JOURNAL OF FOOD COMPOSITION AND ANALYSIS **12,** 63–69 (1999)
Article No. jfca.1998.0805
Available online at http://www.idealibrary.com on IDEAL

Composition of Medium Volatility (Simultaneous Distillation Extraction—SDE) Aromatic Fraction of Pressed, Uncooked Paste Cheese (Mahón Cheese)

Isabel Escriche,*[,1] Juan A. Serra,* Vicente Guardiola† and Antonio Mulet*

*Department of Food Technology, Universidad Politecnica de Valencia, Camino de Vera 14, 46071 Valencia, Spain
†AINIA (Food Industry Research Association), Parc Tecnologic, Paterna, Valencia, Spain

Received October 5, 1998

This paper offers a contribution to the study of the aromatic fraction of uncooked paste cheeses made from cow's milk. Due to the acidic nature of this type of cheese, the main aromatic fraction studied was the acidic aromatic fraction. Research focused on Spanish Mahón cheese of certified origin, manufactured from pasteurized milk and ripened for two months. This study resulted in 28 components being identified, i.e., 11 fatty acids (butanoic acid, 3-methyl butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, 10-undecanoic acid, dodecanoic acid and tetradecanoic acid); six ethyl esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate and ethyl hexadecanoate); one aldehyde (nonanal); four methyl ketones (2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone); three alcohols (2-methyl pentanol, 1-heptanol and 2-6 diterbutyl p-cresol); two n-alkene hydrocarbons (2-methyl 4-nonene and 3-tetradecene) and one aromatic hydrocarbon (limonene). The highest levels were those of non-branched aliphatic fatty acids: decanoic, hexanoic, octanoic and butanoic acids. The volatile fraction was isolated using the combined simultaneous distillation–extraction (SDE) procedure. The components were identified by gas chromatography–mass spectrometry and quantified by applying standard internal procedures. The results obtained were compared with those found in literature, which revealed that the levels of these compounds, which are found in many other varieties of cheese, vary considerably.

INTRODUCTION

The Mahón cheese of certified origin, also known as Minorcan cheese, is manufactured throughout the island of Minorca (Balearic Islands, Spain). Although usually eaten after two month's ripening, it is also consumed fresh, tender and mature. It is made from raw or pasteurized whole cow's milk, uncooked pressed paste and enzymatic coagulation using rennet. The cheeses are parallelepiped-shape with rounded edges, and are usually medium to large-size with weights of up to 4 kg. The rind is smooth and sealed but sticky, and yellow or orange in colour due to the external treatment sometimes applied consisting of one or more of olive oil, paprika and butter. The cheese cross-section is non-springy and sealed, with spontaneous cavities of different sizes; the colour varies from ivory white to deep yellow, with brownish beige edges. It has a unique and characteristic flavour, slightly acidic and salty and not

[1]To whom correspondence and reprint requests should be addressed. Departamento de Tecnologia de Alimentos, Universidad Politécnica de Valencia, Camino de Vera 14, 46071 Valencia, Spain. Fax: +34 96 387 73 69. Email: iescrich@tal.upv.es

0889–1575/99/010063 + 07 $30.00/0

very greasy. It can be milky and moist when fresh, and dry and strong, with a little bite as it ripens (Frau *et al.*, 1994).

The volatile compounds in cheese vary according to the variety and depend on both the manufacturing process and ripening conditions (Roberts and Vichers, 1994; Sable *et al.*, 1997). Numerous studies of the volatile compounds in different varieties have been conducted (Yang and Min, 1994; Preininger and Grosch; 1994, Muir *et al.*, 1997). Only one study which addresses the volatile fraction of Mahón cheese has been located, a comparative study of six European AOC (Certified Origin) cheeses (Bosset and Gauch, 1993).

Different procedures were used to isolate and concentrate the said volatile fraction: the headspace technique (Rafecas *et al.*, 1985; Imhof and Bosset, 1991; Fernandez-Garcia, 1996); direct extraction by solvents (Dunn and Lindsay, 1985; Martelli and Tour, 1990); extraction by supercritical solvents (Toumala and Kallio, 1996); distillation (Gallois and Langlois, 1990; Banks *et al.*, 1992); "Tower" high vacuum distillation and vapour distillation in rotavapour (Klein *et al.*, 1990). Simultaneous vapour distillation and solvent extraction (SDE) was one of the procedures chosen for its speed, economy and effectiveness in isolating cheese volatiles (Godenfroot *et al.*, 1981; De Frutos *et al.*, 1988; Virgili *et al.*, 1994).

The study of the aromatic fraction may contribute to greater knowledge of the specific characteristics of the composition of Mahón cheese and consequently facilitates the process of determining whether a cheese complies with the pertinent certification (European Community Regulations CEE 2·081/92 and 2·082/92). The slightly acidic characteristics of this kind of cheese together with the dearth of knowledge on the acidic compounds in the aromatic fraction are what make the characterization of the volatile fraction of Mahón cheese made from pasteurized milk and ripened for two months so interesting.

MATERIALS AND METHODS

Raw Material

Cheeses manufactured from pasteurized milk and ripened for two months at 12–13°C with a relative humidity of 88%, were provided by the CRDOQM (Mahón Cheese Regulatory Board) in Minorca, Spain. The average moisture content of these cheeses was 0·04 kg of water per kg of cheese, this being the average of 15 readings. Three pieces of approximately 200 g were taken from each of the nine cheeses sent to the laboratory. Each pieces was cut in a pyramid shape from the centre up to the external part of the cheese. Approximately 0·5 cm of the outer zone of the cheese sample, including the rind was discarded. The three pieces of a given cheese were finely grated together and thoroughly blended using a mixer, thus obtaining a representative sample for a single cheese.

All reagents were of analytical grade. Standard compounds for defining GC retention times and mass spectra (MS) were purchased from SIGMA-ALDRICH QUIMICA S.A. (Madrid, Spain).

Isolation of the Aromatic Volatile Components

The volatile components were isolated using equipment manufactured by Fisher Scientific U.K. Ltd (Loughborough, Leics., England), employing the combined simultaneous distillation-extraction procedure based on the method described by Godefroot and colleagues in 1981, using pentane as a solvent. This method is widely used in

cheese analysis (Aishima and Nakai, 1988; De frutos *et al*., 1991; Martinez-Castro *et al*., 1991) although some authors (Werkhoff *et al*., 1998) reported artifacts when boiling took place for 5 h. This problem has probably been minimized by shortening the boiling time when using smaller amounts of material.

In each analysis, a 10 g sample was introduced into a 500 ml round-bottomed flask, to which 100 ml of hot bidistilled water were added together with 50 μg of camphor used as internal standard, as previously described by Frutos *et al*. (1988). The flask was held in an ultrasonic bath for 15 min to facilitate the total disintegration of the sample and was then introduced into the bath of oil heated to 110°C in the extraction equipment. The 50 ml heart flask containing 3 ml of pentane solvent, was introduced into a water bath at 50°C. The steam of both flasks was condensed in the common refrigerated "U-tube" of the equipment. After 30 min distillation all the contents of the U-tube were collected in an airtight, sealed tube and frozen at −18°C to facilitate the separation of the aromatic fraction (which is liquid and has lower density at −18°C) in which all the aromatic compounds were dissolved. This organic phase was concentrated in nitrogen steam up to a final volume of approximately 100 μl.

Gas Chromatography

The concentrated extracts were analysed by an Autosystem Perkin Elmer Gas Chromatograph (Norwalk, Connecticut, U.S.A.) equipped with a flame ionization detector (FID) and a fused silica capillary column Cromlab SL 30 m × 0.25 mm of DB-FFAP phase, specific for free acids, methyl ketones and esters. The oven temperature was programmed: 60°C for 5 min; from 60 to 200°C at 4°C/min; 200°C for 34 min; from 200 to 230°C at 10°C/min. The carrier gas used was nitrogen (2 ml/min). Injections were conducted with a split ratio of 1 : 16. The injected volume was 1 μl. The injector and detector temperature was set at 250°C. The chromatographic data was processed using a Perkin-Elmer Personal Integrator Model 1020 (Norwalk, Connecticut, U.S.A.).

Gas Chromatography–Mass Spectrometry

The volatile components were identified by gas chromatography and mass spectrometry (GC–MS) using a Fisons Trio 1000 GC–MS (Manchester, England) using helium as a carrier gas. Fragmentation was performed by electronic impact EI + at 70 eV, and Scan mode between 50 and 450 mass units. The same chromatographic column and temperature programming mentioned above were applied. The mass spectra obtained for all compounds were compared with several standard mass spectra obtained from the equipment database. The relative retention time of the standards was also compared with those obtained for identified compounds.

Quantification was carried out from peak areas of components and internal standard (camphor). The GC detector response correction of each component was also taken into account. Quantitative results were obtained by calculating the average of nine cheese sample analyses. Coefficients of variation (cv.) were calculated by repeating the SDE extraction and subsequent analysis of a Mahón cheese sample to determine the repeatability of the analysis. The cvs. of most peaks were lower than 15% and never exceeded 20%, which is considered to be a satisfactory result for this type of analysis. The average amount recovered of the internal standard, camphor, was 87%, a figure not taken into account for quantification purposes.

RESULTS AND DISCUSSION

Twenty-eight compounds were identified by GC–MS in this study, of which 21 were quantified by the internal standard procedure (Table 1). The relative retention times were calculated in relation to the internal standard (camphor). The values of the quantified components obtained are shown (μg compound/g dry matter) together with their percentage variation coefficients. Those compounds include 10 fatty acids (butanoic, 3-methyl butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic and tetradecanoic); six ethyl esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate and ethyl hexadecanoate); four methyl ketones (2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone) and one alcohol (1-heptanol). Figure 1 shows a typical GC/FID capillary gas chromatogram of the SDE volatile fraction of Mahón cheese. The peak numbers correspond to the numbers in Table 1.

The main compounds are the decanoic, hexanoic, octanoic, butanoic and dodecanoic fatty acids for which the following average values 110, 91, 53, 48 and 40 (μg/g, dm, respectively, were obtained. The lower values corresponded to the ketones 2-pentanone, 2-undecanone and 2-nonanone with 0·33, 0·4 and 1·1 μg/g dm, respectively. Ethyl esters varied between 1·5 μg/g dm for ethyl hexanoate, and 6.6 μg/g dm

TABLE 1

Components of the SDE volatile fraction in Mahón cheese DO (certified origin) after 60 days of ripening

Component	Peak number	Retention time (min)	Relative retention time[1]	Concentration μg/g dm ($n = 9$)	VC %	Identification
2-methyl pentanone	01	03·83	0·18	0·33	18	GC–MS, GC
Ethyl butanoate	02	04·23	0·21	0·66	14	GC–MS, GC
2-methyl heptanone	03	07·51	0·40	2·0	5	GC–MS, GC
Limonene	04	07·93	0·43			GC–MS
Ethyl hexanoate	05	09·06	0·54	1·5	7	GC–MS, GC
2 methyl 4-nonene	06	09·78	0·58			GC–MS
2 methyl pentanol	07	10·24	0·59			GC–MS
2 methyl nonanone	08	14·43	0·77	1·1	9	GC–MS, GC
Nonanal	09	14·76	0·79			GC–MS
Ethyl octanoate	10	15·99	0·86	3·3	9	GC–MS, GC
3-tetradecene	11	16·63	0·89			GC–MS
1-heptanol	12	16·74	0·90	1·0	8	GC–MS, GC
2-methyl undecanone	13	21·45	1·15	0·4	15	GC–MS, GC
Butanoic acid	14	22·36	1·20	48	12	GC–MS, GC
Ethyl decanoate	15	23·08	1·24	6·6	12	GC–MS, GC
3-methyl butanoic acid	16	23·66	1·27	1·3	15	GC–MS, GC
Pentanoic acid	17	25·74	1·38	1·3	13	GC–MS, GC
Hexanoic acid	18	28·84	1·55	91	9	GC–MS, GC
2-6 diterbutyl p-cresol	19	30·74	1·65			GC–MS
Heptanoic acid	20	31·77	1·71	2·2	14	GC–MS, GC
Ethyl tetradecanoate	21	33·64	1·81	6·6	14	GC–MS, GC
Octanoic acid	22	34·61	1·86	53	6	GC–MS, GC
Nonanoic acid	23	37·24	2·00	1·8	11	GC–MS, GC
Ethyl hexadecanoate	24	39·50	2·12	3·2	0·5	GC–MS, GC
Decanoic acid	25	39·87	2·14	110	9	GC–MS, GC
10-undecanoic acid	26	42·34	2·22			GC–MS
Dodecanoic acid	27	46·06	2·47	40	19	GC–MS, GC
Tetradecanoic acid	28	56·47	3·03	33	18	GC–MS, GC

[1]Calculated in relation to the internal standard camphor retention time.

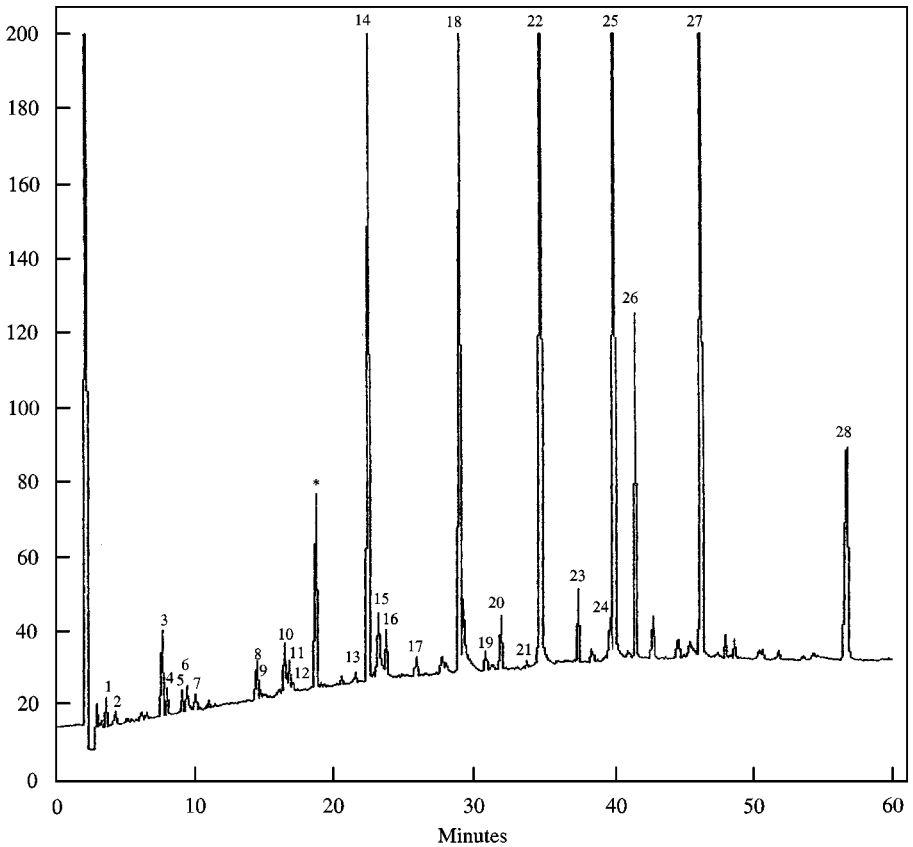

FIGURE 1. Typical capillary gas chromatogram-FID (capillary column DB-FFAP 30 m × 0·25 mm) of SDE volatile fraction components in Mahón cheese. The peak numbers correspond to the numbers of Table 1; (*) internal standard camphor.

for both ethyl tetradecanoate and ethyl hexadecanoate. The only quantified alcohol was found at a level of 1 μg/g dm.

The only reference found to the composition of the volatile fraction of Mahón cheese was the study mentioned earlier (Bosset and Gauch, 1993), which reported a total of 28 volatile compounds including neutral and alkaline compounds and also quantified alcohols, methyl ketones, aldehydes, esters and aromatic hydrocarbons. The compounds identified in that study were found to differ from those described here, probably due to the different extraction procedures employed.

The composition of the aromatic fraction in Manchego cheese (another Spanish cheese with certified origin) was studied (Martinez-Castro *et al.*, 1991) using the simultaneous distillation–extraction procedure (SDE) and the same type of column used in the present study. However, in Manchego cheese, unlike Mahón cheese, the main compounds were ethyl esters and non-fatty acids. Since the manufacturing processes of both cheeses are similar, these differences could be due to the kind of milk used: sheep's milk being used for Manchego and cow's for Mahón cheese.

In a study carried out on the composition of free fatty acids (from C4 : 0 to C16 : 0) in seven known cheese varieties: Camembert, Brie, Port Salut, Monterey Jack, Roquefort, Blue and Limburger (Woo *et al.*, 1984), the levels of almost all the acids

studied were higher in the last three varieties than those obtained in the present study. Mahón cheese on the other hand, had higher levels of hexanoic, octanoic and decanoic acids than the four first varieties.

Seventeen and 27 compounds were identified, respectively, in Cheddar and Swiss cheese ripened for two months (Wesley and Min, 1994). The main aroma constituents were ketones and alcohols, accounting for 92 and 88% of the total constituents of the aromatic fraction, respectively. No acidic compounds were identified in Swiss cheese, however in Cheddar cheese, acids of 4, 5 and 6 carbon atoms were identified, albeit in very small amounts. Both types of cheeses are characterized by the presence of sulphur compounds, which contribute to the aroma of the former (Christensen and Reinnecius, 1995).

In different cooked paste cheeses such as Gruyère (Swiss Gruyère, Greek Gruyère and Comté Gruyère) (Berdague *et al*., 1987; Bosset *et al*., 1993) the main compounds were the fatty acids butanoic, propanoic and ethanoic, however, in Mahón cheese the last two were not found. Nevertheless, the levels of methyl ketones and ethyl esters obtained were approximately the same as those found in Comté Gruyère (Guichard *et al*., 1987). These differences in composition could be related to the type of manufacturing.

From the comparative study carried out it could be concluded that uncooked pressed paste Mahón cheese is characterized by its high volatile fatty acids content. However, these have longer chains (decanoic, octanoic, hexanoic, etc.) than those found in other cheese varieties, such as cooked paste, Gruyère type. Volatile fatty acids, being rather abundant, can contribute considerably to the organoleptic characteristics of Mahón cheese. Methyl ketones and esters contents do not differ significantly from those of many other cheese varieties, except blue cheeses, in which methyl ketones are the main compounds, accounting for between 50 and 75% of the total aromatic profile (Gallois and Langlois, 1990; Taylor, 1995).

REFERENCES

Aishima, T. and Nakai, S. (1987). Pattern recognition of Gc profiles for classification of cheese variety. *J. Food Sci.* **53,** 939–942.

Banks, J. M., Brechany, E. Y. and Christie, W. W. (1992). Volatile components in steam distillates of Cheddar cheese as indicator indices of cheese maturity, flavour and odour. *Food Res. Int*. **25,** 365–373.

Berdague, J. L., Jeunet, R. and Grappin, R. (1987). Affinage et qualité du Gruyère de Comté III. Fermentation lactique et teneur en acides gras volatils des fromages de Comté. *Le Lait* **67,** 249–263.

Bosset, J. O. and Gauch, R. (1993) Comparison of the volatile flavour compounds of six European AOC cheeses by using a new dynamic headspace GC–MS method. *Int. Dairy J*. **3,** 359–377.

Bosset, J. O., Collomb, M. and Sieber, R. (1993). The aroma composition of Swiss Gruyère cheese IV. The acidic volatile components and their changes in content during ripening. *Lebensm. Wiss. Technol.* **26,** 581–592.

Christensen, K. R. and Reinnecius, G. A. (1995). Aroma extract dilution analysis of aged *Cheddar cheese. J. Food Sci.* **60,** 218–220.

De Frutos, M., Sanz, J. and Marinez-Castro, I. (1988). Simultaneous distillation–extraction (SDE) method in the qualitative and quantitative GC analysis of cheese volatile components. *Chromatographia* **25,** 861–864.

De Frutos, M., Sanz, J. and Martinez-Castro, I. (1991). Characterization of artisanal cheeses by GC–MS analysis of their medium volatiles (SDE) fraction. *J. Food Agric. Food Chem* **39,** 534–530.

Dunn, H. C. and Lindsay, R. C. (1985). Comparison of methods for the analysis of higher boiling flavor compounds in Cheddar cheese. *J. Dairy Sci.* **68,** 2853–2858.

Fernandez-Garcia, F. (1996). Use of headspace sampling in the quantitative analysis of artisanal Spanish cheese aroma. *J. Food Agric. Food Chem.* **44,** 1833–1839.

Frau, M., Casals, J., Mestres, J., Mulet, A., Roselló, C. and Squella, J. (1994). Control de calidad del queso Mahón. *Ind. Lacteas Españolas* **187,** 49–54.

Gallois, A. and Langlois, D. (1990). News results in the odours compounds on French cheeses. *Le Lait* **70,** 89–106.

Guichard, E., Berdague, J. L. and Grappin, R. (1987). Affinage et qualité du Gruyère du Comté V. Influence de affinage sur la teneur en composés volatils. *Le Lait* **67,** 319–338.

Godefroot, M., Sandra, P. and Verzele, M. (1981). New method for quantitative essential oil analysis. *J. Chromatogr.* **203,** 325–335.

Imhof, R. and Bosset, J. O. (1991). Comparison of two systems for sample preparation and injection by dynamic headspace GC analysis. *J. High Resolution Chromatogr.* **14,** 621–625.

Klein, B., Gauch, R. and Bosset, J. O. (1990). Comparison of four extraction, concentration and injection techniques for volatiles compounds analysis by GC–MS: An application to the study of the volatile flavor of Swiss Emmentaler cheese. In *Flavour Science and Technology* (Y. Bessière and A. F. Thomas, Eds.), Flavour 90 6th Weurman Symposium. Wiley, Chichester.

Martelli, A. and Tour, M. L. (1990). The aroma of Gorgonzola cheese Note II: differentiation between the volatile components of dolce and nature types. *La Rivista della Societá Italiana di Scienza dell¨Allimentazione* **19,** 9–12.

Martinez-Castro, I., Sanz, J., Amigo, L., Ramos, M. and Martín-Alvarez, P. (1991). Volatile components of Manchego cheese. *J. Dairy Res.* **58,** 239–246.

Muir, D. D., Hunter, E. A. and Banks, J. M. (1997). Aroma of Cheese 2. Contribution of aroma to the flavour of Cheddar cheese. *Milchwissenschaft Milk Sci. Int.* **52,** 85–88.

Preininger, M. and Grosch, W. (1994). Evaluation of key odorants of the neutral volatiles of Emmental cheese by the calculation of odour activity values. *Lebensm. Wiss. Technol.* **27,** 237–244.

Rafecas, M., Boatella, J. and De La Torre, M. C. (1985). Cheese aroma volatile compounds. *Alimentaria*, 29–34.

Regulation CEE No. 2·081/92. Denominaciones de Origen, productos no vínicos. *European Community Regulations.*

Regulation CEE No. 2·082/92. Certificación de las características específicas de los productos agrícolas. *European Community Regulations.*

Roberts, A. K. and Vivhers, Z. M. (1994). Cheddar cheese aging: Changes in sensory attributes and consumer acceptance. *J. Food Sci.* **59,** 328–334.

Sable, S., Letellier, F. and Cottenceau, G. (1997). An analysis of the volatile flavour compounds in a soft raw goat milk cheese. *Biotechnol. Lett.* **19,** 143–145.

Taylor, F. (1995). Bleu cheese flavor production by submerged fermentation with on-line monitoring by gas chromatography. *J. Ind. Microbiol.* **15,** 71–74.

Toumala, T. and Kallio, H. (1996). Identification of free fatty acids and some other volatile flavour compounds from Swiss cheese using on line supercritical fluid extraction–Gas Chromatography. *Z. Lebensmittel-Untersuchung Forschung* **203,** 236–240.

Virgili, R., Parolari, G., Bolzoni, L., Barbieri, G., Mangia, A., Careri, M., Spagnoli, S., Panari, G. and Zannoni, M. (1994). Sensory-chemical relationships in Parmigiano-Reggiano cheese. *Lebensm. Wiss. Technol.* **27,** 491–495.

Werkhoff, P., Güntert, M., Krammer, G., Sommer, H. and Kaulen, J. (1998). Vacuum headspace method in aroma research: flavour chemistry of yellow passion fruits. *J. Food Agricultural Food Chem.* **46,** 1076–1093.

Wesley, T. and Min, D. (1994). Dynamic headspace analyses of volatile compounds of Cheddar and Swiss cheese during Ripening. *J. Food Sci.* **59,** 1309–1312.

Woo, A. H., Kolldge, S. and Lindsay, R. C. (1984). Quantitation of major free fatty acids in several cheese varieties. *J. Dairy Sci.* **67,** 874–878.

Yang, W. T. and Min, D. B. (1994). Dynamic headspace analyses of volatile compounds of Cheddar and Swiss cheese during ripening. *J. Food Sci.* **59,** 1309–1312.