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Food Chemistry 82 (2003) 203–209

Food Chemistry

www.elsevier.com/locate/foodchem

Lipolysis and volatile compounds in low-fat Kefalograviera-type cheese made with commercial special starter cultures

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Received 9 August 2002; received in revised form 22 October 2002; accepted 31 October 2002

Abstract

Two commercially available starter culture systems, Alp DIP and a mixture of Alp DIP D and Joghurt V1, were compared with a regular starter culture, CH-1, for their effects on lipolysis and volatile compounds in a low-fat (9.5%), high moisture (49.6%) Kefalograviera-type cheese during aging. A full-fat control Kefalograviera cheese (30.8% fat, 37.8% moisture) was also made with the regular starter culture. The results indicated that the experimental low-fat cheeses had a higher, but not significantly, total FFA content than the control low-fat cheese and a significantly lower level than the full-fat cheese. The experimental cheeses had also significantly higher levels of acetone at 90 days and acetic acid, diacetyl and acetoin at 180 days than the control low-fat cheese which had significantly higher levels of butan-2-ol and butan-2-one than the former cheeses at both sampling ages.

Keywords: Low-fat cheese; Kefalograviera cheese; Special culture; FFA; Volatile compounds

1. Introduction

Fat has been associated with an increased risk of obesity, certain forms of cancer, atherosclerosis, coronary heart disease, elevated blood pressure and tissue injury diseases associated with lipid oxidation (Dexheimer, 1992; Fenelon & Guinee, 2000). This has created an increased consumer awareness and a dramatic increase in the supply of, and demand for low-fat foods, including cheese.

Generally, reduced- and low-fat cheeses are considered to be less acceptable to consumers than their full-fat counterparts due to texture and flavour defects. The usual textural defects of low-fat cheeses are increased firmness, rubberiness, hardness, dryness and graininess (Emmons, Kalab, Larmond, & Lowrie, 1980; Olson & Johnson, 1990), while the usual flavour defects are low intensity of typical cheese taste and aroma, bitterness, astringency and unclean flavours (Banks, Brechany, & Christie, 1989; Banks, Muir, Brechany, & Law, 1992; Lee, Johnson, & Marth, 1992). It is not clear whether the lack of flavour is due to the lack of precursors from the fat or the lack of the solvent power of the fat or to the different physical structure of the reduced-fat cheese which inhibits certain enzymatic reactions essential for the formation of flavour compounds (Urbach, 1997). Flavour development in cheese is the result of a complex combination of microbial and biochemical activities throughout the ripening period which include the breakdown of milk protein, fat, lactose and citrate and leads to the formation of a heterogeneous mixture of volatile and non-volatile compounds (Fox & Wallace, 1997). The ripening of most cheeses is accompanied by lipolysis, i.e., the hydrolysis of triglycerides to produce free fatty acids (FFA) with chain length $\geqslant$C4. Also, FFA can be produced from the metabolism of carbohydrates and amino acids by bacteria (Fox & Wallace, 1997; Urbach, 1993). FFA contribute to cheese flavour and serve as precursors for a variety of other compounds such as alcohols, esters, aldehydes, ketones and lactones (Fox & Wallace, 1997; Langsrud & Reinbold, 1973; Molimard & Spinnler, 1996; Urbach, 1993). Especially for some cheese varieties, for example, hard Italian and blue-type cheeses, FFAs are the major contributors to the development of their characteristic flavour. Also, methyl ketones and related secondary alcohols originating from FFA are

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0308-8146/03/$ - see front matter
PII: S0308-8146(02)00513-7

important compounds for the development of blue cheese aroma (Fenelon & Guinee, 2000). The catabolism of lactose and citrate produces diacetyl, acetoin and butan-2,3-diol which can be reduced to butan-2-one and then to butan-2-ol. This group of compounds is very important for the characteristic aroma of Cheddar cheese (Urbach, 1993) and fresh cheeses (Fernandez-Garcia, 1996).

Kefalograviera cheese is a very popular hard cheese in Greece. Very few attempts have been made, in experimental studies, to manufacture a low-fat hard cheese similar to the standard Kefalograviera (Katsiari & Voutsinas, 1994). The results of Katsiari and Voutsinas (1994) showed that low-fat Kefalograviera-type cheese (9.7% fat) of acceptable quality can be made from ewe's milk containing 1.5% fat by modifying the conventional cheesemaking procedure. However, the cheese was inferior in flavour quality and physical characteristics to the full-fat (30.8% fat) counterpart made from milk with 6% fat.

Several approaches have been investigated for the potential to improve the flavour and texture of the low-fat cheeses, for example, modification of conventional manufacturing process, use of exogenous enzymes, additives (e.g., fat replacers), specially designed starters or adjunct cultures and combinations of these approaches (Fenelon & Guinee, 2000; Mistry, 2001; Rodriguez, 1998).

Katsiari, Voutsinas, and Kondyli (2002) investigated the use of commercial special starter cultures for improving the sensory quality of a low-fat (9.5% fat) Kefalograviera-type cheese. Their results showed that the low-fat cheeses made with the special cultures Alp DIP and Alp DIP D (plus Joghurt V1) received higher body-texture and significantly higher flavour scores than the control low-fat cheese. Moreover, the former cheeses received body-texture and flavour scores not significantly different from those of the full-fat cheese.

The objective of the present study, which was carried out simultaneously with our previous work (Katsiari et al., 2002), was to determine and compare lipolysis (FFA) and volatile compounds in the low-fat Kefalograviera-type cheese made with different starter cultures to those of full-fat cheese.

2. Materials and methods

2.1. Starter cultures

The commercially available starter cultures used in the cheesemaking of this study were the following:

1. CH-1: a regular (control) thermophilic starter culture consisting of a mixture (1:1) of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Chr. Hansen's Laboratorium, Copenhagen, Denmark). This culture is used commercially for the production of full-fat Kefalograviera cheese.
2. Alp DIP: a special, defined multiple-species starter culture consisting of a mixture of *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Str. thermophilus*, *Lb. helveticus* and *Lb. lactis*. This culture was selected on the basis of preliminary positive results obtained in an initial culture screening for the production of high quality low-fat Kefalograviera-type cheese.
3. Alp DIP D: a special starter culture containing Alp DIP culture and *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*. It was used along with Joghurt V1 culture, a thermophilic, undefined multiple-species culture, consisting of *Str. thermophilus* and *L. delbruckeii* subsp. *bulgaricus*. The selection of Alp DIP D culture was based on the expected good results of Alp DIP culture and on the ability of *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* to produce acetate, diacetyl and acetoin from citrate fermentation (Midje, Bastian, Morris, Martin, Bridgeman, & Vickers, 2000). The Alp DIP, Alp DIP D and Joghurt V1 cultures were gifts from Wiesby GmbH & Co. KG (Niebull, Germany).

2.2. Cheesemaking

Fresh bulk ewe's milk was obtained from the herd of the Agricultural Research Station of Ioannina and standardized to 6.0% fat for the control full-fat cheese and 1.5% fat for the control low-fat, by mixing skim milk and whole milk. Full-fat and low-fat cheeses were made according to the conventional and modified procedures, respectively, of Katsiari and Voutsinas (1994), with the following exceptions: (1) 10 g of $CaCl_2$ were added per 100 kg of low-fat cheese milk instead of 27 and (2) brine salting of low-fat cheeses was done by immersion in 18% (wt./wt.) NaCl solution instead of 20%. Four vats of cheese were made in 1 day. The cheeses were designated as low-fat made with the special culture Alp DIP (cheese A), low-fat made with special culture Alp DIP D supplemented with the Joghurt V1 (cheese B), control low-fat made with the regular lactic culture CH-1 (cheese C) and control full-fat made with the latter culture (cheese, D). The cultures were added directly to the cheese milk at the supplier's recommended levels, i.e., the Alp DIP at a level of 1.0 units/100 l, the Alp DIP D at a level of 0.2 units/100 l, and Joghurt V1 at a level of 0.1 units/100 l cheese milk. The ripening period was 10 min. The control culture was grown in 10% reconstituted, heat-treated (120 °C for 15 min) skim milk powder at 43 °C and was added to the cheese milk at a rate of 0.4% (wt./wt.). The experiment was replicated three times.

Samples from each cheese were taken at various intervals and were analysed for FFA and volatile compounds.

2.3. FFA analysis

Extraction of cheeses lipids, isolation of FFA and determination of FFA concentration by gas chromatography were performed as described by de Jong and Badings (1990). A Shimadzu model GC-17A gas chromatograph equipped with an on-column injector and a flame ionization detector (FID) was used with a fused silica capillary column (length 15 m, inner diameter 0.53 mm) coated with free fatty acid phase OV-351 (bonded poly-glycol-nitroterephthalic, film thickness 1.0 μm). Direct on-column injection took place at 60 °C; the injector temperature was raised from 60 to 230 °C at a rate of 35 °C min^{-1}, and then held at 230 °C for 40 min. Oven temperature was programmed from 60 to 70 °C at a rate of 1 °C $min^{-1,}$ after a 2 min hold at 60 °C, and then to 220 °C at a rate of 10 °C min^{-1} and a 18 min hold at 220 °C. The FID temperature was 225 °C. The carrier gas (helium) flow rate was 8.8 ml min^{-1}. The identification of the individual fatty acids in 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 $C_{9:0}$ as an internal standard and processing the chromatograms with the CLASS-VP™ Chromatography Laboratory Automated Software System (Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

2.4. Analysis of volatile compounds by CG/MS

The headspace volatile compounds were isolated and detected by a dynamic headspace autosampler Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 system (Shimadzu Co, Kyoto, Japan). The cheese samples were grated and 5 g weighed in 20-ml vials; then, the vials were sealed with aluminium-rubber septa. The vials with samples were held at 75 °C for 15 min, purged and pressurised with helium at a flow rate of 35 ml/min. The volatile compounds were driven through the transfer line which was held at 90° C to the injector of the Gas Chromatograph.

The volatile compounds were separated on an HP Innowax capillary column (60 m length×0.25 mm internal diameter, 0.25 μm film thickness) at the following conditions: injector temperature 200 °C; carrier gas helium 0.6 ml/min; temperature program: 35–80 °C at a rate of 5 °C min^{-1}, held for 3 min and go to 200 °C at a rate of 8 °C min^{-1}. The GC column was directly connected without splitting to the ion source of QP 5050 quadrupole mass spectrometric detector which was operating in the scan mode within a mass range of m/z 40–300 at 2 scans/s. The interface line to MS was set at 250 °C. The MS was operating in an electron impact mode at electron energy of 70 eV and was calibrated by autotuning. Identification of the compounds was carried out by computer-matching of their mass spectral data with those of known compounds in the Shimadzu NIST62 Mass spectral Database and by comparing their retention times and mass spectra to those of some standards compounds (when available). Quantification was performed by integrating the peak areas of total ion chromatograms (TIC) by the Shimadzu Class 500 software.

2.5. Statistical analysis

The data of FFA and volatile compounds were subjected to one-way analysis of variance (ANOVA) to test the differences among the four cheeses at each sampling age using the software Statgraphics (Statistical Graphics Corp., Rockville, MD, USA). Statistically significant ($P<0.05$) differences between means were determined using Tukey's test.

3. Results and discussion

3.1. Free fatty acids

Table 1 shows the concentration of the even numbered FFA in the full-fat Kefalograviera and the low-fat Kefalograviera-type cheeses at 90 and 180 days of aging. Palmitic ($C_{16:0}$) and myristic ($C_{14:0}$) acids were the most abundant FFA in all cheeses at both sampling ages. The concentration of isobutyric acid ($iC_{4:0}$) was almost the same in all cheeses while the level of butyric acid ($C_{4:0}$) was significantly ($P<0.05$) lower in the low-fat cheeses than in the full-fat cheese at 180 days of ripening. Chin and Rosenberg (1997) and Dimos, Urbach, and Miller (1996) also found higher butyric acid levels in full-fat than in reduced-fat Cheddar cheese. The concentrations of isovaleric ($iC_{5:0}$), caproic ($C_{6:0}$) and caprylic ($C_{8:0}$) acids did not differ significantly in the four cheeses at both sampling ages while the concentration for capric acid ($C_{10:0}$) were significantly higher in the full fat than in the low-fat cheeses at 90 and 180 days. Other workers (Chin & Rosenberg, 1997; Dimos et al., 1996) found that caproic, caprylic and capric acids were present at higher concentrations in full-fat than in reduced-fat Cheddar cheese. Generally, the levels of butyric, caproic, caprylic and capric acids were low in all cheeses. This trend indicates low lipolytic activity in the cheeses which probably results from the starter microorganisms; the

Table 1
Free fatty acids (mg/100 g cheese) of full-fat Kefalograviera and low-fat Kefalograviera-type cheeses[a,b] made with different starter cultures at 90 and 180 days of aging

Free fatty acid	Age of cheese (days)							
	90				180			
	A	B	C	D	A	B	C	D
$iC_{4:0}$	1.45	1.24	1.30	1.18	1.51	1.50	1.50	1.58
$C_{4:0}$	1.89	1.23	1.52	2.49	1.95[a]	1.64[a]	2.13[a]	3.62[b]
$iC_{5:0}$	1.82	1.87	1.42	2.17	2.19	2.41	1.96	2.97
$C_{6:0}$	4.90	4.58	5.89	6.04	5.99	5.28	5.95	6.64
$C_{8:0}$	1.93	1.73	1.74	2.75	2.58	1.76	1.78	3.79
$C_{10:0}$	2.23a	1.65a	1.67a	5.41b	2.63a	1.72a	1.84a	5.89b
$C_{12:0}$	6.46a	6.32a	5.78a	11.30b	7.54a	6.42a	6.38a	12.68b
$C_{14:0}$	13.15ab	12.10ab	8.16a	15.34b	17.25b	18.55b	8.77a	18.73b
$C_{16:0}$	27.76a	27.82a	23.94a	35.82b	31.92a	31.22a	26.69a	44.55b
$C_{18:0}$	5.22a	5.85a	5.81a	9.74b	6.89a	6.05a	6.21a	12.18b
$C_{18:1}$	6.21a	6.59a	4.50a	10.07b	9.10a	7.06a	6.32a	13.79b
$C_{18:2}$	1.27a	1.20a	1.04a	2.23b	2.12ab	1.44ab	1.27a	2.67b
$C_{18:3}$	nd	nd	nd	0.46	nd	nd	nd	0.84
Total	64.26a	72.18a	62.77a	105.00b	91.67a	85.05a	72.61a	129.75b

Means in a row and at the same age without a letter (a,b) or bearing a common letter (a,b) did not differ significantly.
[a] Means of three trials.
[b] Cheese: A, low-fat with special starter culture ALP DIP; B, low-fat with special starter cultures ALP DIP D and Joghurt V1; C, control low-fat; D, control full-fat. nd: Not detected.

natural lipase of milk was destroyed by the pasteurization process and no lipase was added during cheesemaking. Short- and medium-chain even numbered FFA are more important for the flavour development of the cheese than the long chain FFA, since they have low perception thresholds (Molimard & Spinnler, 1996).

The full-fat cheese and the experimental low-fat cheeses had significantly ($P<0.05$) higher concentrations of myristic acid ($C_{14:0}$) than the control low-fat cheese at 180 days. The concentrations of palmitic, stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids were significantly lower in the low-fat cheeses than in the full-fat cheese at both sampling ages. The concentrations of linoleic acid ($C_{18:2}$) were significantly lower in the low-fat cheeses at 90 days. At 180 days the concentration of linoleic acid in the experimental cheeses did not differ significantly from that in the full-fat cheese. Linolenic acid ($C_{18:3}$) was detected only in the full-fat cheese. The concentration of all individual FFA increased slightly between 90 and 180 days. The concentrations of the FFA found in the cheeses of the present study are in excess of the typical flavour and aroma threshold concentrations reported by Attaie and Richter (1996) and Sable and Cottenceau (1999); therefore, the FFA would be expected to contribute to the organoleptic properties of the cheeses in the current study.

Full-fat cheese had significantly higher values of total levels of FFA than low-fat cheeses (Table 1). It seems therefore, that the special cultures used for the production of the experimental cheeses did not influence, significantly, the production of FFA, since the microorganisms of the used cultures had mainly peptidolytic activity and a weak lipolytic activity (Dellaglio, Torriani, Vlaeminck, & Cornet, 1992).

3.2. Volatile compounds

The most abundant volatile compounds which probably contributed to the flavour of the cheeses at 90 and 180 days of ripening are listed in Table 2 and include, mainly, alcohols (especially short chain alcohols), aldehydes and ketones. These types of compounds have been found in many other cheese varieties such as Parmigiano Reggiano, Mahon, Fontina, Appenzeller and Cheddar (Bosset & Gauch, 1993; Chin & Rosenberg, 1997; Meinhart & Schreier, 1986; Milo & Reineccius, 1997). Some of the volatile compounds found in the cheeses of the present study increased during ripening while others decreased or disappeared. Many of these compounds were common in the four cheeses but they differed quantitatively. Ethanol was the most abundant of the volatile compounds in 90 day-old cheeses with significantly ($P<0.05$) higher levels in the control cheeses than in the experimental ones. The levels of ethanol increased numerically during aging and at 180 days ethanol was the most abundant volatile compound in the full-fat cheese. Dimos et al. (1996) also found higher levels of ethanol in the full-fat than in the reduced-fat Cheddar cheese, in contrast to Chin and Rosenberg (1997), who found the opposite results. Only the low-fat cheeses contained butan-2-ol at both sampling ages. The control low-fat cheese had significantly

Table 2
Volatile compounds (TIC[a] peak area×1000) in full-fat Kefalograviera and low-fat Kefalograviera-type cheeses[b,c] made with different starter cultures at 90 and 180 days of aging

Compounds	Age of cheese (days) 90				180			
	A	B	C	D	A	B	C	D
Alcohols								
Ethanol	29 981a	28 920a	126 096b	169 835c	35 066a	39 547a	166 104b	230 241c
Butan-2-ol	4204b	1220a	5715c		20 532a	29 425a	211 067b	
Pentanol					407b	497b		299a
3-Methylbutanol	295a	257a	566b	322a	358a	429a	1170b	488a
Butan-2,3-diol	63	43	84	55	41a	45a	167b	
Aldehydes								
Acetaldehyde	206a	228a	731b	876c	193a	446a	1411b	1791c
2-Methylpropanal	208				287a	397b	506c	516c
2-Methylbutanal			109	81	127a	143a	406b	446b
3-Methylbutanal	1215b	973b	799b	433a	2051b	2138b	2268b	1866a
3-Hydroxybutanal	51a	25a	65a	87b	92a	122a	107a	198b
Hexanal	28a	21a	47a	91b	166b	64a	79a	102a
Heptanal	121b	43a	42a	119b	190b	105a	116a	161b
Octanal	36a	39a		154b				
Decanal	64	24						
Ketones								
Acetone	1237b	1021b	793a	917a	1312a	2275b	1056a	1143a
Butan-2-one	9356c	1805b	12 619d	333a	35 881a	44 600b	60 711c	
Diacetyl	1968a	5853b	1639a	11 775c	1809b	2776c	1190a	7108d
Acetoin	11 173c	21 995d	515a	1501b	11 468b	25 540d	2840a	12 866c
Other compounds								
Ether			4117	3782	3612b	2827a	4722c	7736d
Ethylacetate			581a	1120b	703a	694a	1040b	1458c
Acetic acid	19 363b	16 333b	19 215b	13 554a	31 200c	39 730c	24 095b	17 195a

Means in a row and at the same age without a letter (a–d) or bearing a common letter (a–d) did not differ significantly.
[a] TIC: total ion chromatograph peak areas in arbitrary units.
[b] Means of two trials.
[c] Cheese: A, low-fat with the special starter culture Alp DIP; B, low-fat with the special starter cultures Alp DIP D and Joghurt V1; C, control low-fat; D, control full-fat.

($P<0.05$) higher levels of 2-butanol than the experimental low-fat cheeses at 90 and 180 days. Dimos (1992) found that the concentration of butan-2-ol increased steadily during maturation and was lower in the full-fat Cheddar cheeses, where it did not appear until about 14 weeks, than in the low-fat cheeses where it appeared at 4 weeks. Pentanol was found in the full-fat and the experimental cheeses only at 180 days of age. The experimental cheeses had significantly higher levels of pentanol than the full-fat cheese. All cheeses contained 3-methylbutanol at levels which increased with age. The control low-fat cheese had significantly higher amounts of 3-methylbutanol than the full-fat cheese and the experimental low-fat cheeses at both sampling ages. All cheeses contained butan-2,3-diol at 90 days and the low-fat cheeses only at the age of 180 days. The control low-fat cheese had significantly ($P<0.05$) higher levels of this alcohol than the experimental low-fat cheeses at the age of 180 days. Chin and Rosenberg (1997) found lower levels of butan-2,3-diol in full-fat cheese in comparison to the reduced-fat Cheddar cheese. Generally, alcohols generate a fruity and nutty flavour in some cheeses and when present at high levels are responsible for flavour defects in Gouda and Cheddar cheeses (Engels, Dekker, de Jong, Neeter, & Visser, 1997).

Acetaldehyde is the most common aldehyde found in fermented dairy products (Langsrud & Reinbold, 1973). It was found in all cheeses and the full-fat cheese and the control low-fat cheese had significantly ($P<0.05$) higher levels than the experimental low-fat cheeses. The level of acetaldehyde increased during aging in all cheeses. All the microorganisms of the cultures used in the cheese manufacture in the present study produce acetaldehyde, and *Str. thermophilus* is claimed by Dellaglio et al. (1992) to produce more acetaldehyde than the others. All cheeses contained 2-methylpropanal only at 180 days. The control low- and full-fat cheeses had significantly higher levels of 2-methylpropanal than the

experimental ones. It was also found that all cheeses contained 2-methylbutanal at 180 days. The control low- and full-fat cheeses had significantly higher levels of this branched chain aldehyde than the experimental cheeses. All cheeses contained 3-methylbutanal at increasing levels as cheese aged. The low-fat cheeses had significantly higher levels of this branched chain aldehyde than the full-fat cheese at both ages. 3-Methylbutanal is responsible for unclean, malty and harsh flavours in Cheddar cheese (Dunn & Linsday, 1985) but is present in good quality Parmesan and Proosdij cheeses (Engels et al., 1997). Hexanal and heptanal were found in all cheeses at different levels, increasing with age. Octanal was found in the experimental low-fat cheeses and the full-fat cheese only at 90 days, while decanal was found only in the experimental cheeses at 90 days and disappeared at 180 days. Oumer, Fernandez-Garcia, Garde, Medina, and Nunez (2001) reported a decrease of the above aldehydes with ripening time in Hispanico cheese. The aldehydes give different flavour notes, for example, acetaldehyde is characterized by its sweet, pungent smell, hexanal is characterized by green, grass like and herbaceous aromas and heptanal gives an oily, woody flavour note (Engels et al., 1997; Milo & Reinecious, 1997; Sable & Cottenceau, 1999).

Ketones are common constituents in most dairy products (Engels et al., 1997). Acetone, a normal constituent of milk and cheese (Urbach, 1993), was found in all cheeses (Table 2) but at different levels. The experimental cheese B had significantly higher levels of acetone than the other cheeses at 180 days. All cheeses contained butan-2-one at 90 days and only the low-fat cheeses at 180 days. The low-fat cheeses had significantly higher levels of butan-2-one than the full-fat cheese at 90 days. Moreover, the control low-fat cheese had the highest level of this ketone among all cheeses. These results are in agreement with the results of Dimos et al. (1996) who also found higher amounts of butan-2-one in reduced-fat than in full-fat Cheddar cheese.

One of the most important ketone flavour compounds is diacetyl, which has a buttery, nut-like flavour (Engels et al., 1997). Diacetyl is formed in dairy products from citrate by various microorganisms and, especially, by *L. lactis* subsp. *lactis* biovar. *diacetylactis* and can be reduced to acetoin (3-hydroxybutan-2-one) and, hence, acetone (Molimard & Spinnler, 1996). Diacetyl was detected in all cheeses at decreasing levels as cheeses aged. Full-fat cheese had the highest levels of diacetyl while the control low-fat had the lowest levels. The low-fat cheeses made with the special starter cultures had intermediate values. Especially, the experimental cheese B made with the culture Alp DIP D which includes the strain*L. lactis* subsp. *lactis* biovar. *diacetylactis* had significantly higher levels of diacetyl than the other low-fat cheeses. Dimos et al. (1996) also found higher levels of diacetyl in full-fat than in reduced-fat Cheddar cheese, while Chin and Rosenberg (1997) did not detect diacetyl in full-fat or reduced-fat Cheddar cheese but they identified its reduction products, i.e., acetoin and butan-2,3-diol. Acetoin, a compound which contributes buttery notes to Cheddar cheese (Chin & Rosenberg, 1997), was found in all cheeses. The control low-fat cheese had the lowest levels of acetoin and the experimental cheese B the highest at both sampling ages, while the other two cheeses had intermediate values. The level of acetoin increased considerably at 180 days of ripening. Other workers (Chin & Rosenberg, 1997; Dimos et al.,1996; Fenelon & Guinee, 2000) reported a decreasing trend in acetoin levels as the level of cheese fat is reduced. This trend was also found in the present study for the full-fat and control low-fat cheeses. The high amounts of acetoin in the experimental cheese B are probably due to the strain*L. lactis* subsp. *lactis* biovar. *diacetylactis,* which is included in the culture Alp DIP D, and produces diacetyl which is converted to acetoin by starter microorganisms (Keen, Walker, & Pederby, 1974).

Diethylether was found only in the control cheeses at 90 days but at 180 days it was detected in all cheeses at different levels. Diethylether is regularly found in the headspace of Cheddar cheese and Swiss Emmental cheese and is not a contaminant (Urbach, 1995). Similarly, ethylacetate was identified only in the control cheeses at 90 days and in all cheeses at 180 days. Full-fat cheese had significantly higher levels of ethylacetate than the low-fat cheeses. Bosset and Gauch (1993) also found ethylacetate in various types of full-fat cheeses. Acetic acid was detected in all cheeses at increasing levels as the cheeses aged. The low-fat cheeses had significantly higher levels of acetic acid than the full-fat cheese at both sampling ages. Other authors also reported higher values for acetic acid in low-fat cheeses in comparison to the full-fat cheeses (Chin & Rosenberg, 1997; Mc Gregor & White, 1990). Acetic acid is characterized by a sour flavour (Milo & Reinecius, 1997) and is the most abundant volatile organic acid in good quality full-fat Kefalograviera-type cheese (Katsiari, Voutsinas, Alichanidis, & Roussis, 2001).

It should be mentioned that Katsiari et al. (2002) found a significant improvement of the sensory quality of the same experimental low-fat cheeses by using the earlier mentioned special cultures compared with the control low-fat cheese made with the regular starter culture. This improvement could be partly attributed to the significantly higher levels of acetone, diacetyl, acetoin and acetic acid in the experimental cheeses.

4. Conclusions

The results of the present study indicate that the use of the special cultures Alp DIP and Alp DIP D (in combination with Joghurt V1 culture) in the manu-

facture of low-fat Kefalograviera-type cheeses increased, but not significantly, the total concentration of free fatty acids in comparison to the control low-fat cheese. The principal volatile compounds found in all cheeses were ethanol, butan-2-one, acetic acid, diacetyl, acetoin, diethylether, 3-methylbutanal and acetone. The experimental low-fat cheeses had higher levels of acetic acid, diacetyl, acetoin and acetone than the control low-fat cheese, compounds which contribute positively to cheese flavour. On the other hand, the low-fat control cheese had excessive amounts of butan-2-ol and butan-2-one.

Acknowledgements

This study was performed as part of NAGREF Project No VI/7 in the framework of the program DEMETRA 95 funded by the National Agricultural Research Foundation of Greece.

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