Journal of Chromatography, 521 (1990) 153–157
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 622

Note

Rapid quantitative analysis of headspace components of green olive brine

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(First received April 18th, 1990; revised manuscript received June 14th, 1990)

In order to study the complex biochemical transformations which take place during the controlled fermentation of vegetables, it is important to have analytical methods available that allow the determination of the main compounds involved. The sugars and the majority of organic acids (fructose, glucose, mannitol, sucrose, lactic acid, acetic acid, malic acid) can be satisfactorily determined by high-performance liquid chromatography [1]. Ethanol, another important component, can also be determined by this technique [2] but, owing to its volatility, gas chromatography (GC), and particularly the headspace technique (HSGC), is more appropriate [3]. This paper describes a simple, rapid method for the simultaneous determination of ethanol and other volatile components in brines of fermented vegetables by HSGC. The study was centred on green table olives, which, together with cucumbers, cabbages and peppers, account for the largest volume of vegetables and fruits commercially brined and fermented in the West [4].

EXPERIMENTAL

Reagents

Analytical-reagent grade anhydrous sodium sulphate (Panreac, Montplet and Esteban, Barcelona, Spain) was used as ionic reagent. Standard substances were obtained from Merck (Darmstadt, F.R.G.) and Fluka (Buchs, Switzerland). An aqueous solution containing 5.682 mg/ml of dioxane (Fluka) was used as an internal standard.

Preparation of the sample

Recovery and reproductibility studies were carried out with a fermentation brine from pickled green olives, prepared in our Department using the classical elaboration process [5]. At the time of this study, 6 months after brining, the latter had pH 4.50, free acidity (expressed as lactic acid) 0.60% and sodium chloride content 5.8%.

Brine (1 ml) and internal standard solution (0.2 ml) were added to a 20-ml vial containing 1.0 g of anhydrous sodium sulphate. This ionic reagent was used to in-

0021-9673/90/$03.50

crease the vapour pressure of volatile compounds in the brine. The vial was closed with a rubber stopper and aluminium cap and immediately placed in a thermostated bath at 60°C for 15 min. Next, a 0.1–0.2-ml sample of vapour was removed through the stopper, using a 0.5-ml gas-tight syringe (Hamilton 1750), and returned to the vial. This operation was repeated three more times, and finally the sample was injected into the gas chromatograph. After each injection, and immediately before making the next, the syringe was cleaned by removing the plunger and passing a current of nitrogen through the interior, at the same time warming the exterior with a hand drier.

Chromatographic conditions

A Perkin-Elmer 3920B gas chromatograph equipped with a flame ionization detector was used. A Supelcowax 10 fused-silica capillary column (30 m × 0.53 mm I.D., 1.0-μm film thickness, Supelco 2-5301) was used for analytical separations. The column was programmed from 50°C (held for 4 min) to 120°C at 8°C/min. The injection port was maintained at 150°C and the detector at 200°C. Nitrogen was used as carrier gas at a flow-rate of 9 ml/min. The chromatograms and peak areas were obtained from a Hewlett-Packard Model 3394A recording integrator.

Peak identification

The major peaks on the chromatograms were identified first by comparison of their retention times with those of authentic standards. The assignments were later confirmed using the syringe reaction technique of Hoff and Feit [6].

Quantification

The response factors of acetaldehyde, methanol, ethanol, 2-butanol and *n*-propanol with respect to the internal standard were determined from individual aqueous solutions at the following concentrations: acetaldehyde 0.187, methanol 8.656, ethanol 1.015, 2-butanol 0.329 and *n*-propanol 1.649 mg/ml. The same procedure was used as for the brine samples, placing 1 ml of solution in the 20-ml vial. The numerical values of the individual response factors (*RF*) determined from a minimum of three injections of each standard were 2.157, 0.329, 0.990, 4.038 and 2.238 for acetaldehyde, methanol, ethanol, 2-butanol and *n*-propanol, respectively, where

$$RF = AW'/WA'$$

A and A' are the total peak areas of component and internal standard, respectively, and W and W' are the weights of component (mg) and internal standard (mg) in the vial.

RESULTS AND DISCUSSION

Analysis of the headspace of the fermentation brine of pickled green olives as described gave the chromatogram shown in Fig. 1. The major peaks were identified as acetaldehyde, methanol, ethanol, 2-butanol and *n*-propanol. The presence of ethanol and acetaldehyde in olive brines has already been demonstrated by Fleming *et al.* [7] using HSGC–mass spectrometry. They also identified methyl sulphide and 2-butanol,

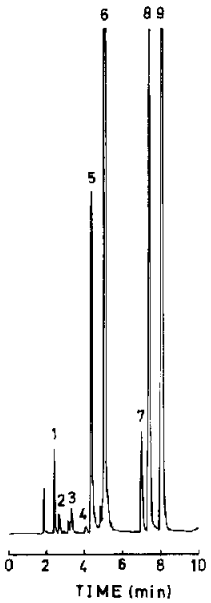

Fig. 1. Headspace gas chromatogram of fermentation brine from pickled green olive. Peaks: 1 = acetaldehyde; 2 = methyl sulphide; 3 = acetone; 4 = ethyl acetate; 5 = methanol; 6 = ethanol; 7 = 2-butanol; 8 = *n*-propanol; 9 = dioxane (internal standard). See text for conditions.

although the latter only in "abnormal" brines. In previous studies, acetone and ethyl acetate were also identified, based on their retention times on three packed columns of different polarity [8]; these compounds were also found by Vlahov *et al.* [9] in brines of pickled green olives and natural black olives.

The wide-bore capillary column used in this work provides sharper and better separated peaks than a packed column and the problem of rapid loss of efficiency with packed columns [8] is eliminated.

The recoveries of different known amounts of compounds added to the fermentation brine of pickled green olives are given in Table I. The graph obtained on plotting the values added against those recovered was linear in all instances with coefficients of determination (R^2) of 0.992, 0.998, 0.999, 0.997 and 0.999 for acetaldehyde, methanol, ethanol, 2-butanol and *n*-propanol, respectively. The precision of the method was measured from eight consecutive analyses of the same brine and the results are given in Table II.

The results were obtained using the individual response factors indicated under *Quantification*. We found that these factors did not show significant statistical differences ($P < 0.05$) with respect to those calculated from a standard mixture of the five components. Consequently, quantification can be effected without any problems from such mixtures, as is usual in multi-component analyses of liquid samples by GC.

We conclude that the method described here permits the simultaneous, reproducible and accurate determination of the main volatile compounds in the headspace (aroma) of a fermentation brine of green olives. The advantages of this method are its

TABLE I

RECOVERY OF KNOWN AMOUNTS OF METHANOL, ETHANOL, *n*-PROPANOL, ACETALDEHYDE AND 2-BUTANOL ADDED TO GREEN OLIVE BRINE

Methanol		Ethanol		*n*-Propanol		Acetaldehyde		2-Butanol	
Added (mg/ml)	Recovery[a] (%)	Added (mg/ml)	Recovery[a] (%)	Added (mg/ml)	Recovery[a] (%)	Added (μg/ml)	Recovery[a] (%)	Added (μg/ml)	Recovery[a] (%)
0.32	113.4	0.34	99.6	0.15	99.9	10.3	103.9	32.9	112.5
0.64	96.4	0.56	95.6	0.29	108.7	18.7	101.0	68.8	104.7
1.08	103.4	1.02	103.9	0.43	113.9	37.8	89.7	99.6	98.4
2.17	104.5	2.02	102.7	0.61	114.5	67.2	101.3	199.2	104.9
	104.4 ± 2.6[b]		100.4 ± 1.4[b]		109.2 ± 2.9[b]		99.0 ± 3.0[b]		105.1 ± 3.1[b]

[a] Each value is the mean of duplicate analyses.
[b] Mean ± standard error.

TABLE II

REPRODUCIBILITY OF THE HSGC METHOD

Compound	Concentration[a] (mg/l)	Relative standard deviation (%)
Acetaldehyde	12.3 ± 1.1	8.9
Methanol	578.8 ± 12.1	2.1
Ethanol	523.1 ± 16.0	3.1
2-Butanol	16.4 ± 0.9	5.5
n-Propanol	154.7 ± 7.5	4.8

[a] Mean ± S.D. of eight determinations using the same brine.

simplicity (sample preparation is minimal) and rapidity (the chromatographic analyses time is less than 10 min). In addition, because the matrix effects are similar, there should be no problem in applying this method to brines of black olives or other fermented vegetable products, such as cucumbers, cabbages and capers.

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