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Methyl carbamate and ethyl carbamate in alcoholic beverages and other fermented foods

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Varying but low levels of ethyl carbamate (EC), also known as urethane, are known to be present in various alcoholic beverages and many other fermented foods. Very little data, however, are available on the levels of methyl carbamate (MC) in such products. In this study, 48 samples of various alcoholic beverages, 12 of bread and toast, 10 of soy sauce, and 14 of yogurt and buttermilk were analyzed for both EC and MC by a newly developed method using gas chromatography thermal energy analyzer (N-mode) for detection and gas chromatography high resolution mass spectrometry for confirmation. Only traces (mostly <5 μg/kg) of MC were detected in some of the products in each category, but much higher levels of EC were detected in some sherries (up to 69 μg/kg), whiskies (up to 247 μg/kg), fruit brandies (up to 432 μg/kg), soy sauces (up to 59 μg/kg), and toasts (up to 29 μg/kg). The levels of EC in the dark toasts (still edible) were significantly higher than those present in the breads or light toasts.

INTRODUCTION

Recent studies have established that low μg/kg to mg/kg levels of ethyl carbamate (EC), also known as urethane, occur in many alcoholic beverages and a few other fermented foods (reviewed by Battaglia *et al.*, 1990). Of various alcoholic beverages, those produced from 'stone' fruits (e.g. peaches, apricots, cherries) or having undergone a heat treatment (as in sherries and some distilled spirits) seem to contain high levels (up to 13 000 μg/kg) of EC (Battaglia *et al.*, 1990; Ough, 1976; Dennis *et al.*, 1989; Christoph *et al.*, 1986; Funch & Lisbjerg, 1988; Hasegawa *et al.*, 1990). Since EC is carcinogenic to laboratory animals (Mirvish, 1968), these findings prompted Health and Welfare Canada to issue guidelines limiting the levels of EC in such beverages (Conacher & Page, 1986). These guidelines take into consideration the average per capita daily consumption levels of various products, and they range from a maximum permissible level of 30 μg/kg EC in table wines, 100 μg/kg in fortified wines and 150 μg/kg in distilled spirits to 400 μg/kg in fruit brandies and liqueurs.

Other fermented foods such as soy sauce, yogurt, bread, and toast also contain traces of EC (Ough, 1976; Zimmerli *et al.*, 1986; Canas *et al.*, 1989; Dennis *et al.*, 1989). Although the concentration of EC in such products is low as compared to that present in some alcoholic beverages, toasted breads and soy sauce seem to contain appreciable levels (up to 14 μg/kg and 76 μg/kg, respectively). Since toast is a common food item in the Western diet, and since the data on EC levels in such products are very limited, there is a need for further research in this area. The available information suggests that there is an approximately 2·5 fold increase in EC levels during normal toasting of breads (Canas *et al.*, 1989), but no data are available on the effect of degree of toasting on EC levels. It is possible that darker toasts might form higher levels of EC than the lightly toasted ones. The situation is analogous to increased formation of EC in heat-treated alcoholic beverages as mentioned above.

Methyl carbamate (MC) is a recently identified contaminant that has been detected in extremely low concentrations in dimethyl pyrocarbonate-treated as well as untreated wines (Sen *et al.*, 1992). The latter finding suggested that like EC, MC might also be produced during fermentation, and, therefore, might be present in various fermented foods and alcoholic beverages. This prompted us to investigate the possible presence of MC in such products. These results along with the corresponding data on EC levels are presented in this report. The paper also reports the development of a new method for the determination of both MC and EC in toast and yogurt that is superior to earlier published methods.

MATERIALS AND METHODS

Reagents and chemicals

All reagents and chemicals used were of analytical grade, and solvents were of glass-distilled variety.Chem

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Elut™ extraction tubes (20 ml), Celite 545 (not acid-washed), neutral alumina for column chromatography, and Sep-Pak Florisil cartridges (2 ml) were purchased from Analytichem International, Harbor City, CA; Fisher Scientific, Nepean, Ontario; ICN Biomedicals, St-Laurent, Quebec; and Waters Associates, Milford, MA., respectively. Neutral alumina and Celite 545 were processed, before use, as described previously (Canas *et al.*, 1988; Sen *et al.*, 1992). EC and MC standards were obtained from Aldrich Chemical Co., Milwaukee, WI, and propyl carbamate (PC) was purchased from ICN K&K Laboratories, St-Laurent, Quebec.

Caution Since both MC and EC are carcinogenic to laboratory animals (Mirvish, 1968; National Toxicology Program, 1987), adequate precaution should be taken while handling or working with these chemicals.

Samples

Various samples of fermented foods (e.g. soy sauce, yogurt, bread and alcoholic beverages were purchased locally either from retail grocery stores or Ontario Liquor Control Board outlets. They were stored under appropriate conditions (wines, yogurts, and breads stored at 4°C) until analysis.

Bread slices (two) were first weighed and then toasted using an electric toaster. Two different settings (light or dark) were used for toasting. Each set of toasts (light or dark) was then weighed, torn into small pieces by hand, and stored in a tightly closed jar at 4°C. Untoasted breads were broken into small pieces and stored as above. If analysis was delayed, the samples were stored at −20°C.

Determination of EC and MC

(a) Alcoholic beverages

The details of the method used for the determination of EC and MC in various alcoholic beverages have been reported previously (Canas *et al.*, 1989; Sen *et al.*, 1992). Basically, it consists of addition of the sample to a Chem Elut™ extraction tube or a Celite + neutral alumina (10% water content) column followed by extraction with dichloromethane (DCM), concentration of the DCM extract to a small volume (~1 ml) using a Kuderna Danish (K-D) concentrator, and determination of EC and MC by gas chromatography using a nitrogen-specific thermal energy analyzer (N mode) as a detector [GC-TEA (N)]. Aliquots taken for analysis varied depending on the alcohol contents of the beverage analyzed. They ranged from 10 ml for wines and 5 ml for sherries, to 2 ml for beverages containing >30% ethanol. In the last two cases, the aliquots were first diluted to ~10 ml with water before proceeding to the actual analysis.

(b) Soy sauce

A 10-g aliquot of the sample was processed by a method similar to that of Canas *et al.* (1989) except that before eluting EC and MC with DCM (from the Celite + alumina column), the column was washed with a 100 ml mixture of *n*-pentane and DCM (80:20), and the washing discarded. Also, about 0.5 ml of either ethanol or ethyl acetate was added, as a keeper, to the DCM eluate before concentration using a K-D concentrator.

(c) Bread and toast

(i) *Extraction and clean-up.* A 10-g aliquot of cut pieces of the sample was processed by the Celite + alumina clean-up method as described by Canas *et al.* (1989) with some modifications. First, 15 ml of NaCl-saturated water was used for initial homogenization of the sample instead of plain distilled water. Secondly, before eluting the carbamates with DCM the sample-loaded column was washed with a 100 ml mixture of *n*-pentane and DCM (80:20) and the washing discarded. Thirdly, the DCM eluate was further purified by a liquid–liquid partitioning technique (to remove fats and lipids) the details of which are described under the next heading.

(ii) *Phase transfer and liquid–liquid partitioning.* The combined DCM eluate was mixed with 0·5 ml ethanol and then concentrated to ~1 ml using a K-D concentrator as described previously (Sen *et al.*, 1992). About 2 ml water and a new boiling chip were added to the concentrated extract, the micro Snyder column was re-fitted on the tube, and the mixture was heated in a water bath (55–60°C) until most of the DCM was driven off. In the end, the Snyder column was taken off and the heating of the mixture was continued for another 2–5 min until the last traces of DCM were gone.

The aqueous extract was quantitatively transferred (using a Pasteur pipette), into a 20-ml separatory funnel; two 2-ml portions each of water and *n*-hexane were used for rinsing. The mixture in the separatory funnel was gently shaken for ~2 min, the two layers were allowed to separate, and the aqueous layer was carefully withdrawn into a 15-ml graduated centrifuge tube. The volume of the aqueous layer was then made up to 10 ml with water, and the solution was saturated with NaCl (~4 g). The mixture was poured on to a 20 ml Chem Elut™ extraction tube, and, following 5 min of equilibration, the extraction tube was eluted with five 20 ml portions of DCM. About 0·5 ml ethanol or ethyl acetate was added to the eluate and the solution was concentrated to ~0·5 ml as reported previously (Sen *et al.*, 1992).

(d) Yogurt

A 10-g aliquot of a well-homogenized sample was mixed with 4 g NaCl in a Sorval Omni Mixer, and the mixture extracted and processed as above for toast.

(e) Reagent blank

A reagent blank was carried out regularly with each new batch of reagents and extraction tubes to check for contamination.

(f) GC-TEA (N) analysis

A Varian GC (model 3400) attached to a TEA (model

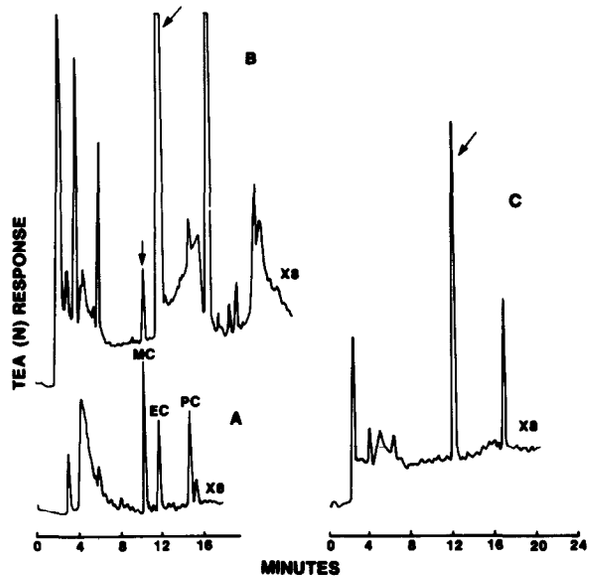

Fig. 1. GC–TEA(N) chromatograms: (A) 200 picograms (pg) MC, 140 pg EC, and 154 pg propyl carbamate (PC) standards; (B) 2 μl/1 ml final extract of a fruit brandy showing the presence of 27·7 μg/kg levels of MC and extremely high levels of EC; (C) 1 μl injection of the above extract (fruit brandy) after dilution to 5 ml to bring the EC peak on scale (2·3 ppm EC).

502) with nitrogen converter (model 610, Thermedics Inc., Woburn, MA) was used for the determination of EC and MC. Two 30-metre DB-wax (1 μm film thickness) fused silica capillary columns (i.d. 0·53 mm) (J&W Scientific Inc., Folsom, CA) connected in series were used for GC analysis. The GC oven temperature was programmed as follows: 110°C for 3 min, then heated to 150°C at 3°C/min followed by further heating to 200°C at 25°C/min (held for 20 min). The injector temperature was at 200°C and the carrier gas (He) flow was 8 ml/min. TEA operating conditions were as described previously (Sen *et al.*, 1992). Under these conditions MC, EC, and propyl carbamate (PC) eluted after 10·7, 12·1, and 15·1 min, respectively (Fig. 1).

(g) GC–Mass spectrometric (GC–MS) confirmation

GC–MS confirmation or determination of MC and EC was carried out as described previously (Sen *et al.*, 1992) using the selected ion monitoring (SIM) mode at a mass resolution of 10 000 (10% valley definition). The ions monitored for this purpose were at m/z 75·0320 $(M)^+$ for MC and those at m/z 62·0242 $(CH_4NO_2)^{+\cdot}$ and m/z 74·0242 $(CH_2 = O\text{–}CONH_2)^{+\cdot}$ for EC (Lau *et al.*, 1989).

Two mass spectrometers, both operating in the electron impact ionization mode, were used for this purpose. The first one, a VG analytical hybrid MS (model 7070 EQ), coupled to a Varian (model Vista 6000) gas chromatograph, was used for the analysis of alcoholic beverage, some bread and toasts, and soy sauce extracts. The second MS was a Kratos Concept 1S high resolution MS attached to a Hewlett Packard 5890, Series II, GC, which was used for the determination of MC and EC in purified extracts of additional toasts and yogurts. A 30-m (i.d., 0·22 mm) DB-Wax 0.25 μm film thickness fused silica capillary column (J&W Scientific Inc.) was used in both cases for GC analysis. Temperature programming was as follows: 60°C for 2 min, then heated to 150°C at 5°C/min followed by further heating to 250°C at 50°C/min. Other conditions were as follows: Injection port, 60°C; GC-MS transfer line, 200°C; carrier gas, He at 20 psi; ion source temperature, 200°C; and electron energy, ~55 eV. Sample size injected was 1 μl in all cases.

RESULTS AND DISCUSSION

The Chem Elut extraction procedure for the determination of MC and EC in wines and that using Celite+ neutral alumina (10% water content) clean-up for the determination of EC in soy sauce, bread and toasts, and yogurt have been studied thoroughly by other researchers (Canas *et al.*, 1989; Dennis *et al.*, 1989; Sen *et al.*, 1992). In this study, the former method was also found to work well for the determination of MC and EC in other alcoholic beverages such as sherries, distilled spirits, and liqueurs. The recoveries of both MC and EC from these substrates at 10 to 50 μg/kg spiking levels ranged between 70 and 114% (Table 1). The latter method (Celite + alumina) worked fairly well for soy sauce but gave poor recoveries (<60%) of MC from bread, toast, and yogurt. It was subsequently determined that because of its polar nature, MC was not efficiently extracted from water which was added to or present in the samples. Saturating the sample extract with NaCl prior to extraction resolved this problem. Also, some of the breads, toasts, and yogurts yielded oily final extracts that interfered with capillary GC analysis. For this reason, the liquid–liquid partitioning clean-up, as described earlier, was developed that removed most of the fats and lipids with minimal losses (<5%) of MC and EC. The overall recoveries of MC and EC from bread, toast, soy sauce, and yogurt at 10 to 20 μg/kg spiking levels were found to vary between 67 and 124% (Table 1). Considering the complexity of the procedure, these recovery values were judged to be satisfactory.

As described previously (Battaglia *et al.*, 1990), various detectors can be used for the GC determination of MC and EC in purified food extracts. The TEA(N) detector was used in this study because of its high sensitivity and specificity to nitrogen-containing organic compounds (Canas *et al.*, 1988; Dennis *et al.*, 1986; Goff, 1987). The technique worked well for the analysis of alcoholic beverages, soy sauce, and yogurt, but was found to be inadequate for the analysis of toasts because of the presence of too many nitrogen-containing organic compounds that eluted near the MC and EC peaks (chromatograms not shown). For this reason, GC–MS high resolution selected-ion monitoring (GC–MS–SIM) was used for the analysis of all breads and toasts. The latter technique was also more sensitive (detection limit, ~0·1 μg/kg) than the former which had a detection limit of about 1–2 μg/kg, GC–MS–SIM was also used for

Table 1. Recoveries of MC and EC from alcoholic beverages, soy sauce, bread and toasts, and yogurt

Sample	Spiking level (ppb)	Method used for analysis[a]	% Recoveries[b]	
			MC	EC
Alcoholic beverages				
Wine	10	A	74	114
Wine	20	A	75	103
Wine	20	A	94	101
Wine	20	A	71	98
Gin	50	B	85	96
Vodka	50	B	94	108
Soy sauce				
Mushroom soy sauce	10	B	60	59
Soy sauce	20	B	98	93
Soy sauce	20	B	120	108
Bread and toast				
White bread	16	C	67	86
White bread toast	16	C	86	90
Scone bread	10	C	88	115
Whole wheat bread toast	16	C	76	71
White bread toast	10	C	96	114
Italian style bread, dark toast	36	C	69	96
Yogurt				
Peach bottom natural yogurt	16	C	71	80
Blueberry bottom yogurt	16	C	83	124

[a] Methods used: A, Chem Elut extraction followed by TEA(N) detection; B, Celite + neutral alumina (10% water content) clean-up followed by GC–MS; C, same as B with additional liquid–liquid partitioning to remove fats and lipids.
[b] Levels of MC and EC present in the unspiked samples were subtracted before calculating recoveries.

confirmation of MC and EC in selected samples of alcoholic beverages, soy sauce, and yogurt. Examples of a few typical chromatograms of the GC–TEA(N) technique are shown in Figs 1 and 2.

The results of the analyses of various fermented foods and beverages are presented in Tables 2–5. Most of the alcoholic beverages (Table 2) contained either no MC, or traces, with the exception of two fruit brandies which contained 9 μg/kg and 27·7 μg/kg. It should be noted that these two samples were old (procured in 1985) and more recent samples were unavailable for analysis. As expected from our previous studies on wines (Sen *et al.*, 1992), most of the alcoholic beverages contained higher levels of EC, but most, except the 1985 samples, were within the Canadian guidelines for EC levels.

On the other hand, the consistent finding of MC in both breads and toasts (Table 3) was unexpected. Although the occurrence of EC in bread and toast has been well documented (reviewed by Battaglia *et al.*,

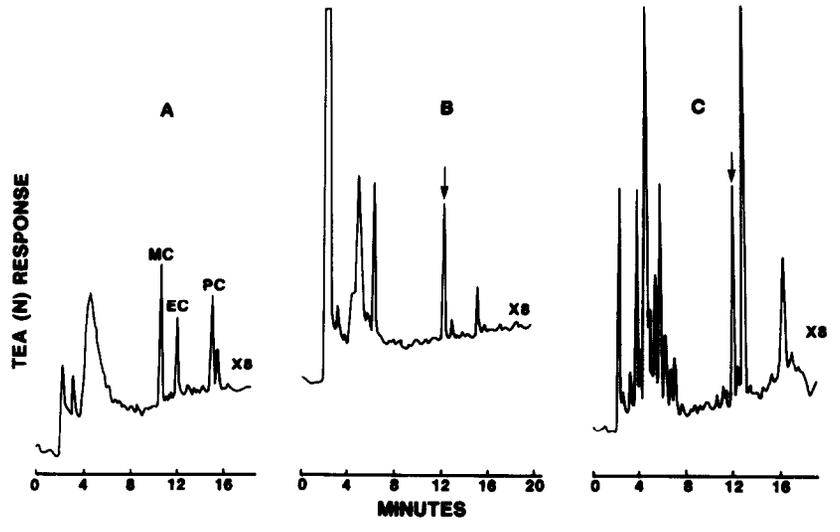

Fig. 2. GC–TEA(N) chromatograms: (A) MC (200 pg), EC (140 pg) and PC (154 pg) standards; (B) 2 μl/1 ml final extract of a bourbon whiskey showing the presence of 67·7 μg/kg levels of EC; (C) 1 μl/2 ml final extract of a soy sauce showing the presence of 61·3 μg/kg EC.

Table 2. Levels of MC and EC detected in various alcoholic beverages

Sample	n	Country of origin	Levels (μg/kg) detected	
			MC	EC
			range (mean)[a]	range (mean)
White wines	3	Canada	N[b]–1	N–2·6
White wines	8	U.S.A	N–2 (<1)	N–24 (5·6)
White wines	2	France	N	1·5–2·9
White wines	3	Germany	N–1·4	2·7–5·6
Red wines	2	Canada	N–1	1·8–4·7
Red wines	5	U.S.A.	N–1 (<1)	1–14 (8·7)
Sake	2	Japan	N	3–29
Sherries	2	Canada	N	29–69[c]
Sherry	1	Australia	N	41
Sherries	3	Spain	N–2·7	28–58[c]
Whisky	6	Scotland	N–0·5[c] (0·1)	26–247[c,d] (75·7)
Rye	1	Canada	N	8[c]
Bourbon	4	U.S.A.	N	44–208[d]
Vodka	1	Canada	N	N
Gin	1	Canada	N	0·5[c]
Rum	1	Canada	N	19[c]
Fruit brandy[d]	1	France	28[c]	2 344[c]
Fruit brandy	1	Austria	9[c]	380[c]
Fruit brandy	1	Canada	1·1[c]	104[c]
Apricot brandy	1	Canada	N	11
Armagnac[d]	2	France	N–0·8[c]	410[c]–432[c]
Other brandies	3	France	N–0·8[c]	25–28[c]

[a] Mean value given only in cases where ≥ 5 samples were analyzed.
[b] N = not detected (detection limit, 1–2 μg/kg).
[c] Confirmed by GC–MS–SIM.
[d] Old samples procured in 1985; newer samples were either not available (e.g. for some of the fruit brandies) or on resampling were found to contain lower levels of EC (e.g. for bourbon and whisky).

1990), this appears to be the first reported occurrence of MC in such products. However, the source of the contamination or the mechanism of its formation is not clear at present. It is possible that like EC, MC is formed as a by-product of fermentation. Further research is desirable in this area.

There appeared to be an approximately 20–30% loss in weights during toasting of bread that might have a slight concentration effect on the EC levels in toasts. Even if one takes this into consideration, the levels of EC in the dark toasts seemed to be significantly ($P < 0·01$) higher than those in the breads and the light toasts when the data were analyzed by Student's T-test (Table 3). There was, however, no significant difference

Table 3. Levels of MC and EC detected in various breads and toasts

Sample	Levels (μg/kg) detected[a]					
	MC			EC		
	Bread	Light toast	Dark toast	Bread	Light toast	Dark toast
White bread	4·1	1·8	1·5	4·1	3·9	22·7
Light rye bread	4·4	2·9	3·8	4·8	4·3	24·1
Potato scone bread	2·2	2·2	3·6	4·4	7·5	29·2
Seven-grain bread	3·0	2·9	2·7	1·8	2·1	11·5
White bread	2·7	3·6	4·5	4·1	10·9	27·9
Whole wheat bread	3·6	2·5	3·0	1·4	1·0	5·7
Whole wheat bread	3·5	1·4	2·6	3·1	2·0	11·8
Italian style bread	3·8	3·3	4·6	3·3	2·0	8·9
Rye bread	3·7	2·2	3·7	2·5	3·8	12·5
Rye bread	2·1	1·5	0·5	1·6	1·8	5·3
Italian style bread	2·2	0·8	1·5	2·3	1·3	4·9
White bread	1·2	0·9	0·3	3·3	10·6	23·6
mean	3·0	2·2	2·7	3·1	4·3	15·7

[a] All results are based on GC–MS–SIM analysis.

Table 4. MC and EC found in soy sauce

Country of origin	Level (μg/kg) detected[a]	
	MC	EC
Thailand	0·1	N[b]
China	0·5	6·5
China	0·2	1·4
China	1·1	1.9
Taiwan	0·2	0·6
USA	0·6	57
USA	0·6	59
Canada	0·1	N
Canada	0·1	N
Canada	N	0·7

[a] All results are based on GC–MS analysis.
[b] N = not detected (<0·1 μg/kg).

between the EC levels detected in breads and the light toasts. The latter values are comparable to those reported by other researchers (Canas *et al.*, 1989; Dennis *et al.*, 1989; Ough, 1976). Our data clearly indicated for the first time an increase in the formation of EC with an increase in the degree of toasting. This observation might be important as the dark toasts, analyzed in this study, still appeared to be edible and might be preferred by some people. The consumption of two slices (average weight 68·6 g) of dark toasts would result in an average intake of 1·07 μg EC and 0·18 μg MC.

Table 5. Levels of MC and EC in yogurt and buttermilk

Sample	Levels (μg/kg) detected	
	MC	EC
Peach bottom natural yogurt (4·5% milk fat)	2·5	N[a]
Yogurt with blueberry (0·1% milk fat)	3·3	N
Strawberry bottom yogurt (2·8% milk fat)	3·6	N
Balkan style natural yogurt (5·9% milk fat)	4·3	N
Fat and cholesterol-free yogurt (0·1% milk fat)	2·1	N
Balkan style blueberry yogurt (4·5% milk fat)	1·2	N
Natural stirred fruit (raspberry) yogurt (1·9% milk fat)	N	N
Strawberry yogurt (0·9% milk fat)	2·0	N
Plain natural yogurt, brand A	1·2	N
Plain light natural yogurt, brand B	1·1	N
Plain natural yogurt, brand C	1·5	0·4
Plain light natural yogurt, brand D	1	0·1
Butter milk	0·3	N
Cultured buttermilk	1·1	N

[a] N = not detected (<0·1 μg/kg).
All results are based on GC–MS analysis.

Table 6. Comparison of GC–TEA(N) data with those obtained by GC–MS

Sample analyzed	Levels (μg/kg) detected			
	GC–TEA(N)		GC–MS	
	MC	EC	MC	EC
Red wine	N[a]	5·8	0·7	8·3
White wine	2·2	4·0	N	4·0
White wine	1·9	24·1	1·9	25
Gin	N	N	N	0·5
Rum	N	19	N	28
Scotch whisky	N	26	N	20·3
Scotch whisky	N	247	0·5	290
Rye	N	8	N	4·0
Fruit brandy	N	104	1·0	180
Armagnac	12·7	432	0·8	554
Armagnac	N	410	1·0	619
Liqueur	N	28·4	0·8	37
Fruit brandy	27·7	2 344	28·8	4 410
Brandy	N	36	N	34
Brandy	N	24·6	N	14·4
Sherry	N	69·4	0·5	58·7
Sherry	N	27·9	0·9	23·3
Soy sauce	N	11·7	0·5	6·5
Soy sauce	N	N	0·2	0·6
Soy sauce	N	50	0·6	56·9
Soy sauce	N	61·3	0·6	59·3
White bread	N	2·8	1·9	3·7
Scone bread	N	N	0·8	0·6

[a] N = not detected (detection limits: about 1–2 μg/kg for GC–TEA(N) and ~0·1 μg/kg for GC–MS).

Of the remaining products analyzed, only some of the soy sauces contained appreciable levels of EC (Table 4). The variation in the levels of EC in various soy sauces may be due to the differences in their processing conditions (some are fermented while some are not) or due to the effect of other ingredients. The levels of MC in the soy sauces were negligible. The yogurt samples (Table 5) were mostly negative for EC but contained traces of (1–4 μg/kg) MC. The latter finding is puzzling and was unexpected. Further research is recommended towards identifying the source of this contamination.

High resolution GC–MS–SIM has been successfully used for the determination of both EC and MC in alcoholic beverages (reviewed by Battaglia *et al.*, 1990; Sen *et al.*, 1992). In this study, two fragment ions at m/z 74 and 62 were monitored at a mass resolution of 8 K for the determination of EC, but only the response of the latter ion was used for quantitation. Also, the response ratio of the above two fragment ions for each sample was calculated and found to be similar to that obtained with EC standard. This was not, however, possible for the determination of MC because there was only one ion (at m/z 75) that could be considered characteristic to MC. The response of this fragment ion at a resolution of 8 K was used for quantitation of MC in all samples. Since MC was detected for the first time in several of the fermented products, we sought a more

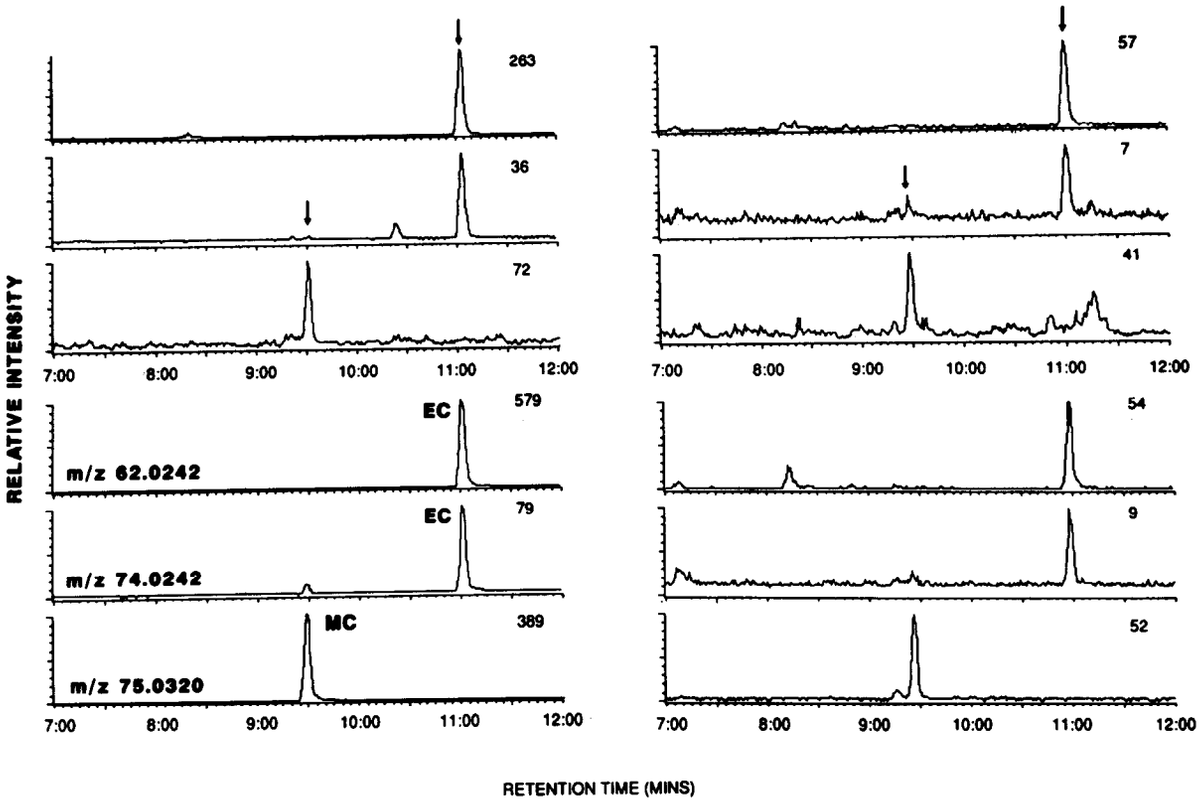

Fig. 3. GC–MS–SIM (high resolution; 8 K) chromatograms: Left bottom—MC (360 pg) at m/z 75·0320 and EC (375 pg) monitored simultaneously for m/z 74·0242 (middle tracing) and 62·0242 (top tracing); Right bottom—similar tracings for an 1 μl/1 ml final extract of a whole wheat bread; Right top—the light toast of the same bread; and left top—the dark toast of the same bread. It should be noted that not all the tracings were produced under the same senstivity settings as can be determined by their relative noise levels. The numbers represent relative height counts. The blank (taken through all the steps) gave very low readings, and its values were subtracted before calculating results.

unequivocal confirmation of the compound. Because of its extremely low levels, it was not possible to obtain a full-scan mass spectrum. GC–MS–SIM in the chemical ionization mode was also considered to be no better because an interfering ion having a similar m/z ratio would be indistinguishable from the corresponding MC fragment. For these reasons, we repeated the GC–MS–SIM (E.I. mode) for two of the toasts under a resolution of 12 K hoping that, at least, this would reduce the possible numbers of interfering ions that would have the same m/z ratio. The levels of MC detected under these conditions were similar to those detected before at lower mass resolution (under 8 K). Therefore, it is highly unlikely that the response at m/z 75 detected in many sample extracts was caused by interfering ions and not by MC. Typical examples of a few GC–MS–SIM chromatograms are presented in Fig. 3. With a few exceptions, the GC–MS–SIM data agreed fairly well with those obtained by GC-TEA(N) (Table 6).

In conclusion, we have developed an improved analytical method for the determination of both EC and MC in a variety of fermented foods that will be useful in future studies. Data presented suggest that in addition to EC, traces of MC may occur in some fermented foods and beverages. The finding of elevated levels of EC in dark toasts should also be of considerable interest. Further studies are recommended towards studying the mechanism of formation of both MC and EC in various fermented foods.

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