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Determination of esters in dry and sweet white wines by headspace solid-phase microextraction and gas chromatography

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Abstract

Headspace solid-phase microextraction (HS-SPME) was studied for the high-resolution gas chromatographic (HRGC) analysis of esters in wines. Five different SPME fibers were tested and the influence of different factors such as temperature and time of desorption, extraction time, extraction technique, stirring, sample and vial volume, sugar and ethanol content were studied and optimised using model solutions. The proposed HS-SPME–GC method is an appropriate technique for the quantitative analysis of esters in dry and sweet white wines.

Keywords: Wine; Food analysis; Solid-phase microextraction; Headspace analysis; Esters

1. Introduction

Wine quality is heavily influenced by flavour compounds. Up to several hundred compounds from different chemical families in different concentrations account for wine flavour [1]. Such compounds come from grapes, fermentation processes and wine ageing. However, both qualitatively and quantitatively, fermentation compounds are the main group, especially esters, which play an important role in white wine aroma.

Classical analytical methods used for high-resolution gas chromatographic (HRGC) analysis of wine aroma compounds such as liquid–liquid extraction, static and dynamic headspace, molecular distillation, solid-phase extraction, etc., have at least one of the following disadvantages: taking a long time, laboriousness, possibility of contamination or loss, artefact formation, use of environmentally hazardous solvents, difficulties in automation, etc. Solid-phase microextraction (SPME) is a relatively novel technique introduced by Pawliszyn [2], which combines direct extraction and pre-concentration without pre-treatment of samples. It is cheap, fast, easily automated, no organic solvents are used and it avoids contact with samples as it works in headspace when volatile compounds are analysed.

This technique has been used to characterise a wide range of wine aroma compounds, including esters [3–9], or some particular family of compounds such as monoterpenes [10–12], or sulphides and disulphides [13–16], or a particular compound like diacetyl [17], or methyl isothiocyanate [18], etc. Most of these studies used manual SPME, while only a few used automatic SPME.

The aim of this work was to apply the HRGC technique combined with automatic headspace (HS) SPME to develop a method to determine a set of esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate,

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ethyl lactate, 2-phenylethyl acetate, benzyl acetate, hexyl acetate, isobutyl acetate, isoamyl acetate) and to study the use of several fibers for the analysis of samples of white wines.

2. Experimental

2.1. Chemicals and reagents

The following ester compounds were studied (CAS number in brackets): ethyl acetate [141-78-6], ethyl butyrate [105-54-4], ethyl hexanoate [123-66-0], ethyl octanoate [106-32-1], ethyl decanoate [110-38-3], ethyl dodecanoate [106-33-2], ethyl lactate [97-64-3], 2-phenylethyl acetate [103-45-7], benzyl acetate [140-11-4], hexyl acetate [142-92-7], isobutyl acetate [110-19-0] and isoamyl acetate [123-92-2]). 4-Methyl-2-pentanol [108-11-2], 2-octanol [4128-31-8], ethyl heptanoate [106-30-9] and ethyl nonanoate [123-29-5] were used as internal standards (I.S.). These standards, with a purity above 99%, were supplied by Aldrich (Steinheim, Germany, and Milwaukee, WI, USA), Riedel-de Haën (Seelze, Germany) and Fluka (Buchs, Switzerland). Sodium chloride [7647-14-5], anhydrous sodium sulphate [7757-82-6] and heptahydrated magnesium sulphate [10034-99-8] were used to control ionic strength. Absolute ethanol (analytical-reagent grade; Merck, Darmstadt, Germany) [64-17-5] and Milli-Q water (Millipore, Bedford, USA) were used as solvents.

Standard solutions of 230 mg/l–720 g/l of each compound were prepared in ethanol and stored at 5 °C. A global standard solution containing all the analytes in the range 0.8 mg/l–8.3 g/l was prepared by mixing an aliquot of each individual solution and diluting with ethanol. A global internal standards solution with all the I.S. in the range 8.2–206.8 mg/l was prepared in ethanol. A concentrated synthetic wine solution of 11 g/l of L(+)-tartaric acid [87-69-4] (analytical reagent grade; Merck) 24% ethanol and water and sodium hydroxide [1310-73-2] (analytical reagent grade; Panreac) to reach pH 3.2 was prepared. In some cases saccharose [57-50-1] (analytical reagent grade; Panreac) was used to reproduce a standard sweet white wine.

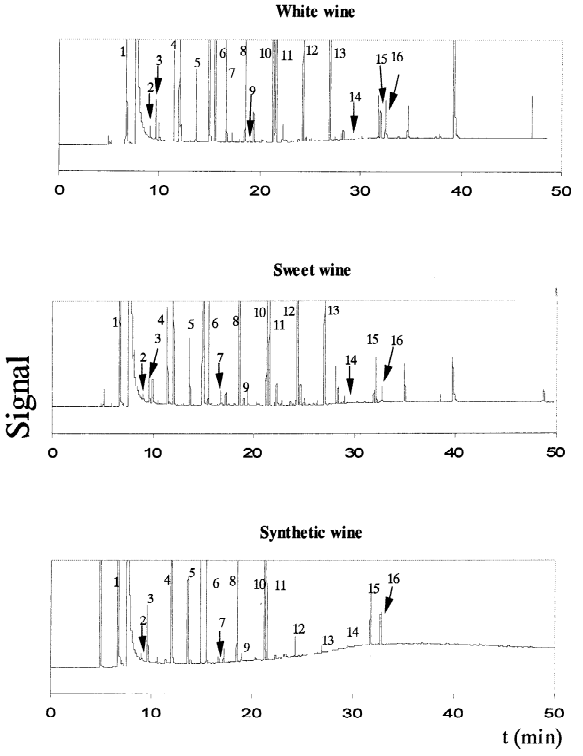

Fig. 1. Chromatograms of a synthetic, sweet and white wines. 1=Ethyl acetate; 2=isobutyl acetate; 3=ethyl butyrate; 4=isoamyl acetate; 5=4-methyl-2-pentanol; 6=ethyl hexanoate; 7=hexyl acetate; 8=ethyl heptanoate; 9=ethyl lactate; 10=2-octanol; 11=ethyl octanoate; 12=ethyl nonanoate; 13=ethyl decanoate; 14=benzyl acetate; 15=2-phenylethyl acetate; 16=ethyl dodecanoate.

2.2. Equipment

Class A volumetric flasks, Gilson pipetmans regularly verified for precision and accuracy, a precision balance (Sartorius BP 210-S), a pH meter (WTW, pH 197-S) and a mechanical shaker (Selecta, Rotabit) were used to prepare solutions.

2.3. SPME fibers

The fibers used (Supelco, Bellefonte, PA, USA) were coated with different stationary phases and various film thicknesses: polydimethylsiloxane 100 μm (PDMS-100), polydimethylsiloxane 7 μm (PDMS-7), polydimethylsiloxane-divinylbenzene 65 μm (PDMS–DVB), polyacrylate 85 μm (PA) and

Carbowax–divinylbenzene 65 μm (CW–DVB). They were conditioned before use by inserting them into the GC injector under the following conditions: PDMS-100, 250 °C for 1 h; PDMS-7, 320 °C for 3 h; PDMS–DVB, 260 °C for 0.5 h; CW–DVB, 250 °C for 0.5 h; and polyacrylate, 300 °C for 2 h.

2.4. Chromatography

The analyses were carried out on a 3400 GC gas chromatograph equipped with an 8200 Standalone autosampler, a 1077 split/splitless injector and a flame ionization detection (FID) system (Varian, Walnut Creek, CA, USA). The injection was made in the splitless mode for 2 min, using a liner of 0.75 mm I.D. which improved the GC resolution. The temperature of the detector was 300 °C and it was fed with 30 ml/min of hydrogen, 300 ml/min of synthetic air and 30 ml/min of nitrogen as make-up gas.

The separations were performed using a CP Wax 57 CB Chrompack capillary column (50 m×0.25 mm I.D., 0.20 μm film thickness) (Varian) with an injector temperature of 250 °C (valid for all the fibers) and an oven temperature programme of 50 °C (15 min), 4 °C/min, 180 °C, 20 °C/min, 220 °C (10 min) The carrier gas was helium with a column-head pressure of 20 p.s.i. (1 p.s.i.=6894.76 Pa).

Fig. 1 shows the chromatograms of a synthetic wine with all the compounds and two real samples of wines where a good separation and resolution among the different peaks can be seen.

Peak identification was accomplished by comparison of the retention times with the standards in the synthetic wine sample.

2.5. Solid-phase microextraction procedure

To prepare solutions for optimisation of the extraction process 4 ml of the concentrated synthetic wine solution and 2.3 g of sodium chloride were added to a 16-ml headspace vial, followed by 200 μl of global standard solution, 100 μl of global internal standard solution and deionized water up to 8 ml total volume (phase ratio: 1). The result was a 5.5-g/l solution in tartaric acid, 12% (v/v), pH 3.2, and the following standard concentrations: ethyl acetate, 207.7 mg/l; ethyl butyrate, 0.52 mg/l; ethyl hexanoate, 1.29 mg/l; ethyl octanoate, 0.31 mg/l; ethyl decanoate, 0.30 mg/l; ethyl dodecanoate, 0.030 mg/l; ethyl lactate, 25.0 mg/l; 2-phenylethyl acetate, 0.24 mg/l; benzyl acetate, 0.02 mg/l; hexyl acetate, 0.10 mg/l; isobutyl acetate, 0.08 mg/l; isoamyl acetate, 2.83 mg/l; 4-methyl-2-pentanol, 2.58 mg/l; 2-octanol, 2.50 mg/l; ethyl heptanoate, 0.53 mg/l; and ethyl nonanoate, 0.10 mg/l. The vial was tightly capped with a PTFE-lined cap and then shaken for 10 min at 200 rpm. The fiber was exposed in the headspace for 40 min with solution shaking and then transferred to the injector to be desorbed (250 °C, 2 min).

All studies were made in triplicate and average values calculated.

3. Results and discussion

3.1. Optimization of desorption conditions

The optimization of thermal desorption has an important influence on precision, sensitivity, retention time and peak shape [19]. We tested the type of injection (split/splitless), desorption time and temperature for each fiber in the injector. The desorption of the analytes was completed using the splitless mode, with 250 °C as injector temperature and 2 min as desorption time, for all the fibers.

3.2. Selection of the fiber

To select the best fiber, the influence of exposure time of each fiber in the headspace was studied. It was observed that PDMS-7 fiber did not extract ethyl lactate or 2-phenylethyl acetate, and CW–DVB fiber did not extract isobutyl acetate or ethyl butyrate. Likewise, the extracted analytes with these two fibers presented lower peak areas than the other fibers. PDMS-100 and PDMS–DVB fibers showed the highest peak areas, but PDMS-100 fiber showed more stable peak areas for all compounds, including internal standards. This fiber was therefore selected for optimization.

In order to optimize the absorption of the PDMS-100 fiber the factors that influence the solution equilibria (extraction time, agitation, sample volume, vial volume, phase ratio, ionic strength (type of salt

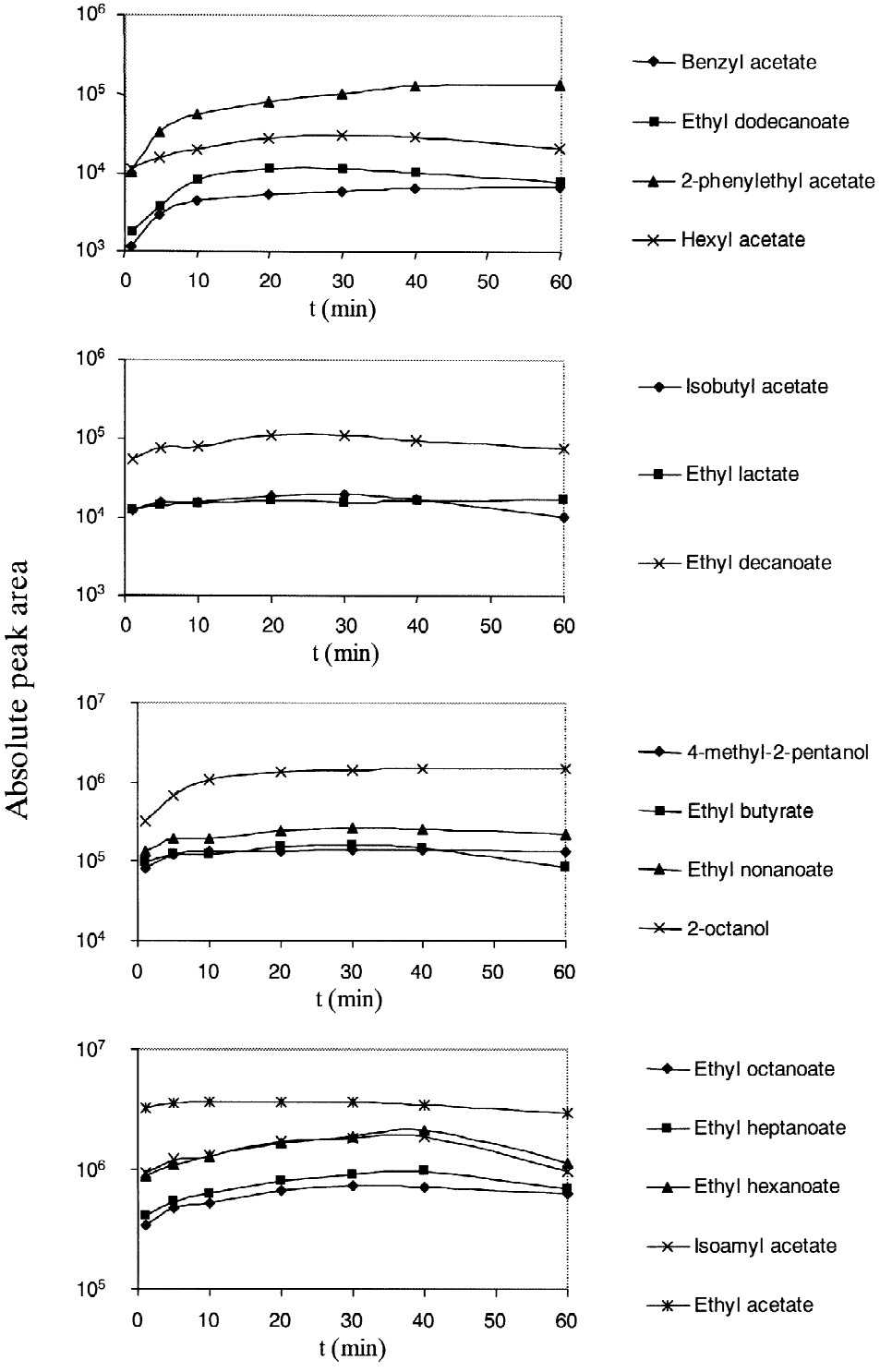

Fig. 2. SPME adsorption time profile determined for the standard mixture of esters and internal standards. Peak areas in a logarithmic scale. For concentrations of the different compounds, see the Experimental section.

and quantity) and matrix effect) were considered. Although the optimisation of temperature is not possible in an 8200 SPME autosampler according to Rocha et al. [8] the absorption of esters showed a statistically significant decrease with the increase in temperature. Thus, working at room temperature, as in our case, will provide better responses than working at high temperature.

3.3. Influence of extraction time

Fig. 2 shows the influence of the extraction time (0–60 min) for every compound including I.S. using the PDMS-100 fiber. The study was performed in 16-ml vials, 12% ethanol, saturated in NaCl, 1:1 phase ratio, with fiber in head space and stirring. As can be seen, after 10 min the increase in peak areas changed very little for most of the compounds, reaching the highest extraction between 20 and 40 min. Absorption of some compounds decreased after 40 min. Subsequent analyses were therefore performed using 40-min exposure time.

3.4. Selection of the extraction technique (headspace vs. direct immersion)

Although headspace has the advantage of avoiding contamination and increasing fiber life time, a comparative study between both the headspace and direct immersion techniques was carried out in order to establish their efficiency. The results obtained for both techniques using the same solution of analytes and 40 min as extraction time are presented in Fig. 3. As can be seen, all extracted compounds showed greater peak areas in the headspace technique with the exception of ethyl lactate and thus the headspace technique was selected for further studies. This behaviour could be explained because there are other compounds in the matrix that compete for fiber-active sites.

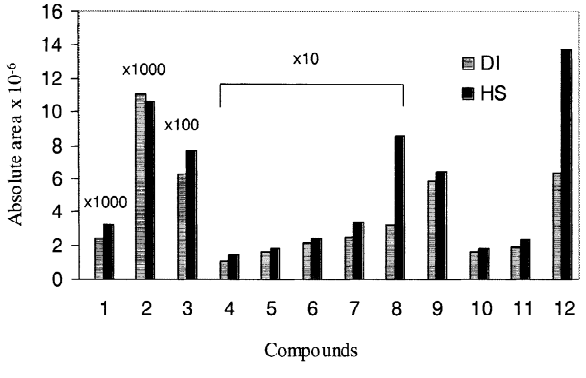

Fig. 3. SPME adsorption peak areas for the different analytes according to the extraction technique. DI, direct immersion; HS, headspace; 1=ethyl dodecanoate; 2=ethyl lactate; 3=ethyl butyrate; 4=2-phenyletyl acetate; 5=isobutyl acetate; 6=hexyl acetate; 7=benzyl acetate; 8=ethyl decanoate; 9=isoamyl acetate; 10=ethyl hexanoate; 11=ethyl acetate; 12=ethyl octanoate.

3.5. Static/stirring

The automatic SPME permits stirring the solution, shaking the vial with the needle of the SPME device, but it does not permit regulation of the stirring speed, so the only possibilities are to shake or not. An extraction study in static and stirring headspace was performed. All compounds presented similar or higher peak areas under stirring. Thus, stirring was subsequently used in all assays.

3.6. Influence of ionic strength (type and concentration of salt)

The effect of various salt concentrations on flavour compounds was described by Yang and Peppard [20]. In this study we tested three types of salts: sodium chloride, sodium sulphate and magnesium sulphate. The results obtained working with saturated solutions of NaCl, Na_2SO_4 and $MgSO_4$ showed that with the exceptions of ethyl acetate and ethyl lactate, for which sodium sulphate was better, sodium chloride was the best salt for the remainder of the compounds to enhance extraction originating the higher peak areas. Magnesium sulphate always produced the minor areas. Thus sodium chloride was chosen to set the ionic strength of the solutions.

Next, a study of the influence of the concentration of sodium chloride in the solution (from 0% to saturation) on the extraction was performed. With the exception of ethyl octanoate, peak areas increased with the amount of salt, attaining maxima when the solution was saturated, thus requiring the addition of 2.3 g of sodium chloride per 16-ml vial.

3.7. Effect of sugar content

Some white wines and especially sweet wines contain significant amounts of sugars and this causes problems when these wines are injected in GC by the direct injection technique, because they can originate caramelization in the injectors and columns causing dirt and damage. The headspace SPME technique avoids contact with samples and thus these problems. To ascertain if sugar can affect the extraction of volatile compounds an extraction study varying the saccharose content (0–200 g/l) in the synthetic wine was performed. The extraction of the compounds is not affected by saccharose content as can be seen in Fig. 4 for six compounds. This is important because the headspace microextraction technique could be applied to sweet wines without interference of the significant amounts of sugar present in these wines.

3.8. Influence of ethanol content

After water, ethanol is the second most important component of wine and like other alcohols it is extracted in the fiber. It effectively competes for fiber active sites, displacing other compounds during the absorption step. Thus it is important to take the ethanol content into account when quantitative analysis is performed. A study of extraction as a function of ethanol content 9–15% (v/v), the range of ethanol for wines, was carried out. Fig. 5 shows that the absolute areas for almost all compounds decreased as ethanol content increased, including the different internal standards. These results are in accordance with those of other authors who also detected the influence of ethanol on extraction [13,21,22].

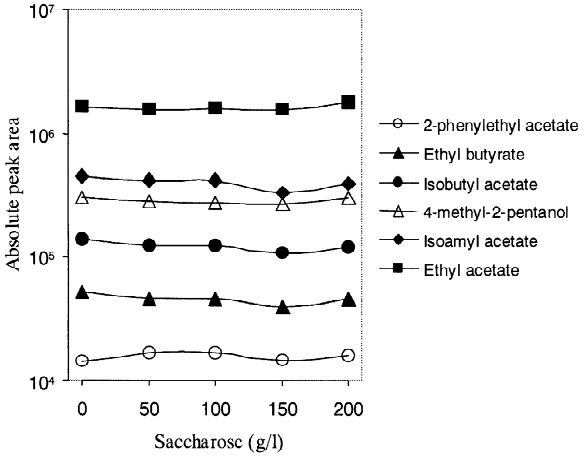

Fig. 4. Peak areas versus concentration of saccharose. Peak areas on a logarithmic scale.

Since peak area of the internal standards also changes with ethanol content, the relative peak areas for every compound with the different internal standards as a function of ethanol content were calculated. According to the results obtained for each analyte the internal standard that gave the lowest change with the percentage of ethanol was chosen. The internal standard selected for each compound is presented in Table 1, and the relative peak areas of each analyte as a function of % ethanol in Fig. 6. In all cases the variation of the relative peak areas were lower than 5% for ±1% of ethanol content.

3.9. Influence of sample volume and vial volume

It is known that sample volume can affect extraction efficiency [19]. Using 16-ml vials we tested 2, 4, 6, 8 and 10 ml of sample volume at the same concentration of analytes, with stirring and 40-min extraction time. The results obtained showed that the peak areas of the different compounds increased from 2 to 4 ml of sample volume and then did not change between 4 and 10 ml (headspace/liquid phase ratio between 1.67 and 0.33) (Fig. 7). This behaviour has been described by Yang and Peppard [23].

Two types of vials for automatic SPME, 2 and 16 ml, are commercially available. To test the influence of the vial size we also tested the 2-ml vial, under the same conditions, but only in the case of phase ratio 1.5 because in the case of the 2-ml vial, to keep the fiber in headspace the volume of sample should be a maximum of 0.8 ml. The obtained results (Fig. 8) showed that a similar or larger amount of all compounds was extracted in the 16-ml vial. Thus, the 16-ml vial was selected for further studies.

3.10. Calibration

The calibrated solutions were prepared in 16-ml vials, 12% ethanol, 1:1 phase rate, saturated in NaCl, with stirring, fiber in headspace and 40-min ex-

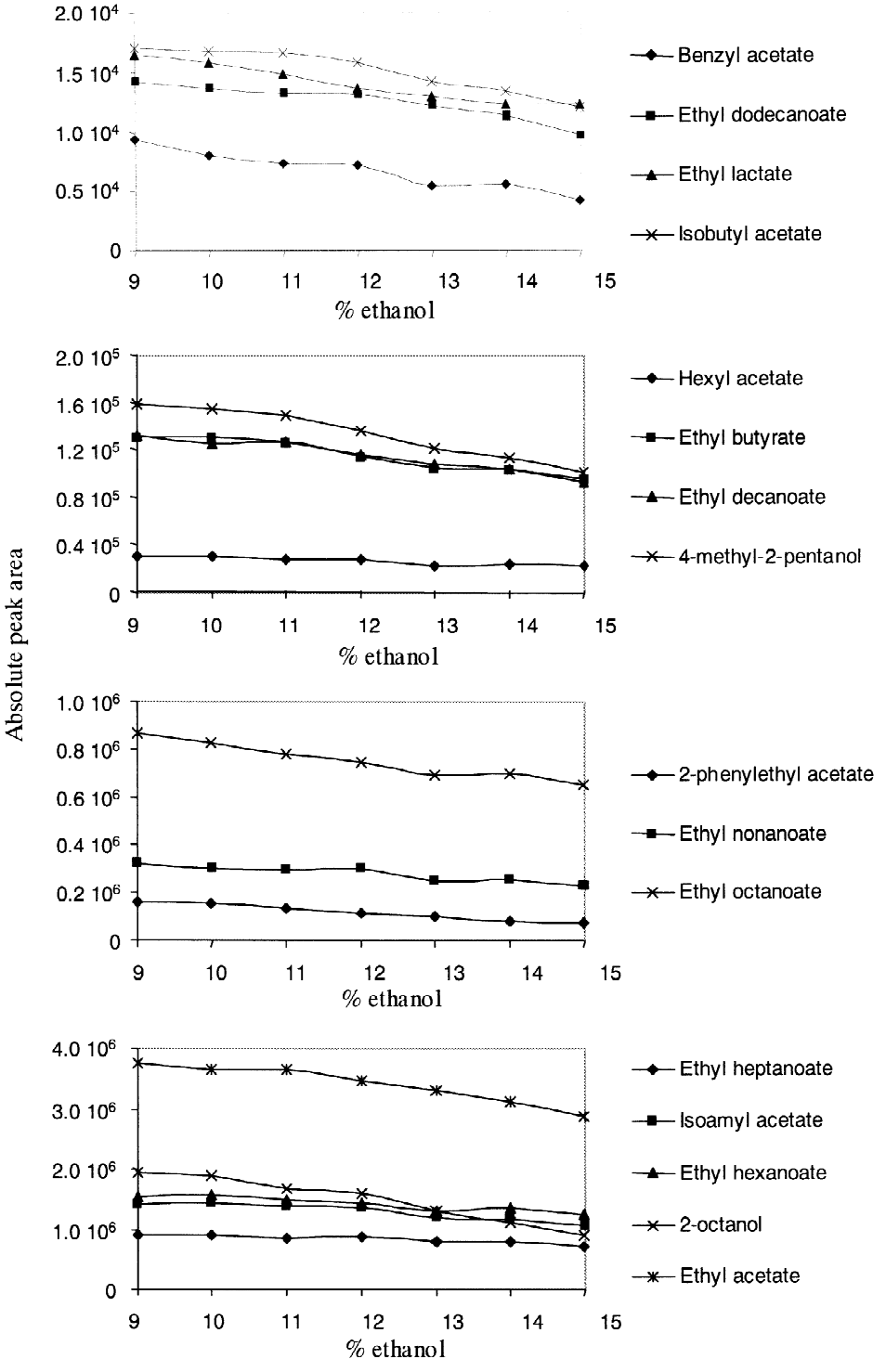

Fig. 5. Absolute peak areas of the different compounds versus ethanol content (%, v/v).

Table 1
Selected internal standard for each analyte

Analyte	Internal standard
Ethyl acetate	4-Methyl-2-pentanol
Isobutyl acetate	Ethyl heptanoate
Ethyl butyrate	Ethyl heptanoate
Isoamyl acetate	Ethyl heptanoate
Ethyl hexanoate	Ethyl heptanoate
Hexyl acetate	Ethyl heptanoate
Ethyl lactate	4-Methyl-2-pentanol
Ethyl octanoate	Ethyl nonanoate
Ethyl decanoate	Ethyl nonanoate
Benzyl acetate	2-Octanol
2-Phenylethyl acetate	2-Octanol
Ethyl dodecanoate	Ethyl nonanoate

traction time. The concentration ranges were selected according to the concentration of these compounds in wines. The range of concentrations studied, limit of detection, intercept, slope, coefficient of regression and repeatability for every compound are presented in Table 2. Limits of detection were determined as three times the noise of five blank injections. The obtained values ranged from 0.001 mg/l for benzyl acetate and ethyl dodecanoate to 2.79 mg/l for ethyl acetate. A linear regression analysis of relative peak areas referred to the respective internal standard versus the analyte concentration was performed. The application of lack of fit test showed that the calculated F-ratio was not significant for all compounds. The values of the correlation coefficients (R^2) were higher than 0.98. The repeatability was estimated by the relative standard deviation (RSD) of the area relative to the selected internal standard for five consecutive solutions. All the values obtained were lower than 10%, ranging from 0.15% for hexyl acetate to 9.0% for isobutyl acetate, with the exception of ethyl dodecanoate (13.3%). These results are similar to or lower than those obtained by other authors [4,7].

3.11. Matrix effect

Considering that in real samples of wine there are compounds other than sugars and ethanol that may interfere in the extraction [3,11,24], a comparative study of slopes was realised between calibration lines in synthetic wines at 12% ethanol and real samples of dry and sweet white wines fortified with several amounts of the analytes (standard additions method). The 95% confidence interval (C.I.) for the slope of the calibration straight lines and the range of slopes for four dry and four sweet white wines are presented in Table 3. As can be seen, the slopes of the regression lines obtained in the standard addition method for the different wines are within the range of the confidence interval of the calibration lines. Thus, it can be concluded that there is no matrix effect.

The developed method was applied to determine the analytes studied in samples of different commercial dry and sweet white wines of the Canary Islands. The results obtained for four dry and four sweet wines are presented in Table 4.

4. Conclusions

A method for the determination of esters in dry and sweet white wines has been optimised using headspace microextraction combined with high-resolution gas chromatography. Five different fibers were tested and the PDMS-100 fiber selected. Different parameters that influence the extraction have been optimised and 40 min extraction time, headspace technique, stirring, saturation in sodium chloride and 16-ml vials were selected. The influence of the alcohol content on the peak areas was resolved by testing different internal standards. Sugar content did not influence the extraction which allows this technique to be applied to sweet wine samples. The developed method has been applied to samples of commercial dry and sweet white wines.

Acknowledgements

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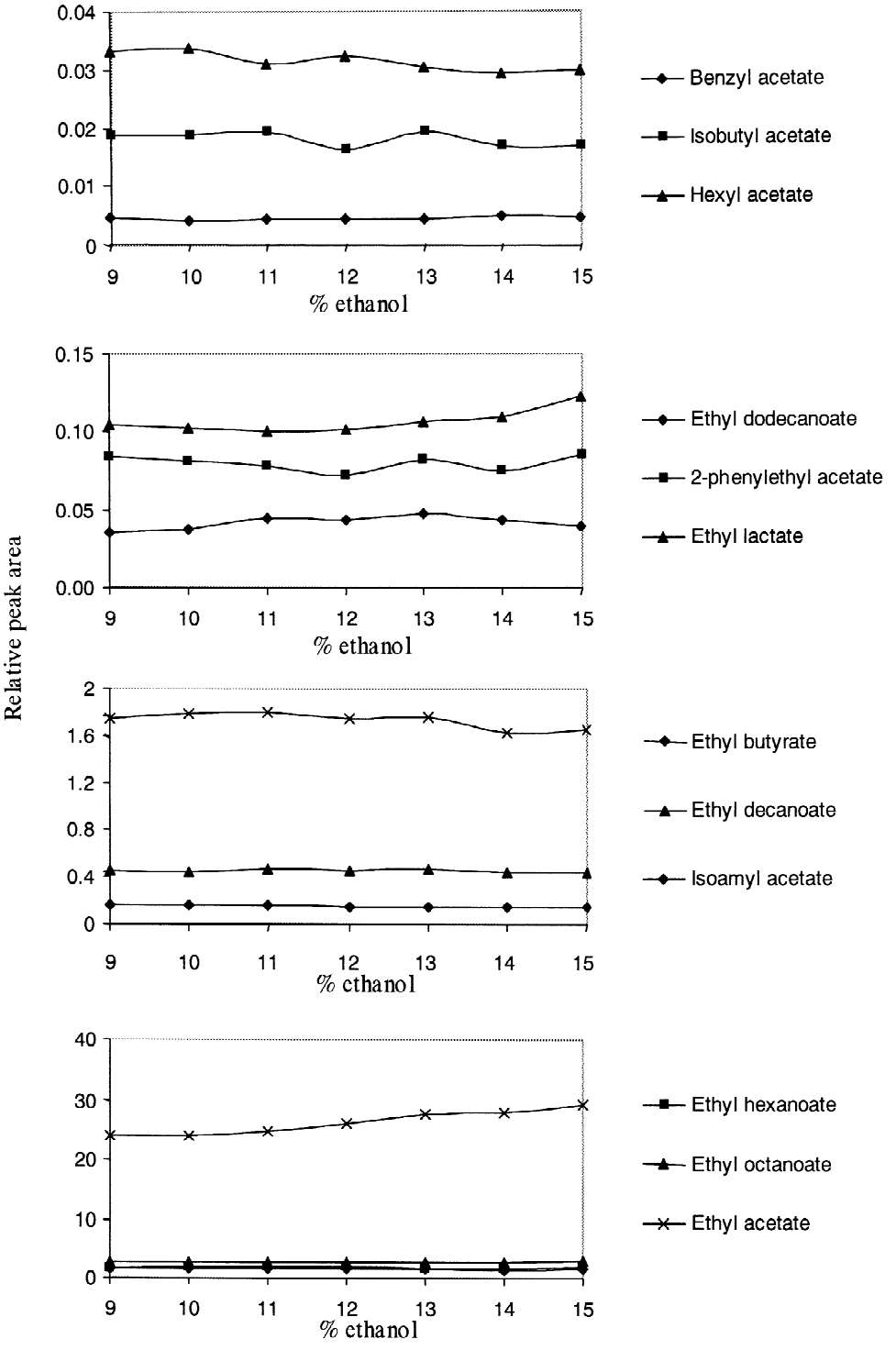

Fig. 6. Relative peak areas of the different compounds versus ethanol content (%, v/v).

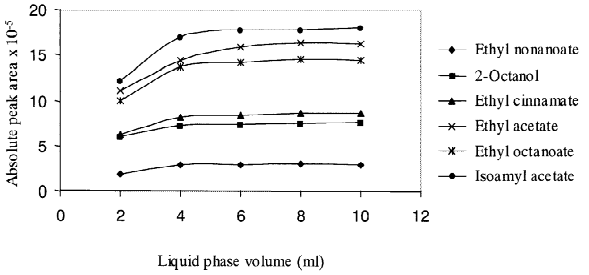

Fig. 7. Absolute peak areas of the analytes versus phase liquid volume.

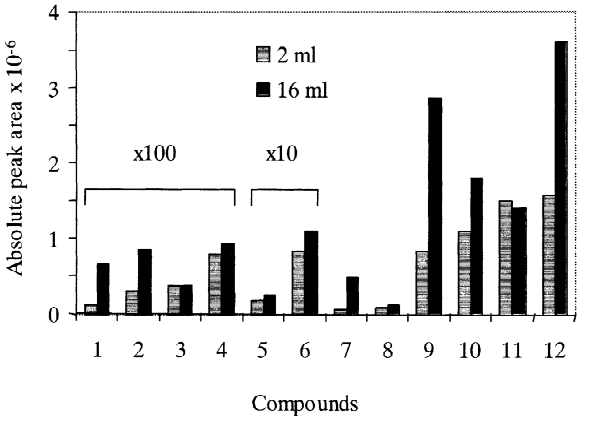

Fig. 8. Absolute peak areas of the solutes versus volume of vial. 1=Ethyl dodecanoate; 2=hexyl acetate; 3=benzyl acetate; 4=isobutyl acetate; 5=ethyl lactate; 6=2-phenyletyl acetate; 7=ethyl decanoate; 8=ethyl butyrate; 9=ethyl octanoate; 10=isoamyl acetate; 11=ethyl acetate; 12=ethyl hexanoate.

Table 3
Comparison of the confidence interval (C.I.) for calibration curves and slopes of standard addition method for real samples of wine. Relative area versus concentration (mg/l)

Analyte	Slope C.I.		Wine slopes	
	Min	Max	Min	Max
Ethyl acetate	0.1259	0.1315	0.1271	0.1307
Isobutyl acetate	0.2363	0.2689	0.2393	0.2551
Ethyl butyrate	0.3015	0.3438	0.3042	0.3348
Isoamyl acetate	0.6050	0.7074	0.6226	0.6699
Ethyl hexanoate	1.4615	1.6849	1.4783	1.5401
Hexyl acetate	1.3637	1.5353	1.3651	1.4680
Ethyl lactate	0.0046	0.0050	0.0046	0.0048
Ethyl octanoate	10.0430	10.9576	10.3525	10.8423
Ethyl decanoate	10.1082	10.8143	10.1978	10.4022
Benzyl acetate	0.2114	0.2339	0.2259	0.2329
2-Phenylethyl acetate	0.3144	0.3507	0.3218	0.3329
Ethyl dodecanoate	6.8952	8.9692	7.0510	7.4026

Table 4
Range of ester contents in commercial white wines

Compound	Mean±SD (mg/l; n=4)	
	Sweet wines	Dry wines
Ethyl acetate	96.56±39.75	85.00±12.54
Isobutyl acetate	0.07±0.02	0.07±0.04
Ethyl butyrate	0.31±0.09	0.41±0.05
Isoamyl acetate	1.81±0.91	2.37±0.62
Ethyl hexanoate	0.87±0.41	1.06±0.19
Hexyl acetate	0.06±0.04	0.14±0.14
Ethyl lactate	13.5±6.6	23.00±18.88
Ethyl octanoate	1.57±0.73	2.11±0.49
Ethyl decanoate	0.65±0.26	0.56±0.06
Benzyl acetate	0.004±0.004	0.003±0.001
2-Phenylethyl acetate	0.23±0.17	0.21±0.05
Ethyl dodecanoate	0.079±0.053	0.021±0.007

Table 2
Range of concentrations, limits of detection (LODs), intercepts (a), slopes (b), regression coefficients and relative standard deviations (RSDs)

	Min (mg/l)	Max (mg/l)	LOD (mg/l)	a	b	R^2	RSDs
Ethyl acetate	18.65	242.5	2.79	−0.3379	0.1287	0.9981	1.14
Isobutyl acetate	0.037	0.984	0.011	−0.0004	0.2526	0.9825	9.03
Ethyl butyrate	0.077	2.006	0.031	−0.0090	0.3227	0.9913	8.56
Isoamyl acetate	0.57	7.49	0.02	−0.0726	0.6562	0.9897	7.83
Ethyl hexanoate	0.16	4.38	0.05	−0.0560	1.5732	0.9934	3.69
Hexyl acetate	0.03	0.79	0.004	−0.0017	1.4495	0.9972	0.15
Ethyl lactate	5.12	66.65	1.68	−0.0058	0.0048	0.9943	4.93
Ethyl octanoate	0.21	2.77	0.07	−0.1414	10.5003	0.9948	1.79
Ethyl decanoate	0.07	2.01	0.02	−0.0082	10.4612	0.9975	2.77
Benzyl acetate	0.003	0.079	0.001	0.0000	0.2227	0.9894	3.61
2-Phenylethyl acetate	0.15	2.00	0.02	−0.0012	0.3325	0.9966	3.33
Ethyl dodecanoate	0.007	0.202	0.001	0.0048	7.9322	0.9842	13.31

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