Volatile Composition of Vinegars. Simultaneous Distillation-Extraction and Gas Chromatographic-Mass Spectrometric Analysis

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Volatile composition of different wine vinegars was investigated by GC-MS. Major volatile compounds were analyzed by direct injection of the sample into the gas chromatograph. The minor volatile components were fractionated by using the simultaneous distillation extraction technique (SDE) before their GC analysis. In both cases, previous neutralization of the sample was not required. Different types of vinegars resulting from conventional acetic fermentation processes as well as vinegars obtained by using the solera system (traditional Spanish process of the Jerez de la Frontera region) were analyzed.

INTRODUCTION

In the past years, several studies on the volatile composition of vinegars resulting from the fermentation of different substrates have been made (Suomalainen and Kangasperko, 1963; Kahn et al., 1966, 1972; Aurand et al., 1966; Jones and Greenshields, 1969, 1970; Cabezudo et al., 1978; Troncoso-González and Guzmán-Chozas, 1987).

From an analytical point of view, the aroma of vinegar is a difficult fraction containing many components covering a wide range of volatilities, polarities, and concentrations. Gas chromatographic analysis of the most volatile compounds (bp < 145 °C) can be performed by direct introduction of the sample because of their relatively high concentrations (major volatiles). However, to characterize as many components as possible in the vinegar, it is necessary to concentrate the minor volatiles prior to analysis. The achievement of a representative aroma concentrate demands a careful selection of the isolation and enrichment steps. In this respect, the elimination of the high proportions of acetic acid and water occurring in the vinegars has been considered of special interest since they could be an important source of interferences in the subsequent gas chromatographic analysis.

Several extraction and concentration methods have been suggested, but various problems have been already reported, mainly concerning eventual losses of volatile compounds or chemical changes of some components caused during sample preparation.

Different solvents such as trichlorofluoromethane (freon 11) and dichloromethane have previously been recommended for liquid-liquid extraction and subsequent analysis of food flavors (Hardy, 1969; Rapp et al., 1976; Brander et al., 1980; Fagan et al., 1982). However, some volatile components can be lost in the required concentration step with a decrease in their recoveries.

The simultaneous steam distillation-solvent extraction technique (SDE) (Likens and Nickerson, 1964; Nickerson and Likens, 1966; Godefroot et al., 1981, 1982) has already proved its usefulness for carrying out the adequate enrichment of various types of samples without demanding a subsequent concentration step (Núñez et al., 1984; de Frutos et al., 1988; Núñez and Bemelmans, 1984; Blanch et al., 1991). However, to our knowledge, this procedure has not been applied to date to the analysis of vinegars.

As far as the chromatographic analysis is concerned, the use of micropacked columns (Cramers and Rijks, 1979) could be advantageous for the study of the major volatiles of vinegars since they provide high specific efficiencies and do not demand any special introduction system. Moreover, the high sample capacities exhibited by micropacked columns in comparison to that of capillary columns is of great interest for the analysis of trace compounds (Reglero et al., 1985; Herraiz et al., 1987). The use of a micropacked column loaded with a stationary phase of adequate selectivity for the separation of volatile major compounds resulting from alcoholic and acetic fermentation has been already reported (Cabezudo et al., 1978; Reglero et al., 1986). On the other hand, analysis of the more complex medium-volatility fraction demands the use of a highly efficient capillary column to obtain acceptable resolutions.

The purpose of this work was to attempt to identify volatile components of conventional wine vinegars and sherry wine vinegars by GC-MS, without samples being neutralized prior to the chromatographic analysis. The minimization of the formation of artifacts due to degradation products and the reduction of eventual losses of volatile compounds occurring during the treatment of the sample were also intended. To this aim, the possibility of using the SDE procedure for the isolation and concentration of trace compounds was investigated.

EXPERIMENTAL PROCEDURES

Samples. Samples analyzed in this study can be classified into two main groups: (a) three conventional vinegars, including two wine vinegars obtained in our laboratory and one commercial vinegar; (b) three commercial sherry wine vinegars, obtained by following the so-called "solera system", according to the traditional Spanish process of the Jerez de la Frontera region, which is distinguished by a secondary growth of a yeast film, "flor", and simultaneous fractional blending of samples of different ages. This process is known to result in samples of a distinctive aroma.

Simultaneous Distillation-Extraction (SDE). Sample concentration was carried out by using a Chrompack micro steam distillation-extraction apparatus in the high-density solvent configuration. A 250-mL volume of vinegar is heated by applying an oil bath at 120 °C. At the same time, 1.5 mL of dichloromethane distills by heating with a water bath at 45 °C. Vapors of solvent and sample are condensed by a cold finger refrigerated at -5 °C. The construction of the apparatus is such that vapor- and liquid-phase extractions are achieved and continuous reflux of water and solvent is maintained during the extraction time (1.5 h). A subsequent concentration step was not required. SDE extracts were maintained, for less than 48 h, in the freezer until

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analyzed. In this time, no changes in the sample composition were observed.

Gas Chromatographic–Mass Spectrometric Analysis. Major volatile compounds were analyzed by direct injection of 1 μL of untreated vinegars since acetic acid concentration of the samples does not affect significantly the performance of the chromatographic column. A 0.5-μL volume of 3-pentanol (added with a microsyringe to a 10-mL volume of sample) was used as internal standard. Calibration was performed by injection of three synthetic solutions of the standards in a range of concentrations similar to that expected for real samples. Correlation coefficients were higher than 0.99 in all cases.

A Perkin-Elmer Sigma-3 gas chromatograph equipped with a standard injector (internal diameter of the glass liner, 1 mm) and having the suitable software to integrate peak areas was used. Analytical data were obtained from a 4 m × 0.85 mm i.d. micropacked column coated with a mixture of Carbowax plus bis-(2-ethylhexyl) sebacate (92:8), 4% on desilanized Volaspher A-2, 100–125 μm, which was made from deactivated stainless steel in our laboratory. Hydrogen (80 psig) served as carrier gas. The injector and detector (FID) temperatures were 180 °C, and the column oven temperature was maintained at 60 °C. Because of the simplicity of this fraction and the specific selectivity of the stationary phase, identifications were only accomplished by matching peak retention times with those of pure standards.

Minor volatile compounds were investigated by GC–MS analysis of the SDE extracts. Quantification was carried out through peak areas obtained from total ion chromatograms. In most cases, response factors were calculated by calibration with pure standards. If these standards were not available, response factors of similar compounds were used. Methyl octanoate was used as internal standard by adding a 10-μL volume of a solution (2% in ethanol) to the sample, prior to the extraction process. Because of the relatively low solubility of acetic acid in the solvent used, neutralization of the sample prior to the chromatographic analysis was not required. Finally, a volume of 3 μL was sampled into the chromatograph.

To confirm peak identification, two instruments with different fragmentation properties were used: (a) A Perkin-Elmer 8320 gas chromatograph, provided with a PTV injector, linked to a Perkin-Elmer ITD-50 ion trap detector (EI 70 eV) was used. The chromatograph was equipped with a SGE 50 m × 0.22 mm i.d. fused silica capillary column coated with a 0.25-μm layer of cross-linked BP-21. Helium (30 psig) was used as carrier gas. The separation was carried out by programming the column from 70 °C (5 min isothermal) at 5 °C/min to 180 °C; the temperature was maintained there for 10 min. The injector was held at 30 °C upon injection; subsequently, it was ballistically heated to 300 °C, and this temperature was kept for 5 min. The detector was set at 250 °C.

(b) A Konik KNK-200 gas chromatograph, equipped with a split/splitless injector, coupled to a VG 12-250 quadrupole mass spectrometer (EI 70 eV) was also used. Column and chromatographic conditions were as mentioned above. Injector and detector temperatures were kept at 250 °C, and the split ratio was 1/40.

Compounds were identified by comparison of spectra with those of the NBS (National Bureau of Standards) library. Moreover, most components were identified by matching their spectral data with those of authentic reference compounds analyzed under identical conditions.

RESULTS AND DISCUSSION

Figures 1 and 2 show, respectively, the chromatograms obtained by direct injection of a vinegar and a SDE vinegar extract. It should be stressed that the proposed method allows the analysis of the major volatile compounds, in 35 min (retention time of 3-hydroxybutan-2-one, the last eluted compound), because no additional time is employed in an isolation and concentration step.

Table I lists the compounds identified in the various samples studied, either by direct injection into the gas chromatograph (major volatile compounds) or by GC–MS analysis of the extracts obtained by applying the SDE technique (minor volatile compounds). The different samples belonging to each of the two groups mentioned under Experimental Procedures showed very similar volatile compositions. Because of that, information given in Table I is only headed with "conventional wine vinegar" and "sherry wine vinegar" and no further distinction is made between the samples included in each group.

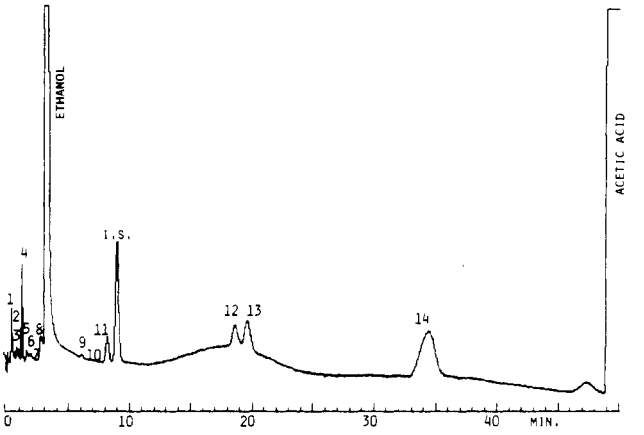

Figure 1. Chromatogram of the major volatile fraction of a sherry wine vinegar obtained by direct injection (see conditions in the text). Peak identification: 1, ethanal; 2, ethyl formate; 3, methyl acetate; 4, ethyl acetate; 5, 1,1-diethoxyethane; 6, ethyl propanoate; 7, propyl acetate; 8, methanol; 9, 1-propanol; 10, isoamyl acetate; 11, 2-methyl-1-propanol; 12, 2-methyl-1-butanol; 13, 3-methyl-1-butanol; 14, 3-hydroxybutan-2-one; I.S., 3-pentanol.

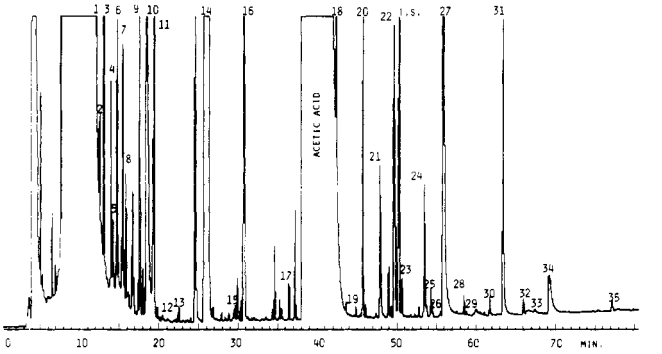

Figure 2. Chromatogram of the SDE extract of the minor volatile fraction of a sherry wine vinegar (see conditions in the text). Peak identification: 1, butanone; 2, methyl *tert*-butyl ether; 3, ethyl 2-methylpropanoate; 4, methyl isopropyl ketone; 5, isobutyl acetate; 6, ethyl butyrate; 7, isopropyl propanoate; 8, butyl acetate; 9, ethyl 3-methylbutanoate; 10, isoamyl acetate; 11, 2-methyl-1-propanol; 12, 2-pentanol; 13, 1-butanol; 14, 2-methyl-1-butanol plus 3-methyl-1-butanol; 15, hexyl acetate; 16, 3-hydroxybutan-2-one; 17, ethyl 2-hydroxypropanoate; 18, furan-2-carboxaldehyde; 19, furyl methyl ketone; 20, benzaldehyde; 21, propanoic acid; 22, 2-methylpropanoic acid; 23, butanoic acid; 24, pentane-2,4-dione; 25, ethyl decanoate; 26, diethyl succinate; 27, 3-methylbutanoic acid; 28, benzyl acetate; 29, pentanoic acid; 30, phenylacetaldehyde; 31, 2-phenethyl acetate; 32, hexanoic acid; 33, benzyl alcohol; 34, phenylethanol; 35, furfuryl alcohol; I.S., methyl octanoate.

The relative abundance of each compound as well as the type of sample in which it was detected is also indicated in Table I. There were identified 61 volatile components, including carbonyl compounds, ethers, ethyl esters, lactones, alcohols, and acids, occurring in concentrations ranging from 0.1 to 1000 mg/L. As expected, high concentrations were found for characteristic compounds resulting from the acetification process such as ethyl acetate and acetic acid. In general, there appear to be few differences in the qualitative composition of wine vinegars and sherry wine vinegars, although several compounds, such as butanone, furyl methyl ketone, pentane-2,4-di-

Table I. Identified Compounds in the Samples Analyzed

compound	ID	conventional wine vinegar[a]	sherry wine vinegar[a]
Carbonyl Compounds:			
ethanal	GC	++++	++++
butanone	MS		+++
methyl isopropyl ketone	MS	++	+++
furan-2-carboxaldehyde	MS, GS	++	+++
furyl methyl ketone	MS		++
benzaldehyde	MS, GC	++	+++
phenylacetaldehyde	MS, GC	+	++
pentane-2,4-dione	MS		+++
3-hydroxybutan-2-one	MS, GC	++++	+++++
Ethers:			
methyl *tert*-butyl ether	MS		++
di-*n*-butyl ether	MS	+	
Acetals:			
1,1-diethoxyethane	GC	+++	+++
Esters:			
ethyl formate	GC	++	++
methyl acetate	GC	+++	+++
ethyl acetate	GC	++++	++++++
ethyl propanoate	GC	+++	+++
propyl acetate	GC	++	++
isobutyl acetate	MS, GC	++	++
methyl 2-methylpropanoate	MS, GC	+	
ethyl 2-methylpropanoate	MS, GC	+	+++
ethyl butyrate	MS, GC	+	+++
isopropyl propanoate	MS, GC		+++
butyl acetate	MS, GC	+	++
ethyl 3-methylbutanoate	MS, GC		+++
isoamyl acetate	MS, GC	++	++++
ethyl hexanoate	MS, GC	+	+
hexyl acetate	MS, GC	+	+
ethyl 2-hydroxypropanoate	MS, GC	++++	++
ethyl octanoate	MS, GC	+	
diethyl succinate	MS, GC	+	+
benzyl acetate	MS	++	++
2-phenethyl acetate	MS, GC	+	+++
ethyl decanoate	MS, GC	+	+
Lactones:			
γ-butyrolactone	MS, GC	++	
Alcohols:			
methanol	GC	++++	++++
ethanol[b]	GC	0.2–0.8	1.0–1.2
2-butanol	MS	+	
1-propanol	MS, GC	+++	+++
2-methyl-1-propanol	MS, GC	+++	++++
2-pentanol	MS		+
1-butanol	MS, GC	+	+
2-methyl-1-butanol	MS, GC	++++	+++
3-methyl-1-butanol	MS, GC	++++	++++
1-pentanol	MS	+	
1-hexanol	MS, GC	+	+
cis-3-hexen-1-ol	MS, GC		+
benzyl alcohol	MS, GC	+	+
phenylethanol	MS, GC	++	++
furfuryl alcohol	MS	+	++
phenol	MS	++	++
3-(methylthio)-1-propanol	MS	++	
Acids:			
acetic acid[c]	MS, GC	10.9–11.7	12.3–12.5
propanoic acid	MS, GC	+++	++++
2-methylpropanoic acid	MS, GC	++++	++++
butanoic acid	MS, GC	++	+++
3-methylbutanoic acid	MS, GC	++++	+++++
pentanoic acid	MS, GC	++	++
hexanoic acid	MS, GC	+++	+++
heptanoic acid	MS, GC	++	
octanoic acid	MS, GC	++	+
decanoic acid	MS, GC	+	+

[a] Concentration: +, <0.1 mg/L; ++, 0.1–1 mg/L; +++, 1–10 mg/L; ++++, 10–100 mg/L; +++++, 100–1000 mg/L; ++++++, >1000 mg/L. [b] % v/v. [c] % w/v.

Table II. Mean Values and Coefficients of Variation of the Normalized Areas Obtained from Five SDE Extracts of a Wine Vinegar

compound	X, mg/L	CV, %
isoamyl acetate	8.55	7.39
isoamyl alcohol	28.63	4.37
ethyl 2-hydroxypropanoate	11.39	7.61
1-hexanol	8.23	3.20
benzaldehyde	7.78	11.69
propanoic acid	13.64	6.40
γ-butyrolactone	3.06	7.36
hexanoic acid	4.83	3.76
phenylethanol	9.00	9.51
octanoic acid	4.84	5.37

one, isopropyl propanoate, and ethyl 3-methylbutanoate among others, were only detected in sherry wine vinegars, whereas 3-(methylthio)-1-propanol and heptanoic acid do not occur in this type of sample.

With regard to those compounds occurring in both conventional wine vinegars and sherry wine vinegars, the highest amounts were generally found in the samples resulting from the solera system. It is worthwhile pointing out that formation of different compounds during the ageing process of sherry wine vinegar could be followed by the proposed method.

Concerning data obtained for ethanal, it should be mentioned that high values for this component have been already reported in aged vinegars (Troncoso-González and Guzmán-Chozas, 1987).

Table II gives the mean values and the coefficients of variation of relative (normalized) peak areas obtained for several compounds representative of the volatile composition of vinegars. Coefficients of variation of less than 12% (calculated from five SDE extracts) were obtained. It must be stressed that peak size on the chromatograms is dependent not only upon the actual concentration of the component represented by the peak but also upon the solubility of each compound in the extracting solvent. Therefore, careful studies about percentage recoveries of the compounds to be analyzed should precede the use of the SDE technique for the quantitative analysis of the volatile composition of vinegars. Formation of artifacts originated during SDE was not suspected since the presence of the identified compounds could be explained by the use of sherry wine or by the acetification and ageing processes. Moreover, the aroma of the SDE extracts was very similar to that of the original sample.

The use of the simultaneous distillation extraction technique seems to be an adequate approach for the comparison of vinegars of different types. Therefore, the proposed method could be of interest for further research concerning the influence of several factors such as the substrate used, the elaboration procedure, and the ageing process in the vinegar obtained.

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