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Short communication

Determination of flavour and off-flavour compounds in orange juice by on-line coupling of a pervaporation unit to gas chromatography–mass spectrometry

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Abstract

A method for the analysis of several volatiles compounds responsible for orange juice flavour (ethyl butanoate, limonene, linalool, α-pinene, geranial, neral and α-terpineol) has been developed. The isolation of this compounds was carried out by pervaporation (PV) followed by on-line gas chromatography–mass spectrometry (GC–MS). The coupling PV–GC–MS has been successfully applied to fresh hand-squeezed orange juices and frozen concentrated ones in order to establish differences in their flavour profile. Method has been validated with recovery spike experiments which showed its applicability for a wide range of concentrations.

Keywords: Orange; Juice; *Citrus sinensis*; Flavour analysis; Authentication; Pervaporation

1. Introduction

The fruit juice industry has become one of the world's major agricultural businesses with world trade in fruit juices annually exceeding $ 10 billion [1]. A key characteristic of this product is the flavour. Extensive research over three decades has been focused at identifying, quantifying and organoleptically evaluating the different components of fresh orange juice, important to aroma or flavour, to quality assurance testing, as well as in adulteration monitoring [2–7].

Sweet orange (*Citrus sinensis*) typical aroma is attributed to alcohols (about 22%), hydrocarbons (22%), esters (20%) and aldehydes (18%) considering solely the number of compounds involved. Among these compounds, citral, limonene, linalool, α-pinene, ethyl butanoate, acetaldehyde and octanal have been identified as most contributing to orange flavour, and can be used in orange juice authentication [1]. In addition, some orange juice off-flavours can be developed as a result of chemical, microbiological or biochemical activity. Interaction of the juice and its packaging may also be concerned. α-Terpineol is a well known off-flavour compound present in stored citrus products formed from D-limonene or linalool. This compound exceeds the taste threshold level under practical processing and storage conditions [8].

Gas chromatography has usually been the analytical technique under selection for orange essence oil aroma identification [4,9]. The chromatographic analysis of flavours and off-flavours in food usually requires sample pre-treatment to remove as many interfering compounds as possible. Procedures to isolate flavour compounds usually involved some form of distillation (vacuum, stream distillation) or extraction, or a combination of both, solvent assisted flavour evaporation (SAFE) [10] or supercritical fluid extraction (SFE) [11]. In the meantime, headspace analysis assists greatly in

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providing a suitable extract for analysis which contains substances causing the olfactory stimuli responsible for a perceived odour [1]. Both static [12] and dynamic purge and trap headspace [13] are promising techniques for this purpose, although the last one is more sensitive. Other fast-recovery systems have been proposed for volatile compounds analysis in foodstuff. Likewise, solid-phase microextraction (SPME) has been widely used to analyze flavour compounds in oranges [14–16], apples [17,18], tomatoes [19], strawberries [19], off-flavour in wine [20] and so on.

In this work, a method based on the pervaporation (PV) approach has been developed. This technique has long been used in industry in competition with other separation techniques such as distillation, extraction and adsorption [21]. Analytical pervaporation constitutes a reliable alternative to headspace for volatile compounds isolation and preconcentration before their introduction in some analytical determinative instrumental device, especially gas chromatography [22,23]. In the analytical field, PV has been proposed for the speciation of contaminants [24–26] in soil and sewage sludge. In food analysis, pervaporation is a good alternative for urea and ammonia [27], and total and volatile acidity monitoring in wines [28], off-flavour compounds in wines [29] and the selective determination of pectinesterase activity in fruits [30]. In relation with flavour compounds, it has been proposed for producing apple juice and apple juice aroma concentrates [31] and the influence of feed flow velocity on pervaporative aroma recovery from a solution of apple juice has been stated [32]. However, pervaporation has not been used to the analysis of flavour compounds in orange juice.

In the present study, a pervaporation device has been on-line coupled to a gas chromatograph–mass spectrometer (PV–GC–MS) to perform a rapid, simple and reliable determination of some volatile substances representative of the most important types of compounds responsible for orange flavour. These were: terpene hydrocarbons (limonene and α-pinene), alcohols (linalool and α-terpineol), aldehydes (citral: neral plus geranial) and esthers (ethyl butanoate). They were selected to test the applicability of this technique to their quantitative isolation from orange juice. Differences between processed packed juice submitted to treatments and fresh hand-squeezed juice have also been established.

2. Materials and methods

2.1. Samples

Oranges for fresh juice samples and processed juices (sold as frozen concentrated orange juice) were obtained from a local market. Oranges were hand-squeezed at the laboratory using a kitchen juicer.

Juices were reconstituted to a single strength (12 °Brix) just prior to analysis.

2.2. Chemicals

Ethyl butanoate (99.7%), limonene (99%), α-terpineol (97%), α-pinene (99.5%), citral (96%), linalool (98.5%) and *n*-dodecanol (99.5%) were purchased from Aldrich (Steinheim, Germany).

2.3. Extraction of volatile compounds by pervaporation

The pervaporation module consisted of a lower compartment, where the sample was injected through an injection port, an upper compartment in which the carrier gas collected the volatile analytes, and a hydrophobic membrane (PTFE membrane, 1.5 mm thick and 40 mm in diameter, Trace Biotech AG, Braunschweig, Germany) that separates both compartments placed on a support. The two chambers were aligned with the membrane support using two metallic bars. The whole module was placed between two aluminium supports and four long screws closed the system tightly. The upper chamber of the pervaporator was located on the loop of a high pressure injection valve (Rheodyne, USA) in order to keep the acceptor gas static or circulating to the chromatographic column. The flow manifold tubing was of 0.8 mm i.d. (Supelco, Bellefonte, PA, USA). This coupling has been described in other studies and reported elsewhere [35], however, in the present work the pervaporation module has been lightly modified since it is not necessary to introduce large volumes of sample by using a peristaltic pump. The pervaporation coupling for flavour compounds isolation from orange juice is shown in Fig. 1.

A 500 μl aliquot of juice (either processed packed juice or fresh hand-squeezed) were injected into the lower chamber of the pervaporation unit (homemade device) using a hypodermic needle. A spacer was placed below the membrane in order to create a headspace above the liquid sample. Pervaporation device was placed in a water bath at 60 °C and sample was submitted to pervaporation process for 5 min. Finally, the high pressure valve was switched and a Helium

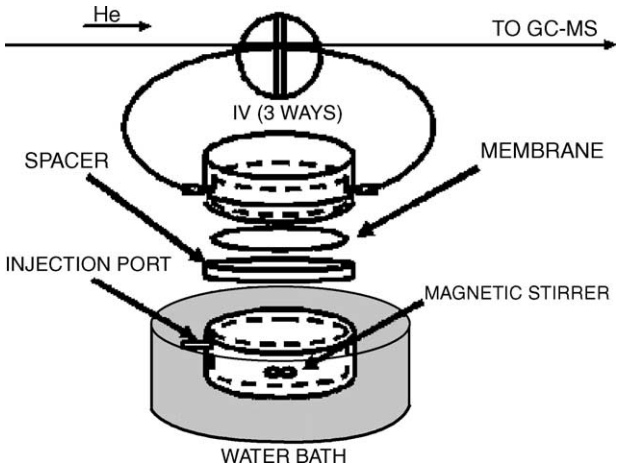

Fig. 1. Scheme of the pervaporation approach.

stream (60 ml min^{-1}) drove the pervaporated analytes to the chromatograph–mass spectrometer. Sampling time was fixed at 10 s, however this parameter depends of the flow manifold dimensions.

2.4. Gas chromatography–mass spectrometry (GC–MS)

Pervaporation outlet was directly coupled to the injector port of a Varian Model 3800 gas chromatograph paired with a Saturn 2000 ion-trap mass spectrometry detector (Varian, Sunnyvale, CA, USA). The gas chromatograph was fitted with a fused-silica capillary column with a VF-5 ms stationary phase and dimensions: 30 m × 0.25 mm i.d., 0.25 μm film thickness (Factor Four CPSIL-8, Varian Ibérica). The carrier gas was helium at a flow-rate of 1 ml min^{-1}. The injector operated in the split mode (20:1) at an initial temperature of 50 °C ramped to 75 °C at 100 °C/min, held at 75 for 20 min and finally increased to 220 °C at 100 °C/min. The oven temperature was set at 30 °C, subsequently increased to 130 °C at 6 °C/min, then to 155 °C at 30 °C/min, then to 200 °C at 50 °C/min and finally held at 200 °C for 5 min.

The temperature of the GC–MS transfer line was maintained at 280 °C. Full scan electron impact ionization data were acquired under the following conditions: solvent delay 2.5 min, 70 eV electron impact energy, emission current 70 μA, scan time 1 s $scan^{-1}$, and manifold and trap temperatures 50 and 150 °C, respectively. The automatic gain control was switched on with a target fixed at 20,000 counts. The overall run time consisted of 2.5 min of delay and one segment, scanning the following range (*m*/*z*): 40–200 from 2.5 to 25.4 min.

2.5. Identification and quantitation

The peaks were identified by running solutions of reference compounds. Library mass spectra [33] were also used when concentration allowed.

For quantitation, a juice base were prepared by reconstitution to 11.8 °Brix of concentrated orange juice (evaporator pump-out) that contained no significant amounts of these volatile constituents which was confirmed by gas chromatography analysis.

The reference compounds were quantified using regression equations determined by injecting four different concentrations added to a juice base to obtain integrated peak area ratios (peak area analyte/peak area internal standard) which were calculated and plotted against the concentration of the analytes. The concentrations of the standards in the juice base were: 2.5, 1.0, 0.1 and 0.01 mg l^{-1} for α-pinene, linalool, α-terpineol, ethyl butanoate and citral. A separate quantitation was carried out for limonene following the same procedure, except that the concentrations of added limonene were 200, 100, 50 and 10 mg l^{-1}. The internal standard used for quantitation was *n*-dodecanol.

Each juice sample was quantitatively analyzed in triplicate by PV–GC–MS.

3. Results and discussion

3.1. Volatile compounds extraction from orange juice using pervaporation variables optimization

The most important variable affecting pervaporation process is temperature, which enhanced the compounds releasing from the matrix when it increases. Therefore, it induces an increase in sensitivity. A temperature of 60 °C was selected as optimum (Table 1), since higher temperatures did not improve the signal.

Preconcentration time of the analytes in the static gas volume of the chamber headspace was also studied. It is not surprising that longer time yielded better results. The temperature and stirring applied to the sample during this time produce an efficient and continuous diffusion of the analytes from the air gap above the sample though the selective membrane. When the pervaporation time was increased, the analyte recoveries also increased. Insignificant improvements were obtained with time longer than 5 min, which was chosen as optimum. Selected values for these parameters are shown in Table 1.

The use of a high carrier gas flow (helium) improves the signal due to the higher amount of analyte introduced in the chromatograph, however, the optimum value was obtained at 60 ml min^{-1} (at atmospheric pressure) due to the limitations of chromatograph injector dynamic for high flow values. For both temperature and sweeping gas flow optimization, the resulting peak areas for a range of values are shown in Table 2.

3.2. Applicability of pervaporation to volatile compounds analysis in orange juices

Recovery assays were conducted at the 0.5 mg l^{-1} level of ethyl butanoate, α-pinene, linalool, α-terpineol and citral and at the 100 mg l^{-1} level of limonene in a juice base. Beforehand, the dearomatized orange juices were measured gas chromatographically and it contained no significant amounts of volatile compounds. The averaged recoveries in the spike experiments were higher than 80% for all the analytes. Results are summarized in Table 3.

In order to study the differences between hand-squeezed and frozen concentrated orange juice the approach proposed was applied to these types of samples. For this purpose, 15

Table 1
Variables range and optimum values

Parameter	Range studied	Optimum value
Bath temperature (°C)	30–80	60
Pervaporation time (min)	3–15	5
He flow (ml min^{-1})	10–70	60
Sampling time (s)	5–20	10

Table 2
Optimization of temperature and sweeping gas flow

	Relative peak area[a] ± S.D. (%)						
	Ethyl butanoate	α-Pinene	Limonene	Linalool	α-Terpineol	Neral	Geranial
Temperature (°C)							
30	42 ± 6.7	38 ± 3.8	41 ± 6.4	47 ± 3.9	55 ± 4.2	32 ± 4.7	33 ± 3.8
40	74 ± 4.5	52 ± 5.1	53 ± 4.5	61 ± 4.5	64 ± 3.6	67 ± 5.1	67 ± 4.5
50	100 ± 5.3	69 ± 2.1	71 ± 3.8	69 ± 2.6	79 ± 5.2	89 ± 3.9	93 ± 2.7
60	97 ± 3.6	100 ± 3.9	100 ± 3.2	100 ± 3.3	82 ± 4.9	100 ± 3.0	100 ± 2.0
70	89 ± 2.8	98 ± 2.6	96 ± 2.6	98 ± 2.1	100 ± 2.9	65 ± 2.7	99 ± 5.6
80	56 ± 3.9	98 ± 3.0	98 ± 4.1	96 ± 4.0	98 ± 3.5	73 ± 4.9	97 ± 5.2
He flow (ml min^{-1})							
10	45 ± 3.2	35 ± 3.5	41 ± 4.7	47 ± 2.9	59 ± 3.8	68 ± 2.5	78 ± 3.3
20	61 ± 5.5	56 ± 2.8	53 ± 6.1	68 ± 2.4	62 ± 4.5	67 ± 3.8	82 ± 4.6
30	65 ± 2.9	68 ± 3.2	75 ± 5.3	63 ± 3.5	73 ± 3.4	69 ± 2.8	79 ± 2.2
40	97 ± 6.1	82 ± 4.9	89 ± 5.0	82 ± 3.9	85 ± 3.8	88 ± 5.7	86 ± 3.9
50	100 ± 2.0	99 ± 2.2	97 ± 3.8	95 ± 3.8	94 ± 2.9	98 ± 4.9	99 ± 4.1
60	95 ± 3.4	100 ± 3.9	100 ± 2.9	100 ± 5.1	100 ± 3.6	100 ± 5.3	100 ± 2.0
70	99 ± 4.5	99 ± 4.0	98 ± 5.1	99 ± 2.6	97 ± 4.4	94 ± 4.7	97 ± 3.2

[a] Relative peak area = (peak area/maximun peak area) × 100; n = 5 replicates.

Table 3
Recovery trials for a dearomatized orange juice spiked with the analytes

Analyte	Spike (mg l^{-1})	Juice base	
		Mean of measured concentration[a] after spike $\bar{X} \pm \sigma$ (mg l^{-1})	Mean recovery of spike (%)
Ethyl butanoate	0.5	0.53 ± 0.25	106
α-Pinene	0.5	0.44 ± 0.13	88
Limonene	100	110 ± 8.72	110
Linalool	0.5	0.41 ± 0.12	82
α-Terpineol	0.5	0.55 ± 0.10	110
Citral-a geranial–b neral	0.5	0.42 ± 0.45	84

[a] n = 5 replicates; internal standard: n-dodecanol.

samples of both fresh hand-squeezed orange juice and frozen concentrated orange juice were analyzed with PV–GC–MS. Seven volatile substances representative of the most important types of compounds responsible for orange flavour, such as terpenes (limonene and α-pinene), alcohols (linalool and α-terpineol), aldehydes (citral: neral plus geranial) and esthers (ethyl butanoate), were selected to test the applicability of pervaporation to their quantitative isolation from these matrices. Results obtained can be seen in Table 4.

Mean values for individual constituents in natural and processed juices exhibit important differences. Freshly made orange juice contains considerably less limonene, α-pinene and linalool than processed ones. These results can be related to the large contribution of peel oil efficiently extracted in processed juices [1]. α-Terpineol, which constitute a negative contribution to juice flavour, was present at relatively high levels in a number of processed juices. This fact would be expected since this is a degradation product of limonene [34]. α-Terpineol is also considered as an indicator of the age of orange juice and its presence becomes a problem at levels higher than 2 μg g^{-1} [35]. Ethyl butanoate is considered as an indicator of quality in the aromatic fraction of a juice, consequently a reduction in the concentration of this component resulted in an impoverishment of the aromatic quality [36].

Table 4
Amounts (mg l^{-1}) of volatile constituents in fresh and processed orange juices

Analyte	Fresh hand-squeezed orange juice		Frozen concentrated orange juice	
	Mean[a]	Range	Mean[a]	Range
Ethyl butanoate	0.26	0.2–0.9	0.16	0.01–0.6
α-Pinene	0.55	0.4–1.3	1.55	0.6–1.9
Limonene	61.9	29–80	151	99–256
Linalool	0.69	0–1.9	0.80	0.3–1.6
α-Terpineol	0.19	0.05–1.9	0.39	0–1.7
Citral-a geranial–b neral	0.20	0.1–0.5	0.50	0.06–0.6

[a] 15 samples of each type of orange juice; internal standard: n-dodecanol.

According to our results using PV–GC–MS, this compound was found at lower levels in processed juices than in freshly made ones.

4. Conclusions

In spite of pervaporation has been widely used in industry, at laboratory scale it is not frequent in comparison with other techniques such as solid-phase microextraction, solvent extraction or distillation. In this work, a pervaporation method for the separation of flavour compounds from orange juices has been performed. The proposed approach presents good recoveries for target compounds and it is simple, fast and cheap with high sample throughput. With the proposed method, only 5 min were needed to isolate the compounds under study, thereby SPME consumes about 15 min [7,14–18]. In addition, when SPME is used for the analysis of volatile compounds in food, the complexity of this matrix (the mostly cases for food) could damage the fibre. Moreover, taking into account that this work has been performed by using a home-made device, results could be in future studies considerably improved.

Further shortcoming studies should focus on the study of other compounds present in orange juice as well as in other food matrices.

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