Journal of Chromatography, 594 (1992) 259–267
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 852

Analysis of zapatera olives by gas and high-performance liquid chromatography

Alfredo Montaño*, Antonio de Castro, Luís Rejano and Antonio-Higinio Sánchez

Unidad E.I. Biotecnología de Alimentos, Instituto de la Grasa y sus Derivados (CSIC), Apartado 1078, 41012 Seville (Spain)

(First received July 19th, 1991; revised manuscript received October 29th, 1991)

ABSTRACT

Gas chromatography and high-performance liquid chromatography were applied to normal and "zapatera" olive brines obtained from typical fermentation brines of green table olives after different treatments. The zapatera samples were obtained by pH adjustment to 5.1 followed by inoculation with a suspension of sediment from a zapatera brine and incubation at 30°C for 40 days. The compounds determined were lactic acid, C_2–C_6 fatty acids, acetaldehyde, methanol, ethanol, 2-butanol and *n*-propanol. Normal and zapatera brines were compared to identify components that indicated spoilage. One of these components was found in the gas chromatogram of the volatile fatty acids from the zapatera samples and identified as cyclohexanecarboxylic acid by gas chromatography–mass spectrometry. A comparison of the corresponding aromagrams revealed quantitative differences in aroma composition. Various relationships calculated from the peak areas of selected unknown components in these aromagrams were so distinct as to provide a basis for characterizing zapatera spoilage.

INTRODUCTION

The table olive is an important product for Spain, during 1989 128 000 tonnes valued at $3 \cdot 10^{10}$ pesetas being exported [1]. The most important of the different commercial preparations is the Spanish-style green olive, characterized by a lactic acid fermentation. At present, owing to the lack of a pure culture fermentation that guarantees the uniformity and safety of the product, these olives are still prepared by natural, spontaneous fermentation. One risk of such a practice is that there will be an increased likelihood of microbial spoilage, including the clearly differentiated and defined "gas pocket", putrid fermentation, butyric fermentation and "zapatera" spoilage [2]. The last type of spoilage is frequent in Spanish-style green olives and causes large losses to the industry. It is characterized by the development of a very penetrating, unpleasant odour in olives undergoing fermentation. It seems that the spoilage results from decomposition of organic acids at a time when little or no sugar is present and the lactic acid fermentation stops before the pH has decreased below 4.5 [2]. Zapatera spoilage seems to be caused by the participation of species of at least two genera of bacteria, *Clostridium* and *Propionibacterium* [3]. Although the characteristic odour of zapatera samples is different to that of other malodorous fermentations (*e.g.*, butyric fermentation), there is sometimes confusion over the term "zapatera", with a tendency to classify as such most olives with abnormal flavour [2].

Detection of zapatera samples by sensory methods depends on both the olfactory detection threshold of the taster and the stage of development of the spoilage. Detection is almost impossible in zapatera olives corrected by dilution and/or masked by addition of aroma-giving substances, a frequent type of fraud in the olive industry.

Traditional microbiological methods for detection spoilage in food present two main problems: (a) they take days, or even weeks, to carry out and (b) the microorganisms responsible may not be detected on analysis. However, the chemical changes

0021-9673/92/$05.00

originating from microbial activity remain detectable independently of whether the microorganisms are viable or not [4].

The foregoing more than justifies the proposed aims of this work, namely to separate by chromatographic methods the volatile component or components contributing significantly to the typical unpleasant smell of zapatera olives, and to establish an analytical method to detect the spoilage.

EXPERIMENTAL

Samples

A typical fermentation brine of green olives from our laboratory was used. It had the following physico-chemical characteristics: pH 4.50, titratable acidity (expressed as lactic acid) 0.60%, sodium chloride content 5.8% and volatile acidity (expressed as lactic acid) 0.62%. Aliquots of this brine (200 ml) were placed in ten flasks each of 300-ml capacity, and were treated as shown in Table I. A 1-l volume of a zapatera brine supplied by the industry was centrifuged at 16 300 *g* for 10 min and the sediment obtained was washed with saline solution (1% NaCl) and finally resuspended in 25 ml of the same solution. A 3-ml portion of this suspension was used as inoculum.

In addition, 24 zapatera brines and 20 normal brines from different olive-processing plants were analysed.

Apparatus

The following instruments were used: a gas chromatograph (Perkin-Elmer Model 3920B) fitted with a flame ionization detector coupled to a recording integrator (Hewlett-Packard Model 3394A), a liquid chromatograph (Perkin-Elmer Series 4) with a manual injector (Rheodyne Model 7125), in combination with a spectrophotometric detector (Perkin-Elmer Model LC-85B) coupled to a recording integrator (Hewlett-Packard Model 3390A), and a mass spectrometer (AEI MS-30/VG-70) with a VG11-250 data system connected to a gas chromatograph (Hewlett-Packard Model 5890).

Analytical methods

Chemical characteristics. Titratable acidity, pH, volatile acidity and sodium chloride content of the brines were determined by the usual methods used in our laboratory [2].

Volatile acids by gas chromatography (GC). The following procedure was used to determine the volatile acids by GC: 20 ml of the sample were placed in a Kjeldhal apparatus used for the determination of volatile acidity, collecting *ca.* 250 ml of distillate. After neutralization with 0.2 *M* NaOH, it was transferred to a porcelain capsule and evaporated to dryness in a water-bath at 100°C. Immediately, 2 ml of 2 *M* H_2SO_4 were added to the residue and extracted twice with 25-ml portions of diethyl ether. After adding 0.5 ml of isocaproic acid solution (105 ppm in diethyl ether) as internal standard, the extract was dried over anhydrous sodium sulphate and concentrated *in vacuo* at 30°C to a volume of 0.5 ml or less. An aliquot (0.2–0.3 μl) of this concentrate was taken for GC analysis. A Supelcowax 10 fused-silica capillary column (30 m × 0.53 mm I.D., 1.0-μm film thickness) (Supelco) was used for

TABLE I

TREATMENTS CARRIED OUT WITH THE AIM OF REPRODUCING ZAPATERA SPOILAGE

Treatment	Sample				
	S1–S2	S3–S4	S5–S6	S7–S8	S9–S10
pH adjustment[a]	No	Yes	Yes	Yes	Yes
Inoculation[b]	No	No	Yes	Yes	No
Addition of glucose and pasteurisation[c]	No	No	No	Yes	Yes
Storage at 30°C[d]	Yes	Yes	Yes	Yes	Yes

[a] Concentrated NaOH was added to pH 5.1.
[b] 3 ml of inoculum, prepared as described in the text, were added.
[c] 3 g of glucose were added before pasteurization in a water-bath at 80°C for 10 min.
[d] Paraffin oil was added previously to form a surface layer *ca.* 1 cm thick.

the analysis of the volatile acids. The oven temperature was maintained at 150°C, the injector at 200°C and the detector at 230°C. Nitrogen was used as the carrier gas and auxiliary gas at flow-rates of 9 and 60 ml/min, respectively. Identification of C_2–C_6 volatile acids was made by comparing their retention times with authentic standards on different chromatographic columns. These columns and the conditions were as follows: (1) Supelcowax 10, as described above; (2) 2 m x 1/4 in.- O.D. glass column packed with 0.3% Carbowax 20M + 0.1% H_3PO_4 on 60–80-mesh Carbopack C at 140°C with nitrogen as carrier gas at 50 ml/min; and (3) 1.5 m × 1/8 in. O.D. stainless-steel column, packed with 20% sebacic acid on 80–100-mesh Chromosorb W AW at 135°C with nitrogen (saturated with formic acid) as carrier gas at 30 ml/min. The GC results were calculated from the peak areas obtained from the integrator, using the internal standard method. Identification of the "key compound" in the chromatograms of volatile acids from the zapatera brines was carried out by GC on a Supelcowax 10 column (30 m × 0.25 mm I.D.) (Supelco) with helium as carrier gas at 150°C for 5 min, then programmed to 180°C at 6°C/min, in conjunction with mass spectrometric analysis (GC–MS). Peak identification was confirmed by comparing the GC retention time and mass spectrum with that of an authentic standard.

Headspace components. Analysis of the major volatile components of the brines was carried out by the headspace method of Montaño *et al.* [5]. Quantification was effected using the peak areas using the internal standard method with dioxane as internal standard.

Volatile compounds by GC. The volatile compounds (excluding the organic acids) responsible for the flavour were separated from the brines by extraction with diethyl ether and analysed by GC. A determined volume of brine (>50 ml) was neutralized to pH 7–8 with solid magnesium oxide and the aid of a pH meter and magnetic stirrer. The precipitate was separated by filtration and the filtrate (50 ml) extracted three times with 25-ml portions of diethyl ether, stirring slowly to prevent emulsion formation. An internal standard, 0.5 ml of a dodecane solution in diethyl ether (0.054%), was added to the extract, which was immediately dried over anhydrous sodium sulphate. Most of the solvent was removed *in vacuo* at 30°C and an aliquot (0.2–0.3 μl) of the concentrate injected into the gas chromatograph. The same analytical column was used as in the analysis of volatile acids, under the following operating conditions: carrier gas (nitrogen) flow-rate, 8 ml/min; auxiliary gas flow-rate, 60 ml/min; injector temperature, 120°C; detector temperature, 270°C; oven temperature, 60°C for 4 min, then programmed to 230°C at 4°C/min.

Volatile and non-volatile acids by high-performance liquid chromatography (HPLC). The organic acids, both volatile and non-volatile, were monitored together by HPLC using a cation-exchange column (Aminex HPX-87H, 300 × 7.8 mm I.D.) (Bio-Rad Labs.), with a cation-exchange guard column and UV detection at 220 nm. In this instance, sample preparation included only a dilution stage (1:10 with deionized water) followed by passage through a minicolumn containing a cation-exchange resin (2-ml bed of Amberlite IR-120H) to remove Na^+. To avoid dilution on being passed through the resin, 6 ml of sample were added and discarded before collecting 2 ml for chromatographic analysis. An aliquot (50 μl) of this fraction was injected into the chromatograph after filtration through a 0.45-μm membrane filter. The analytical column was kept at 65°C during analysis, in which 0.005 *M* H_2SO_4 was used as mobile phase at a flow-rate of 0.8 ml/min.

RESULTS AND DISCUSSION

For the satisfactory use of GC or HPLC in the investigation of food spoilage, it is necessary to compare the compositions of spoiled samples with those of normal samples of the same product lot in order to characterize the spoiled samples [4]. Owing to the difficulty of obtaining brines of zapatera olives with their corresponding unchanged controls, we tried to reproduce spoilage in the laboratory by applying the treatments shown in Table I. An initial exploratory examination of the odd-numbered samples, carried out after 20 days of storage, revealed the presence of the typical zapatera smell in one of them (S5). Moreover, a considerable increase (0.8%) was obtained in the volatile acidity of this sample compared with the remainder, which did not show any apparent signs of spoilage.

TABLE II

PHYSICO-CHEMICAL ANALYSIS AND OTHER OBSERVATIONS OF THE SAMPLES AFTER 40 DAYS OF STORAGE

Sample	pH	Volatile acid[a]	Colour[b]	"Zapatera" smell[c]
S1	4.67	0.66	NY	−
S2	4.67	0.66	NY	−
S3	5.24	0.88	NY	−
S4	5.26	0.80	NY	+
S5	5.39	1.58	NY	+ +
S6	5.39	1.52	NY	+ +
S7	4.06	0.64	DY	−
S8	4.06	0.62	DY	−
S9	4.24	0.66	DY	−
S10	4.37	0.70	DY	−

[a] Expressed as % lactic acid.
[b] NY = Normal Yellow; DY = dark yellow.
[c] − = Not detected; + = intense; + + = very intense.

Determination of organic acids and headspace components

Chemical and chromatographic analyses of the samples were performed after 40 days of storage. Sample S5 and its replicate S6 were detected by smell as clearly spoiled, and brine S4 (but not its replicate) was weakly so. Apart from smell, S5 and S6 were appreciably different from the others in the values of their physico-chemical characteristics pH and volatile acidity, and also in the concentration of some of the compounds determined in this study (Tables II and III). Thus, lactic acid was not detected in the zapatera samples (S5 and S6), which on the other hand showed a large increase in the concentration of the C_2–C_6 volatile acids. Similar results were obtained by Fleming *et al.* [6] in studies of butyric acid spoilage of fermented cucumber, although the concentrations of the major volatile acids (propionic and butyric acids) were clearly different to those in the zapatera olive brines. Cucumber brine after butyric acid spoilage contained a concentration of butyric acid of about 3500 ppm [6], more then fifteen times as much as in the zapatera samples S5 and S6. The concentration of this acid in the latter samples was similar to that obtained in an abnormal fermentation of sauerkraut, characterized by a "cheese-like" off-odour [7]. In contrast, the concentration of propionic acid in the zapatera samples (*ca.* 10 000 ppm) was much higher than those of both the butyric cucumber brine and off-odour sauerkraut (600 and 7 ppm, respectively). Degradation of lactic acid and concomitant formation of volatile acids in the zapatera samples S5 and S6 caused a rise in pH (Table II) owing to the lower dissociation constants of these acids compared with that of lactic acid, as observed by Borbolla and Re-

TABLE III

DETERMINATION OF LACTIC ACID AND THE VOLATILE C_2–C_6 FATTY ACIDS AFTER 40 DAYS OF STORAGE

Sample	Acid (ppm)						
	Lactic[a]	Acetic[a]	Propionic[b]	*n*-Butyric[b]	Isovaleric[b]	*n*-Valeric[b]	*n*-Caproic[b]
S1	8750	4962	1628	2.0	0.6	ND[c]	0.4
S2	10882	3601	1080	2.2	0.5	Tr[d]	0.1
S3	5622	3920	4280	1.1	0.7	Tr	0.2
S4	6331	3902	3896	1.2	0.8	Tr	0.2
S5	ND	5550	11023	225.2	2.3	292.1	8.0
S6	ND	5291	9462	189.0	2.1	240.3	6.4
S7	19771	3340	321	0.7	0.2	Tr	0.1
S8	17790	3031	339	1.0	0.3	ND	0.2
S9	17971	6162	597	0.9	0.6	ND	0.2
S10	14972	3910	443	1.4	0.7	ND	ND

[a] Determined by the HPLC procedure described in the text, using the external standard method.
[b] Determined by the GC procedure described in the text, using the internal standard method.
[c] ND = Not detected.
[d] Tr = Trace amounts (<0.1 ppm).

TABLE IV

DETERMINATION OF THE MAJOR HEADSPACE COMPONENTS OF THE BRINES

Sample	Compound (ppm)				
	Acetaldehyde	Methanol	Ethanol	2-Butanol	*n*-Propanol
S1	4.2	647.7	546.6	17.0	452.9
S2	5.1	685.2	569.1	15.3	430.5
S3	4.7	690.1	470.5	19.0	144.8
S4	10.9	633.5	513.9	23.8	201.5
S5	9.3	938.9	ND[a]	17.9	ND
S6	11.8	954.3	ND	18.9	ND
S7	4.5	646.2	532.4	15.3	157.1
S8	4.8	628.1	518.0	14.7	146.1
S9	3.3	594.3	2968.1	15.4	153.4
S10	4.2	572.7	1962.1	14.7	149.8

[a] ND = Not detected.

jano [8]. C_4–C_6 fatty acids were also detected in the normal samples, although in small amounts. These were not detected by Delmouzos *et al.* [9], undoubtedly owing to the lower sensitivity of the chromatographic techniques that they used.

Another important difference was observed in the concentration of the major headspace components of the brines (Table IV). The absence of ethanol and *n*-propanol and the larger amounts of methanol and acetaldehyde in the zapatera brines S5 and S6 are noteworthy. In turn, the other (weakly) zapatera sample, S4, did not differ significantly from its replicate S3 with respect to the organic acids and volatile components, except perhaps in the higher concentrations of acetaldehyde and *n*-propanol.

Comparison of the profiles of organic acids and volatile components.

In order to find any differences between the normal and the zapatera samples, an examination of the chromatograms of organic acids and headspace components was carried out. In addition, the chromatograms of volatile compounds responsible for flavour (aromagrams) were compared.

Organic acids (HPLC). HPLC, using a cation exchange column (Aminex HPX-87H), has been used previously in a qualitative way to obtain profiles of microbial metabolites [10]. The chromatograms obtained as "fingerprints" for the brines S3 and S5 are shown in Fig. 1. The zapatera samples S5 and S6 show distinct characteristics, mainly because of the different concentrations of organic acids. However, the zapatera brine S4 hardly differed from its replicate in this type of analysis, limiting the application of this method for the detection of zapatera spoilage.

Volatile fatty acids (GC). The profile of volatile fatty acids obtained by GC presented a peculiarity common to the three zapatera samples that differentiated them clearly from the others, namely an intense peak at a retention time of 35 min (Fig. 2). Apparently the concentration of the compound responsible was directly related to the intensity of the typical smell of the spoilage, with an appreciably smaller area of the peak in sample S4 corresponding to a less intense smell. This "key compound" was identified as cyclohexanecarboxylic acid by GC–MS. When added to normal olive brines, taste panelists at our laboratory remarked that this compound imparted a "zapatera-like" odour to the brine. In addition, a large peak (retention time 35 min) was also detected in all the zapatera brines supplied by industry, but not detected (or detected as a small peak) in the normal samples analysed. This peak can also be attributed to cyclohexanecarboxylic acid, although a mass spectrometric analysis to confirm this point was not carried out.

This is the first report of cyclohexanecarboxylic acid as a constituent of zapatera olives. Additional work is necessary to establish both its detection threshold and its limit of detection by the GC method used. However, it is possible to speculate on the

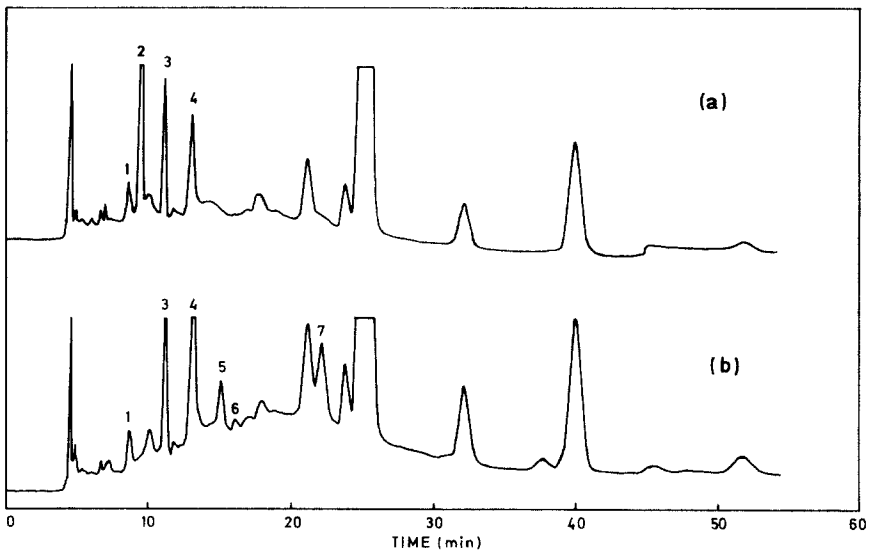

Fig. 1. HPLC of organic acids (Aminex HPX-87H column) from (a) normal brine S3 and (b) zapatera brine S5. Peaks: 1 = succinic acid; 2 = lactic acid; 3 = acetic acid; 4 = propionic acid; 5 = isobutyric acid; 6 = *n*-butyric acid; 7 = *n*-valeric acid.

origin of this compound in zapatera brines. Phenolic compounds from olives appear as constituents in olive brines (0.20–0.35% expressed as caffeic acid) and their concentrations remain almost unchanged during the fermentation process [11]. Anaerobic biodegradation of phenols, using a sewage sludge inoculum, has been reported to produce cyclohexanecarboxylic acid, and several pathways have been proposed to explain this finding [12]. Therefore, it could be that the presence of this acid in zapatera brines is due to biodegradation of phenolic compounds by the anaerobic bacteria involved in this spoilage (*Clostridia* and *Propionibacteria*).

Headspace components (GC). Although the profiles of the volatile components of the headspace (Fig. 3) of the zapatera samples S5 and S6 differed appreciably from the rest, mainly owing to the absence of ethanol and *n*-propanol, the fact that no important differences were observed between S3 and S4 limits the application of the method, as in the HPLC method described above. It is necessary to bear in mind that direct injections of a very dilute vapour sample (headspace gas) produce peaks only for those major components which possess relatively high vapour pressures and are present in sufficient amounts to activate the detector [13]. The minor components may also be extremely important in the flavour of a food, and in some instances more important than the major components owing to their lower detection threshold [14]. Therefore, the use of a dynamic method (purge and trap) may be advisable for the analysis of the headspace components, including the minor ones.

Volatile components (GC). The high proportions of acetic and propionic acids relative to the amounts of other volatile compounds in olive brine interfere with the separation of the latter components, present only in trace amounts. Consequently, volatile acids were eliminated by neutralization prior to extraction. Magnesium oxide was used as a neutralizing agent, following the procedure of Kahn *et al.* [15]. Fig. 4 shows the gas chromatograms of volatile components extracted with diethyl ether from samples S3 and S6. Addition of an internal standard (*n*-dodecane, as used by Lafuente *et al.* [16] in juices) helped to assign the peaks and allowed checking of the good reproducibility of the method for a future determination of the principal components. No significant ($p<0.05$) differences were found between the ratios of the areas A (peak)/A (dodecane) in three consecutive analyses using the same brine.

Comparison of the chromatograms revealed clear, mainly quantitative, differences in the compo-

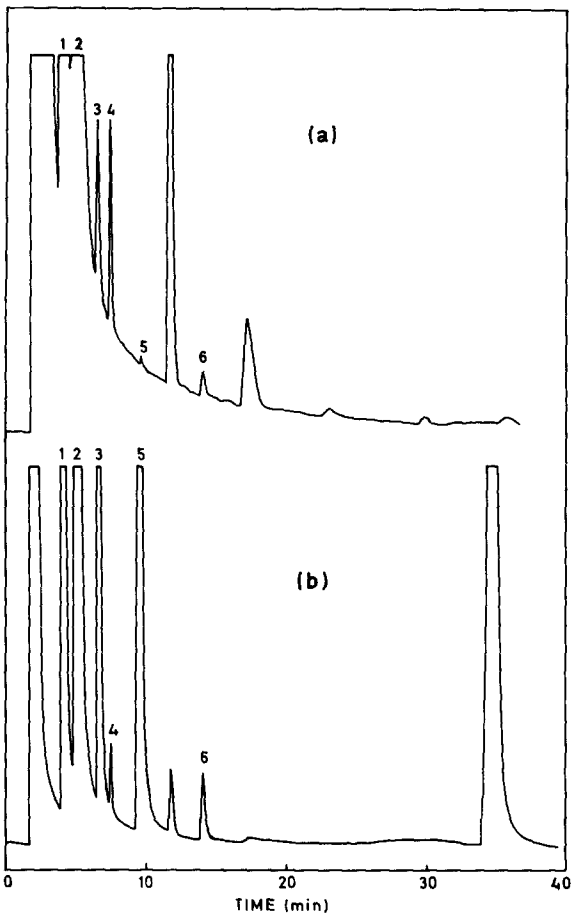

Fig. 2. GC of volatile acids from (a) normal brine S3 and (b) zapatera brine S6. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = *n*-butyric acid; 4 = isovaleric acid; 5 = *n*-valeric acid; 6 = *n*-caproic acid. The last peak in the zapatera sample was identified as cyclohexanecarboxylic acid.

sition of aroma, but at the same time it raised the problem of recognizing, among the many components, that or those specifically associated with the spoilage. To simplify this task, the chromatograms were divided into three well differentiated sections: (A) from the start until 20 min; (B) from 20 until 40 min; (C) from 40 min until the end. The main differences are apparently in section B, and the key substances within it are indicated by numbers (Fig. 4). Comparison of the peak areas of these substances in each of the samples gave the ratios shown in Table V. It can be observed that there are marked differences between the ratios calculated for the zapatera samples (S4, S5 and S6) and those for the unspoiled samples (S2, S3, S7 and S9). The same was seen with brines supplied by different companies (data not shown). Thus, of the zapatera brines analysed, the ratio of components *A* (peak 5)/*A*(peak 2) was never lower than 200, whereas for the normal samples it never exceeded 20. Consequently, this type of study could be of great use for the detection of spoilage, in the same way that it has been applied successfully to the characterization by GC of different varieties of grape [17].

In summary, it is deduced that it is possible to differentiate clearly between as zapatera and a normal olive brine, either from the gas chromatograms of volatile acids or from the gas chromatograms of the volatile components responsible for flavour (aromagrams). Cyclohexanecarboxylic acid, in combination with other volatile acids, seems to be

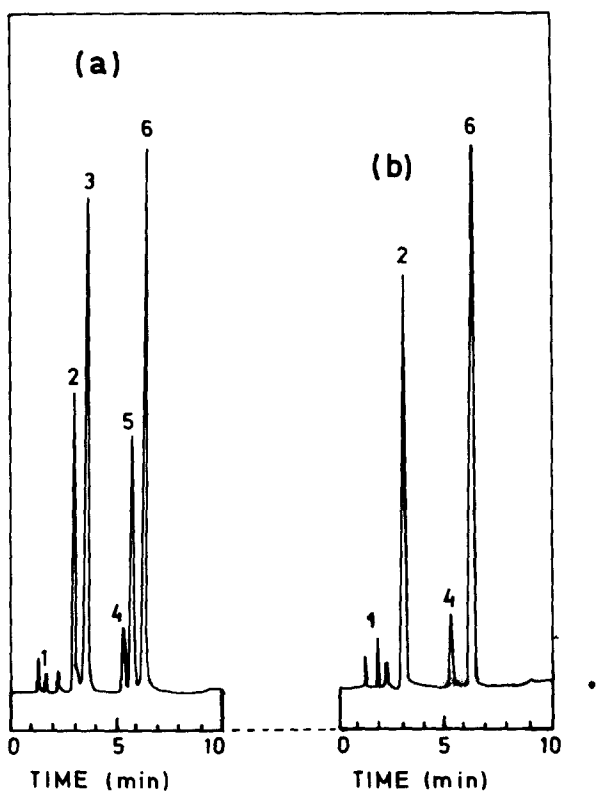

Fig. 3. GC of headspace components from (a) normal brine S3 and (b) zapatera brine S5. Peaks: 1 = acetaldehyde; 2 = methanol; 3 = ethanol; 4 = 2-butanol; 5 = *n*-propanol; 6 = dioxane (internal standard).

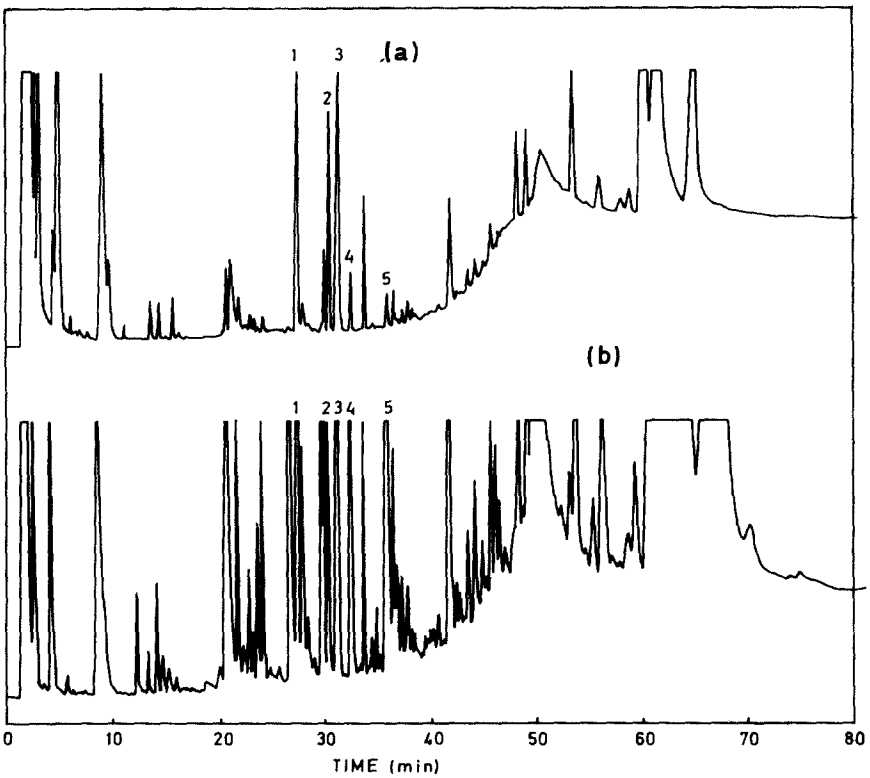

Fig. 4. GC of volatile components extracted with diethyl ether from (a) normal brine S3 and (b) zapatera brine S6. The numbered peaks correspond to the selected key compounds.

responsible for the unpleasant, typical smell of zapatera olives. This suggests that the determination of this compound by GC could be used to check the quality of suspect samples at the point of purchase. This would undoubtedly help to detect possible commercial fraud in which there is an attempt to mask spoilage either by dilution or by addition of aroma-giving substances.

ACKNOWLEDGEMENTS

The authors thank J. L. Rios for the mass spectrometric analysis and interpretations. A. M. is grateful to the Ministerio de Educación y Ciencia of the Spanish Government for a grant (Beca de Perfeccionamiento para Doctores y Tecnólogos). This work is part of the research project ALI88-01151-

TABLE V

RATIOS CALCULATED FROM THE PEAK AREAS OF SELECTED KEY COMPONENTS

Ratio of area × 100.

Ratio of components[a]	Sample						
	S2	S3	S4	S5	S6	S7	S9
5:1	20	10	270	1960	690	4	1
5:2	50	20	480	2320	3780	15	17
5:3	30	10	350	1160	2100	6	7
5:4	180	70	2220	390	790	60	60

[a] Peak numbers shown in Fig. 4.

CO2-01 supported by the Spanish Government through CICYT.

REFERENCES

1 ACEMESA, XXIV Asamblea de Miembros del Instituto de la Grasa y sus Derivados, Seville, May 1990.
2 M. J. Fernández Díez, Biotecnología de la Aceituna de Mesa, CSIC Seville, 1985.
3 R. H. Vaughn, in J. G. Carr, C. V. Cutting and G. C. Whiting (Editors), *Lactic Acid Bacteria in Beverages and Food*, Academic Press, New York, 1975, Ch. V. 3, P. 307.
4 M. J. Eyles and R. F. Adams, *Int. J. Food Microbiol.*, 3 (1986) 321.
5 A. Montaño, A. H. Sánchez and L. Rejano, *J. Chromatogr.* 521 (1990) 153.
6 H. P. Fleming, M. A. Daeschel. R. F. McFeeters and M. D. Pierson, *J. Food Sci.*, 54 (1989) 636.
7 M. L. Vorbeck, L. R. Mattick, F. A. Lee and C. S. Pederson, *J. Food Sci.*, 26 (1961) 569.
8 J. M. R. Borbolla and L. Rejano, *Grasas Aceites*, 32 (1981) 103.
9 J. G. Delmouzos, F. H. Stadtman and R. H. Vaughn, *J. Agric. Food Chem.*, 1 (1953) 333.
10 R. F. Adams, R. L. Jones and P. L. Conway, *J. Chromatogr.*, 336 (1984) 125.
11 A. Vazquez Roncero and M. L. Janer del Valle, *Grasas Aceites*, 29 (1978) 23.
12 A. W. Obayashi and J. M. Gorgan, *Management of Industrial Pollutants by Anaerobic Processes*, Lewis, Chelsea, MI, 1985.
13 W. G. Jennings and M. Filsoof, *J. Agric. Food Chem.*, 25 (1977) 440.
14 D. A. Cronin, in I.D. Morton and A. J. MacLeod (Editors), *Techniques of Analysis of Flavours –Food Flavours, Part A Introduction*, Elsevier, Amsterdam, 1982, Ch. IIa.
15 J. H. Kahn, G. B. Nickol and H. A. Conner, *J. Agric. Food Chem.* 20 (1972) 214.
16 B. Lafuente, J. Benedito, A. Mansanet and M. I. Nadal, *Rev. Agroquim. Tecnol. Aliment.*, 16 (1976) 89.
17 A. Rapp, H. Hastrich, L. Engel and W. Knipser, in G. Charalambous and G. E. Inglett (Editors), *Flavor of Foods and Beverages –Chemistry and Technology*, Academic Press, New York, 1978, Ch. 25, p. 391.