

## Characterization of Agarwood Incense using Gas Chromatography – Mass Spectrometry (GC-MS) coupled with Solid Phase Micro Extraction (SPME) and Gas Chromatography – Flame Ionization Detector (GC-FID)

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### ABSTRACT

*This paper presents the application of Solid Phase Micro Extraction (SPME) coupled with Gas Chromatography – Mass Spectrometry (GC-MS) and Gas Chromatography – Flame Ionization Detector (GC-FID) in characterizing the agarwood incense. The work involved three types of SPME fibres at 30 minute sampling time. The fibres are 50/30  $\mu\text{m}$  divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS), 65  $\mu\text{m}$  polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 85  $\mu\text{m}$  carboxen-polydimethyl siloxane (CAR-PDMS). The results showed that among the many compounds extracted by GC-MS coupled with SPME, six compounds were substantially found in high quality agarwood incense due to their high percentage area (%). They are  $\beta$ -maaliene,  $\alpha$ -elemol,  $\beta$ -selinene, 10-epi- $\gamma$ -eudesmol, agarospirol and caryophellene oxide. The finding offers a new approach for establishing the volatile profile of agarwood incense components as well as for agarwood grading and discrimination.*

**Keywords:** agarwood incense, high quality, GC-MS coupled with SPME, GC-FID.

### INTRODUCTION

Agarwood or gaharu is resin-impregnated heartwood of the plant genus *Aquilaria* (*Thymelaeaceae*). Agarwood commands a high demand in the perfumery industry as well as for the manufacturing of incense, soap and shampoo [1]. Its essential oil is very popular, notably in the European countries and Egypt [2]. Agarwood beads and bracelets are valuable 'natural art' in Japan, Korea and Taiwan [1].

Agarwood is traded depending on its quality. It has been recognised that chemical profiles affect the quality of agarwood oil [3]. Typically, agarwood quality is graded based on their physical properties such as colour and odour. However, this grading method has drawbacks such as low productivity, time consuming and the result is not standardized as grading is done by human trained grader [4]. In this paper, the characterisation of the agarwood chemical compounds is proposed as an initial study for the agarwood grading and discrimination.

Numerous methods may be used to extract volatile compounds from agarwood incense [2]. Solid phase micro-extraction (SPME) is one of the latest chromatography techniques to

extract volatile compounds from essential oils and incense [5]. The SPME is a simple sorbent extraction technique that is fast and does not require the use of solvents [6]. It produces particles which are small enough to allow mobility in a field [7]. The SPME is a substitute for conventional techniques of extracting volatile organic compounds. It combines the extraction of analytes from the sample matrix into the fibre coating and forwarded into an analytical instrument [8-10].

Some researchers have studied the effect of SPME fibre coating polarity and its extraction mechanism to identify volatile compounds in any type of matrix [11-13]. Another study was a comparison between the various types of fibre coatings; polydimethylsiloxane (PDMS), polyacrylate (PA), PDMS/divinylbenzene (DVB), PDMS/carboxen and DVB/carboxen/PDMS [14]. This study indicates that PDMS has a good sensitivity towards non polar compounds, and PDMS/DVB has better sensitivity towards polar compound of low volatility.

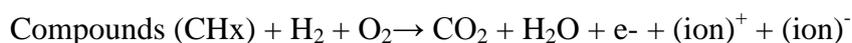
Several instruments, such as gas chromatography (GC) and High Performance Liquid Chromatography (HPLC) have been used to analyse compounds. The contemporary GC was developed by James and Martine in 1952. The GC is introduced to mixture analysis from near zero until over 700 K boiling point of compounds. It is a standard analytical method for research, development and quality control in many industries including petrochemical, drug and forensics [15].

Gas Chromatography-Flame Ion Detector (GC-FID) is an important analysis instruments especially in the natural product and perfumery industry, and is widely used to analyze essential oil, fatty acid and the terpene group monoterpene and sesquiterpene [16, 17]. The FID detector is commonly used in gas chromatography as a detector due to its sensitivity towards carbon-hydrogen bond molecule. Therefore, it is favoured in the analysis of general hydrocarbon chain bonds. FID has a stable response and avoids contamination or column bleed. Even though the process is straightforward and stout, the application of hydrogen diffusion flame to ionize compounds will destroy the sample during the process [17].

The purpose of this research is to resolve complex mixture of volatile agarwood smoke using the most effective technique of GC-MS coupled with SPME, followed by analysis using GC-FID. Section II of this paper presents the theory of FID and Kovalt Indices. Section III describes the methodology of this study, followed by results and discussion in Section IV and conclusion in Section V.

## THEORY

The theory of FID can be abridged as organic substances eluting from the column go through the degradation reaction in a Hydrogen (H<sub>2</sub>) – rich region, forming a group of single carbon (C) species. As the flow mixes at the reaction zone in the presence of oxygen (O<sub>2</sub>), the following reaction occurs [18]:



After combustion, ions are accumulated by a brace of polarized electrodes within the detector, producing a current that is amplified by the electrometer and switched into a voltage. The voltages are delivered by a recording gadget.

The retention times differ according to the column length, its film thickness, and diameter of the column, carrier gas velocity and pressure as well as annulled time. It produces different values when measured by different analytical laboratories [18].

Kovats Index can be typically demonstrated in a mathematic equation.

The first equation for isothermal chromatography is shown in Equation (1) [18]:

$$I = 100 \left[ n + (N - n) \frac{\log(t'_{r(\text{unknown})}) - \log(t'_{r(n)})}{\log(t'_{r(N)}) - \log(t'_{r(n)})} \right] \quad (1)$$

Where,

*I* = Kovats retention index,

*n* = the number of carbon atoms in the smaller alkane,

*N* = the number of carbon atoms in the larger alkane,

the number of carbon atoms in the smaller alkane,

*t'*<sub>r</sub> = the adjusted retention time.

Equation (2) is for temperature programmed chromatography [18]:

$$I = \left[ \frac{(t_{r(\text{unknown})}) - (t_{r(n)})}{t_{r(N)} - t_{r(n)}} \right] * (100 * z) + (100 * n) \quad (2)$$

Where,

*I* = Kovats retention index,

*n* = the number of carbon atoms in the smaller alkane,

*N* = the number of carbon atoms in the larger alkane,

*z* = the difference of the number of carbon atoms in the smaller and larger alkane,

*t*<sub>r</sub> = the adjusted retention time.

## METHODOLOGY

### A. Agarwood Sample Preparation

High quality agarwood chipwood used in this study was purchased from Gua Musang, Kelantan. A total of 100 g dry samples is ground into milled agarwood and re-dried (40 °C) in the oven until constant weight is obtained. The dried samples are then used for extraction.

### B. The SPME Method

The extraction of agarwood chipwood was done using the solid phase microextraction (SPME). The SPME equipment was purchased from Supelco Inc., Bellefonte, PA, USA. Three different types of fibres were chosen for extraction of volatile compounds; 50/30  $\mu\text{m}$  divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS), 65  $\mu\text{m}$  polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 85  $\mu\text{m}$  carboxen-polydimethyl siloxane (CAR-PDMS). As recommended by a previous study [19], the headspace volatile of incense is chosen for the extraction method. A total of 0.2 g milled sample is transferred into a 4 mL clear glass vial with a screw cap and PTFE and silicone septum (Supelco Inc., Bellefonte, P.A, USA). Then, the extraction is performed manually for 30 minutes, using a SPME holder (Fig. 1). These set-ups are applied for all three types of SPME fibres.

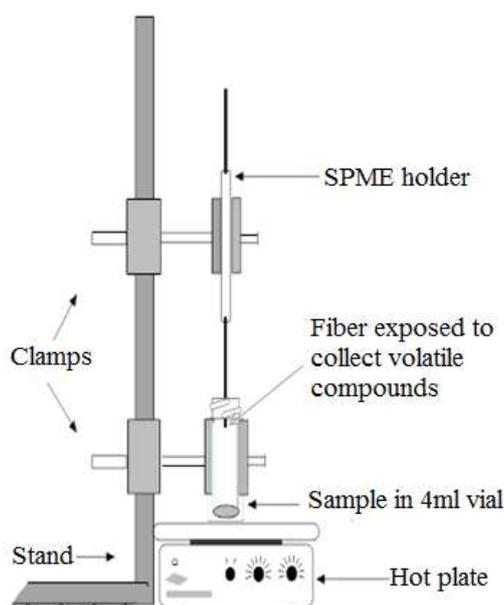


Fig. 1 The schematic diagram of headspace volatile for SPME

### C. The GC-FID Analysis: GC-MS coupled with SPME

In GC-FID analysis, the analysis of vapour is carried out by GC-MS coupled with SPME. Incense sample analysis was performed using an Agilent 7890 GC-FID, equipped with a 30 m long fused silica DB-1 capillary column (0.25 mm I.D., 0.25  $\mu\text{m}$  film thicknesses). Helium was used as a carrier gas with a flow rate of 1.2 ml/min. Injector and detector temperatures were set at 220  $^{\circ}\text{C}$  and 250 $^{\circ}\text{C}$ , respectively. The manual SPME injector was set to the splitless mode using a narrow SPME inlet liner. The oven temperature was programmed at 60  $^{\circ}\text{C}$  initially, and increased at a rate of 3  $^{\circ}\text{C}/\text{min}$  to a final temperature of 250  $^{\circ}\text{C}$  maintained for 5 minutes. The identification of compounds were done by comparing their retention time independent constant, namely, Kovats indices (KI) or Retention Indices (RI) with RI published data. The RI was calculated using a linear hydrocarbon (C<sub>8</sub>-C<sub>22</sub>).

## RESULT & DISCUSSION

Table I lists the chemical compounds present in high quality agarwood by using three kinds of SPME fibres, namely, CAR-PDMS, PDMS-DVB and DVB-CAR- PDMS for a 30- minute extraction, using GC-FID analysis. The observation showed that the highest contribution was from the oxygenated sesquiterpene group. These compounds;  $\alpha$ -elemol, caryophellene oxide, 10-epi- $\gamma$ -eudesmol and agarospirol were marker compounds in agarwood vapour due to their high content in agarwood especially in high quality agarwood.

Table I: Chemical composition (% relative peak area) of high quality agarwood vapour (Headspace volatile)

Compound	Extraction method	<sup>1</sup> RI	CAR-FIMS	FDMS-DVB	DVB-CAR-FIMS	Identification
<b>Monoterpene Hydrocarbons</b>						
fenylacetaldehyde		830	0.00	0.00	0.02	<sup>2</sup> RI
benzaldehyde		935	1.56	0.7	0.44	RI <sup>3</sup> MIS
phenol		956	0.11	0.02	0.00	RI
p-cymenol		1002	0.00	0.00	0.00	RI
pinenol		1062	0.05	0.1	0.06	RI
acetophenone		1066	0.00	0.03	0.01	RI
4-phenyl-2-butanone		1210	0.55	0.48	0.35	RI, MIS
p-vinylpinenol		1286	0.00	0.01	0.00	RI
3,4-dimethoxybenzal		1312	0.02	0.03	0.02	RI, MIS
vanillin		1367	0.23	0.26	0.15	RI
<b>Sesquiterpene Hydrocarbons</b>						
$\beta$ -cadinene		1414	1.08	1.20	0.86	RI, MIS
$\alpha$ -guaiene		1440	0.68	0.70	0.57	RI, MIS
aromadendrene		1443	0.87	0.91	0.73	RI, MIS
$\gamma$ -guaiene		1472	0.65	0.62	0.50	RI
$\beta$ -agarofuran		1474	0.54	0.50	0.43	RI
$\beta$ -selinene		1486	7.93	8.39	7.42	RI, MIS
$\alpha$ -cadinolene		1496	0.18	0.18	0.17	RI
$\gamma$ -guaiene		1499	0.65	0.67	0.63	RI
$\alpha$ -bulbolenene		1503	0.72	0.72	0.70	RI
<b>Oxygenated Sesquiterpene</b>						
$\alpha$ -elemol		1530	12.23	12.54	12.72	RI
cur-hydroagarofuran		1555	10.56	9.98	10.99	RI, MIS
tricholol		1561	0.74	0.71	0.85	RI
epoxybulbolenene		1572	0.21	0.20	0.21	RI
caryophyllene oxide		1600	16.26	15.70	16.63	RI
pinol		1603	1.34	1.40	1.61	RI
humulene oxide II		1606	1.84	1.83	1.96	RI
1,5-epoxy-cur-hydroguaiene		1614	1.71	1.69	1.80	RI
10-epi- $\gamma$ -eudesmol		1619	9.50	9.87	10.73	RI
agropyronol		1631	4.36	4.45	4.85	RI, MIS
epi- $\alpha$ -cadinol		1640	0.19	0.21	0.22	RI
isochloro-eudesmol		1643	0.32	0.36	0.38	RI
linalool		1650	0.24	0.22	0.24	RI
$\alpha$ -eudesmol		1652	0.85	0.82	0.94	RI
bulbolenol		1664	0.09	0.20	0.10	RI
dihydroisochloro-eudesmol		1673	0.43	0.50	0.56	RI
epi- $\alpha$ -binobolol		1678	0.05	0.06	0.07	RI
$\alpha$ -binobolol		1683	0.00	0.07	0.07	RI
selina-3,11-dien-9-one		1687	0.14	0.11	0.13	RI
pentadecanol		1695	0.06	0.09	0.09	RI
rotundone		1703	0.02	0.03	0.04	RI
selina-3,11-dien-9-ol		1721	0.02	0.01	0.02	RI
selina-4,11-dien-14-oic acid		1728	0.13	0.11	0.13	RI
selina-3,11-dien-14-ol		1735	0.03	0.04	0.05	RI
9,11-epoxybulbolenene-8-one		1740	0.03	0.09	0.11	RI
selina-3,11-dien-14-ol		1750	0.00	0.00	0.00	RI
pinin-1(10),11-dien-9-one		1752	0.02	0.02	0.02	RI
selina-4,11-dien-14-ol		1758	0.00	0.02	0.03	RI
pinin-1(10),11-dien-15-ol		1770	0.32	0.00	0.00	RI
selina-3,11-dien-14-oic acid		1775	0.00	0.51	0.83	RI
microfuranol		1776	0.01	0.01	0.01	RI
dihydroselinone		1799	0.00	0.00	0.00	RI
pinin-1(10),11-dien-15-oic acid		1811	0.00	0.00	0.00	RI
pentadecanoic acid		1842	0.00	0.03	0.05	RI
hexadecanol		1865	0.02	0.02	0.02	RI
eudesmol		1880	0.00	0.00	0.00	RI
valerianic acid		1912	0.00	0.00	0.04	RI
2-hydroxy-pinin-1(10),11-dien-15-oic acid		1932	0.31	0.05	0.09	RI
pinin-1(10),11-dien-15,2-oxide		2019	0.00	0.02	0.02	RI
<b>Monoterpene Hydrocarbons</b>						
		2.53		1.63	1.65	
<b>Sesquiterpene Hydrocarbons</b>						
		13.28		13.89	11.99	
<b>Oxygenated Sesquiterpene</b>						
		62.82		61.89	66.60	
<b>Total</b>		<b>77.83</b>		<b>77.61</b>	<b>79.64</b>	

<sup>1</sup>KI: Kovats retention indices on DB-1 column, <sup>2</sup>RI: linear retention indices relative to the retention time on DB-1 column of a homologous series of n-alkanes (C8-C22),  
<sup>3</sup>MS: identification by comparison of MS with those of the NIST library

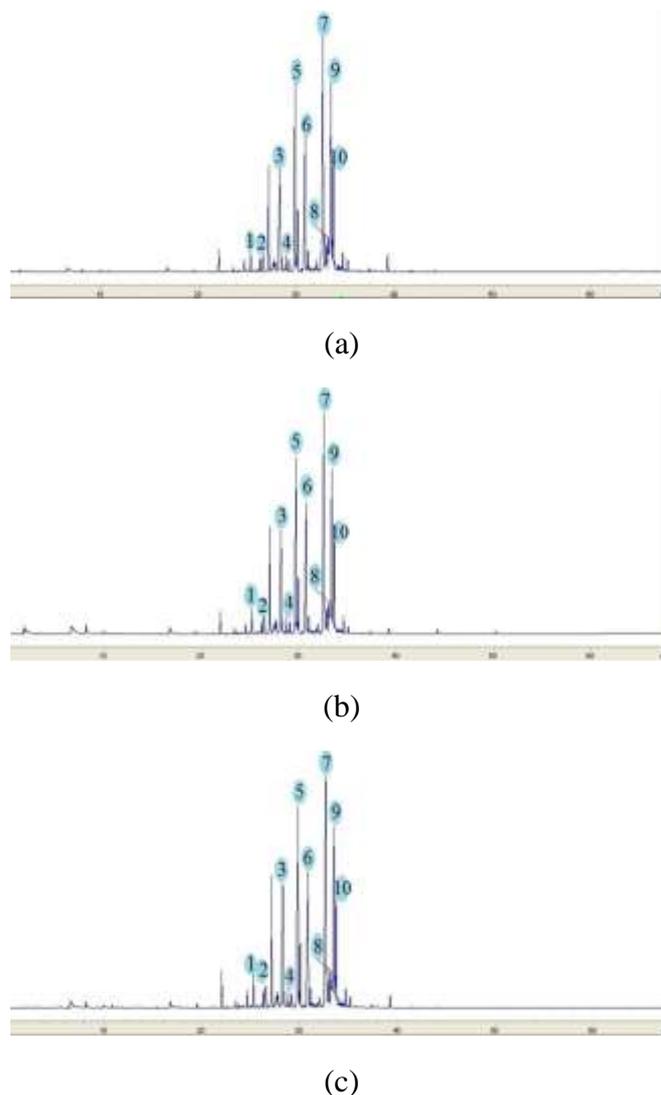
In addition, the result also revealed that the capability of different kinds of SPME fibres to absorb the volatile compounds of agarwood vapour is varied. Apparently, the DVB-CAR-PDMS fibre is considered as the best absorption fibre because of its capability to extract more compounds and gives a high percentage area, ranging between 72.17 %-81.30 %. This observation is in line with Pawliszyn's theory of SPME [7]. Meanwhile, the PDMS-DVB absorbed analytes in the range of 77.26 % to 77.95 % and the CAR-PDMS absorbed the volatile compounds in the range of 74.34 % to 78.18 %.

The findings are summarized in Table 1 and Fig. 2. It can be seen that there are five peaks of significance exist in the graph. The peaks correlate to  $\beta$ -selinene,  $\alpha$ -elemol, nor-ketoagarofuran, caryophellene oxide and 10-epi- $\gamma$ -eudesmol. All these compounds are from the sesquiterpenes group, and they (except for  $\beta$ -selinene) extracted the highest percentage area with the DVB-CAR-PDMS fibre. For  $\beta$ -selinene, the highest percentage area is by PDMS-DVB extraction.

Fig. 3 shows the GC-FID chromatogram of high quality agarwood extraction using three kinds of SPME fibres and a 30-minute extraction process. Ten compounds were chosen due to the high percentage area and their chromatogram patterns. These compounds were  $\beta$ -maaliene,  $\alpha$ -guaiene,  $\beta$ -selinene,  $\gamma$ -guaiene,  $\alpha$ -elemol, nor-ketoagarofuran, caryophellene oxide, 1,5-epoxy-nor-ketoguaiene, 10-epi- $\gamma$ -eudesmol and agarospirol. Generally, the chromatogram pattern of these compounds was consistent and quite similar to each other. The caryophellene oxide has the highest percentage area (%) among others. The marker compounds in high quality agarwood were consistently present, despite the use of application of different fibres during extraction.

Fig. 4 shows the chemical structure of marker compounds in agarwood incense by headspace volatile;  $\beta$ -maaliene,  $\alpha$ -elemol,  $\beta$ -selinene, 10-epi- $\gamma$ -eudesmol, agarospirol and caryophellene oxide. It shows that every compound has different and unique carbon-hydrogen bond molecule. Caryophellene oxide was observed to have a complex structure with three hydrogen and one oxygen separately in its bond, providing the highest percentage area during extraction.





**Note:** (1) $\beta$ -maaliene; (2) $\alpha$ -guaiene; (3) $\beta$ -selinene; (4)  $\gamma$ -guaiene;(5)  $\alpha$ -elemol; (6) nor-ketoagarofuran; (7)caryophellene oxide; (8)1,5-epoxy-nor-ketoguaiene;(9) 10-epi- $\gamma$ -eudesmol;(10)agarospirol.

Fig.3 GC-FID chromatogram of high quality agarwood vapour using (a) DVB- CAR-PDMS, (b) CAR-PDMS, (c) PDMS-DVB fibres

## V. CONCLUSION

The characterisation of high quality agarwood incense using GC-MS coupled with SPME and GC-FID analysis has been successful presented in this paper. Results showed that among many compounds extracted by GC-MS coupled with SPME, six compounds were found to significantly exists in high quality agarwood incense due to their high percentage area (%). These compounds were  $\beta$ -maaliene,  $\alpha$ -elemol,  $\beta$ -selinene, 10-epi- $\gamma$ -eudesmol, agarospirol and caryophellene oxide. The finding introduced the use of volatile profiles from agarwood incense components, which is beneficial for agarwood grading and discrimination.

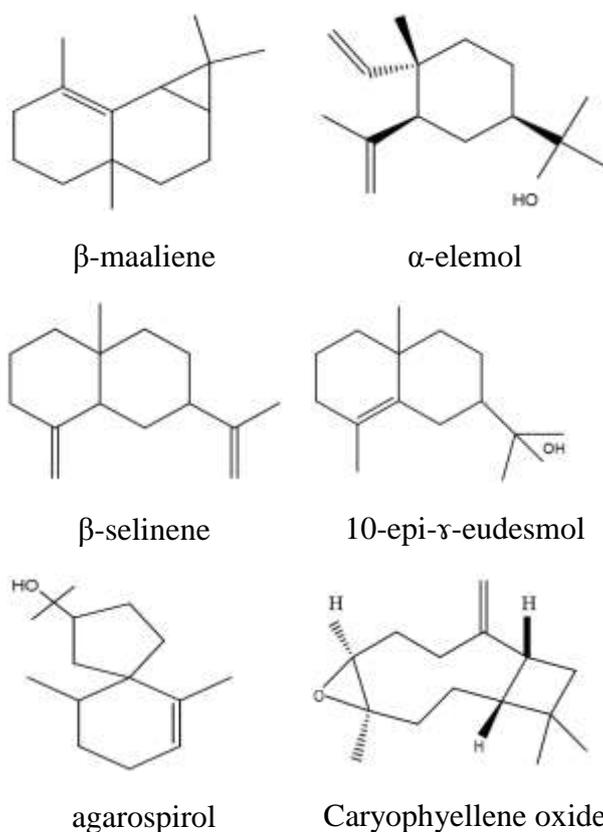


Fig. 4 Chemical structure of major compounds in agarwood incense by headspace volatile

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#### REFERENCES

- [1] A. P. Gerard, 2007. "Agarwood: the life of a wounded tree," *IIAS Newsletter*, vol. 45, pp. 24-25.
- [2] H. Heuveling van Beek and D. Phillips, 1999. "Agarwood: Trade and CITES Implementation in Southeast Asia," *Unpublished report prepared for TRAFFIC Southeast Asia, Malaysia*.
- [3] M. N. I. Bhuiyan, J. Begum, and M. N. H. Bhuiyan, 2008. "Analysis of essential oil of eaglewood tree (*Aquilaria agallocha* Roxb.) by gas chromatography mass spectrometry," *Bangladesh Journal of Pharmacology*, vol. 4, pp. 24-28.
- [4] R. Naef, 2011. "The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: a review," *Flavour and Fragrance Journal*, vol. 26, pp. 73-87.

- [5] N. Ismail, M. H. F. Rahiman, M. N. Taib, N. A. M. Ali, M. Jamil, and S. N. Tajuddin, 2014. "Application of ANN in agarwood oil grade classification," in *2014 IEEE 10th International Colloquium on Signal Processing & its Applications (CSPA)*, pp. 216-220.
- [6] H. Prosen and L. Zupančič-Kralj, 1999. "Solid-phase microextraction," *Trends in Analytical Chemistry*, vol. 18, pp. 272-282.
- [7] J. Pawliszyn, "Theory of solid-phase microextraction, 2000." *Journal of chromatographic science*, vol. 38, pp. 270-278.
- [8] S. Liou, C. Chen, T. Yang, and J. Lin, 2008. "Determination of particulate-bound formaldehyde from burning incense by solid phase microextraction," *Bulletin of environmental contamination and toxicology*, vol. 80, pp. 324-328.
- [9] J. Richter and I. Schellenberg, 2007. "Comparison of different extraction methods for the determination of essential oils and related compounds from aromatic plants and optimization of solid-phase microextraction/gas chromatography," *Analytical and bioanalytical chemistry*, vol. 387, pp. 2207-2217.
- [10] S. Dehghan Abkenar, Y. Yamini, F. Shemirani, and Y. Assadi, 2006. "Solid phase microextraction with gas chromatography–mass spectrometry: a very rapid method for identification of volatile organic compounds emitted by *Carum copticum*," *Natural product research*, vol. 20, pp. 850-859.
- [11] E. Carasek and J. Pawliszyn, 2006. "Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber," *Journal of agricultural and food chemistry*, vol. 54, pp. 8688-8696.
- [12] J. A. Field, G. Nickerson, D. D. James, and C. Heider, 1996. "Determination of essential oils in hops by headspace solid-phase microextraction," *Journal of agricultural and food chemistry*, vol. 44, pp. 1768-1772.
- [13] J. Rohloff, 1999. "Monoterpene composition of essential oil from peppermint (*Mentha × piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis," *Journal of agricultural and food chemistry*, vol. 47, pp. 3782-3786.
- [14] D. Roberts, P. Pollien, and C. Milo, 2000. "Solid-phase microextraction method development for headspace analysis of volatile flavor compounds," *Journal of agricultural and food chemistry*, vol. 48, pp. 2430-2437.
- [15] P. Kusch and G. Knupp, 2004. "Headspace-SPME-GC-MS identification of volatile organic compounds released from expanded polystyrene," *Journal of Polymers and the Environment*, vol. 12, pp. 83-87.
- [16] K. D. Bartle and P. Myers, 2002. "History of gas chromatography," *Trends in Analytical Chemistry*, vol. 21, pp. 547-557.
- [17] A. Van Asten, 2002. "The importance of GC and GC-MS in perfume analysis," *Trends in Analytical Chemistry*, vol. 21, pp. 698-708.
- [18] D. Joulain and W. A. König, 1998. *The atlas of spectral data of sesquiterpene hydrocarbons*: EB-Verlag.
- [19] T. C. Tran and P. J. Marriott, 2007. "Characterization of incense smoke by solid phase microextraction—Comprehensive two-dimensional gas chromatography (GC × GC)," *Atmospheric Environment*, vol. 41, pp. 5756-5768.