Pertanika J. Sci. & Technol. 25 (S): 167 - 174 (2017)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Optimization of Headspace Solid Phase Microextraction (HS-SPME) for the Extraction of Volatile Organic Compounds (VOCs) in Mangoes (*Harumanis* cv.) Using 2 Stages Multivariate Analysis

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ABSTRACT

Headspace solid phase microextraction (HS-SPME) was used to isolate volatile compounds (VOCs) from mangoes (*Harumanis* cv.). Among the four SPME fibres investigated, the mixed phase coating, 65 µm polydimethyl siloxane–divinylbenzene (DVB/PDMS) showed the highest efficiency in extracting VOCs as 26 compounds were detected with the total area of 9.6 x 109. The optimization of SPME factors was conducted in 2 stages using multivariate design. The first stage involved screening of the significant factors using the Plackett–Burman (P–B) design, followed by the optimization of the significant factors utilizing Central Composite Design (CCD). The experimental design for both P-B and CCD design was generated using Design-Expert version 6.0.4 (Stat Ease Software). Extraction time and temperature appeared to be the most significant factors in extracting VOCs in mangoes, with the optimum conditions prevailing at 55°C and 34 minutes respectively.

Keywords: CCD, *Harumanis*, HS-SPME, VOCs

ARTICLE INFO

Article history:
Received: 28 September 2016
Accepted: 03 February 2017

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ISSN: 0128-7680

INTRODUCTION

Mango (*Mangifera indica* L.) is a popular fruit that is rich in nutrients and a desirable taste, belonging to the Anacardiaceae family it is also known as the King of Fruits due to its aroma and sweetness. The pleasant aroma and flavour of the fruit is derived from various volatile compounds such as esters, lactones, monoterpenes and sesquiterpenes as identified in previous studies (Ansari et al., 2004; Kulkarni et al., 2012).

The volatile organic compounds (VOCs) refers to the molecules that have appreciable vapour pressure at room temperature with molecular weight lesser than 300 Dalton (El Hadi et al., 2013). The VOCs can be used to develop a volatile profile of mangoes and used for authentication and quality control studies (Shyam et al., 2013). The isolation of VOCs in fruit is crucial as the VOCs are generally present in trace amounts and the complexity of the fruit as the matrix. Additionally, VOCs are also thermolabile requiring rapid and effective isolation technique that can extract, concentrate and clean them in a single step (Cheng et al., 2011).

Solid-phase microextraction (SPME) has proven to be a useful extraction method that is rapid, versatile and simple (Pawliszyn, 1999). Previous studies (Laohaprasit et al., 2011; Liang et al., 2012; Chidley et al., 2013) reported that use of SPME to extract VOCs in fruit matrix, however there is no clear conclusion on the choice of fibre especially in non-targeted analysis. Thus, it is important to find the most efficient fibre prior to optimization study. Apart from using the most efficient fibre, there are many other factors such as extraction time, extraction temperature, stirring effect, desorption temperature and time, sample amount, and salt addition t may affect the efficiency of HS-SPME (Ouyang & Pawliszyn, 2008). Optimizing these factors is crucial in order high SPME efficiency in extracting VOCs from mangoes is achieved.

Optimisation is a process of balancing several aspects of a system to attain the best result for a set of criteria. Traditionally, the optimization of SPME was done using univariate optimization method; each factor being optimized and studied in isolation (Massart et al., 1997). The drawback of this technique is it leaves interaction between factors unexamined. Multivariate design is able to overcome this shortcoming by using experimental software to identify the significant factors of the SPME parameters and thereby maximize the response of an experiment. Thus, in addition to saving time as the number of experiments done can be cut down, the chromatographic response of the analytes can be improved as interference can be eliminated (Miller & Miller, 2010). One of the widely used multivariate designs to optimize extraction of VOCs in food is the Response Surface Methodology (RSM) (Stalikas et al., 2009). To directly optimize all the factors that may affect the efficiency of SPME screening using Plackett-Burman (PB) should be done, thus reducing number of experiments to estimate the significant factors (Scheppers, 1999).

The aim of this study is to initially screen the significant factors affecting HS-SPME extraction of VOCs in *Harumanis* mangoes using PB design and optimize the significant factors through RSM.

METHOD

Chemical reagents

All chemicals used in this study were of analytical quality and all solvents were HPLC grade. Deionized water was obtained from a Mili-Q water purification system (Milipore, Bedford, PA, USA). Sodium chloride used in screening experiments was supplied by Merck (Darmstadt, Germany). All the SPME fibres used together with clear glass screw cap vials (PTFE/silica septa) were purchased from Supelco.

Sample preparation

Ripe *Harumanis* mangoes were obtained from local orchards in Perlis, Malaysia. For the volatile analysis of homogenized mango pulp, fresh mango will be sliced and homogenized using a blender. The homogenized mango pulp was stored in a freezer with temperature less than 0˚C and analysed in triplicate.

Fibre selection

4 commercially available SPME fibres differing in the thickness and solvent phase coating were tested and compared. The tested fibres were non-polar 100 ɱm polydimethyl (PDMS), semi polar fibres, 65 ɱm polydimethyl siloxane-divinylbenzene (PDMS-DVB) and 75 ɱm CAR-PDMS and also polar fibre, polyacrylate (PA) to study the efficiency of different fibre in extracting the aroma volatiles. Prior to extraction, each of the chosen fibre was conditioned at the GC injection port according to the guidelines given by the manufacturer. The criteria used to select the best fibre is the fibre with the highest total area and the highest number of compounds detected.

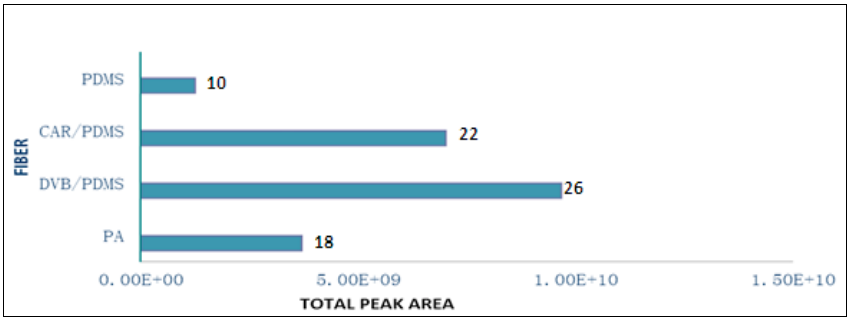

Figure 1. Efficiency of each fibre on the VOCs extraction by HS-SPME

SPME optimization

The optimization of SPME was done in 2 stages; screening stage and optimization stage using multivariate analysis.

Screening of significant factors

The screening of SPME significant factors was carried out using Plackett–Burman (P–B) design matrix with a 2^{7-4}. Seven factors were selected for the screening stage using 2^{7-4} Plackett–Burman design matrix include: extraction temperature (30°C and 60°C), time (10 min and 40 min) salt addition (0 g and 1 g), stirring rate (0 and 500 rpm), desorption time (1 min and 5 min) and desorption temperature (100°C and 240°C). The significant factors given by P-B design were further optimized using RSM.

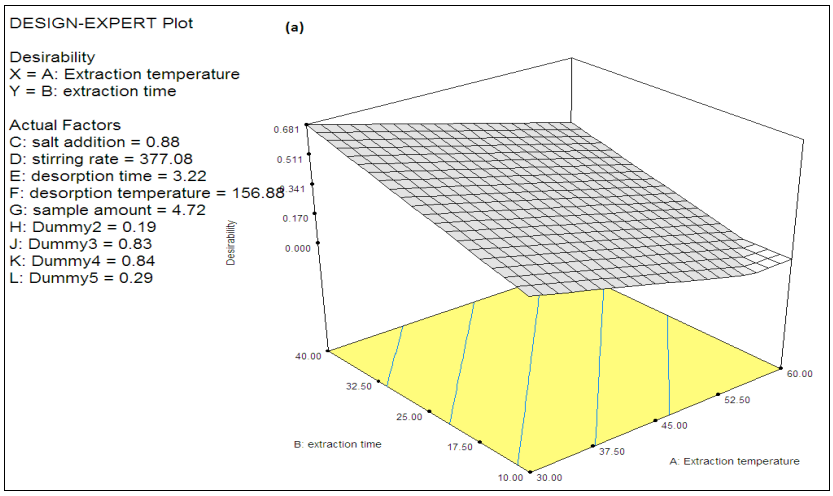

Figure 2. 3D surface plot from Plackett-Burman design

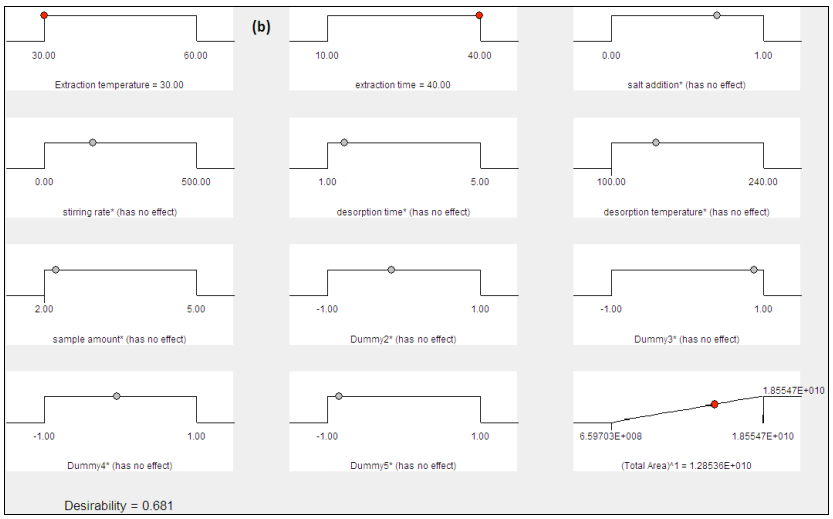

Figure 3. The ramps from Plackett-Burman design

Optimization of significant factors

The extraction temperature and time found to be significant in the screening stage was further optimized using RSM (Central Composite Design). Three responses will be evaluated, that is, total area, trans-beta ocimene and 2, 4, 6-octatriene. Trans-beta-ocimene and 2,4,6-octatriene was selected as the response for the CCD design in view of their having recorded the highest frequency of occurrences in early preliminary studies.

GC-MS conditions

The volatile compounds were analysed using a GC- MS system (Agilent Technologies 5977 Inert Mass Selective Detector and Agilent 7890B) under the following operating conditions: HP-5MS capillary column (Agilent 19091S-433, 0.25 mm 30 m 0.25 m) was used. GC/MS detection was done using ionization energy of 70 eV in the 50– 550 a.m.u. mass range. Column temperature was increased from 40°C to 250°C with temperature program: starting at 40°C withhold time 2mins, to be subsequently increased 150°C (5°C min−1) with hold time of 1min. Finally, the temperature was increased to 250°C (10°C min−1) with hold time of 5 mins and total run time of 40 minutes. The injector temperature and mode was 280°C with split ratio of 1:00. The ion source temperature was 230°C and the interface temperature was 280°C. At a flow rate of 1.2 mL/min, Helium was used as the carrier gas. The VOCs in *Harumanis* mangoes were identified by comparing their spectra with those available in the NIST14 digital library.

Experimental design approach

The experimental designs for both screening and optimization stages were generated using Design-Expert version 6.0.4 (Stat Ease Software).

RESULTS AND DISCUSSIONS

Fibre selection

Different kinds of analytes may require different type of SPME fibres as each fibre provides different absorption/adsorption properties. To use the correct fibre is very important for the SPME method to ensure highest efficiency can be achieved. Therefore, to obtain the highest efficiency for the extraction of VOCs from mangoes using the HS-SPME-GCMS, 4 commercially available SPME fibre differing in the thickness and solvent phase coatings were tested (PDMS, PDMS/DVB, CAR/PDMS and PA). The overall efficiency of the selected SPME fibre was assessed by examining the total area of GC-MS peaks, in addition to examining the total area, the number of VOCs detected from each of the fibre was assessed.

As shown in Figure 1, DVB/PDMS fibre recorded the highest total peak area of 9.6×10^9 besides also showing that the most number of VOCs recorded i.e. 26 peaks was chosen as the most efficient fibre in this study, and used for the optimization study using PB and RSM. A study of VOCs extraction from tomato plant using SPME (Sousa et al., 2006) also showed the same trend. This may due to the fact that DVB part of DVB/PDMS fibre consist of wide pores (meso and macro) that may contribute to fast equilibrium and lead to the efficient extraction. The results show both mixed phase coatings (DVB/PDMS and CAR/PDMS) recorded higher total area when compared with single coating fibres (PA and PDMS). Mixed phase coatings expressed complementary properties in contrast with single phase films because they managed to adsorb a broad range of analytes with different chemical properties.

Screening of significant factors

The screening of SPME significant factors were carried out using Plackett–Burman (P–B) design matrix with a 2^{7-4}. Seven SPME factors (extraction temperature, extraction time, salt addition, stirring rate, desorption time, desorption temperature and sample amount) were tested with two runs of high and low level for each factor.

According to the results shown in Figure 2 and Figure 3, the significant factors for this study were extraction temperature and extraction time. As presented in the 3D surface plot of Figure 2, as the extraction temperature and extraction time increased from low value to high value extraction efficiency also increased (Abdulra'uf et al., 2015). Other factors such as the addition of salt, sample size, stirring rate, desorption time and desorption temperature showed no significant effect, allowing it to be fixed at any value, since their adjustment will have equal effect on extraction efficiency. Clicia et al. (2014) obtained a similar result when using fractional factorial design (2^{4-1}) to find the most HS-SPME significant factors in extracting VOCs from varieties of mangoes in Brazil. Hence, only these two factors were further evaluated in the optimization process by means of the Response Surface Methodology (CCD).

Optimization of Extraction Temperature and Time

In the first stage of the optimization method, PB design indicated that only extraction temperature and extraction time were the significant factors that needed to be further optimized using Central Composite Design in RSM. 14 experiments were run in 3 blocks of points and the CCD experiments were run in a random manner to reduce the effect of uncontrolled variables. Three responses were used to optimize the extraction time and temperature for the total area of all detected compounds, area of trans-beta ocimene and also the area of 2,4,6- octatriene. Trans-beta ocimene and 2,4,6- octatriene was chosen as the response due to the fact that these two compounds recorded the highest frequency of occurrences in preliminary experiments (data not shown here) including the fibre selection experiment. The results obtained from CCD experiments are summarized in Table 1.

Table 1
Summary of Central Composite Design for HS-SPME of VOCs in mangoes

Response	Transform	Model	Lack Of Fit	R^2	Equation	Significant
Total area	Square root	Quadratic significant	Not significant	0.8305	Sqrt(TotalArea) = +71264.06+16957.4A+30761.33 B-12686.95A2-28439.83B2-4612.69AB	A^2, A
Trans-beta ocimene	None	Quadratic significant	Not significant	0.8826	Trans-beta ocimene = +5.429E+008+1.858E+008A+ 4.354E+008B-5.723E+007A2-3.873E+ 008B2-4.381E+007AB	A, B^2
2,4,6-octatriene	Square root	Linear significant	Not significant	0.5317	Sqrt(2,4,6,octatriene) = +13568.79+2383.14A-1222.11B	A

The value of coefficient of determination (R^2) for the total area of the compounds, trans-beta-ocimene and 2,4,6-octatriene was 0.8305, 0.8826, and 0.5317 respectively. The ANOVA showed that the statistical analysis of the data was significant ($p<0.05$). In addition, there was a non-significant ($p>0.05$) lack of fit in all the 3 responses which validate the models. In optimizing the extraction conditions, the goals were set at the maximum level of total area of the compounds, trans-beta-ocimene and 2,4,6-octatriene. Extraction time of 34 minutes and extraction temperature of 55°C are found to be the optimum conditions with desirability of 0.992. The value of desirability higher than 0.80 and approaching unity is good. The total area of the compounds obtained from this study also increased with temperature but it started to decrease after extraction temperature reached 55°C as shown in Figure 4. Trans-beta ocimene also recorded the same trend.

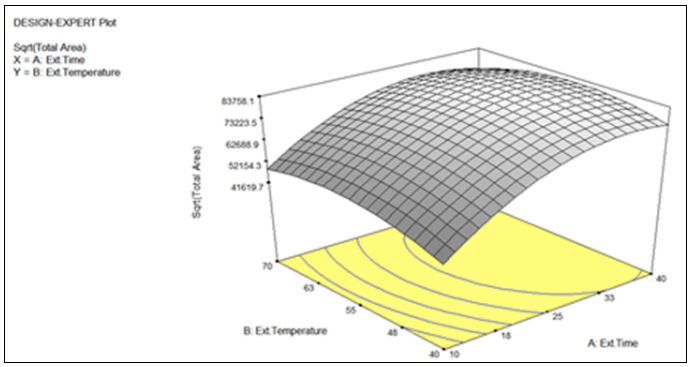

Figure 4. 3D surface plot for total area from Central Composite Design

CONCLUSION

Mixed coating phase, DVB/PDMS has demonstrated to be an efficient fibre in isolating VOCs from mangoes. Results from this study also indicated the successful utilization of the two-stage multivariate analysis to screen and optimize the significant factors for HS-SPME extraction. Total area of compounds found from the optimization results along with trans-beta ocimene and 2,4,6-octatriene were significantly influenced by extraction temperature and time with optimum conditions of 55°C for 34 minutes respectively with 0.992 desirability.

ACKNOWLEDGEMENTS

Authors are grateful to the Ministry of Higher Education, Malaysia for funding this study (Project number: 600-IRMI/FRGS 5/3 (70/2016)).

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