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Ultrasonic nebulization headspace ionic liquid-based single drop microextraction of flavour compounds in fruit juices

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ABSTRACT

The ultrasonic nebulization headspace ionic liquid-based single drop microextraction was developed for the extraction of flavour constituents from fruit juices. The GC–MS was applied to the separation and detection of the constituents. The parameters affecting extraction performance, such as microdrop volume, extraction time, enrichment time, extraction temperature, and position of microdrop, were investigated and optimized. The optimized system was: ionic liquid, 1-hexyl-3-methylimidazolium tetrafluroborate; solvent microdrop volume, 12.5 μL; extraction time, 5 min; enrichment time, 20 min; extraction temperature, 80 °C; the height of the microdrop above the solution surface, 1 cm; and, the pH value of sample solution did not affect the species, and solubility of analytes. The recoveries were in the range of 80.4–115.0%, and the relative standard deviations were lower than 9.1%. The present method is a simple and sensitive method for the determination of flavour constituents in fruit juices.

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. Aroma is a decisive criterion for evaluating fruit juice quality [1]. Volatile flavour constituents directly affect the sensorial quality of fresh and processed fruit products [2]. A considerable amount of methods have been developed for characterizing flavour compounds.

The sample pretreatment is a critical step for the determination of flavour compounds. The methods to extract flavour compounds usually involved distillation, extraction, combination of distillation and extraction, and solvent assisted flavour evaporation [1]. Recently, some enrichment methods have been proposed for the determination of volatile compounds. The solid-phase extraction (SPE) [3], solid-phase microextraction (SPME) [4–6], and single drop microextraction (SDME) [7] have been developed. SDME has the advantages of simplicity, rapidity and inexpensiveness, and was applied in extracting analytes from spice [8], water [9], vegetable [10], and so on [11]. To meet a variety of analytical requirement, SDMEs were divided into direct immersion (DI)-SDME [12] and headspace (HS)-SDME. In HS-SDME, the microdrop was suspended in the headspace of extraction vessel, and the extraction efficiency for each constituent in sample depends on its volatility and distribution ratio between the gas phase and the suspended solvent. So, HS-SDME, which was usually combined with stirring extraction, ultrasonic extraction and microwave assisted extraction, is an effective method to extract flavour constituents from foodstuffs. When the HS-SDME was applied, the nonvolatile compounds were not extracted, which is of crucial importance in flavor compound isolation from such matrices as foodstuffs [13]. The HS-SDME was applied for the extraction of volatile aldehydes from cucumber [14] and flavors from clove buds [15]. HS-SDME was applied for the extraction of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in wine samples [16] and volatile sulphur compounds in beer and beverage [17]. Ionic liquid-based-SDME coupled with ion mobility spectrometry (IMS) was applied for the determination of 2,4,6-trichloroanisol in water and wine samples [18].

HS-SDME coupled with ultrasonic nebulization extraction was first studied for extraction of the essential oil from *Cuminum cyminum* L [19]. The description of ultrasonic nebulization extraction has been reported in literature [20,21]. These experiments confirmed that HS-SDME coupled with ultrasonic nebulization extraction is a feasible and alternative method for the extraction of active constituents from samples.

The ionic liquids (ILs) are semi-organic molten salts which consist of organic cations and organic or inorganic anions. The ILs emerge as possible "green" solvents [22–24], because they have the advantages of immeasurably low vapor pressure, high stability, large viscosity, moderate dissolvability of organic compounds, as well as adjustable miscibility and polarity [25–27].

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ILs were recently proposed as the hopeful substitutes of extraction solvents. In recent years, the ILs have attracted increasing interest and are used more and more as attractive alternatives to environmentally friendly solvents in sample preparation [28–30]. ILs are promising solvents in the preparation of various active constituents from medicinal plants, such as essential oils [31], alkaloids [32,33], terpene lactones [34], and polyphenolic compounds [35].

Because IL has immeasurably low vapor pressure, the evaporation of the ILs are difficult. If GC is going to be applied after SDME, the ILs can contaminate the GC column. This drawback limits the application of the IL-based SDME in GC analysis [36].

In this paper, the ionic liquid 1-hexyl-l-3-methylimidazolium tetrafluoroborate ($[C_6MIM][BF_4]$), which is hydrophilic, was used as extraction solvent in SDME. However, the IL is not suitable to be introduced into the GC system, because the IL can contaminate the GC column. With the aim of contributing to exploiting the advantages of ILs as extraction solvents for the microextraction of flavour in fruit juices, the present work focuses on the development of a simple and available approach for the use of IL-based SDME prior to GC system.

2. Experimental

2.1. Chemicals and sample preparation

Fruit juices (samples 1, 2 and 3) used in this experiment were purchased from local supermarkets in Changchun, Jilin Province, China. Sample 1, 2 and 3 are orange, mango and peach juices, respectively. The content of fruit juice in sample 1 is higher than 10%. Sample 2, 3 contain 30 and 36% fruit juices, respectively. The pH value of the three samples is 2.5.The samples were kept at 4 °C before analysis. In this study, all experiments were carried out with sample 1 except for the experiment mentioned in Section 3.2.2 in which the three samples were used.

The standards, hexyl acetate (99%) and geranyl acetate (98%) were purchased from Acros Company (New Jersey, USA). The standard Limonene (> 98%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). *n*-Decane was used as internal standard and purchased from Acros Company (New Jersey, USA). 1-Ethyl-3-methylimidazolium tetrafluoroborate ($[C_2MIM][BF_4]$), 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($[C_6MIM][BF_4]$) were obtained from Chengjie Chemical Co., Ltd. (Shanghai, China). Chromatographic grade *n*-hexane was purchased from Fisher Scientific Company (UK).

The standard stock solutions of target compounds and internal standard were prepared by dissolving the compounds in *n*-hexane. These stock solutions were stored in the dark at 4 °C. Pure water was obtained with Milli-Q water purification system (Millipore Co., USA). The other reagents purchased from Beijing Chemical Factory (Beijing, China) are all of analytical grade.

2.2. Instruments and apparatus

The extraction and concentration system was assembled in our laboratory. A schematic diagram of the system is shown in Fig. 1. An ultrasonic humidifier (Beijing Branson Ultrasound Co. Ltd., China) working at 1.7 MHz with maximum output power of 35 W was employed as the ultrasonic source. The extraction vessel was a self-made glass flask (100 mL). The port on the bottom of the extraction vessel was sealed with the PVC film and the size of the port was the same as that of the piezocrystal. The space between the ultrasonic nebulization extraction and the piezocrystal was full of coupling water. A heating tape connected with a

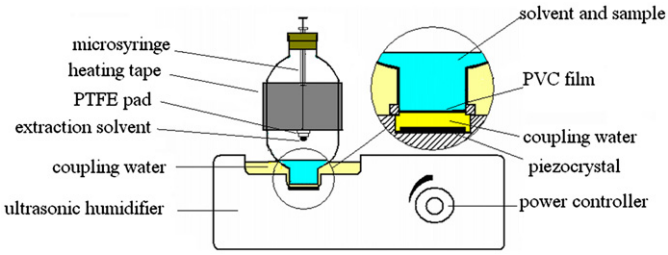

Fig. 1. UN-HS-IL-SDME system.

thermocouple sensor (XMTD-2001 Xinghua AOTE Temperature Instrument Co., Xinghua, China) was used for heating the extraction vessel and can regulate the heating temperature. A 25 μL microsyringe (Zhenhaisan'ai Instrument Co. Ltd, Ningbo, China) was used for microextraction and sample injection. It is not possible to suspend a large volume of microdrop of $[C_6MIM][BF_4]$ from the tip of a bare needle. In order to suspend a large volume of drop on the needle of the microsyringe, the tip of the microsyringe needle was sheathed with a polytetrafluoro-ethylene (PTFE) pad as shown in Fig. 1. In this way, the probability of drop detachment decreased and a large volume of microdrop can be suspended at the tip of syringe with good stability.

2.3. GC-–MS analysis

The sample solution was analyzed using a GC–MS QP 2010 (Shimadzu, Kyoto, Japan). Chromatographic separation was conducted with a DB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Ultra-high-purity helium (99.999%) was used as the carrier gas at a constant flow of 1.0 mL min^{-1}. The temperature program was set initially at 60 °C for 2 min to 140 °C at a rate of 10 °C min^{-1} (held for 1 min), and then to 220 °C at a rate of 3 °C min^{-1} (held for 1 min). The injector temperature was maintained at 250 °C, and the injection volume was 1.0 μL in the split mode (split ratio 1:5). The ion source temperature, interface temperature, and electron impact ionization energy were 200 °C, 250 °C, and 70 eV, respectively. The mass spectrometer was operated in a selected ion monitoring (SIM) mode for quantitative analysis. Full-scan MS data were acquired in the range of *m/z* 50–550 to obtain the fragmentation spectra of the target analytes.

2.4. UN-HS-IL-SDME

Fruit juice was centrifuged at 3000 rpm for 5 min before extraction. 5 mL of fruit juice and 5 mL of water were added into the extraction vessel shown in Fig. 1. The needle of the 25 μL microsyringe was passed through the plug and was clamped at a fixed position in the extraction vessel. The height of the tip of the microsyringe above the surface of the sample solution was approximately 1 cm. The extraction vessel was wrapped with a heating tape and the extraction temperature was controlled at 80 °C. When the nebulizer was switched on, the ultrasonic energy was transferred through coupling water to the sample solution and the sample solution spurted up from the bottom of the extraction vessel. The "ultrasonic fountain" appeared and the extraction vessel was full of aerosol. The analytes in fruit juice were transferred from extraction solvent to headspace by the help of ultrasonic fountain. Ultrasonic nebulization can be used to produce very fine aerosol, and the analytes can be enriched and accumulate in the aerosol [37]. All the experiments were performed in triplicate. In the headspace, the analytes were transferred from the aerosol into the gas phase due to the gas–liquid distribution equilibrium. After ultrasonic nebulization extraction

for 5 min, the nebulizer was turned off. The nebulizer was at a standstill for about 1 min. Then 12.5 μL of ionic liquid was pushed out and suspended from the PTFE pad above the sample solution. Mass transfer of the analytes from gas phase to the suspended IL microdrop continued until the enrichment was completed. After enrichment for 20 min, the extract was withdrawn into the microsyringe. Then the extract was injected into a 1.5 mL concentration tube and a small amount of anhydrous sodium sulfate was added into the tube. After addition of 20 μL *n*-hexane containing 0.002% *n*-decane (internal standard) to the tube, the tube was shaked for 1 min. The target analytes were extracted from ionic liquid phase into the *n*-hexane phase. Then 1.0 μL of the resulting extract was injected into GC–MS system for analysis.

3. Results and discussion

3.1. Optimization of UN-HS-IL-SDME

In order to achieve an adequate extraction performance, several parameters, including the type of the IL, the pH value, microdrop volume, extraction time, enrichment time, extraction temperature, and the position of microdrop were optimized. When one parameter was changed, other parameters were fixed at their optimal values.

3.1.1. Influence of the type of IL

The structures of ILs have significant influence on their physicochemical properties, which might greatly affect the extraction efficiency of the target analytes. Three kinds of ILs, including $[C_2MIM][BF_4]$, $[C_4MIM][BF_4]$, and $[C_6MIM][BF_4]$ were used as the extraction solvents in single drop microextraction. The experimental results are shown in Fig. 2. With the increase of alkyl chain length from ethyl to hexyl, the relative peak areas of the three analytes dramatically increase. This phenomenon could be attributed to the fact that increasing alkyl chain length can lead to the decrease of the solubility of the ILs and increase of the solution viscosity, which can result in the increase of the solubility of the analytes in the IL. So $[C_6MIM][BF_4]$ was chosen as suspended solvent in this work.

3.1.2. Influence of the pH value

The pH value plays an important role in analysis of fruit juices. The influence of pH value of the sample solution on extraction efficiency was studied. The pH value of the fruit juice itself is 2.5. It can be found that the change trend of the relative peak areas of three target analytes is not significant with the increase of pH value from 2.5 to 8.5. This may because the pH value of sample solution did not affect the species and solubility of analytes. So the pH value of the fruit juice sample was not adjusted in the following studies.

3.1.3. Influence of solvent microdrop volume

The effect of microdrop volume on the relative peak area, which is the peak area ratio of the target analyte to internal standard, is shown in Fig. 3. It can be seen that when the solvent microdrop volume increases from 5 to 15 μL, the relative peak areas of three target analytes increase. To examine the effect of the microdrop volume, the student's *t* test was applied. The statistical analysis indicated that there was significant difference ($p < 0.1$) among the relative peak areas for the analytes obtained with 5, 7.5, and 10 μL of the solvent. However, the difference between the peak areas for hexyl acetate obtained with 10 and 12.5 μL of solvent was significant ($p < 0.1$) and that for limonene and geranyl acetate obtained with 10 and 12.5 μL of solvent was not significant ($p < 0.1$). When solvent microdrop volume was 15 μL, the microdrop seemed to be unstable and easily released during the extraction. Based on the experimental results, 12.5 μL was selected as the solvent microdrop volume in the work.

3.1.4. Influence of extraction time

The effect of extraction time on yields of the flavour compounds was tested in the range of 2–20 min. The experimental results are shown in Fig. 4. It can be found that the relative peak areas of hexyl acetate and limonene increase with the increase of the extraction time from 2 to 5 min, but decrease when the time is longer than 5 min. The relative peak area for geranyl acetate increases significantly with the increase of the extraction time ranging from 2 to 5 min and then increases marginally after 5 min. It was found that the concentration of some analytes in the droplets of the ultrasonically generated aerosol was significantly higher than that in the solution being nebulized [38]. The analytes

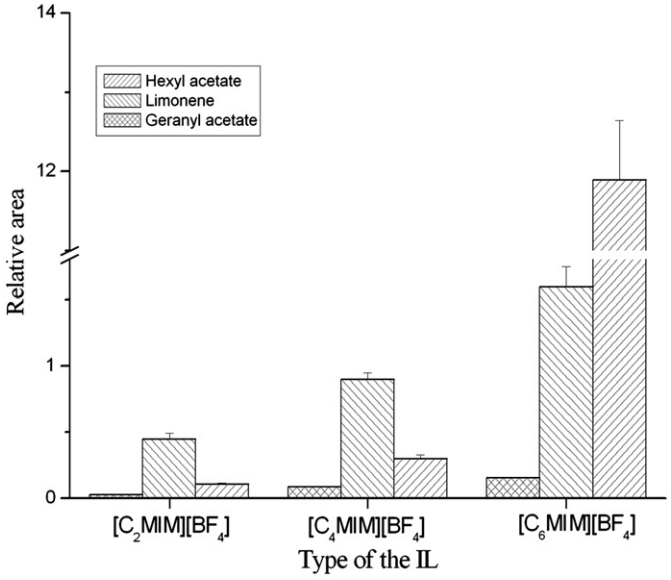

Fig. 2. Effect of the type of the IL. Solvent microdrop volume, 12.5 μL; extraction time, 5 min; enrichment time, 20 min; extraction temperature, 80 °C; the height of the microdrop above the solution surface, 1 cm.

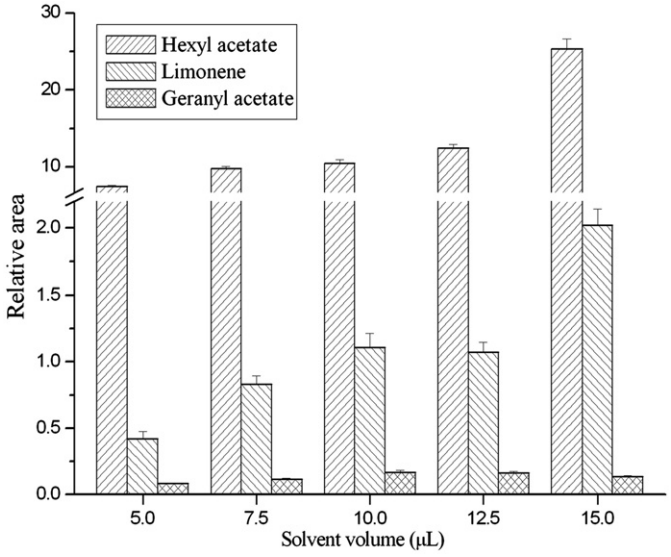

Fig. 3. Effect of solvent microdrop volume. Extraction time, 5 min; enrichment time, 20 min; extraction temperature, 80 °C; the height of the microdrop above the solution surface, 1 cm.

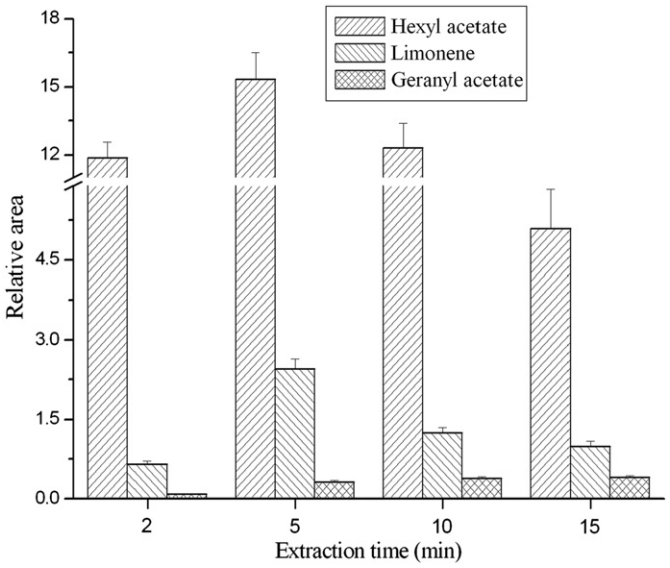

Fig. 4. Effect of extraction time. Solvent microdrop volume, 12.5 μL; enrichment time, 20 min; extraction temperature, 80 °C; the height of the microdrop above the solution surface, 1 cm.

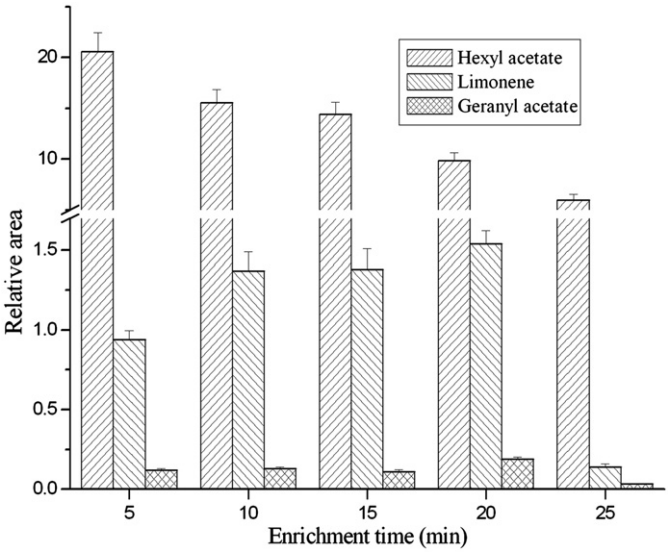

Fig. 5. Effect of enrichment time. Solvent microdrop volume, 12.5 μL; extraction time, 5 min; extraction temperature, 80 °C; the height of the microdrop above the solution surface, 1 cm.

were transferred from the droplets into the gas phase. The concentrations of the analytes in gas phase should be higher than those without nebulization because of the enrichment of the analytes in the aerosol droplets. However, hexyl acetate and limonene are volatile compounds and geranyl acetate is a kind of semivolatile compound. The transfer rates of volatile and semivolatile compounds are different. The volatile compounds are more easily transferred from the aerosol phase into the gas phase than the semivolatile compounds. The compounds in the gas phase may be adsorbed onto the inner wall of the extraction vessel. The adsorbed amount increases with the increase of extraction time within a certain period. The amount of the volatile compounds adsorbed onto the wall should be larger than that of the semivolatile compounds. So, to obtain high extraction yields, the extraction time for the volatile compounds should be shorter than that for the semivolatile compounds. A compromise time, 5 min was selected.

3.1.5. Influence of enrichment time

The enrichment time is the time of exposure of the microdrop in the headspace. The amount of the target analytes transferred into the microdrop reaches their maximum when the equilibrium is established. The effect of enrichment time (5, 10, 15, 20, 25 min) was tested, and the experimental results are shown in Fig. 5. The relative peak area of hexyl acetate decreases with the increase of enrichment time. However, the peak areas of limonene and geranyl acetate first increase with the increase of the enrichment time and then decrease when the time is longer than 20 min. The reason may be the back-extraction from the microdrop into the headspace. The HS-SDME was not an exhaustive extraction method, and the extraction efficiency did not always increase with the increase of enrichment time [39]. The analytes are distributed among the sample phase, the headspace and the microdrop. Thus, the amount of the analyte transferred into the microdrop reaches its maximum when the equilibrium is established [40]. As soon as the concentration of the analyte in headspace is lower than the equilibrium value, the analyte molecules begin to diffuse from the microdrop into the gas phase. Finally, 20 min was chosen to be the enrichment time.

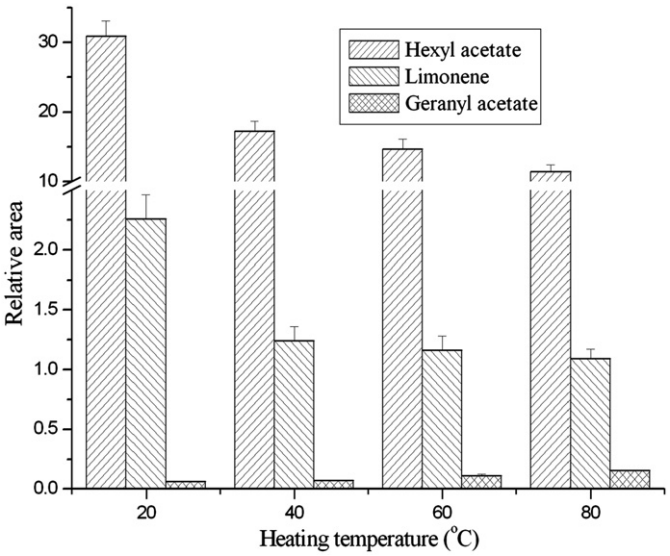

Fig. 6. Effect of extraction temperature. Solvent microdrop volume, 12.5 μL; extraction time, 5 min; enrichment time, 20 min; the height of the microdrop above the solution surface, 1 cm.

3.1.6. Influence of extraction temperature

The influence of extraction temperature on extraction efficiency of target analytes were studied and the experimental results are shown in Fig. 6. The relative peak areas of hexyl acetate and limonene decrease and that of geranyl acetate increases with increase of the extraction temperature. The effect of the extraction temperature on the mass transfer of the analytes from the aqueous phase to gas phase and from the gas phase to extraction solvent is significant. The relative peak areas of volatile analytes with low boiling points, such as hexyl acetate and limonene, decrease with increase of the temperature. This may be because the analytes tend to be in gas phase. The increase of temperature should be beneficial to the transfer of semivolatile analytes, such as geranyl acetate, from the gas phase into suspended solvent (ionic liquid). If the temperature is higher than 80 °C, the suspended microdrop is unstable. So 80 °C was chosen as the extraction temperature.

3.1.7. Influence of position of microdrop

The effect of the height of the microdrop above the surface of the sample solution was investigated. The experimental results are shown in Fig. 7. Because the height of the extraction vessel and the maximum power of the nebulizer were limited, the height of the fountain was also limited. Therefore, the concentration of analytes may be different at different height above the surface of the sample solution. As shown in Fig. 7, the relative peak areas of the analytes decrease with the increase of the height of the microdrop. When the extraction vessel is heated, the water in the surface of the sample can evaporate and be adsorbed onto the microdrop. Thus, when the height of microdrop was less than 1 cm, the microdrop was unstable and easily released. Therefore, the tip of the microsyringe was fixed at a height of 1 cm above the surface of the sample solution.

The optimized parameters are shown in Table 1.

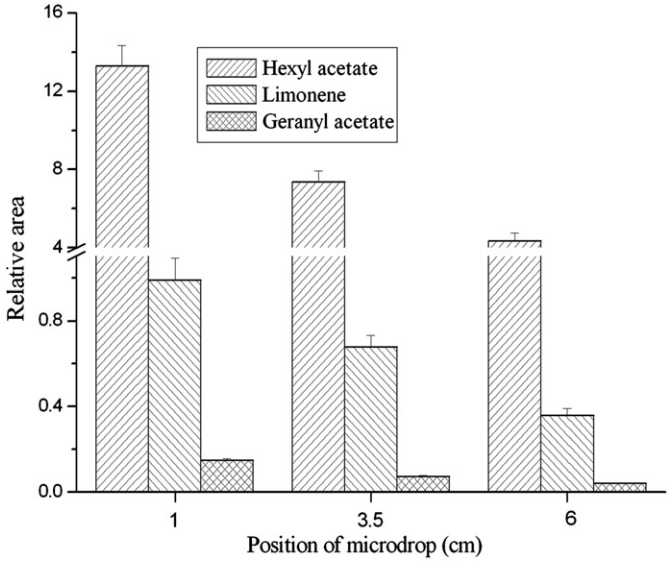

Fig. 7. Effect of position of microdrop. Solvent microdrop volume, 12.5 μL; extraction time, 5 min; enrichment time, 20 min; extraction temperature, 80 °C.

3.2. Method validation

To evaluate the performances of the present method, the linear regression equations, limits detection and quantification, accuracy, and precision were tested.

3.2.1. Analytical figures of merit

The calibration curves were made by plotting relative peak areas versus the concentrations of the analytes under the optimized conditions. The linear regression equations, linear ranges, and correlation coefficients were listed in Table 2. The limits of detection (LODs) and quantification (LOQs) are the analyte concentrations producing signal/noise ratio of 3 and 10, respectively. As can be seen in Table 2, the LODs for hexyl acetate, limonene and geranyl acetate are 0.0971, 0.00437, and 0.0989 μg mL^{-1}, respectively.

3.2.2. Analysis of samples

The present method was applied to the extraction of flavour compounds from fruit juices and the extracts were analyzed by GC–MS. In order to test the accuracy and applicability of the present method, three kinds of fruit juices were analyzed. The analytical results are shown in Table 3. The recoveries range from 80.4 to 115.0% with relative standard deviations ranging from 1.5 to 9.1%. These results could be acceptable.

3.3. Comparison of the present method with other methods

A comparison of the present method and other methods reported in literature, including headspace solid-phase microextraction (HS-SPME), headspace trap (HT), simultaneous distillation extraction (SDE), stir bar sorptive extraction (SBSE), headspace-solvent microextraction (HSME), and pervaporation (PV) was made. The results are shown in Table 4. When SPME is

Table 1
The optimized parameters.

Type of the IL	The pH value	Solvent volume (μL)	Extraction time (min)	Enrichment time (min)	Heating temperature (°C)	Position of microdrop (cm)
[C_6MIM][BF_4]	2.5	12.5	5	20	80	1

Table 2
Analytical figures of merit.

Analytes	Calibration curve	Linear range (μg mL^{-1})	Correlation coefficient (r)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Hexyl acetate	A=5.316C+14.156	0.35–7.05	0.9986	0.097	0.32
Limonene	A=0.930C+0.016	0.02–1.72	0.9998	0.0044	0.015
Geranyl acetate	A=12.000C−1.458	0.36–7.16	0.9995	0.099	0.33

Table 3
Analytical results of spiked samples.

Sample	Hexyl acetate					Limonene					Geranyl acetate				
	Content (μg mL^{-})	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD (%)	Content (μg mL^{-1})	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD (%)	Content (μg mL^{-1})	Added	Found (μg mL^{-1})	Recovery (%)	RSD (%)
1	0.70	0.88	1.71	115.0	1.5	0.72	0.88	1.71	114.1	2.4	1.43	0.88	2.26	92.4	4.2
2	0.33	0.86	1.05	82.2	2.8	0.29	0.86	1.11	94.5	7.9	0.83	0.86	1.55	80.4	8.5
3	0.36	0.89	1.36	113.2	8.1	0.20	0.89	0.98	91.2	9.1	0.98	0.89	1.70	81.1	6.7

Table 4
Comparison of the present method with other methods.

Extraction method	Matrices	Device, material and reagent	Analytes	Extraction time (min)	Extraction temperature (°C)	Analytical method	LOD/LOQ ($\mu g\ mL^{-1}$)	Ref.
HS-SPME	Citrus juice	SPME fibre	Volatile	120	40	Qualitative	–	[41]
Headspace—HRGC/MS	Apple juice	Turbo matrix 40 headspace trap	Aroma	101.9	100	Quantitative	0.0012–0.0205/ 0.003–0.0616	[42]
SDE	Grape juice	SDE apparatus, dichloromethane	Volatile	90	60	Qualitative	–	[43]
SBSE	Grape juice	Tir bar, acetone	Volatile	120	20	Qualitative	–	[43]
HSME	Orange juice	*n*-Hexadecane and benzyl alcohol	Volatile	36	70	qualitative	–	[44]
PV	Orange juice	Pervaporation module	Flavour	5	60	Qualitative	–	[45]
UN-HS-IL-SDME	Fruit juice	Ionic liquid	Flavour	25	80	quantitative	0.0044–0.099/ 0.015–0.33	This work

applied for the extraction of volatile compounds, the complex sample matrices, such as most foods, could damage the fibre, which is expensive. In these methods reported, HS-SPME, SDE, SBSE, HSME, and PV were only applied to the qualitative analysis of the volatile compounds. In HT, SDE, SBSE, and PV the special device was used. Compared with these methods, the present method was simpler in operation and the special device is not required. Compared with HS-SPME, HT, SDE, SBSE, HSME, the extraction time is shorter, when the present method was applied.

4. Conclusions

In this study, UN-HS-IL-SDME for extraction and concentration of flavour compounds from fruit juices was developed. First the hydrophilic IL was used as extraction solvent and the analytes were extracted from the sample into the IL phase. Then *n*-hexane was used as extraction solvent and the target analytes were extracted from IL phase into *n*-hexane because the *n*-hexane is suitable for the GC system. Using the IL-based system prior to the GC system, the contamination of the GC column was prevented. By heating the extraction vessel, the transfer of the semivolatile compounds from the sample to the microdrop was improved. Moreover, in the present method large volume of ionic liquid was used as extraction solvent. The results indicated that UN-HS-IL-SDME is a feasible and alternative method for extracting flavour compounds from fruit juices.

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