Food Chemistry **48** (1993) 313–316
Analytical Methods Section

Determination of ethyl carbamate in soy sauces

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(Received 5 August 1992; revised version received and accepted 23 January 1993)

The potential carcinogen ethyl carbamate was determined in 18 soy sauces, which were classified in three groups: fermented, non-fermented and those with unclear production process. The sample preparation included a solid-phase extraction with Extrelut. Non-polar compounds were separated by rinsing the column with *n*-pentane before dichloromethane elution. The concentrated extracts were measured by gas chromatography–selected ion monitoring–mass spectrometry (GC–SIM–MS) using chemical ionization with methane as reagent gas. Ethyl carbamate as well as propyl carbamate as internal standard were detected by two representative mass fragments down to a detection limit of 1 ppb. The fermented sauces showed a tendency to higher ethyl carbamate concentrations than the non-fermented sauces.

INTRODUCTION

Ethyl carbamate (EC) is a well-known carcinogenic compound. It causes benign and malignant tumours in various species of experimental animals, especially in lungs and liver (Mirvisch, 1968; Schmähl *et al.*, 1977). Because it is regarded as a potential carcinogen to humans, it is generally undesirable in foods and its content should be as low as possible.

As it occurs in many fermented products, EC also may be present in soy sauces. Ough (1976) described the potential occurrence of EC in soy sauces at the low ppb level. Canas *et al.* (1989) investigated 12 soy sauces and found 84 ppb EC as the highest concentration, in a Japanese sauce. Also, Hartmann and Rosen (1989) reported on the occurrence of EC in fermented and non-fermented soy sauces. The fermented sauces showed up to 100 ppb EC, whereas the carcinogen was not detectable in non-fermented sauces. A recent study by Hasegawa *et al.* (1990) has demonstrated that soy sauces containing ethanol as preservative may yield EC concentrations up to 50 ppb.

Fat-free soy flour and roasted, squeezed wheat are often used for the production of soy sauces. After a long fermentation with suitable microorganisms salt is added up to a concentration of 18%. During fermentation the microorganisms produce extra-cellular hydrolases, which split the proteins, carbohydrates and nucleic acids in the raw products. Low-quality products may be blended with sauces produced with acid hydrolysates (Belitz & Grosch, 1987).

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Food Chemistry 0308-8146/93/$06.00 © 1993 Elsevier Science Publishers Ltd, England. Printed in Great Britain

METHOD

The procedure used to prepare the sauces was a modification of the method of Baumann and Zimmerli (1986). The sample preparation included a solid-phase extraction with Extrelut. Non-polar compounds were eluted with *n*-pentane followed by a dichloromethane extraction to deliver the EC fraction.

Preparation of the Extrelut column

Dried Na_2SO_4 (5 g) was placed in the column. The material of a commercially available Extrelut column (10 ml) was mixed with dried NaCl (5 g) and added on top of the Na_2SO_4 layer. Approximately 5 g of soy sauce was weighed, with a precision of ±10 mg. Internal standard solution (25 μl) (250 ng of propyl carbamate in 25 μl of ethanol) was added. The sauce was transferred with distilled water (5 ml) into the Extrelut column. After 15 min the column was rinsed twice with *n*-pentane (2 × 10 ml) at a drop rate of 1–2 drops s^{-1}. After elution of the unwanted non-polar components the extraction was performed with dichloromethane (15 ml), and was repeated three times. The eluate was concentrated under reduced pressure at 30°C in a Rotavapor to a volume of about 10 ml. The extract was transferred with a 2 ml keeper solution (iso-octane–1-propanol; 80/20, v/v) and dichloromethane (2 ml) into a graduated conical flask. The volume was reduced to 1 ml under a gentle nitrogen stream. The addition of the 'keeper' had been found to be necessary to prevent solvent evaporation during on-column injection. The extract (1 μl) was analysed by gas chromatography– selected ion monitoring–mass spectrometry (GC–MIS–MS) with chemical ionisation (Fauhl & Wittkowski, 1992).

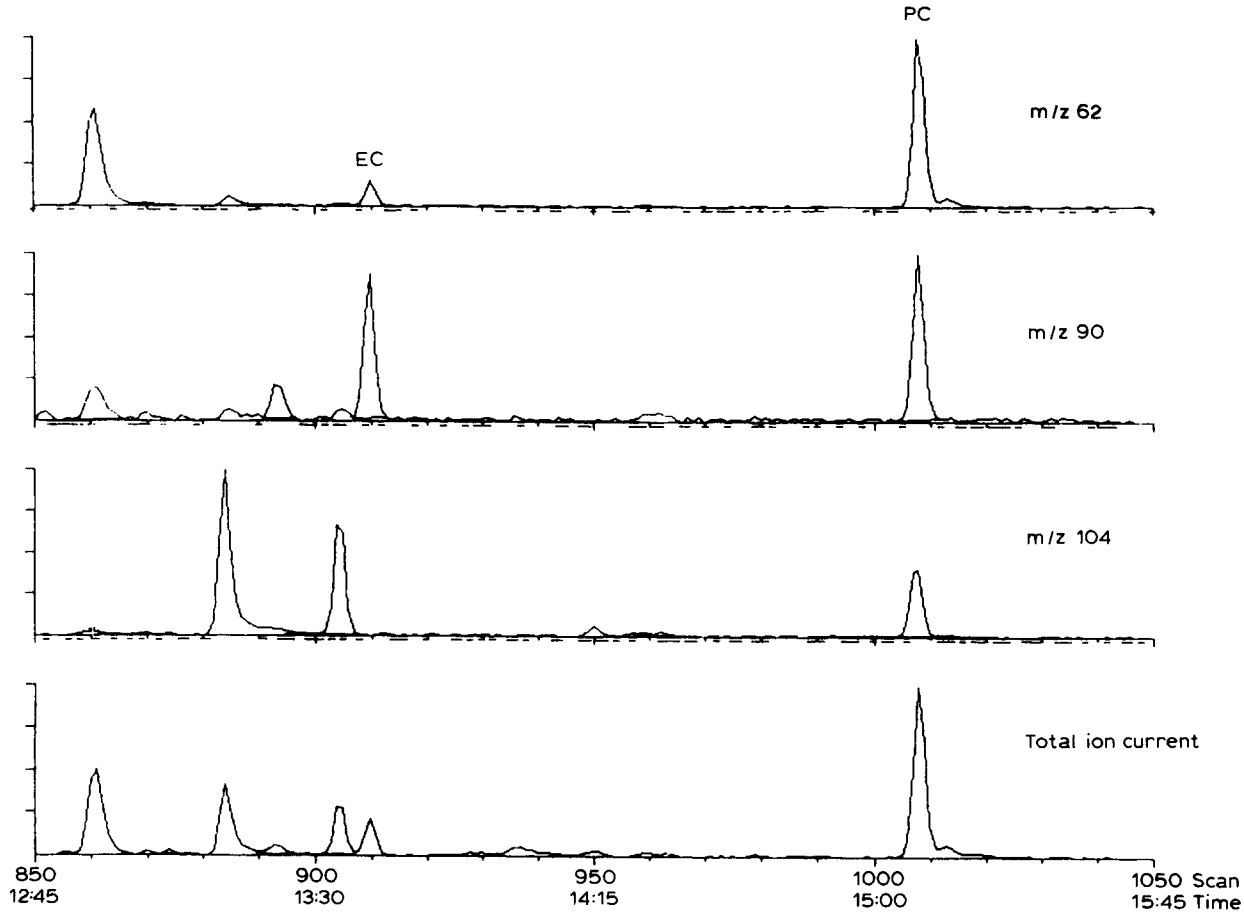

Fig. 1. Mass chromatograms of a soy sauce containing 8 ppb ethyl carbamate (EC) spiked with 48 ppb propyl carbamate (PC). Each chromatogram was normalized to the largest peak in the retention time window.

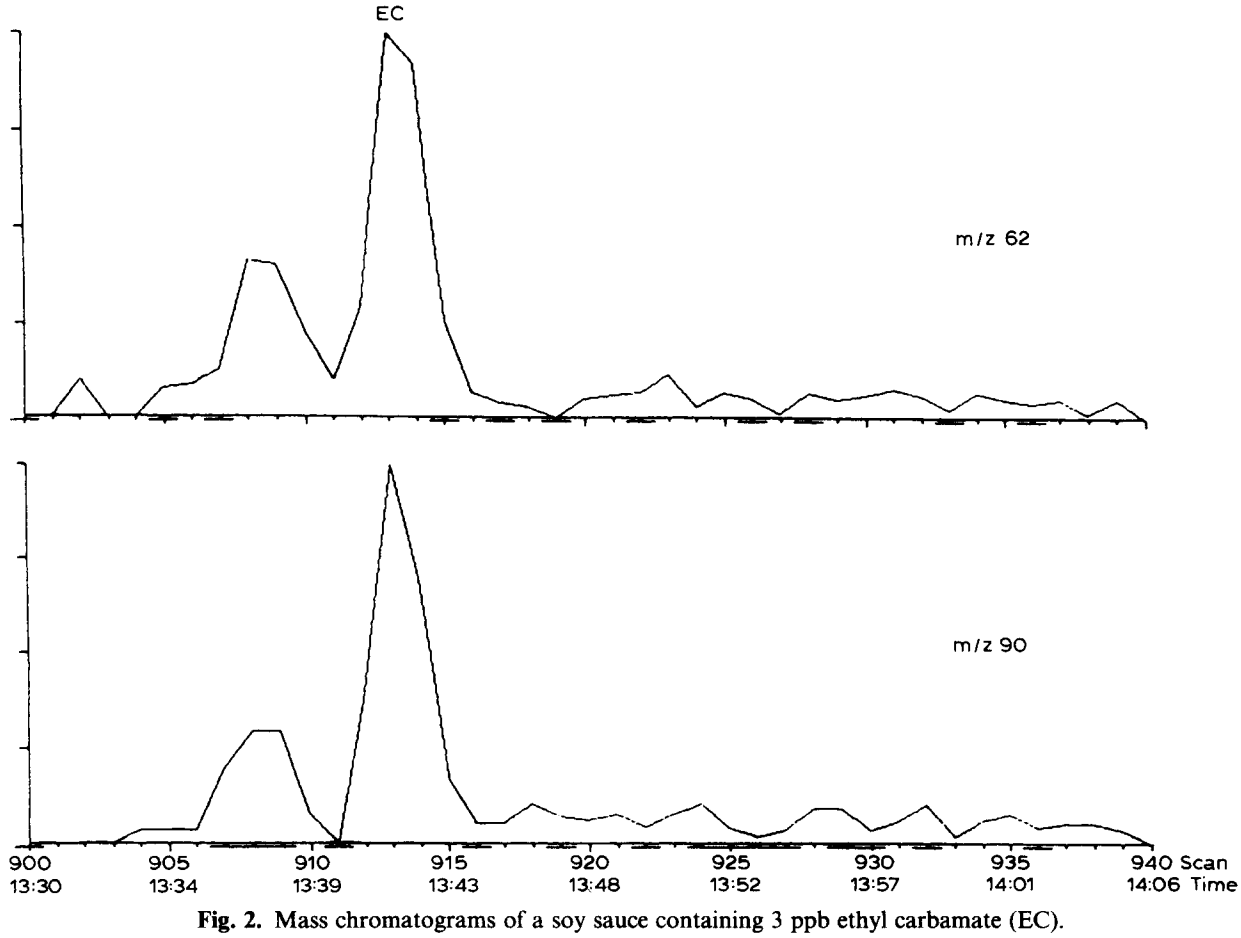

Fig. 2. Mass chromatograms of a soy sauce containing 3 ppb ethyl carbamate (EC).

Gas chromatography and mass spectrometry

Gas chromatography

Instrument:	Hewlett Packard 5890
Injector:	Hewlett Packard on-column
Carrier gas:	He, at 26 cm s^{-1}
Column:	55 m × 0·32 mm DB Wax, film thickness 0·25 μm connected to a 1 m × 0·53 mm fused silica retention gap
Temperature programme:	70°C held for 2 min; 8°C min^{-1} to 180°C, held for 3 min, 20°C min^{-1} to 220°C, held for 15 min.

Mass spectrometry

Instrument:	Finnigan 4000 quadrupole mass spectrometer
Temperature:	transline 250°C; ion source 120°C
Reagent gas:	methane
Pressure:	0·40 Torr fore pressure; $1·3 \times 10^{-5}$ Torr ion source pressure
Selected ion monitoring:	*m/z* 62, 90, 95 and 104; each fragment was registered for 200 ms per scan

Ethyl carbamate was detected by fragments *m/z* 62 and *m/z* 90 (M + 1). Those two fragments appeared in a ratio of 1:1. The internal standard propyl carbamate was measured by three characteristic fragments at *m/z* 62, 90, and 104. The additional detection of *m/z* 95 was included as control. Quantification was achieved by calculating the peak areas of fragment *m/z* 62 for EC and also for propyl carbamate with inclusion of the response factor.

RESULTS AND DISCUSSION

The method described above was applied to the analysis of various soy sauces to evaluate differences in the EC content of fermented and non-fermented soy sauces. The detection of two mass fragments (*m/z* 62 and *m/z* 90) with the same intensity for EC and three mass fragments (*m/z* 62, 90 and 104) for propyl carbamate guaranteed a close fit for the substance/peak identity. Figure 1 shows the mass chromatograms obtained by analysis of a soy sauce containing 8 ppb EC and 48 ppb propyl carbamate as internal standard. In Fig. 2 the mass fragment traces *m/z* 62 and 90 represent a soy sauce containing 3 ppb EC. The detection limit is 1 ppb at a signal-to-noise ratio of 3:1.

Table 1. EC concentrations in soy sauce

Not fermented		Production unclear		Fermented	
Sample no.	ppb	Sample no.	ppb	Sample no.	ppb
1	8	11	7	14	78
2	19	12	3	15	19
3	3	13	45	16	9
4	18			17	11
5	12			18	23
6	5				
7	11				
8	4				
9	3				
10	4				

Table 2. Influence of ethanol addition on EC concentrations

Sample no.	EC (ppb) before addition	EC (ppb) after addition and storage
1	8	9
3	3	5
11	7	13
14	78	83

The values measured in 18 soy sauces are listed in Table 1, and are classified into those with no reference to fermentation, or those with a reference to traditional production and those with a clear reference to fermentation on their labels. Descriptions on the labels such as 'traditional production', etc., are listed separately because a final decision on their production process cannot be made. In one case (sample 13) it was mentioned only that soy sauces in general have been produced for over 2000 years.

The analysed soy sauces contained EC up to 78 ppb. This result confirms previous statements in the literature. Despite the values for 'Production unclear' and the fact that we cannot claim the samples to be representative, there is a tendency to lower values of EC in non-fermented products and to higher values in fermented sauces. Two of the three highest values of over 20 ppb (samples 14 and 18) are clearly indicated as fermented, and the third (sample 13) cannot be classified. None of the non-fermented soy sauces exceeded a value of 20 ppb. It is not known how far some of the samples under investigation have been blended with acid hydrolysates. However, before a final statement can be given on this subject, it would be desirable to analyse samples for which the production processes are exactly known.

One possible formation pathway of EC in soy sauce had been thought to be from ethanol added as a preservative (Hasegawa *et al.*, 1990). To establish the extent of this influence, samples 1, 3, 11 and 14 were adjusted to 5% ethanol (w/w) and stored for 10 days in darkness at 40°C. Table 2 clearly indicates that the values increased slightly.

ACKNOWLEDGEMENT

The assistance of W. Blaas is gratefully acknowledged.

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