Optimization of headspace sampling using solid–phase microextraction (SPME) for volatile components in Starfruit juice

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ARTICLE INFORMATION

ABSTRACT

Volatile compounds are important flavor compounds of fruit juices and beverages. In this study, a headspace solid-phase microextraction (SPME) gas chromatography-mass spectrometry (GC-MS) was used to analyze volatile components in starfruit juices. Several experimental parameters (e.g., adsorption temperature, adsorption time and sample volume) were optimized to improve sampling efficiency in two aspects: maximum adsorption and selective adsorption of volatile components onto SPME fiber. The following conditions were found to be optimal for selectivity and sensitivity: adsorption temperature of 50°C for 30 min with a 65 μm divinylbenzene/polydimethylsiloxane (DVB/PDMS) coated fiber and a sample volume of 15 g in a 30 ml vial. The proposed technique could be applied for the analysis of volatile compounds that contribute to starfruit juice flavor in different cultivars and also their ripening stages.

Keywords
Optimization
SPME
Starfruit
Volatile compounds
GC-MS

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1. Introduction

Starfruit or carambola is a popular tropical fruit of the east. Carambola is sweet and slightly acidic, succulent and juicy with attractive flesh and distinctive flavour. The carambola (Averrhoa carambola L.) belongs to the family Oxalidaceae. They are cultivated in many tropical and subtropical countries in the world, like Indonesia, Israel, Malaysia, Florida and countries from Central America (Pino, Marbot, Rosado, & Bello, 2004). The fruit is fleshy, very attractive in appearance, and very particular in shape with 5 longitudinal ribs, which provides a star shape in the cross section. Its skin is thin, waxy and yellowish. The flesh is light to dark yellow, without fiber and very juicy. Five color indices are used to indicate different stages of maturity for carambola fruit, namely: color index 1: green; color index 2: trace of yellow to less than 25% yellow; color index 3: 25% to 75% yellow; color index 4: 75% to 100% yellow; color index 5: full orange or golden yellow. The color indices 1 and 2 refer to as unripe fruit, 3 and 4 as ripe fruit and 5 as overripe (Law & Abdullah, 1984). The flavor is variable and ranges from light sour to sweet. The fruit is low in calories and a good potassium source. Furthermore, these fruits are often relatively inexpensive. Carambola is rich in vitamins such as Vitamin A and Vitamin C with more than 25 mg per 100 g fresh fruit (Siong, 1985). They are usually eaten fresh and also served as fresh juices or used as flavored ingredients in juice blends. However, to improve and widen its marketability, especially for the export market, efforts have been made to produce value added products from the fruit. Among the potential products which are expected to receive

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favourable response amongst the consumers is clarified juice.

SPME offers certain advantages over other sampling techniques. It is inexpensive, avoids the use of solvents and does not require extended sample preparation, resulting in time saving (Roberts, Pollien, and Milo, 2000). During headspace analysis by SPME, exhaustive adsorption does not occur; instead, equilibrium is reached between the matrix and the stationary phase coating the fibre (Zhang and Pawliszyn, 1993). There are many factors that affect SPME fibre performance, such as the choice of stationary phase and adsorption conditions (Prosen and Zupean Č-Krajl, 1999). Several types of stationary phase of different thicknesses and polarities are available. Generally, a polydimethylsiloxane (PDMS) phase is preferable for non-polar analytes and a polyacrylate phase for more polar compounds. The PDMS phase is available in various thicknesses, with a thicker phase being more suitable for volatile compounds and a thinner phase more efficient for larger molecules. There are also other more selective phases such as Carboxen and divinylbenzene that are used with PDMS and are more suited for the analysis of low-molecular-weight volatile compounds. It is necessary to take into account that competitive effects between volatile compounds may skew the results when one compound with a high affinity to the fibre is present at high concentration (Roberts, Pollien, and Milo, 2000). The use of SPME to analyse the volatile compound composition of various foods and drinks has been widely reported (Marsili, 1999; Bazemore, Goodner and Roeself, 1999; Elmore, Erbahadir, and Mottram, 1997; Chin, Bernhard and Rosenberg, 1996).

SPME is an extraction technique based on the equilibrium among a three-phase system that included sample either in liquid or solid form, headspace above the sample, and the fiber coating. The amount of analyte extracted by the fiber coating, \( n_f \), can be expressed as Equation 1:

\[
\frac{n_f}{V_s} = \frac{K_{fs} V_f C_o}{K_{fs} V_f + K_{hs} V_h + V_s}
\]

where \( K_{fs} \) is the distribution constant or partition coefficient of an analyte between the fiber coating and the sample phase; \( K_{hs} \) is the partition coefficient of an analyte between the headspace and sample phase; \( V_f \), \( V_h \) and \( V_s \) are the volumes of the headspace, sample and fiber coating respectively; \( C_o \) is the initial concentration of the analyte in the sample (Zhang and Pawliszyn, 1993). Efficiency of SPME is influenced by the factors mentioned in this relationship.

Flavour is critical factor in evaluation of fruit products (Shewfelt, 1986; Askar, et. al., 1993). According to Shewfelt, 1986, flavour is constituted of taste and aroma components. Other researchers also stressed that fruit flavour is a vital attribute which makes the juice a desirable ingredient for many formulated beverages and products (Chen, et. al, 1982; Chen, Wu and Wu, 1987; Yu and Chiang, 1986; Lue and Chiang, 1989; Arjona and Matta, 1991) and hence serves as a natural concentrate due to its unique flavour and high acidity. The unique flavour is attributed to several volatile compounds (Shimbamoto and Tang, 1990). Therefore it is important to identify the volatiles in the fruit juice.

In this study, experimental parameters (adsorption time, adsorption temperature and sample volume) were optimized for SPME headspace sampling in the determination of volatile compounds in starfruit juice samples.

2. Materials and Methods

2.1 Chemicals

Methanol GC grade diluent was purchased from Fischer Scientific (UK) while sodium chloride salt (NaCl) was obtained from Merck (Germany). An internal standard solution (IS) of propyl butanoate (Sigma-Aldrich, MI, USA) was prepared at a concentration of 1000 mg/L and stored at 4°C prior to analysis.

2.2 Starfruit juice preparation

Fresh carambola fruits (Carambola Averrhoa L.) were obtained and purchased from a local market, Selangor, Malaysia. Color index 3 (25% to 75% yellow) was chosen for the ripeness of the fruit in Kajang for this study. 500g ripe carambola fruits were deseeded and processed using a juice extractor (Model PJ-39, Pensonic, Malaysia) to obtain 350g juice. 15g starfruit juice was then transferred into a 30ml glass vial containing 5g of NaCl. The vials were subsequently sealed with PTFE-silicon septa (Supelco, Bellefonte, PA, USA) after spiked with 15μg propyl butanoate solution as internal standard in the analysis.

2.3 SPME

The SPME used in this study was the manual type, comprising of a SPME fiber holder, and a 65 μm divinylbenzene /polydimethylsiloxane (DVB/PDMS) coated fiber (Supelco, Bellefonte, PA, USA). The fiber was provided attached to a stainless steel plunger sheathed by a protective needle. During analysis the needle passes through the septum of the sample container and is depressed to expose the fiber to the headspace for analyte adsorption. After a specified period of incubation time, the fiber is retracted into the needle and then ejected from the sample vial prior to being inserted into the GC injector port for analytes desorption onto the column. All fibers were preconditioned in the injection port of the gas chromatograph according to the instructions provided by the supplier, prior to analysis.
2.4 Chromatography
The samples were analysed using an Agilent model 6890 GC system fitted with a fused silica capillary column. The injector was maintained at split mode with ratio 10:1 under 250°C. Helium gas with purity 99.99% was used as carrier gas at 1.0 ml/min constant flowrate. Oven temperature was programmed as follow: 40°C initially for 1.5 minutes, then increased to 240°C at 50°C/min and held for 2 minutes.

A Leco Pegasus III time-of-flight mass spectrometer which connected with GC through a transfer line heated at 220°C was used for the detection of volatile compounds. The analyte ionization was programmed at 70eV electron impact at 200°C. 1500V detection voltage and 30 spectra per second data acquisition rate with a mass range of 33 to 400 m/z. Acquired data was processed using Chromatof software while the mass spectral identification were made based on mass spectral library from National Institute of Standards and Technology (NIST) version 2.0. The relative amounts of volatile compounds were expressed as their peak area ratio to peak area of the internal standard (1 µg per g propyl butanoate), using a response factor of 1.

The sample vials were equilibrated for 10 min at 50°C under magnetic stirring, followed by fiber exposure to the headspace of the solution to adsorb the analytes for 30 min at 30°C. The effects of adsorption time (15 min, 30 min, and 60 min), adsorption temperature (30°C, 50°C, and 65°C) and sample size (1g in 2ml vial, 5g in 10ml vial, 15g in 30ml vial) with sample to headspace ratio maintained at 1:2 were examined for six selected volatile compounds. Also, the salt addition was investigated. Subsequently the stainless steel needle in which the fiber is housed was pushed through the vial septum, allowing the fiber to be exposed to the headspace of the sample. The fiber was then pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. Sample vials were prepared in triplicate from the similar batch.

3. Results and Discussion
3.1 Effect of SPME variables
SPME with headspace sampling was necessary in the this study in order to avoid any interfering contaminants such as sugars (fructose and sucrose in fruit) and protein existing in the durian homogenates that would also deteriorate the efficiency of fiber coating (Scheppers Wercinski and Pawliszyn, 1999). To investigate the effect of the SPME variables, major volatiles in starfruit that included methyl butanoate, 2-hexenal, methyl hexanoate, methyl heptanoate, methyl octanoate and methyl benzoate were monitored.

3.1.1 Effect of fiber adsorption time and temperature
The reproducibility and sensitivity of headspace volatile compounds analysis by SPME technique are greatly influenced by the vapor pressure of the volatiles in the vial. Temperature and time are the two most important factors that affecting the vapor pressure and equilibrium of the volatile compounds in the vial sample (Liu and Yang, 2002).

Figure 1 shows the effect of adsorption time on the adsorptivity of the selected volatile compounds from the starfruit juice. The efficiency of the adsorption are displayed as the peak areas of selected volatile compounds after different exposure times of the fiber to the starfruit juice sample headspace for 15, 30, and 60 min. For this study, adsorptivity is defined as the ability of the volatile compounds to adhere to the SPME fiber under the conditions studied and was measured using GC-MS detector response peak area (Achouri, Boye and Zamani, 2006). The rate of adsorption was highest for 60 min of fiber exposure for methyl butanoate, 2-hexenal, methyl hexanoate, methyl heptanoate, methyl octanoate and ethyl benzoate. These results further showed that for all of these volatile compounds only reached their adsorption capacity after 30 min. For practical applications, a 30 min adsorption time might, therefore be appropriate to be chosen due to rapid adsorption and isolation purposes of all selected volatile compounds from the starfruit juice sample.

The effect of adsorption temperature on the adsorptivity on three selected temperature of 30, 50, and 65°C are shown in Figure 2. Maximum adsorptivity was observed for temperature 50 and 65°C. Desorption of volatile compounds from the fiber with increasing temperature during headspace SPME analysis has already been reported and been interpreted to result from a competition on the fiber between the different compounds released in the headspace ((Achouri, Boye and Zamani, 2006; Lambropoulou and Albanis, 2002; Lecanu, et. al., 2002; Liu and Yang, 2002). Hence, higher adsorption temperature increased the liberation of volatile compounds into headspace and consequently enhanced the adsorption by SPME but reduced the adsorption of volatile compounds with smaller molecules included methyl butanoate and methyl hexanoate. An adsorption temperature of 50°C, therefore appears to be optimal for the recovery of the all the volatile compounds been studied.
Figure 1. Effect of adsorption time on the adsorption efficiency of PDMS/DVB fiber in the starfruit juice sample.

Figure 2. Effect of adsorption temperature on the adsorption efficiency of PDMS/DVB fiber in the starfruit juice sample.

3.1.2 Effect of sample size and salting out

Three different vial sizes were tested for the sample size effect but the highest amount of volatiles was extracted using a 30ml vial that contained 15g starfruit juice homogenate (Figure 3). Lesser amount of volatiles extracted from the 5g sample in a 10ml vial was expected since \( n_f \) is less when \( V_s \) is smaller. Although larger sampling size, \( V_s \), would enhance SPME sensitivity, the amount of extracted volatiles was less when 50g of sample was kept in a 100ml vial at similar sampling period. The greater the sample size, the greater the quantity of analytes that will be adsorbed onto the fiber, and shorter time for an equilibrium to be reached (Achouri, Boye and Zamani, 2006). Larger sampling size with similar relative volumes of headspace and sample, \( V_s/V_h \), is not recommended because longer time is needed for equilibrium among the phases.
(Eisert and Pawliszyn, 1997) even though sampling with a larger amount is more representative. For low volatility fractions, a larger sample volume is much preferred for better detection and quantification (Achouri, Boye and Zamani, 2006). Similar findings have been reported elsewhere (Achouri, Boye and Zamani, 2006; Matisóva, et. al., 2002). Therefore, sample volume of 15 g starfruit juice sample in 30 ml vial was chosen.

Figure 3. Effect of sample size on the adsorption efficiency of PDMS/DVB fiber in the starfruit juice sample.

Dissolution of salt into sample matrix is a way to enhance the $K_{hs}$ of polar compounds (Lee et al., 2003; Rocha et al., 2001; Steffen and Pawliszyn, 1996; Penton, 1999). To study the salt addition and stirring effect, 5 g of NaCl salt was dissolved to saturate the sample matrix while 5 g of distilled water was added to act as a control. Figure 4 shows that increment of the extracted amount was observed in all six analytes. Consequently, the solubility of volatile compounds in the matrix decreased and the $K_{hs}$ value declined saturated with salt. When salt was dissolved into the matrix that contained water, electrolytes existed (Yang and Peppard, 1994). Salting out of volatiles from the matrix had altered the volatiles distribution in headspace and thus the equilibrium phases between fiber coating and headspace accordingly. Absorption of propanethiol and diethyl disulfide by the fiber coating declined due to the competition of other volatiles with greater concentration distributed in the headspace (Zhang and Pawlisyn, 1993). Meanwhile, sodium salt showed a greater improvement for the SPME absorption as electrolytes from sodium have higher affinity with negative ions in water than from calcium and so better salting out effect was observed. A similar effect was also reported by Lee and coworkers (2003).

3.2 SPME analysis of starfruit juice volatiles

Based on the results obtained, the following conditions were selected for the analysis of volatile compounds in the freshly prepared starfruit juice samples: adsorption temperature of 50°C, adsorption time of 30 min and a sample volume of 15 g of starfruit juice in a 30 ml vial.

Table 1 lists the volatile compounds identified in the freshly prepared starfruit juice samples in two different column namely, DB-5(10m×0.18mm i.d.,0.18µm film) from J&W Scientific, Folsom, CA, USA at 1.0 ml/min constant flowrate and Supelcowax-10 (10m×0.1mm i.d.,0.1µm film) from Supelco, Inc., Bellefonte, PA, USA which with constant flowrate of 0.4 ml/min. A J&W DB-5 column is nonpolar, low bleed column and excellent general purpose column for wide range of applications. The Supelcowax 10 is a general purpose polar column that offers a highly polar and bonded phase. A total of more than 15 volatile compounds were identified. The main constituents of volatile compounds been identified were ester groups followed by alcohols, aldehydes and carotenoid precursor (Macleod and Ames, 1990).

Conclusion

In conclusion, the effect of several experimental parameters (e.g. adsorption temperature, adsorption time and sample size) on the efficiency of SPME headspace sampling for starfruit juices were studied. The results obtained have demonstrated that for starfruit juices, a good optimal conditions is to perform the SPME analysis using an adsorption temperature of 50°C for 30 min with a 65 µm divinylbenzene /polydimethylsiloxane (DVB/PDMS) coated fiber with and sample volume of 15 g in a 30
The technique used is very convenient and fast. This technique, SPME, thus can be used to characterize and monitor quality changes of volatile compounds that contribute to starfruit juice flavor in different cultivars and also their ripening stages.

Figure 4. Salting-out effect on SPME sensitivity

Table 1. Identification of volatile compounds in starfruit juice using SPME GC-MS using different types of column

<table>
<thead>
<tr>
<th>DB-5</th>
<th>SupelcoWax-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 2-methylpropanoate</td>
<td>Methyl butanoate</td>
</tr>
<tr>
<td>Methyl butanoate</td>
<td>Methyl hexanoate</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>Ethyl hexanoate</td>
</tr>
<tr>
<td>Methyl isobutyl ketone</td>
<td>Methyl heptanoate</td>
</tr>
<tr>
<td>2-Hexen-1-ol</td>
<td>2-Hexenyl acetate</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>Methyl octanoate</td>
</tr>
<tr>
<td>Methyl hexanoate</td>
<td>Methyl octanoate</td>
</tr>
<tr>
<td>1-hepten-3-ol</td>
<td>2-Hexen-1-ol</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>1-hepten-3-ol</td>
</tr>
<tr>
<td>2-Hexenyl acetate</td>
<td>Megastigma-4,6,8-triene</td>
</tr>
<tr>
<td>Methyl heptanoate</td>
<td>Methyl benzoate</td>
</tr>
<tr>
<td>2-ethyl-1-hexanol</td>
<td>Ethyl benzoate</td>
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<tr>
<td>Methyl benzoate</td>
<td>Ethyl benzoate</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>Ethyl benzoate</td>
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<tr>
<td>Nonanal</td>
<td>Ethyl benzoate</td>
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<td>Ethyl benzoate</td>
<td>Ethyl benzoate</td>
</tr>
<tr>
<td>Methyl Salicylate</td>
<td>Ethyl benzoate</td>
</tr>
<tr>
<td>Megastigma-4,6,8-triene</td>
<td>Ethyl benzoate</td>
</tr>
</tbody>
</table>

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