profiles in Serra da Estrela cheese throughout ripening. *International Dairy Journal, 10*, 255–262.

Dumont, J. P., & Adda, J. (1978). Flavour formation in dairy products. In D. G. Lund & H. E. Nursten (Eds.), *Progress in flavour research* (pp. 245–262). London: Applied Science.

Dunn, H. C., & Lindsay, R. C. (1985). Evaluation of the role of microbial Strecker-derived aroma compounds in unclean-type flavours of Cheddar cheese. *Journal of Dairy Science, 68*, 2859–2874.

Elmore, J. S., & Mottram, D. S. (1998). Extraction by headspace trapping onto Tenax of novel thiazoles and 3-thiazolines in cooked beef. In C. J. Mussinan & M. J. Morello (Eds.), *Flavour analysis: Developments in isolation and characterization. ACS symposium series* (Vol. 705, pp. 69–77). Washington, DC: American Chemical Society.

Engels, W. J. M., Dekker, R., de Jong, C., Neeter, R., & Visser, S. (1997). A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. *International Dairy Journal, 7*, 255–263.

Fox, P. F., & Wallace, J. M. (1997). Formation of flavour compounds in cheese. *Advances in Applied Microbiology, 45*, 17–85.

Fox, P. F., Law, J., McSweeney, P. L. H., & Wallace, J. (1993). Biochemistry of cheese ripening. In P. F. Fox (Ed.), *Cheese: Chemistry, physics and microbiology* (2nd ed., Vol. 2, pp. 389–438). London: Chapman & Hall.

Gripon, J. C. (1993). Mould-ripened cheeses. In P. F. Fox (Ed.), *Cheese: Chemistry, physics and microbiology* (Vol. 2, pp. 111–136). London: Chapman & Hall.

Griffith, R., & Hammond, E. G. (1989). Generation of Swiss cheese flavour compounds by the reaction of amino acids with carbonyl compounds. *Journal of Dairy Science, 72*, 604–613.

Horwood, J. F., Lloyd, G. T., & Stark, W. (1981). Some flavour compounds of Feta cheese. *Australian Journal of Dairy Technology, 36*(3), 34–37.

Imhof, R., & Bosset, J. O. (1994). Quantitative GC–MS analysis of volatile flavour compounds in pasteurised milk and fermented milk products applying a standard addition method. *Lebensmittel-Wissenschaft und Technologie, 27*, 265–269.

Izco, J. M., & Torre, P. (2000). Characterization of Roncal cheese volatile flavour compounds extracted by the purge & trap method and analysed by GC–MS. *Food Chemistry, 70*, 409–417.

Izco, J. M., Irigoyen, A., Torre, P., & Barcina, Y. (2000). Effect of added enzymes on the free amino acids and sensory characteristics in Ossau-Iraty cheese. *Food Control, 11*, 201–207.

Keen, A. R., Walker, N. J., & Peberdy, M. F. (1974). The formation of 2-butanone and 2-butanol in Cheddar cheese. *Journal of Dairy Research, 41*, 249–257.

Kondyli, E., Katsiari, M. C., Masouras, T., & Voutsinas, L. P. (2002). Free fatty acids and volatile compounds of low-fat Feta-type cheese made with a commercial adjunct culture. *Food Chemistry, 79*, 199–205.

Larsen, T. O. (1998). Volatile flavour production by *Penicillium caseifulvum*. *International Dairy Journal, 8*, 883–887.

Law, B. A. (1999). Cheese ripening and cheese flavour technology. In B. A. Law (Ed.), *Technology of cheesemaking* (pp. 163–192). Sheffield, England: Sheffield Academic Press Ltd..

Martinez-Castro, I., Sanz, J., Amigo, L., Ramos, M., & Martinez-Alvarez, P. (1991). Volatile compounds of Manchego cheese. *Journal of Dairy Research, 58*, 239–246.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheese during ripening: A review. *Lait, 80*, 293–324.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. *Journal of Dairy Research, 65*, 317–333.

Molimard, P., & Spinnler, H. E. (1996). Review: Compounds involved in the flavour of surface mold-ripened cheeses: Origins and properties. *Journal of Dairy Science, 79*, 169–184.

Monnet, V., Condon, S., Cogan, T. M., & Gripon, J.-C. (1996). Metabolism of starter cultures. In T. M. Cogan & J.-P. Accolas (Eds.), *Dairy starter cultures* (pp. 47–99). Cambridge, UK: VCH.

Sable, S., & Cottenceau, G. (1999). Current knowledge of soft cheeses flavor and related compounds. *Journal of Agriculture and Food Chemistry, 47*, 4825–4836.

Seth, R. J., & Robinson, R. K. (1988). Factors contributing to the flavour characteristics of mould-ripened cheese. In R. K. Robinson (Ed.), *Developments in food microbiology* (Vol. 4, pp. 23–47). London, UK: Elsevier Applied Science.

Tzanetakis, N., & Litopoulou-Tzanetaki, E. (1992). Changes in numbers and kinds of lactic acid bacteria in Feta and Teleme, two Greek cheeses from ewe's milk. *Journal of Dairy Science, 75*(6), 1389–1393.

Tzanetakis, N., Hatzikamari, M., & Litopoulou-Tzanetaki, E. (1996). Yeasts of the surface microflora of Feta cheese. In *Yeasts in the dairy industry: Positive and negative aspects. Proceedings of the symposium organised by Group F47-IDF, Copenhagen, Denmark, September 2–3, 1996*.

Urbach, G. (1995). Contribution of lactic acid bacteria to flavour compounds formation in dairy products. *International Dairy Journal, 5*, 877–903.

Urbach, G. (1997). The chemical and biochemical basis of cheese and milk aroma. In B. A. Law (Ed.), *Microbiology and biochemistry of cheese and fermented milk* (2nd ed., pp. 253–298). London: Blackie Academic & Professional.

Zygouris, M. P. (1952). *The dairy industry* (2nd ed.). Athens, Greece: Ministry of Agriculture.