Investigation of Volatile Flavor Compounds in Fresh and Ripened Domiati Cheeses†

Sonia Collin,*‡ Magdy Osman,§ Sabine Delcambre,‡ Ali I. El-Zayat,§ and Jean-Pierre Dufour‡

Unité de Brasserie et des Industries Alimentaires, Université Catholique de Louvain, Place Croix du Sud 2/Bte 7, B-1348 Louvain-la-Neuve, Belgium, and Department of Food Science, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

The volatile compounds of three fresh and six 3-months-ripened Domiati cheeses were investigated with a dynamic headspace GC–MS system. Unlike the amino acid degradation products and the diketones, acrolein, propan-1-ol, butan-2-one, butan-2-ol, and a large number of esters appear during maturation. Moreover, various sulfur compounds contribute significantly to the overall cheese aroma, particularly when bad ripening occurs. Most of the volatile compounds are synthesized after 2 months of maturation.

INTRODUCTION

Domiati cheese is a white pickled soft cheese widely produced in Egypt. It differs chiefly from other pickled varieties such as feta, teleme, and brinza in being salted at the very first in its manufacture (5–15%, salt weight × 100/milk weight) (El-Zayat and Omer, 1987). It is mainly produced from buffalo and cow milks. Since it is common practice to pasteurize to destroy the pathogenic microflora in milk, the addition of starters is recommended. However, raw milk is occasionally used. Domiati cheese has a distinctive mild and rather salty flavor when fresh. It acquires its characteristic flavor and suitable composition after a period of 3–6 months in salt whey. Domiati cheese flavor has been improved by many investigators through various additions either to the milk or to the pickling brine, e.g., glutamate (Ghaleb, 1977), rennet substitutes (Wahba, 1979), sweet or hot green pepper and sour orange peel (Mahmoud et al., 1979), pepsin and trypsin (Ismail et al, 1980), lipases (Abd El-Salam et al., 1981), free fatty acids (El-Safty and Ismail, 1982), milk hydrolysate (Said and Mohran, 1984), β-galactosidase (El-Safty et al., 1985), and curd cell extract of *Lactobacillus* spp. (Abou-Donia, 1986).

A large number of studies on the technological aspect of the manufacturing (Fahmi and Sharara, 1950; Said and Mohran, 1984; Omer and El-Shibiny, 1985) and the physicochemical (El-Erian et al., 1974; Hofi et al., 1975; El-Zayat, 1987) and microbiological (El-Zayat et al., 1984; El-Safty et al., 1985; El-Zayat, 1986) characteristics of the cheese have been published. However, except for the study on free fatty acid composition (El-Shibiny et al., 1974), no data are available on the volatile flavor compounds of Domiati cheese.

In the present work, we have identified and quantified the main volatile compounds which could contribute to the Domiati flavor, using a dynamic headspace injector directly coupled to a gas chromatograph–mass spectrometer system. The analyses of three fresh and six 3-months-ripened cheeses and of cheeses sampled during maturation are presented.

* Author to whom correspondence should be addressed.

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‡ Université Catholique de Louvain.

§ Suez Canal University.

MATERIALS AND METHODS

Cheese Samples. Three fresh cheeses (age; 7 days), made from pasteurized (samples I and II) or unpasteurized (sample III) milk, and six 3-months-ripened cheeses (samples IV–VIII, optimal ripening; sample IX; presence of off-flavors) were kindly provided by local Domiati manufacturers (II and IV–IX from Miser Milk and Food Co., Ismailia, Egypt; I and III from Suez Canal University, Ismailia, Egypt) and used immediately for analysis. Additional fresh cheese samples from unpasteurized milk (X and XI) were maintained in small containers under salted whey at room temperature (18–22 °C) (this is referred to as "laboratory maturation" in the text).

Reagents. Acetaldehyde, acrolein, butyraldehyde, butan-2-one, caproaldehyde, diacetyl, ethyl propionate, pentane-2,3-dione, pentan-2-one, and valeraldehyde were from Fluka Chemika. Butyl acetate, dimethyl sulfide, ethyl formate, heptanal, hexan-2-one, 2-methylbutyraldehyde, methyl mercaptan, 2-methylvaleraldehyde, pentane, propyl acetate, propyl propionate, *m*-xylene, *p*-xylene, and *o*-xylene came from Aldrich Chemical. Acetone, dimethyl disulfide, ethyl butyrate, heptan-2-one, isoamyl acetate, isobutyraldehyde, 3-methylbutyraldehyde, 2-methylbutan-1-ol, 3-methylbutan-1-ol, nonane, and octane came from Janssen Chimica. Butan-2-ol, ethanol, 2-methylpropan-1-ol, pentan-2-ol, and propan-1-ol came from Polyscience Corp. Ethyl acetate and toluene were from UCB Chemical.

Sample Preparation. One gram of cheese and 9 mL of volatile-free, deionized water (Milli-Q water purification system, Millipore, Bedford, MA) were poured into the purge vessel maintained on ice. The sample was then manually shaken. As an internal standard, 25 μL of 2-methylvaleraldehyde (9 ppm in water) was added. All of the preparation steps were carried out at 4 °C.

Dynamic Headspace Injector Operator Conditions. A purge-and-trap injector from Chrompack was used. Chromatographic injection was achieved in the following three steps.

(*1*) *Precooling of the Cold Trap (Metal Capillary).* The trap was cooled for 1 min with a stream of liquid nitrogen.

(*2*) *Purge of the Sample.* The temperature of the purge vessel was set at 70 °C. Optimization experiments on cheese samples have shown that identical concentrations are determined whatever the purge temperature, in the investigated range (20–70 °C), enabling us to conclude that no significant decomposition reaction occurs. The sample was purged with nitrogen gas (10 mL/min) for 15 min. The gas stream was successively passed through a condenser kept at −15 °C by means of a cryostat (Colora WK 15) to remove water vapor and then through an oven at 200 °C. The volatiles were finally concentrated in the cold trap maintained at −95 °C (liquid nitrogen).

(*3*) *Desorption of the Volatiles.* Cooling was stopped, and the surrounding metal capillary was immediately heated to 220 °C for 5 min. The carrier gas swept the trapped compounds into the analytical column.

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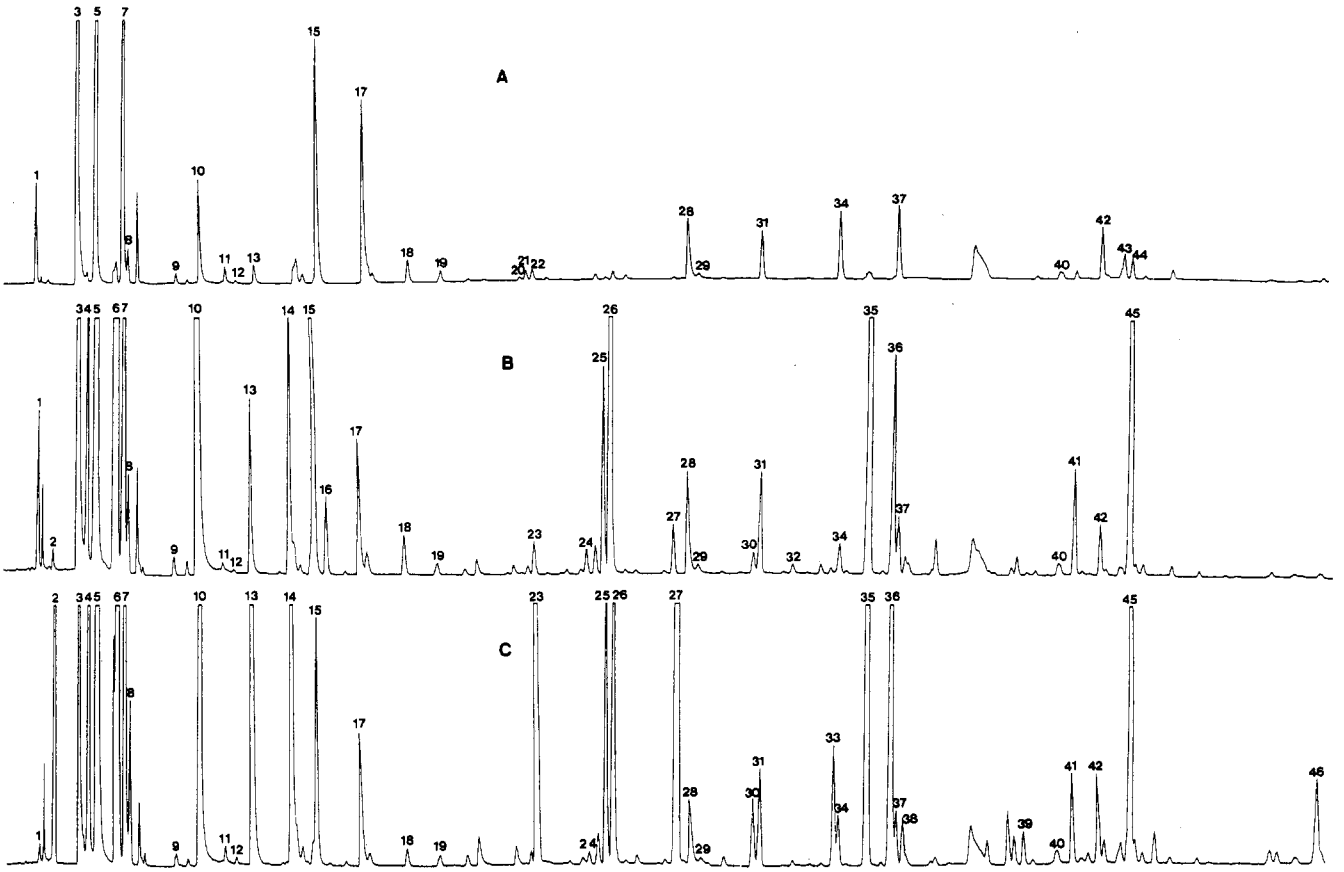

Figure 1. GC traces of (A) a fresh cheese (sample I); (B) a good 3-months-ripened cheese (sample VII); (C) a bad 3-months-ripened cheese (sample IX). The numbering is the same as in Table I.

Gas Chromatography Analytical Conditions. A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an integrator (Shimadzu C-R3A) was used. Analysis of cheese volatile compounds was carried out on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) apolar CP-SIL5 CB capillary column (film thickness, 1.2 μm). Oven temperature, initially kept at 30 °C for 15 min, was programmed to rise from 30 to 100 °C at 2 °C/min, remaining at the maximum temperature for 15 min thereafter. Nitrogen carrier gas was used at a flow rate of 1.5 mL/min. Injection and detection temperatures were 200 and 220 °C, respectively. All analyses were done in duplicate. The minimum peak area for data acquisition was set at 500 μV. The assessment of the technique reproducibility was made in analyzing five times the same mixture of standard compounds (each at a concentration of 50 ppb in the purge vessel, except ethanol which was at 1 ppm) [see the coefficients of variation (CV) in Table I].

Gas Chromatography–Mass Spectrometry Analytical Conditions. The column (see above) was directly connected to an HP 5988 quadrupole mass spectrometer. The carrier gas was helium. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C MS Chemstation analytical workstation. Identification was done on the basis of peak enhancement by coinjection with authentic standard compounds and with the help of the NBS/EPA/NIH mass spectra library. Injections of standard compounds in the GC-FID system (with nitrogen as carrier gas) revealed identical peaks sequence.

Calibration for Quantitative Analysis. Recovery factors (RF given in Table I, extraction yield plus detector response) for the various volatile constituents were determined by adding standard amounts (six points, in duplicate) of pure compounds to cheese samples (Domiati cheese I) prior to analysis, from stock solutions in volatile-free, deionized water (except for heptanal, heptan-2-one, isoamyl acetate, toluene, octane, and xylene, whose stock solutions were prepared in acetone). Accordingly, added amounts were adjusted to approximate the expected concentrations. Under these conditions, the RF factors were obtained for the total extraction and analysis process.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of the most volatile compounds from a fresh (sample I), a good 3-months-ripened (sample VII), and a bad 3-months-ripened (sample IX) Domiati cheese. Over 50 compounds were clearly separated, 44 of which could be identified with various degrees of certainty. The volatile compounds belonged to six major groups: aldehydes, ketones, alcohols, esters, sulfur compounds, and hydrocarbons. The identification and concentration of most of them are summarized in Table I.

Comparative analysis of the volatile compounds of fresh and ripened Domiati cheeses points out several marker compounds of maturation: acrolein (propenal), butan-2-one, propan-1-ol, butan-2-ol, ethyl propionate, propyl acetate, ethyl butyrate, propyl propionate, propyl butyrate, and pentane. The origin of these compounds could be chemical or biological degradation of proteins and lipids. As previously described (El-Shibiny et al., 1974; El-Zayat, 1987; Zein et al., 1979), the content of most amino acids and fatty acids drastically increases during Domiati maturation. Various microbiological genera that have been isolated from our samples (see Table II) are probably involved in these pathways.

Among the aldehydes, acetaldehyde and propenal (acrolein) were the two major compounds present in the cheese, the latter appearing during maturation. Branched aldehydes such as isobutyraldehyde, 3-methylbutyraldehyde, and 2-methylbutyraldehyde (from Strecker or microbiological degradations of amino acids) and linear aldehydes such as butyraldehyde, valeraldehyde, caproaldehyde, and heptanal (typically derived from lipid oxidation) were detected in very low concentration in Domiati cheese (see Table I), as were their corresponding primary alcohols. The intense production of acrolein (up to 1 ppm)

Table I. Compounds Identified in Domiati Cheeses[a]

	PN	RT		RF	CV	fresh I	fresh II	fresh III	good ripening IV	good ripening V	good ripening VI	good ripening VII	good ripening VIII	bad IX
aldehydes, ppb														
acetaldehyde	1	4.6	GC, MS	1.61	7.9	978	1098	1532	550	406	1218	1430	767	310
acrolein	4	6.7	GC, MS	10.0	3.1	–	–	–	290	370	1024	552	520	1300
isobutyraldehyde	9	10.3	GC, MS	41.5	4.1	–	–	21	–	–	–	–	–	–
butyraldehyde	12	12.7	GC	30.1	3.7	–	–	–	–	–	–	–	–	–
3-methylbutyraldehyde	18	19.7	GC, MS	52.7	4.4	10	13	41	15	19	10	18	20	–
2-methylbutyraldehyde	19	21.1	GC	65.6	4.4	–	–	9	–	–	–	–	–	–
valeraldehyde	22	24.8	GC	52.6	4.9	–	–	–	–	–	–	–	–	–
caproaldehyde	34	37.5	GC, MS	42.0	5.0	40	77	36	36	36	31	19	26	28
heptanal	43	48.5	GC	26.0	5.7	–	–	–	–	–	–	–	–	–
ketones, ppb														
acetone	5	7.1	GC, MS	13.7	6.6	1355	3246	2879	1216	2707	5849	6223	6768	8260
diacetyl	11	12.3	GC	4.6	7.6	–	–	–	–	–	–	–	–	–
butan-2-one	13	13.5	GC, MS	19.5	5.2	–	–	–	43	52	138	171	57	1569
pentan-2-one	20	24.2	GC	31.3	7.1	–	–	–	–	–	–	–	–	–
pentane-2,3-dione	21	24.6	GC	7.5	4.6	–	–	–	–	–	–	–	–	–
hexan-2-one	32	36.2	GC	32.9	7.7	–	–	–	–	–	–	–	–	–
heptan-2-one	42	48.0	GC, MS	27.1	3.3	42	–	30	26	52	86	42	36	77
alcohols, ppm														
ethanol	3	6.3	GC, MS	0.22	6.8	230	165	457	260	237	423	476	341	190
alcohols, ppb														
propan-1-ol	10	11.2	GC, MS	2.3	6.4	766	700	343	7712	10636	9112	33456	24184	17132
butan-2-ol	14	15.1	GC, MS	4.3	6.2	–	–	–	460	520	263	1652	1164	4528
2-methyl-propan-1-ol	17	17.9	GC, MS	2.2	6.5	1987	1518	1539	1294	1203	1730	1502	1460	1518
pentan-2-ol	24	27.1	GC, MS	6.2	7.1	–	–	–	245	106	183	96	96	–
3-methylbutan-1-ol	28	31.0	GC, MS	2.4	6.7	440	495	1713	504	891	1122	1128	950	690
2-methylbutan-1-ol	29	31.8	GC	6.6	6.6	–	–	–	–	–	–	–	–	–
esters, ppb														
ethyl formate	7	8.2	GC, MS	13.5	3.6	781	800	730	901	1043	1045	1338	1215	1014
ethyl acetate	15	16.0	GC, MS	31.9	2.9	169	33	561	306	327	450	388	406	183
ethyl propionate	25	28.2	GC, MS	52.3	5.4	–	–	–	56	93	20	87	61	127
propyl acetate	26	28.6	GC, MS	52.0	5.1	–	–	–	217	437	96	446	317	185
ethyl butyrate	35	38.6	GC, MS	47.7	4.3	–	–	58	646	660	714	673	571	635
propyl propionate	36	39.7	GC, MS	48.2	4.5	–	–	–	198	116	23	109	51	403
butyl acetate	38	40.1	GC	35.0	4.6	–	–	–	–	–	17	–	–	38
isoamyl acetate	41	47.0	GC, MS	24.0	4.6	–	–	88	113	111	65	101	91	89
propyl butyrate	45	49.2	GC, MS	53.0	4.8	–	–	–	634	557	127	459	341	1212
sulfur compounds, ppb														
dimethyl sulfide	8	8.4	GC, MS	36.5	1.6	–	–	–	–	–	–	–	–	230
dimethyl disulfide	27	30.7	GC, MS	30.0	4.8	–	–	–	24	39	–	37	–	1639
sulfur compounds, area μV														
methyl mercaptan	2	5.1	MS			–	–	–	–	–	–	–	–	8414
methyl thioacetate	23	25.1	MS			–	–	–	–	536	–	747	–	26378
methyl thiopropionate	33	37.3	MS			–	–	–	–	–	–	–	–	2777
methyl thiobutyrate	39	45.4	MS			–	–	–	506	–	–	–	–	757
dimethyl trisulfide	46	57.0	MS			–	–	–	–	–	–	–	–	2797
hydrocarbons, ppb														
pentane	6	7.9	GC, MS	9.1	6.5	–	196	–	6331	8540	6952	6907	9070	2731
toluene	31	34.3	GC, MS	71.8	3.2	22	20	23	37	31	91	32	31	31
octane	37	39.8	GC, MS	3.7	5.8	488	–	379	438	548	575	355	493	337
p-, *m*-xylene	40	46.3	GC, MS	23.3	4.5	–	–	–	–	–	25	–	–	25
o-xylene	44	48.8	GC, MS	31.8	5.3	22	31	16	55	18	17	–	–	21
unknown, area, μV														
	16	16.2				–	–	–	1400	2274	576	1714	2169	–
	30	34.1				–	–	–	580	529	–	504	–	1596

[a] Peak numbering (PN) gives the order of elution through the column; RT, column retention time (min); GC, gas chromatographic retention data compared with those of authentic samples; MS, mass spectral data compared with those of library compounds and/or those of authentic samples; RF, peak area (μV)/concentration in the cheese sample (ppb) (including extraction yield + detector response); CV, coefficient of variation obtained for five analyses of the same sample, standard deviation × 100/mean, %); concentrations or areas are means of duplicates; (–), area < 500 μV.

Table II. Microbiological Genera That Have Been Isolated from the Nine Domiati Samples

	no. of distinct isolates		no. of distinct isolates
Lactobacillus sp.	27	*Brevibacterium linens*	7
L. casei	1	*Propionibacterium acidipropionici*	1
L. alimentarius	10	*Microbacterium lacticum*	7
L. farciminis	16		
Enterococcus sp.	5		
E. faecalis	3		
E. faecium	2		

and propan-1-ol (up to 33 ppm) presumably arose from the metabolism of methionine as shown for Cheddar cheese (Abd El-Salam et al., 1981). The laboratory maturations indicated that production of propan-1-ol and acrolein essentially started after 2 months of ripening (Figure 2).

Production of butan-2-one (up to 171 ppb) and its corresponding secondary alcohol, butan-2-ol (up to 1.65 ppm), was also demonstrated. Both compounds increased after 2 months of ripening (Figure 3). These compounds should logically arise from β-oxidation of fatty acids. Other methyl ketones such as pentan-2-one or hexan-2-one were detected in trace amounts only (unquantified, lower than 15 ppb). All of these compounds are commonly found in mold-ripened cheeses, such as blue cheese, to which they impart a characteristic aroma (Gallois and Langlois, 1990).

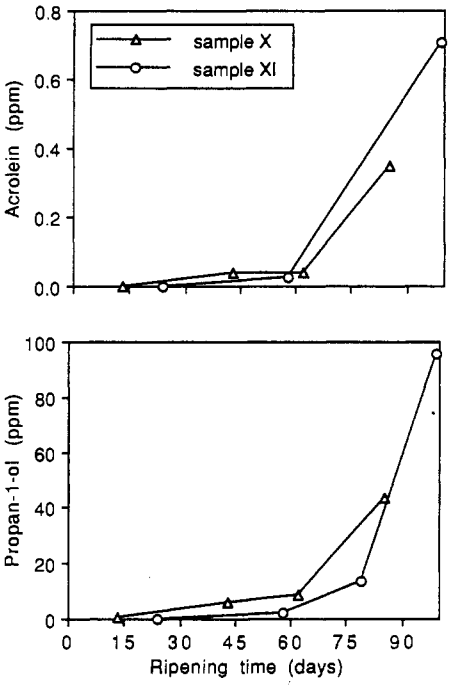

Figure 2. Evolution of acrolein and propan-1-ol in samples X and XI during laboratory maturation.

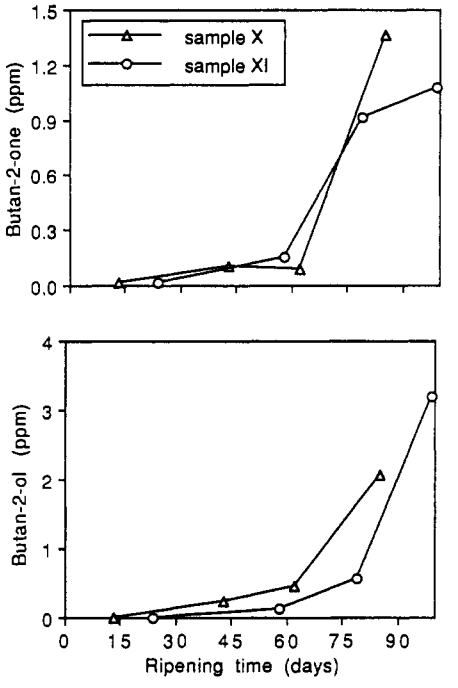

Figure 3. Evolution of butan-2-one and butan-2-ol in samples X and XI during laboratory maturation.

Propan-1-ol, butan-2-ol, and butan-2-one, which appeared during Domiati maturation, were also reported as three major aroma compounds in feta cheese (Horwood et al., 1981).

Near-constant and low concentrations (lower than 30 ppb) of diacetyl and pentane-2,3-dione were measured at all stages of cheese ripening. This can probably be linked to the absence of *Leuconostocs* in all Domiati samples here studied (see Table II). This bacterial genus is usually referred to as the most important diacetyl producer in cheese, producing, for instance, the typical flavor of cream cheese (Devoyod and Poullain, 1988).

Significant production of various esters such as ethyl propionate (up to 93 ppb), propyl acetate (up to 446 ppb),

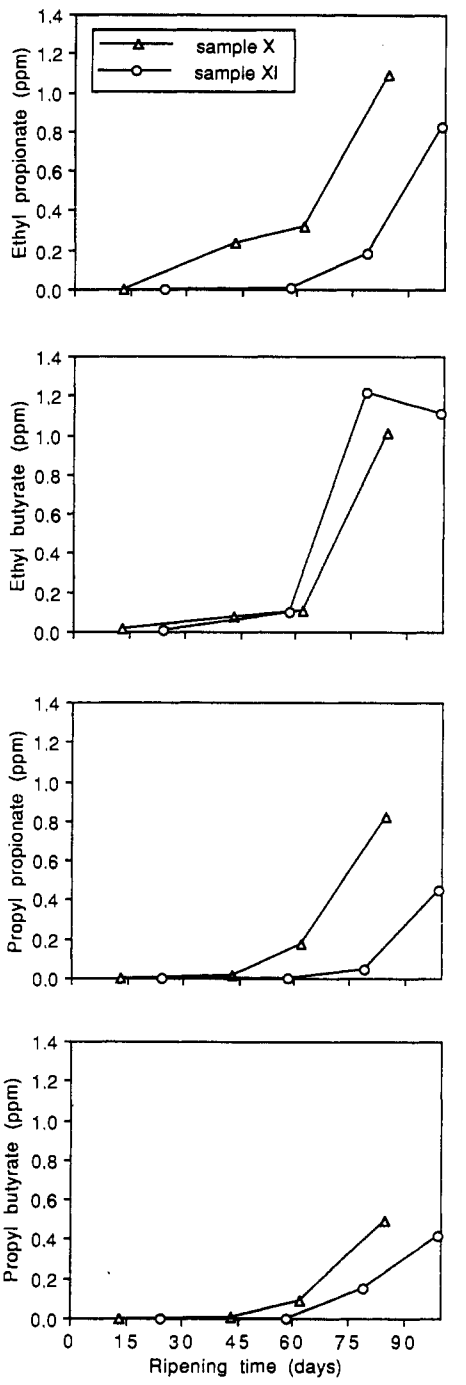

Figure 4. Evolution of ethyl propionate, ethyl butyrate, propyl propionate, and propyl butyrate in samples X and XI during laboratory maturation.

ethyl butyrate (up to 714 ppb), propyl propionate (up to 198 ppb), and propyl butyrate (up to 634 ppb) was observed (Table I). This behavior is presumably attributable to the parallel increase of the short- and medium-chain fatty acid concentration during the corresponding period (El-Shibiny et al., 1974; Zein et al., 1979). Most of them arise from fat hydrolysis, probably carried out by *Streptococcus* and *Lactobacillus*, both genera being well represented in the Domiati samples under investigation (Table II). As illustrated by the laboratory maturations (Figure 4), the ester content significantly increased after 1.5–2 months of ripening. Similar ester synthesis is well-known in various cheeses (Dumont and Adda, 1978). Provided they are not overconcentrated, esters possess interesting fruity notes which contribute favorably to the cheese aroma by minimizing the sharpness imparted by fatty acids.

High pentane levels were also produced during ripening (up to 9 ppm), probably through oxidative breakdown of unsaturated fatty acids (Grosch, 1982).

Various sulfur compounds such as dimethyl disulfide, dimethyl trisulfide, methyl thioacetate, methyl thiopropionate, methyl thiobutyrate, and methyl mercaptan were also identified. Most of them were totally absent from the fresh cheeses and, if present, detected in low concentration in the five good 3-months-ripened cheeses. Unlike the good-quality ripened cheeses (samples IV–VIII), the badly matured cheese (sample IX) contained numerous sulfur compounds at very high concentrations. Methyl mercaptan probably derived from methionine, which was in higher concentration in this sample (161.6 ppm, to be compared to 35.6–116.0 ppm in the five good samples; not detected in the three fresh samples). Its esterification logically led to the presence of various thioesters. Corynebacteria [detected in Domiati (see Table II)] proved to be very efficient in this pathway. By this mechanism, methyl thioacetate imparts a characteristic cooked-cauliflower flavor to Limburger cheese (Parliment et al., 1982). As most thioesters are characterized by very low thresholds (parts per billion and subparts per billion), there is no doubt that they are responsible for the off-flavors detected in cheese sample IX. Dimethyl disulfide, known for its onion-tomato-like flavor, and higher polysulfides were also detected (from methyl mercaptan oxidative reactions). The 1.6 ppm dimethyl disulfide concentration in cheese IX also contributed to the bad aroma [a 3 ppb threshold level in water (Shankaranarayana et al., 1974)]. Much higher amounts of various other volatile compounds (acrolein, butan-2-one, butan-2-ol, propyl propionate, and propyl butyrate) in sample IX should also be noted. In particular, 1.2 ppm (much higher than the 18 ppb threshold level, in water) of propyl butyrate could also explain the off-flavor of this cheese.

Our results clearly show how the dynamic headspace method makes it quick and easy to monitor cheese ripening. We are currently attempting to identify the flavor-active compounds among those described here by means of the CHARM technique (Acree et al., 1984).

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