

Solid-Phase Extraction and Determination of Trace Aroma and Flavour Components in Cider by GC-MS

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Key Words

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Summary

Minor volatile compounds are responsible for the aromas of cider. A simple technique for the analysis of these components is described based on solid-liquid phase extraction followed by quantitation by gas chromatography-mass spectrometry (GC-MS). The method is quantitative for analysis of alcohols, esters, lactones, phenols, and medium and long chain-length fatty acid.

Introduction

Volatile components play an important role in the quality of cider, therefore it is very important to understand the formation of chemical flavours during its making. The analysis of flavour constituents, particularly the lipophilic compounds, requires their removal from the media, their purification and concentration. Different methods have been used in several complex media in order to achieve this, namely: the headspace technique [1], steam distillation and supercritical fluid extraction [2], trapping over porous polymer [3-4], solid-liquid extraction over resins [5-7], purge-extraction techniques [8], simultaneous distillation-extraction [9] and batch and continuous solvent extraction [10].

Although the static headspace technique is a very attractive method in the analysis of food and beverage aromas and volatile pollutants, its primary disadvantage is that of low sensitivity with respect to low volatile components and trace compounds. On the other hand, sensitivity may be increased by purge and trap techniques. Simultaneous distillation-extraction is not time-consuming, but artefact formation due to thermally induced changes is possible. Likewise, distillation and liquid-li-

quid extraction are well fitted for monitoring the components responsible for aroma, but are time-consuming and also, the stability of the emulsion produced is quite significant, making separation of the phases difficult. The absorption of analytes onto hydrophobic resins, such as, XAD-2 and C₁₈, has been widely employed [11] because of their power to extract organic components from aqueous media, but a high level of interference by ethanol (the major component in alcoholic beverages) occurs. An alternative system is to employ hydrophilic resins to retain water and high polarity compounds and to then elute the low polarity organic components with appropriate solvents.

The aim of this work was to evaluate a solid-phase extraction procedure in order to develop a quantitative method for monitoring flavour components of cider.

Experimental

Analytical-grade samples of different standards and solvents were from Fluka-Chemika (Buchs, Switzerland), Aldrich-Chemie (Steinheim, Germany), Sigma (St. Louis, USA), and Lab-Scan (Barcelona, Spain) and were used without further purification.

Raw Material

Two typical ciders, supplied by traditional makers from the Region of Asturias, with different sensory profiles (fruity and sharp aromas) were employed in this study. The cider was prepared from the juice of a mixture of cider apples with different sensory properties endowing the resulting juice with an overall acidic nature. The processing of this mixture included the following steps: milling with a hammer mill, slow pressing and fermentation by wild yeast.

Extraction

Extrelut-20 columns (Merck, Darmstadt, Germany) filled with large-pore kieselguhr of granular structure supported by a frit glass for the extraction of lipophilic compounds of cider, were employed. The sample (20 mL) and the internal standard (16.69 mg L⁻¹ 2-

ethyl-1-hexanol), adjusted to pH 3.00 with hydrochloric acid, were degassed using an ultrasonic bath for 3 min and percolated through the kieselguhr and allowed to soak for 15 min until totally absorbed. Immediately after, 80 mL dichloromethane were eluted through the column by manifold vacuum for 30 min. Anhydrous sodium sulphate was used to dehydrate the organic phase. Next, 50 mL organic extract were collected and then concentrated. A helical gas flow (nitrogen) by vortexing action (Turbo Vap™ Evaporator-Zymark) was employed for solvent removal to a final volume of 0.5 mL; which was monitored using microswitch and optical sensing. The concentration temperature was kept at 30 °C by a water bath.

GC-MS

GC-MS was on a Hewlett-Packard 5970A mass selective detector (MSD) interfaced with a Hewlett-Packard 5790 gas chromatograph (Palo Alto, CA) fitted with a column (FFAP, 50 m × 0.22 mm i.d.; phase thickness, 0.33 μm) inserted into a split/splitless injector (splitless time: 1 min) and terminated at the MSD. Chromatographic conditions were as follows: initial temperature, 40 °C; program rate, 3 °C min⁻¹; final temperature, 220 °C; injector and detector temperature, 250 °C; MS interface temperature, 160 °C; carrier gas, He at 1 mL min⁻¹. Injection (1 μL) was by means of an autosampler. Analyses were performed in the electron impact (EI) mode and ionisation voltage was 70 eV. Both scan and ion-monitoring modes for detection were chosen.

Results and Discussion

The GC-MS method used enabled us to identify 36 cider aroma components in Asturian ciders with a confidence level of more than 72 %: including twelve alcohols (1-propanol, isobutanol, 2-propenol, 1-butanol, amyl alcohols, 3-ethoxy-1-propanol, 1-hexanol, 2,3-butanediol, 2-(methylthio)-1-propanol, benzyl alcohol, 2-phenylethanol, tyrosol); eight esters (ethyl lactate, diethyl succinate, ethyl caproate, ethyl caprylate, ethyl caprate, ethyl laurate, ethyl myristate, ethyl palmitate); two ketones (acetoin, γ-butyrolactone); two phenols (4-ethylguaiaicol, 4-ethylphenol) and twelve organic acids (acetic, propanoic, isobutyric, butanoic, caproic, caprylic, capric, benzoic, lauric, phenylacetic, myristic, palmitic).

Quantitation of the aroma compounds was by using the internal standard method. 2-Ethyl-1-hexanol was chosen as the standard for all volatile compounds, and its concentration in the initial solutions was 16.69 mg L⁻¹. The use of undecanoic acid as standard for volatile compounds eluted in the last part of the chromatogram did not improve the results obtained. The relative retention time, the response (peak area) ratio, and relative response factor of each volatile against 2-ethyl-1-hexanol were determined at concentrations of each component ranging from the quantitation limits to

threetimes the amount present in cider. The relative response factor was obtained by dividing the peak area of each volatile by the peak area of the internal standard. The relative response factor divided by the concentration ratio (volatile compound:2-ethyl-1-hexanol) yielded the relative response factors for each compound. These response factors were around 1.4–1.5 for ethyl esters of long-chain fatty acids and diethylsuccinate, about 1.1 for acetate esters and aromatic alcohols, close to 1 for ethyl esters of the middle aroma fraction and long-chain monocarboxylic acids, close to 0.8 for higher alcohols and near 0.5 for γ-butyrolactone, 2-3 butanediol and ethyl lactate.

Recovery studies were carried out to determine the accuracy of the method. Known amounts (between 50 % and 150 % of the amounts found in cider) of each volatile belonging to the different chemical types above, were added to a variety of samples and the resulting spiked samples were subjected to the entire analytical sequence. All analyses were carried out in triplicate at three concentration levels. For the recovery studies, the mass spectrometer was operated in the selected-ion monitoring mode. The characteristic ions, namely: $m/z = 60(\text{C}_2\text{H}_4\text{O}_2^+)$ and $73(\text{C}_3\text{H}_5\text{O}_2^+)$ for aliphatic organic acids, $m/z = 88(\text{C}_4\text{H}_8\text{O}_2^+)$ for fatty acid ethyl esters, $m/z = 43(\text{C}_2\text{H}_3\text{O}^+)$ for acetates, $[\text{M}-15]^+$ for 4-ethyl phenol derivatives, $[\text{M}-31]^+$ for β-hydroxyethyl phenol derivatives and $[\text{M}-44]^+$ for lactones, were employed in quantitative studies. The alcohols and ketones were quantified by means of appropriate ions in order to minimise interference of other analytes.

The average recoveries (Table I) for alcohols, hydroxyacid esters, lactones, phenols and fatty acids are acceptable with a relative standard deviation of less than

Table I. Recovery of some volatile compounds added to various ciders.

Compound	% Recovery ± RSD
1-Propanol	104.83 ± 2.95
Isobutanol	78.73 ± 2.45
Amyl alcohols	100.03 ± 1.40
1-Butanol	102.01 ± 5.30
2,3-(R,R-S,S)-Butanediol	12.30 ± 4.73
2,3-Butanediol(meso form)	5.07 ± 3.26
2-Phenylethanol	105.33 ± 5.39
Acetoin	84.2 ± 6.20
Ethyl lactate	88.31 ± 0.98
Diethyl succinate	90.32 ± 4.20
Benzyl alcohol	92.10 ± 2.50
γ-Butyrolactone	103.00 ± 3.96
3-(Methylthio)-1-propanol	77.50 ± 6.53
Ethyl caproate	65.70 ± 4.80
Ethyl caprylate	69.23 ± 4.40
Ethyl caprate	68.53 ± 4.56
Ethyl laurate	67.54 ± 7.58
Propanoic acid	0
2-Phenylethyl acetate	92.00 ± 3.15
Caprylic acid	94.82 ± 6.01
4-Ethylphenol	87.51 ± 3.42
Isoamyl acetate	83.00 ± 5.00
Capric acid	94.37 ± 4.06
Benzoic acid	110.41 ± 4.19

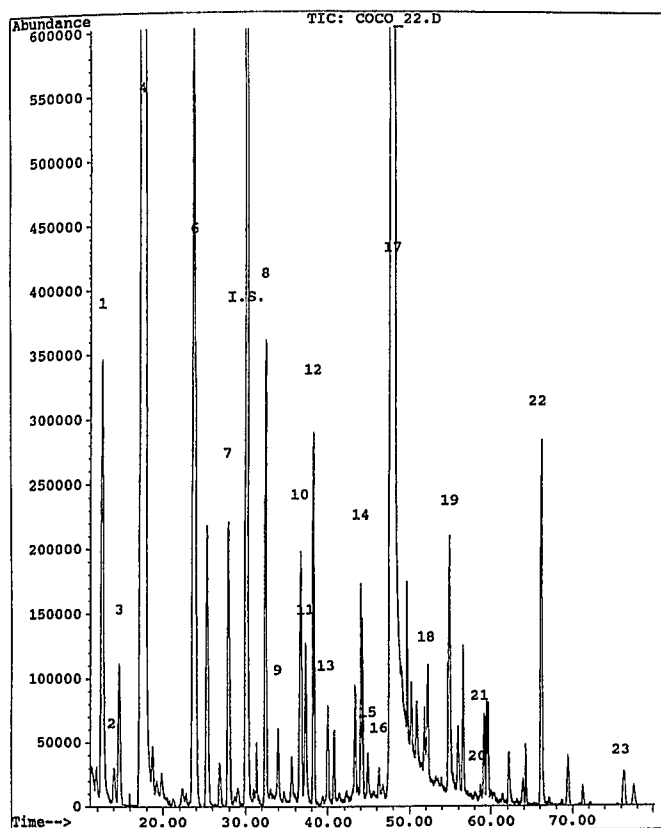


Figure 1
Total ion chromatogram of volatile compounds in fruity cider; **1** = isobutanol; **2** = isoamyl acetate; **3** = butanol; **4** = amyl alcohols; **6** = ethyl lactate; **7** = ethyl caprylate; **8** = 2,3 (R,R and/or S,S)-butanediol; **9** = 2,3-butanediol (meso form); **10** = ethyl caprate; **11** = dihydro-2(3H)-furanone; **12** = diethyl succinate; **13** = 3-(methylthio)-1-propanol; **14** = 2-phenylethyl acetate; **15** = ethyl laurate; **16** = benzyl alcohol; **17** = 2-phenylethanol; **18** = caprylic acid; **19** = 4-ethylphenol; **20** = ethyl palmitate; **21** = capric acid; **22** = lauric acid; **23** = myristic acid.

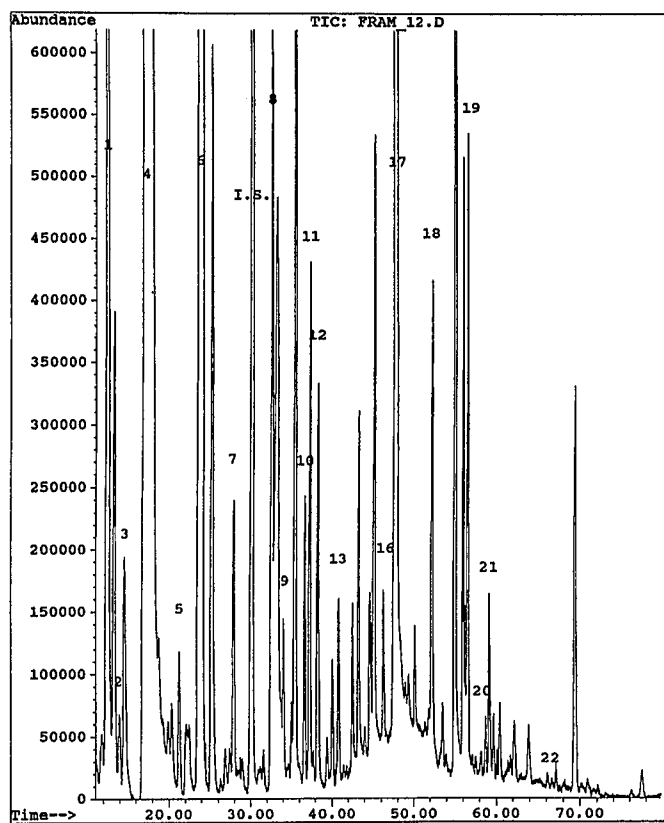


Figure 2
Total ion chromatogram of volatile compounds in sharp cider; **1** = isobutanol; **2** = isoamyl acetate; **3** = 1-butanol; **4** = amyl alcohols; **5** = acetoin; **6** = ethyl lactate; **7** = ethyl caprylate; **8** = 2,3 (R,R and/or S,S)-butanediol; **9** = 2,3-butanediol (meso form); **10** = ethyl caprate; **11** = dihydro-2(3H)-furanone; **12** = diethyl succinate; **13** = 3-(methylthio)-1-propanol; **16** = benzyl alcohol; **17** = 2-phenylethanol; **18** = caprylic acid; **19** = 4-ethylphenol; **20** = ethyl palmitate; **21** = capric acid; **22** = lauric acid.

6 %, indicating that the proposed method is suitable for their analysis. However, recoveries for fatty acid ethyl esters and butanediol are lower, and organic acids of low molecular mass, for example, propanoic acid, were not extracted. The short-chain esters were not detected under our experimental conditions because they are associated with the solvent front.

This chromatographic method was used for studying the flavour of two ciders with different sensory profiles: fruity and sharp aromas (Figures 1 and 2). As regards to higher alcohol levels, more amyl alcohols (typical nose-catching aromas) [12], 1-butanol, and isobutanol were detected in the cider with a sharp aroma (Table II); however, the concentration of 2-phenylethanol, an alcohol associated with a scented aroma according to Williams' criteria [12], was higher in the fruity cider. On the other hand, although benzyl alcohol or phenylmethanol is not a common by-product in fermentation by yeast, it was found in both ciders monitored. The concentration of this alcohol was higher in the cider with sharp aromas. Isoamyl acetate, long associated

with banana odour by chemists [13], is present in the ciders tested, but 2-phenylethyl acetate (an analyte associated with scented aromas) was only noticed in the fruity cider (Table II), which is due to a higher level of phenethyl alcohol. In relation to the ethyl esters of hydroxy and dicarboxylic acids, more ethyl lactate was detected in the sharp aroma cider, which may be due to esterification by lactic acid bacteria strains; the same diethyl succinate level was detected in both ciders. On the other hand, it has been pointed out that the contribution of ethyl lactate in alcoholic beverage aromas may be low because of their odour threshold values of 250 ppm [14].

Several esters of long-chain fatty acids (ethyl esters of caprylic, capric, lauric and palmitic acids) were detected in the ciders; these have long been associated with fruity (the first members of this group), soap-scented (intermediate members of this group) and stearine (C₁₆ and C₁₈ ester acids) aromas [15]; we found a higher level of ethyl esters of caprylic, capric, and lauric acids in the fruity cider than in the cider with sharp aroma (Table

Table II. Content (mg L⁻¹ ± RSD) of volatile components of two ciders determined by solvent extraction and Extrelut resin.

Component	R _t (min)	Cider	
		Fruity	Sharp
1-Butanol (3)	14.65	2.24 ± 4.09	3.88 ± 4.60
Amyl alcohols (4)	17.43	134.29 ± 3.46	170.85 ± 5.50
Isobutanol (1)	12.59	7.73 ± 3.37	21.92 ± 7.55
2-Phenylethanol (17)	47.95	185.24 ± 4.24	57.30 ± 5.90
Benzyl alcohol (16)	46.25	0.53 ± 5.60	1.54 ± 8.83
Ethyl lactate (6)	23.68	11.86 ± 4.80	100.27 ± 3.87
Diethyl succinate(12)	38.42	2.27 ± 4.80	2.32 ± 3.28
Ethyl caprylate (7)	27.88	2.22 ± 3.50	1.92 ± 6.43
Ethyl caprate (10)	36.73	3.39 ± 7.80	2.15 ± 5.89
Ethyl laurate (15)	44.92	0.68 ± 7.00	N.D.
Ethyl palmitate(20)	58.74	0.38 ± 3.66	0.66 ± 4.14
i-Amyl acetate (2)	13.98	0.28 ± 6.30	0.58 ± 9.50
2-Phenylethyl acetate (14)	44.01	1.21 ± 5.15	N.D.
Dihydro-2(3H)-furanone (11)	37.21	3.50 ± 5.45	7.90 ± 2.94
Acetoin (5)	21.20	N.D.	1.92 ± 4.88
2,3(R,R-S,S)-Butanediol (8)	32.44	37.63 ± 6.14	90.89 ± 4.84
2,3-Butanediol(meso form)(9)	33.98	22.10 ± 6.30	75.34 ± 7.92
3-(Methylthio)-1-propanol(13)	40.00	1.41 ± 8.88	1.80 ± 6.17
4-Ethylphenol (19)	56.49	1.37 ± 8.92	3.33 ± 5.56
Caprylic acid (18)	52.23	1.22 ± 8.03	3.51 ± 4.39
Capric acid (21)	59.13	1.25 ± 8.10	2.20 ± 2.42
Lauric acid (22)	66.16	4.88 ± 5.00	0.77 ± 8.18
Myristic acid (23)	76.40	1.91 ± 4.80	N.D.

N.D.= not detected

II). However, a lower ethyl palmitate level was detected in the cider with a fruity aroma.

Certain typical compounds produced in the spoilage of fermented products were also monitored, namely acetoin, and 2,3 (S,S) and/or 2,3 (R,R) butanediol and butanediol, meso form. As seen in Table II, acetoin was only detected in the sharp aroma cider as well as a higher level of butanediol, which indicates a higher level of spoilage.

Acetaldehyde, long associated with sharp flavours [12], was quantified by the GC method optimised by the authors [16]; the above GC-MS technique could not be employed in acetaldehyde analysis because the solvent front did not allow accurate quantitation of this analyte. Between 20 and 45 ppm acetaldehyde were detected in both ciders. The level of this component cannot, therefore, explain sensorial differences determined, since its sensory threshold is rather high, and hence small changes in acetaldehyde level can hardly exert an influence on the odour.

The concentration of γ -butyrolactone(dihydro-2(3H)-furanone) was higher (2.25 times) in the sharp cider than in the fruity one. On the other hand, a higher concentration of 3-(methylthio)-1-propanol(methionol) was detected in the sharp cider. Methionol may be considered quantitatively as an important compound in alcoholic beverages; it has a typically sulphur flavour (cabbage water aroma) [17].

The 4-ethylphenol (odour threshold value in water at 15 % ethanol, 1000 ppb) appeared to a high degree in the sharp cider; this compound is associated with spicy, phenolic and bittersweet odours [12].

In relation to the medium-chain fatty acid levels (caprylic and capric), which have a key role in causing stuck fermentations, this was more important in the sharp cider than in the fruity one. However, a greater concentration of long-chain fatty acids (lauric and myristic) was detected in the fruity cider.

Conclusions

Using the GC-MS method proposed, good recoveries with adequate accuracy for alcohols, esters, lactones, phenols and fatty acids are obtained. However, the short-chain fatty acids are not extracted. The analytical technique outlined is simple, is not time-consuming and the analysis of minor volatile compounds in cider by the proposed method, enables the sensorial profile to be explained.

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