Talanta 84 (2011) 924–930

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Gas chromatographic determination of 29 organic acids in foodstuffs after continuous solid-phase extraction

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ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form 15 February 2011
Accepted 20 February 2011
Available online 24 February 2011

Keywords:
Organic acids
Foodstuffs
Continuous solid-phase extraction
Gas chromatography–mass spectrometry

ABSTRACT

A simple and expeditious method based on continuous solid-phase extraction and gas chromatography–mass spectrometry (GC–MS) was reported for the direct determination of 29 organic acids in food and beverages. A sorbent column packed with 80 mg of LiChrolut EN–Supelclean ENVI-18 (1:1) was employed for extraction and clean-up purposes. After elution with 200 μL of methanol, the methanolic extract was directly injected into the GC–MS without prior derivatization. The method provided good linearity (0.5–1000 $\mu g\,kg^{-1}$) and fairly good precision for all compounds (RSD lower than 6.2%). The recoveries of the organic acids from diluted samples that were spiked at three different concentrations (10, 40 and 100 $\mu g\,kg^{-1}$) ranged from 93 to 98%. The applicability of the method was demonstrated by analyzing the target compounds in a wide variety of foodstuffs including beer, wine, fruit juice, soy sauce, soya milk and honey samples.

1. Introduction

Although organic acids have been used to counteract pathogens in food for many years, there is a glaring need to assess and improve their continued effectiveness and sustainability. There is also a growing demand for food samples to be produced using milder treatments (e.g. less heat, salt, sugar, and chemicals) and for newer technologies to prevent the growth of dangerous bacteria [1]. Organic acids play a pivotal role in maintaining the quality and nutritional value of food [2]. These compounds can be added as acidulants or stabilizers (e.g., citric, sorbic, benzoic, fumaric and malic acids). In cases of inadequate sterilization and/or microbial contamination during storage, sugar fermentation results in the formation of volatile acids (C_2–C_{12}) and impairs the quality of some products [3,4]. Moreover, as components of food, organic acids contribute to the organoleptic properties (flavor, color and aroma) of foodstuffs [5–7]. Thus, the quantitative determination of organic acids in these types of samples is of interest in many industrial and research fields because it can be used in the quality control of wine as an indicator of deterioration due to storage or aging (because the classes and content of organic acids give a characteristic taste to wine) or even to determine authenticity and to ensure that food is safe for consumption.

In general, the great complexity of food samples demands an appropriate sample preparation technique before analysis. As a rule, beverages usually entail a simple pretreatment such as dilution and/or filtration [7,8], but for other food the potential interference of matrix compounds (e.g. fats, vitamins, proteins, polysaccharides) require the employment of more complex pretreatment and clean-up procedures. Traditional methods such as stream distillation and liquid–liquid extraction [2] are time consuming and environmentally unfriendly. The above-mentioned problems could be successfully resolved by solid-phase extraction (SPE) [6,9–12], which can be implemented via flow systems, resulting in a dramatically increased throughput and reduced analytical cost through decreased reagent consumption. Other alternatives such as single-drop microextraction [13], solid-phase microextraction [14] and stir-bar sorptive extraction [15,16] have also been successfully applied to the analysis of short and medium-chain fatty acids and preservatives in vinegar, beverages and dairy products.

Although the applicability of ion exchange chromatography [10], liquid chromatography [7,11,14,17–19] and capillary electrophoresis [8,20,21] for the determination of organic acids has been clearly demonstrated, gas chromatography (GC) coupled with flame ionization [3,4,6,16,22] or mass spectrometric (MS) detectors [13,15] is an attractive alternative due to its simplicity, separation efficiency and excellent sensitivity and selectivity. Many short-chain organic acids are thermostable and sufficiently volatile, thus fulfilling key requirements for GC measurement [2]. However, other acids should be derivatized to convert these compounds into less polar and stable derivates suitable for their GC determina-

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doi:10.1016/j.talanta.2011.02.031

tion [6,13,22]. To avoid the derivatization process of organic acids, some authors have successfully employed capillary GC columns coated with polar stationary phases such polyethylene glycol or nitroterephthalic acid modified polyethylene glycol. When using these columns it is possible to obtain a good chromatographic resolution, avoiding peak tailing [4,16].

The aims of this research were to: (1) developed an automatic SPE system combined with the GC–MS technique for the determination of organic acids (29 compounds) using complex samples like food; (2) study the effect of common interferences present in the food matrixes on the sorption of the organic acids into the sorbent column.

2. Experimental

2.1. Standards and reagents

Standards of the 29 organic acids were supplied from Sigma–Aldrich (Madrid, Spain) and were of >99% purity. Sacharose, fructose, glucose, tannic acid, gallic acid, acetonitrile, 2-*tert*-butyl-4-methylphenol, methanol, ethanol and LiChrolut EN (particle size 40–120 μm) were purchased from Merck (Darmstadt, Germany). Silica-reverse phase sorbent with octadecyl functional groups (Supelclean ENVI-18) was supplied from Supelco (Madrid, Spain).

Stock standard solutions of individual organic acid (10 g L^{-1}) were prepared in methanol or ethanol and stored in glass-stopped bottles at 4 °C in the dark until use. Standard working solutions containing all organic acids were prepared daily by dilution of the stock in water purified with a Milli-Q System (Millipore, Bedford, MA, USA). Methanol containing 2 mg L^{-1} of 2-*tert*-butyl-4-methylphenol as internal standard (IS) was used as eluent.

2.2. Instruments and apparatus

Analyses were performed using a Focus GC instrument (Thermo Electron SA, Madrid, Spain) interfaced to a DSQ II mass spectrometer controlled by a computer running XCalibur software. GC separations were performed with a 30 m HP-INNOWax capillary column of 0.25 mm I.D., packed with a polyethylene glycol stationary phase (0.25 μm film thickness) from J & W (Folson, CA, USA). Helium (purity 6.0) was used as the carrier gas at a flow rate of 1.5 mL min^{-1}. The column temperature was initially set at 60 °C for 4 min and then raised at 10 °C min^{-1} to 200 °C, held for 1 min and raised up to 250 °C at 8 °C min^{-1} (held for 5 min). The GC injection port and transfer line temperatures were kept at 250 °C. The ion source temperature was 200 °C for the 70 eV electron impact ionization mode. The mass spectrometer was operated in the selected ion monitoring mode (SIM), selecting three *m/z* values for each organic acid, which are included along with the analytical figures of merit of the proposed method (see Table of analytical features). The MS was set in full scan mode (40–300 amu) for identification purposes. Samples were injected using an AI/AS 3000 autosampler (Thermo Electron SA) in the split mode (1:20). The time for solvent delay was set to 4 min.

The proposed continuous SPE system consisted of a Gilson Minipuls-3 peristaltic pump (Villiers-le-Bel, France) fitted with poly (vinylchloride) tubes, two Rheodyne (Cotati, CA, USA) 5041 injection valves and a PTFE laboratory-made sorption column containing 80 mg of the mixture LiChrolut EN/Supelclean ENVI-18 (1:1) sorbents. The column was conditioned first with 1 mL of acetonitrile–methanol (1:1) and later with 1 mL of purified water. In these conditions the column remains serviceable for at least 2 months with no change in its properties.

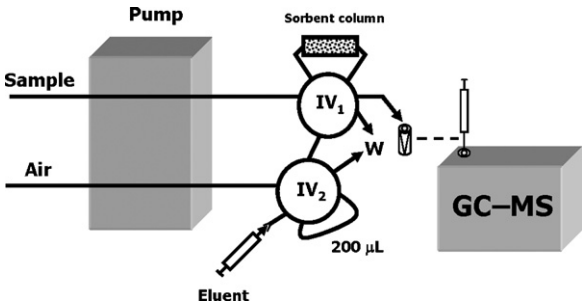

Fig. 1. Continuous module for the preconcentration/clean-up of organic acids in foodstuffs. IV injection valve, W waste.

2.3. Samples

All samples (beer, wine, fruit juice, honey, soy sauce and soya milk) were purchased at local supermarket in Spain. In the laboratory, the samples were kept cold (4 °C), stored in the darkness until analysis and the seal of each bottle was broken before its analysis. Beer samples were degassed in an ultrasonic bath for 5 min and filtered through 0.45 μm membrane filters (mixed cellulose esters, Millipore Ibérica, Spain); the other samples were directly filtered. Then, for all samples the pH were adjusted at 1.3 with an appropriate volume of dilute HCl. In order to avoid the blockage of the flow system, the honey sample (0.1 g) was diluted with 1 mL of purified water and stirred for 5 min at room temperature before filtration.

2.4. Analytical procedure

The SPE system used for the determination of organic acids in foodstuffs is shown in Fig. 1. Volumes of 1 mL of each sample (or the diluted honey) or standard solutions at pH 1.3 (adjusted with 0.1 mL of 0.5 M HCl) containing between 0.5 and 1000 μg kg^{-1} of each organic acid were aspirated through the sorbent column (located in the loop of the injection valve, IV_1) at 4 mL min^{-1}. All organic acids were sorbed and the sample matrix was sent to waste; simultaneously the loop of the second IV_2 was filled with the eluent (methanol containing 2 mg L^{-1} of 2-*tert*-buty-4-methylphenol as IS) by means of a syringe. Any residual water remaining inside the column and connectors was flushed with an air stream for 1 min. Next, IV_2 was switched to pass the loop contents (200 μL) through the column, in the opposite direction of the sample, in order to elute organic acids. The whole extract was collected in a conical glass insert (0.3 mL) inside a 2 mL amber glass GC vial which was tightly sealed and aliquots of 1 μL were injected into the gas chromatograph for analysis.

3. Results and discussion

3.1. Solid-phase extraction unit

The high complexity of food samples demands efficient clean-up and extraction steps to eliminate matrix interferences. In a previous work [23], we developed a flow system for the simultaneous preconcentration of some aliphatic and aromatic organic acids in water samples. In this method the best sorbent was a mixture of LiChrolut EN/Supelclean ENVI-18 (1:1) materials, and methanol was the most efficient eluent. This system was initially adopted to determine 29 organic acids in food samples, but by virtue of the complexity of the sample matrix and increasing number of analytes, the effect of the chemical and flow variables influencing the extraction, preconcentration and elution process were investigated. When an analyte is in neutral form, it becomes more hydrophobic, and retention strengthens under reverse-phase conditions. For organic acids,

Table 1
Chemical and flow variables selected for the proposed SPE method.

Variable	Optimum range (selected value)
Sample pH	1.3
Amount of LiChrolut EN–Supelclean ENVI-10 (1:1 ratio) sorbent (mg)	75–85 (80)
Breakthrough volume (mL)	≤60 (1)
Sample flow rate (mL min^{-1})	0.5–5.0 (4.0)
Eluent volume (μL)	150–200 (200)
Air flow rate (mL min^{-1})	2–4 (3)

when the sample pH is adjusted at 2 units below the pK_a values, the compounds are neutralized. The pK_a values of the studied organic acids ranged between 3 and 5, therefore the food samples or standards solutions were adjusted at pH 1.3 by adding diluted HCl before the introduction in the continuous SPE system. On the other hand, the breakthrough volume depends on the packing efficiency of the sorbent bed and on the strength with which the analytes are retained, and is crucial in SPE methods because it is directly related to the sensitivity of the method. The effect of this variable was examined by using standard solutions containing 100 ng of each organic acid in variables volumes of purified water (from 10 to 100 mL) for insertion into the SPE system. Sorption efficiency of ca. 100% was obtained with aqueous volumes up to 60 mL. However, due to the sensitivity of the method and according to the levels of organic acids normally found in foodstuffs, 1 mL of sample was chosen. The optimum values of other variables affecting the sorption/elution process of the acids (amount of sorbent, eluent volume, sample and air flow rates) were listed in Table 1.

The effect of some species commonly found in food matrixes, which might affect the retention of the organic acids in the sorbent column, was assessed by using standard solutions containing 20 μg kg^{-1} of each organic acid. Firstly, the influence of the ethanol content was examined by using standard solutions prepared in water–ethanol medium with proportions of ethanol that ranged from 0 to 50%. From the results obtained, it can be concluded that ethanol had no effect on retention in proportions up to 20% for all organic acids, which is higher than the content present in the alcoholic beverages analyzed (<15%). Higher ethanol concentrations, however, resulted in dramatically decreased retention of organic acids in the sorbent. This can be ascribed to this particular mechanism of sorption which involves the partitioning of moderately polar organic compounds from a polar phase (water) into the sorbent via polar interactions between the acid group and the underlying sorbent surface. When the aqueous sample contains an ethanol concentration higher than 20%, the solvent breaks the bonds and solubilizes organic acids, reducing their sorption. Secondly, the potential interference of the sugars (glucose, fructose and sacharose) was examined, due to their presence in honey and some beverage samples, using proportions of 30 g kg^{-1}. From the results obtained, the influence of these sugars on the retention of the 29 organic acids was negligible at the higher concentrations assayed, which were higher than what are typically present in the beverages analyzed. Tannins, one of the main constituents of beer and wine, caused no interference at a concentration of 2 g kg^{-1} (higher than those present in these types of beverages). Finally, other compounds including cations (Fe, Cu, Ca, Mg, Na and K), glycerol and phosphates were also assayed at concentrations of 200 mg kg^{-1}. No interference was detected, probably because none of these substances were retained in the sorbent column.

3.2. Analytical performance

The performance and reliability of the proposed SPE–GC–MS method (Fig. 1) was assessed by determining the linear range, analyte detectability and precision for the 29 organic acids studied. Table 2 shows the figures of merit of the proposed method. Although the sensitivity of the method was high considering that

Table 2
Analytical figures of merit of the proposed SPE–GC–MS method for the determination of organic acids in foodstuffs.

Organic acid	Linear range (μg kg^{-1})	Detection limit (μg kg^{-1})	Precision (RSD, %)		m/z[a]
			Within-day	Between-day	
Acetic	1.0–1000	0.4	4.5	5.9	**43**, 45, 60
Propionic	6.0–1000	2.0	4.8	6.2	45, **74**, 75
Butyric	3.0–1000	1.0	6.2	6.4	**60**, 73, 88
2-Methylbutyric	1.5–1000	0.4	4.4	5.7	57, **74**, 102
Pentanoic	0.5–1000	0.1	5.5	6.5	**60**, 73, 87
Hexanoic	1.0–1000	0.3	5.8	6.8	**60**, 73, 116
Octanoic	1.0–1000	0.4	3.4	4.4	**60**, 73, 144
Nonanoic	2.5–1000	0.8	5.2	6.8	**60**, 73, 158
Decanoic	2.5–1000	0.7	5.8	6.5	**60**, 73, 172
Dodecanoic	0.5–1000	0.2	4.5	5.9	60, **73**, 200
Myristic	1.0–1000	0.4	5.6	6.3	60, **73**, 228
Palmitic	1.0–1000	0.4	5.1	6.6	41, **55**, 254
Heptadecanoic	1.0–1000	0.4	5.8	6.5	60, **73**, 270
Stearic	1.0–1000	0.4	5.0	6.5	43, **73**, 274
Oleic	2.0–1000	0.7	5.7	6.4	**55**, 69, 282
Linoleic	2.5–1000	0.8	5.9	6.7	**67**, 81, 280
Sorbic	1.0–1000	0.3	5.4	6.5	67, **97**, 112
Benzoic	0.5–1000	0.2	5.2	6.8	77, **105**, 122
o-Toluic	1.5–1000	0.5	5.6	6.9	**91**, 119, 136
m-Toluic	1.5–1000	0.5	4.9	5.4	**91**, 119, 136
p-Toluic	1.5–1000	0.5	5.0	6.5	**91**, 119, 136
Phenylacetic	1.5–1000	0.5	4.8	5.7	65, **91**, 136
Phthalic	2.5–1000	0.8	5.8	6.5	76, **104**, 148
Lactic	6.0–1000	2.0	5.4	6.0	43, **45**, 90
Malic	6.5–1000	2.0	5.0	6.5	**43**, 89, 134
Succinic	1.5–1000	0.4	6.1	6.9	55, **45**, 118
Fumaric	1.5–1000	0.4	5.2	6.8	45, **98**, 116
Levulinic	3.0–1000	1.0	5.8	6.5	**43**, 56, 116
Citric	0.5–1000	0.2	5.9	6.7	43, **129**, 192

[a] Base peaks used for quantification are boldfaced; m/z for IS (2-*tert*-butyl-4-methylphenol): 121, **149**, 164.

it is possible to use up to 60 mL of sample volume (breakthrough volume) and to elute with 0.2 mL of eluent (preconcentration factor ~300), only 1 mL of sample was chosen depending on the levels or organic acids in foodstuffs. Thus, the analytical curves were constructed by using 1 mL of aqueous standard with variable amounts of each acid (0.5–1000 μg kg^{-1}), obtaining good correlation coefficients in all instances (over 0.995 in all cases). Limits of detection (LODs) ranged from 0.1 to 2.0 μg kg^{-1} after being defined at the concentration of the analyte that provides a chromatographic peak equal to three times the regression standard deviation ($S_{y/x}$) divided by the slope of each calibration graph. Similar LODs were obtained using 12 individual standard solutions containing 10 μg kg^{-1} of each organic acid through their mean values and standard deviations. The precision of the proposed method, like RSD, was evaluated by analyzing 11 individual standard mixtures containing a 20 μg kg^{-1} concentration of each organic acid on the same day (within-day) as well as on three different days (between-day). The results obtained were satisfactory, with RSD values ranging from 3.4 to 6.2% (within-day precision) and 4.4 to 6.9% (between-day precision). The good degree of precision can be ascribed to the automatization of the sample treatment (SPE unit) and the use of an internal standard to correct chromatographic errors.

In order to validate the proposed method, a recovery study was conducted by analyzing diluted samples (diluted from twice to 50-times depending on the food sample) fortified with 10, 40 and 100 μg kg^{-1} of each organic acid in triplicate (n = 3). All samples (beer, white and red wines, fruit juice, soy sauce, soya milk and honey) contained some organic acids, thus recovery percentages were obtained after subtracting the previously quantified endogenous compounds from total contents. All compounds were accurately identified and the average recoveries (93–98%) were acceptable for all samples (Table 3). Therefore, matrix interferences were completely suppressed during the clean-up step in the SPE unit.

3.3. Analysis of foodstuffs

The proposed SPE–GC–MS method was applied to determine organic acids in several types of samples, namely: beers, wines, fruit juice, soy sauce, soya milk and honey. Samples were analyzed in triplicate by using the analytical procedure described in Section 2. When the concentration of some organic acids lay outside the linear range of the method (Table 2), the sample concerned was diluted with purified water before analysis.

The method was applied for most beers of different origins (10 countries), with varying amounts of alcohol (0–6%) and produced from barley or wheat. Organic acids are originated by yeast during fermentation and contribute to beer flavor; these results are listed in Table 4. As can be seen, up to 16 organic acids have been found in the 29 tested beers. There were noticeable variations in concentrations from one sample to another which may be an indication of differences in fermentation and maturation conditions [16]. Acetic, propionic, butyric and 2-methylbutyric acids were detected at concentrations ranging between 0.083 and 2.5 mg kg^{-1}. Hexanoic, octanoic and decanoic acids, which are associated with rancid flavor characteristics, were present at concentrations (0.1–2.0 mg kg^{-1}) lower than those previously found in pilsner-type beers (1.6–6.7 mg kg^{-1}) [5]. Long-chain acids (palmitic, stearic, oleic), which are of great importance because their degradation may lead to the formation of a characteristic aging flavor, were found in the majority of the beers (except for 3 beers of both wheat and barley) at concentrations under 1.7 mg kg^{-1}. Finally, dodecanoic, phenylacetic, lactic, fumaric, levulinic and citric acids were also detected at concentrations similar to those mentioned. The barley beer from Argentina had the lowest number of organic acids and these were found at lower concentrations (6 acids at concentrations ≤0.7 mg kg^{-1}).

Table 3
Average recoveries (±SD, n = 3) of organic acids spiked to foodstuffs samples.

Organic acid	Beer			Wine			Fruit juice			Soy sauce			Soya milk			Honey		
	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}	10 μg kg^{-1}	40 μg kg^{-1}	100 μg kg^{-1}
Acetic	95(6)	96(5)	98(5)	94(6)	96(5)	98(5)	95(6)	96(5)	96(5)	96(6)	98(6)	98(5)	97(6)	98(6)	98(5)	97(6)	98(5)	98(5)
Propionic	95(6)	95(6)	96(5)	93(5)	97(5)	95(6)	96(6)	95(5)	98(5)	93(5)	93(5)	96(6)	96(6)	98(5)	97(6)	96(6)	97(5)	98(5)
Butyric	94(6)	97(6)	96(6)	93(5)	96(6)	95(6)	94(5)	96(6)	97(6)	94(5)	96(6)	98(6)	96(6)	95(6)	97(5)	96(6)	95(6)	98(5)
2-Methylbutyric	93(5)	94(5)	96(6)	93(5)	95(6)	97(5)	98(6)	98(5)	98(5)	97(6)	98(5)	98(5)	93(5)	95(5)	95(5)	96(6)	98(5)	98(5)
Pentanoic	95(6)	96(6)	96(6)	95(6)	96(5)	97(6)	93(6)	97(6)	98(6)	94(6)	96(6)	98(5)	95(6)	96(6)	97(6)	94(5)	98(6)	96(6)
Hexanoic	96(6)	95(6)	98(6)	96(6)	98(6)	98(6)	94(6)	96(6)	96(5)	93(5)	95(6)	97(6)	95(6)	98(6)	98(6)	94(5)	97(6)	98(6)
Octanoic	94(5)	96(4)	97(5)	93(5)	94(5)	96(5)	93(5)	98(5)	97(5)	93(5)	95(5)	96(5)	93(5)	96(5)	97(5)	96(5)	98(5)	98(5)
Nonanoic	94(6)	98(5)	98(5)	97(6)	98(5)	98(6)	96(6)	98(6)	98(5)	93(5)	97(6)	97(5)	93(5)	95(5)	95(6)	93(5)	95(5)	97(5)
Decanoic	96(6)	97(6)	98(6)	96(6)	98(6)	98(6)	94(6)	96(6)	98(6)	95(6)	96(6)	97(6)	93(5)	93(5)	96(6)	98(6)	98(6)	98(6)
Dodecanoic	94(6)	96(5)	97(5)	96(6)	96(6)	94(5)	93(5)	95(5)	97(6)	94(6)	98(5)	98(5)	94(5)	96(5)	98(5)	96(6)	97(5)	98(5)
Myristic	93(5)	98(6)	96(6)	93(5)	95(5)	96(6)	93(5)	96(5)	98(6)	96(6)	97(6)	98(6)	95(6)	96(6)	98(6)	94(6)	96(6)	97(5)
Palmitic	94(5)	96(6)	98(5)	94(5)	96(6)	98(5)	93(5)	97(6)	98(6)	93(6)	95(5)	94(5)	95(6)	97(5)	97(5)	93(5)	98(6)	96(5)
Heptadecanoic	94(5)	96(6)	97(6)	95(6)	98(6)	98(6)	96(6)	96(6)	97(6)	96(6)	98(6)	97(6)	94(6)	98(6)	97(6)	94(6)	98(6)	98(6)
Stearic	93(5)	95(5)	98(5)	96(6)	98(5)	98(5)	93(5)	94(5)	96(5)	94(5)	98(5)	96(5)	93(5)	94(5)	97(5)	96(6)	98(5)	98(5)
Oleic	93(6)	94(6)	96(6)	96(6)	95(6)	94(5)	94(5)	96(6)	98(6)	94(6)	96(6)	98(6)	95(6)	97(6)	96(6)	96(6)	97(6)	97(5)
Linoleic	94(6)	96(6)	98(6)	96(6)	98(6)	98(6)	98(6)	98(6)	98(6)	96(6)	98(6)	98(6)	96(6)	98(6)	98(6)	93(5)	95(6)	96(6)
Sorbic	96(6)	98(5)	98(5)	94(5)	97(6)	96(5)	93(5)	93(5)	96(6)	96(6)	95(6)	97(5)	94(6)	98(6)	96(6)	93(5)	95(6)	96(6)
Benzoic	93(5)	95(5)	96(6)	94(5)	96(5)	98(5)	93(5)	95(6)	98(6)	93(5)	96(5)	98(6)	94(5)	97(6)	98(6)	94(6)	96(6)	98(5)
o-Toluic	94(6)	96(6)	98(6)	94(5)	96(6)	98(6)	95(6)	96(6)	97(6)	94(5)	96(6)	98(6)	97(6)	98(6)	98(6)	95(6)	96(6)	96(6)
m-Toluic	93(5)	95(6)	95(5)	97(6)	97(5)	98(6)	97(6)	98(5)	98(5)	95(6)	96(6)	98(6)	96(6)	98(6)	97(6)	93(6)	97(5)	98(5)
p-Toluic	93(5)	96(6)	97(5)	94(6)	96(6)	98(6)	93(5)	96(5)	98(5)	95(6)	95(6)	96(6)	96(6)	97(6)	97(5)	94(5)	96(6)	97(6)
Phenylacetic	93(5)	95(6)	96(6)	93(5)	94(5)	96(6)	94(6)	97(5)	98(6)	94(6)	98(6)	96(6)	95(6)	96(6)	97(6)	96(6)	98(5)	98(5)
Phthalic	95(6)	96(6)	97(6)	95(6)	96(6)	96(6)	93(5)	95(6)	97(6)	93(5)	94(5)	98(6)	93(6)	93(6)	96(6)	96(6)	97(6)	98(6)
Lactic	96(6)	98(6)	98(6)	95(6)	97(6)	98(6)	93(5)	96(6)	95(6)	95(6)	96(6)	97(6)	94(5)	96(6)	98(6)	94(6)	96(6)	97(5)
Malic	96(6)	98(5)	98(5)	97(6)	98(6)	98(6)	93(5)	95(5)	95(5)	96(6)	97(5)	98(5)	93(5)	94(5)	96(6)	93(5)	98(56)	96(5)
Succinic	94(6)	95(6)	96(5)	96(6)	98(6)	98(6)	93(6)	95(6)	95(6)	94(6)	98(6)	98(6)	95(6)	96(6)	97(6)	95(6)	96(6)	98(6)
Fumaric	96(6)	98(6)	98(6)	97(6)	98(5)	98(5)	93(5)	96(6)	97(6)	96(6)	98(5)	98(5)	96(6)	97(6)	98(6)	95(6)	96(5)	96(5)
Levulinic	94(5)	98(6)	96(6)	96(6)	98(6)	98(6)	93(6)	98(6)	97(6)	96(6)	95(6)	97(5)	94(6)	96(6)	97(6)	94(6)	98(6)	98(6)
Citric	94(5)	96(6)	98(6)	96(6)	95(6)	97(6)	93(6)	94(5)	96(6)	96(6)	97(6)	98(6)	93(6)	95(6)	95(6)	93(6)	94(6)	96(6)

Table 4
Organic acids found in beer samples (±SD, mg kg^{-1}, $n = 3$).

Organic acid	Country raw material ethanol (%)										
	Germany barley (4.9)	Holland barley (5.8)	Spain barley (0.0)	Spain 2 barley (5.4)	Brazil barley (4.3)	Mexico barley (4.5)	Czech republic barley (4.4)	Belgium barley (5.0)	Germany wheat (5.0)	Belgium wheat (4.9)	Argentina barley (4.9)
Acetic	0.90 ± 0.06	1.0 ± 0.1	0.95 ± 0.06	0.95 ± 0.06	0.70 ± 0.05	0.85 ± 0.06	0.15 ± 0.01	0.15 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	–
Propionic	0.50 ± 0.03	0.20 ± 0.01	0.25 ± 0.02	0.25 ± 0.02	0.30 ± 0.02	–	0.20 ± 0.01	–	–	0.30 ± 0.02	–
Butyric	0.60 ± 0.04	2.0 ± 0.1	0.45 ± 0.03	1.3 ± 0.1	1.5 ± 0.1	0.95 ± 0.06	1.1 ± 0.1	0.80 ± 0.05	2.5 ± 0.2	1.1 ± 0.1	0.45 ± 0.03
2-Methylbutyric	0.65 ± 0.04	0.45 ± 0.03	0.09 ± 0.01	0.65 ± 0.04	0.25 ± 0.02	–	0.95 ± 0.06	0.40 ± 0.03	–	–	–
Hexanoic	0.30 ± 0.02	0.85 ± 0.06	0.10 ± 0.01	1.2 ± 0.1	0.30 ± 0.02	0.40 ± 0.03	1.7 ± 0.1	0.30 ± 0.02	0.70 ± 0.05	1.1 ± 0.1	0.20 ± 0.01
Octanoic	0.65 ± 0.04	1.95 ± 0.12	0.20 ± 0.01	2.0 ± 0.1	0.85 ± 0.05	0.35 ± 0.02	1.5 ± 0.1	0.80 ± 0.06	1.7 ± 0.1	1.6 ± 0.1	0.70 ± 0.04
Decanoic	0.35 ± 0.02	0.65 ± 0.04	0.30 ± 0.02	0.50 ± 0.03	0.30 ± 0.02	0.50 ± 0.03	0.55 ± 0.04	0.35 ± 0.02	–	0.50 ± 0.03	0.35 ± 0.02
Dodecanoic	0.70 ± 0.05	0.30 ± 0.02	0.15 ± 0.01	0.20 ± 0.01	–	–	–	–	–	–	–
Phenylacetic	1.1 ± 0.1	–	0.70 ± 0.05	0.90 ± 0.06	1.1 ± 0.1	0.95 ± 0.06	0.80 ± 0.05	1.1 ± 0.1	1.9 ± 0.1	1.0 ± 0.1	0.55 ± 0.03
Palmitic	0.55 ± 0.04	0.35 ± 0.02	–	0.35 ± 0.03	–	0.55 ± 0.04	–	–	–	–	–
Stearic	1.4 ± 0.1	0.50 ± 0.04	–	–	1.1 ± 0.1	0.80 ± 0.05	1.7 ± 0.1	1.0 ± 0.1	0.60 ± 0.04	–	–
Oleic	–	–	–	0.55 ± 0.04	–	–	–	–	–	–	–
Lactic	0.85 ± 0.05	0.40 ± 0.04	0.15 ± 0.01	0.85 ± 0.05	0.12 ± 0.01	0.15 ± 0.01	0.45 ± 0.03	0.20 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	–
Fumaric	1.25 ± 0.08	1.10 ± 0.07	0.80 ± 0.05	0.90 ± 0.06	0.65 ± 0.04	0.75 ± 0.05	0.20 ± 0.01	0.90 ± 0.06	–	–	–
Levulinic	–	0.07 ± 0.01	0.95 ± 0.06	0.60 ± 0.04	–	–	–	–	0.30 ± 0.02	–	–
Citric	0.15 ± 0.01	0.45 ± 0.04	–	–	–	0.20 ± 0.01	–	0.20 ± 0.01	–	–	0.35 ± 0.02

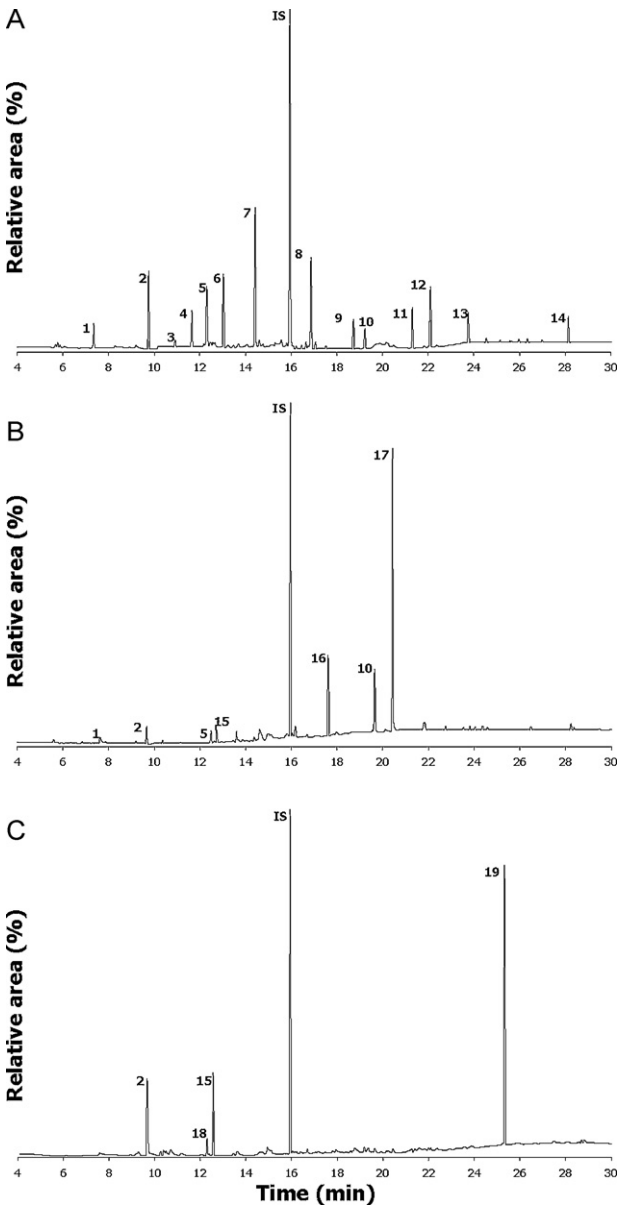

Fig. 2. GC–MS chromatograms obtained in the analysis of diluted Spanish beer 2 (A) soy sauce 2 (B) and honey 1 (C) samples. 1: lactic; 2: acetic; 3: propionic; 4: butyric; 5: 2-methylbutyric; 6: fumaric; 7: hexanoic; 8: octanoic; 9: decanoic; 10: levulinic; 11: dodecanoic; 12: phenylacetic; 13: palmitic; 14: oleic; 15: succinic; 16: sorbic; 17: benzoic; 18: malic; 19: citric acids; IS, internal standard (2-*tert*-butyl-4-methylphenol).

With regard to wine samples, up to 10 acids were found of the 29 tested; organic acid content can vary largely depending on the type of wine (white or red). As could be expected, the major acids were malic, citric, lactic, succinic and acetic for white wines and lactic, citric and acetic acids for red ones. Fumaric, hexanoic, octanoic and decanoic acids were found in lower quantities (Table 5). Sorbic and phenylacetic acids were only found in white wines. These values are within the range described in the literature [7,8,10,12] and depend mainly on the origin/variety of the grape and aging of the wine.

Two commercial peach and apple juice samples were also analyzed. Malic, citric, and succinic acids were found in higher quantities (110–1100 mg kg^{-1}). Other organic acids such as propionic, 2-methylbutyric, hexanoic, octanoic, decanoic and fumaric acids were also found although in lower amounts (Table 5). These values are within the range described in the literature [24]. In both samples, benzoic and sorbic acids were not found (preservative-

Table 5
Organic acids found in foodstuffs (±SD, mg kg^{-1}, n = 3).

Organic acid	White wine 1 (11.5)[a]	White wine 2 (11)[a]	Red wine 1 (13)[a]	Red wine 2 (12)[a]	Peach juice	Apple juice	Soy sauce 1	Soy sauce 2	Soya milk	Honey 1	Honey 2
Acetic	140 ± 7	135 ± 9	150 ± 10	200 ± 10	3.7 ± 0.2	3.6 ± 0.2	7.5 ± 0.4	4.1 ± 0.3	0.70 ± 0.05	22 ± 1	58 ± 3
Propionic	–	–	–	–	1.5 ± 0.1	–	1.5 ± 0.1	–	1.4 ± 0.1	–	–
2-Methylbutyric	–	–	–	–	–	1.8 ± 0.1	3.3 ± 0.2	2.0 ± 0.1	0.40 ± 0.03	–	–
Hexanoic	1.7 ± 0.1	0.55 ± 0.04	0.30 ± 0.02	–	2.0 ± 0.1	–	–	–	0.60 ± 0.04	–	–
Octanoic	–	2.0 ± 0.1	0.20 ± 0.01	2.0 ± 0.1	–	1.7 ± 0.1	–	–	0.50 ± 0.03	–	–
Decanoic	0.80 ± 0.05	–	0.40 ± 0.03	–	3.0 ± 0.1	0.55 ± 0.03	–	–	0.60 ± 0.04	–	–
Sorbic	60 ± 3	70 ± 4	–	–	–	–	22 ± 1	18 ± 1	4.2 ± 0.3	–	–
Benzoic	–	–	–	–	–	–	15 ± 1	46 ± 3	18 ± 1	–	–
Phenylacetic	0.45 ± 0.03	0.40 ± 0.03	–	–	–	–	1.1 ± 0.1	–	3.4 ± 0.2	–	–
Lactic	470 ± 30	610 ± 40	1400 ± 90	1200 ± 80	–	8.5 ± 0.5	0.90 ± 0.05	2.3 ± 0.1	10.2 ± 0.6	–	–
Malic	1400 ± 80	1200 ± 70	13 ± 1	15 ± 1	290 ± 20	1100 ± 60	–	–	–	20 ± 1	27 ± 2
Succinic	410 ± 30	320 ± 20	45 ± 3	80 ± 5	380 ± 20	110 ± 7	–	7.5 ± 0.5	–	24 ± 1	–
Fumaric	4.8 ± 0.3	3.8 ± 0.2	1.7 ± 0.1	2.3 ± 0.1	1.8 ± 0.1	0.95 ± 0.06	–	–	–	–	0.15 ± 0.01
Levulinic	–	–	–	–	–	–	52 ± 4	48 ± 3	29 ± 2	–	–
Citric	510 ± 30	750 ± 50	110 ± 7	250 ± 20	740 ± 40	140 ± 9	–	–	–	40 ± 2	59 ± 4

[a] Ethanol (%).

free) but other authors have found sorbic (<210 mg kg^{-1}) and benzoic acids (<150 mg kg^{-1}) in several fruit juices below the imposed maximum concentrations [12,19,25].

Organic acid content was also evaluated in 2 soy sauces and 1 soya milk (Table 5). Acetic, 2-methylbutyric, lactic and levulinic acids were detected in all samples as components. This is consistent with their formation as by-products of microorganism metabolism during the elaboration of these products. In addition, hexanoic, octanoic and decanoic acids were also found in soy milk. The three samples analyzed contained sorbic and benzoic acids as preservatives (from 4 to 46 mg kg^{-1}) at values below the concentrations allowed for these compounds by the European Union Directives [25] and the Food and Drug Administration (FDA) [26].

The applicability of the proposed method was finally tested by analyzing two honey samples (the results are also shown in Table 5). The compounds found in major concentrations were acetic, malic, succinic and citric acids (20–60 mg kg^{-1}), which are similar to those published by other authors [9,20], but mainly depend upon the floral and geographical origin of the honey [11,17]. Finally, Fig. 2 shows the chromatograms obtained from the analysis of Spanish barley beer 2, diluted twice with purified water (A), as well as of soy sauce 2 (B) and honey 1 (C), both diluted 50 times with water. As can be seen, all the compounds were clearly identified with no significant interferences from the sample matrix, which demonstrates how well the clean-up step using the sorbent column performs.

4. Conclusions

In the absence of a comprehensive method to determine a wide range of organic acids by GC in food without derivatization, the proposed SPE–GC–MS method may be an appropriate procedure for the simultaneous determination of up to 29 organic acids in different types of foodstuffs due to its operational efficiency (simplicity, repeatability, robustness, low time consumption and low cost). The proposed aims have been fully achieved; thus this method can be used to control different types of food from several points of view: (i) nutritional quality, (ii) to explore the continuous effectiveness of organic acids as a natural preservative in most foodstuffs, (iii) to evaluate the influence of storage/aging, and (iv) to establish the authenticity of the food.

Acknowledgements

The authors would like to thank to the DGI of the Spanish Ministry of Science and Innovation for financial support awarded in the form of Grant CTQ2010-17008. Moreover, the Junta of Andalucian has also promoted the present investigation (PO7-FQM-02493). This work was also funded by Grant D/023/85/09 from Agencia Española de Cooperación Internacional y Desarrollo (AECID). FEDER also provided additional funding.

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