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) to charaterize agarwood incense. The work involved three types of SPME fibres at 30 minutes sampling time. The fibres are 50/30 µm divinylbenzene-carboxen-polydimethysiloxane (DVB-CAR-PDMS), 65 µm polydi methylsiloxane-divinylbenzene (PDMS-DVB) and 85 µm carboxen-polydimethyl siloxane (CAR-PDMS). The results showed that among many compounds extracted by GC-MS coupled with SPME, six compounds were found significantly presence in high quality agarwood incense due to their high percentage area (%). They are β-maaliene, α-elemol, β-selinene, 10-epi-γ-eudesmol, agarospirol and caryophellene oxide. The finding offers a new approach for establishing the volatile profile of agarwood incense components which is beneficial for agarwood grading and discrimination.

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

INTRODUCTION

Agarwood or gaharu is a resin-impregnated heartwood from Karas tree genus *Aquilaria* (Thymelaeaceae). Agarwood command a high demand in the perfumery industry as well as for manufacturing incense, soap and shampoo [1]. Its essential oils are very popular notably in the European countries and Egypt [2]. Beads as well as bracelets of agarwood pieces 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, the agarwood quality is graded based on their physical properties such as colour and odour. However, this method has drawbacks such as low productivity, time consuming and the result is not standard as the 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 sorbent extraction where it is a simple technique, fast and solvent free extraction [6], and it is in small size which can be mobiled on field [7]. The SPME is a substitute of conventional technique for extracting volatile organic compounds; it is combination step of extract analytes from sample matrix into the fibre coating and forward into an analytical instrument [8-10].

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

There are several instruments used to analyze a sample such as gas chromatography and HPLC. In year 1952, the contemporary GC was made-up by James and Martine. 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 forensic analysis [15].

Gas Chromatography-Flame Ion Detector (GC-FID) is one of important analysis instruments especially in the natural product and perfumery industry, and widely used to analyze essential oil, fatty acid and terpene group: monoterpene and sesquiterpene [16, 17]. The FID detector is commonly used in gas chromatography as a detector due to its sensitivity onto carbon-hydrogen bond molecule. Therefore, it is favored to analyze the general hydrocarbon chain bond. FID has a stable response and sustain from 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 using GC-MS coupled with SPME and analyze it using GC-FID. The paper is organized as follows: Section II presents the theory of FID and Kovalt Indices. It is followed by Section III where methodology in this study is described. After that is result and discussion in Section IV before conclusion is made in Section V.

Theory

The theory of FID can be abridged as: organic substance eluting from the column go through the degradation reaction in this Hydrogen (H_2) – rich region, forming a group of single carbon (C) species. As the flow mix at the reaction zone, with oxygen (O_2) available, the following reaction occurs [18]:

$$\text{Compounds (CHx)} + H_2 + O_2 \rightarrow CO_2 + H_2O + e- + (\text{ion})^+ + (\text{ion})^-$$

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

The retention times differ onto entity chromatography practice, for instance it is considered to the column length, its film thickness, and diameter of the column, carrier gas velocity and pressure as well as annulled time. This has caused the different value measured by different analytical laboratories [18].

Kovats Index typically demonstrated in mathematic equation.

There two equations, first for isothermal chromatography is shown in Eqn. (1) [18]

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

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,*
t'_r = *the adjusted retention time.*

While Eqn. (2) is for temperature programmed chromatography [18]

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

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_r = *the adjusted retention time,*

METHODOLOGY

The Agarwood Sample Preparation

The high quality agarwood chipwood used in this study was purchased from Gua Musang, Kelantan. A 100 g of dry samples was ground into milled agarwood and dried again (40°C) in oven until the weight was constant and stored prior to extraction.

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 fibre were chosen to extract volatile compound: 50/30 µm divinylbenzene-carboxen-polydimethysiloxane (DVB-CAR-PDMS), 65 µm polydi methylsiloxane-divinylbenzene (PDMS-DVB) and 85 µm carboxen-polydimethyl siloxane (CAR-PDMS). As recommended by [19], the headspace volatile of incense was chosen for extraction method. Every milled sample was weighed at 0.2 g before it was being transferred into a 4 mL of clear glass vial with a screw cap and PTFE and silicone septum (Supelco Inc., Bellefonte, P.A, USA). Then, the extraction was performed manually by SMpe holder as shown in Fig. 1. for 30 minutes sampling time. These set-ups are applied for all three types of SPME fibre.

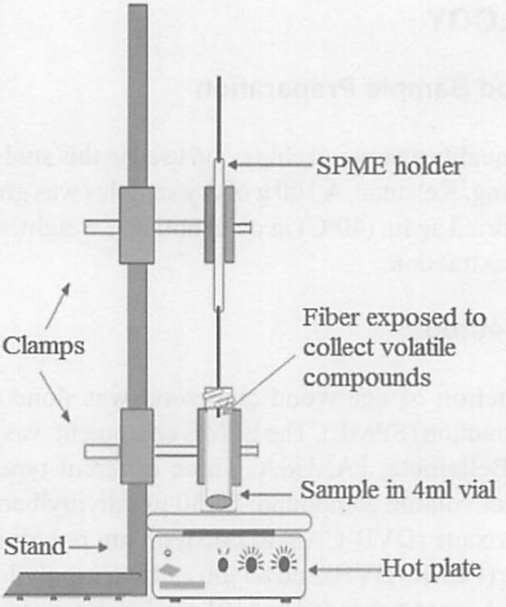


Figure 1: The Schematic Diagram of Headspace Volatile for SPME

The GC-FID Analysis: GC-MS Coupled with SPME

In GC-FID analysis, the analysis of vapour was carried out using GC-MS coupled with SPME where the incense sample 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 μm film thicknesses). Helium was used as a carrier gas with a flow rate 1.2 ml/min. The injector and detector temperature are set at 220 ˚C and 250˚C, respectively. The manual SPME injector was set in splitless mode using a narrow SPME inlet liner. The oven temperature was programmed at 60 ˚C initially, and increased at a rate of 3 ˚C/min to a final temperature of 250 ˚C and maintain for 5 minutes. The identification of compounds was done by comparison of their retention time independent constants, namely, Kovats indices (KI) or Retention Indices (RI) with RI published data. The RI was calculated using a linear hydrocarbon (C_8-C_{22}).

RESULT AND DISCUSSION

Table I listed the chemical compounds present in high quality agarwood in three kinds of SPME fibres, namely, CAR-PDMS, PDMS-DVB and DVB-CAR- PDMS at 30 minutes extraction using GC-FID analysis. The observation showed that the highest contribution is from an oxygenated sesquiterpene group. These compounds, α-elemol, caryophellene oxide, 10-epi-γ-eudesmol and agarospirol were probably marker compounds in agarwood vapour due to their high contribution in agarwood especially in high quality agarwood.

Table 1: Chemical Composition (% Relative Peak Area) of High Quality Agarwood Vapour (Headspace Volatile)

Extraction method Compound	[1]KI	CAR-PDMS	PDMS-DVB	DVB-CAR-PDMS	Identification
Monoterpene Hydrocarbon					
furfuryl alcohol	830	0.00	0.00	0.02	[2]RI
benzaldehyde	935	1.56	0.7	0.44	RI [3]MS
phenol	965	0.11	0.02	0.00	RI
p-methylanisol	1002	0.00	0.00	0.00	RI
guaiacol	1062	0.05	0.1	0.06	RI
acetophenone	1066	0.00	0.03	0.01	RI
4-phen yl-2-butanone	1210	0.55	0.48	0.35	RI, MS
p-vinylguaiacol	1286	0.00	0.01	0.00	RI
3, 4-d imethoxyph enol	1312	0.02	0.03	0.02	RI,MS
vanilin	1367	0.23	0.26	0.15	RI
Sesquiterpernes Hydrocarbon					
β- maaliene	1414	1.08	1.20	0.86	RI, MS
α- guaiene	1440	0.68	0.70	0.57	RI, MS
aromadend rane	1443	0.87	0.91	0.73	RI, MS
γ- gurjunen e	1472	0.65	0.62	0.50	RI
β- agarofuran	1474	0.54	0.52	0.43	RI
β- selinene	1486	7.93	8.39	7.42	RI, MS
α- muurolene	1496	0.18	0.18	0.17	RI
γ- guaiene	1499	0.65	0.67	0.63	RI
α- bul nesene	1503	0.72	0.72	0.70	RI
Oxygenated Sesquiterpernes					
α- elemol	1530	12.23	12.54	12.72	RI
nor-ketoagarofuran	1555	10.56	9.98	10.99	RI, MS
tridecanol	1561	0.74	0.71	0.85	RI
epoxybulnesene	1572	0.21	0.20	0.21	RI
caryoph ellene oxide	1600	16.26	15.70	16.63	RI
guaiol	1603	1.34	1.40	1.61	RI
humulene epoxide II	1606	1.84	1.83	1.96	RI
1, 5-epoxy-nor-ketoguaiene	1614	1.71	1.69	1.80	RI
10-epi-γ-eudesmol	1619	9.50	9.87	10.73	RI
agarospirol	1631	4.36	4.45	4.85	RI, MS

epi-α-cadinol	1640	0.19	0.21	0.22	RI
jinkoh-eremol	1643	0.32	0.36	0.38	RI
kusun ol	1650	0.24	0.22	0.24	RI
α-eudesmol	1652	0.85	0.82	0.94	RI
bulnesol	1664	0.09	0.20	0.10	RI
dehydrojinkoh-eremol	1673	0.43	0.50	0.56	RI
epi-α-bisabolol	1678	0.05	0.06	0.07	RI
α-bisabolol	1683	0.00	0.07	0.07	RI
selina-3, 11-dien-9-one	1687	0.14	0.11	0.13	RI
pentadecanal	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-al	1735	0.03	0.04	0.05	RI
9, 11-eremophiladien-8-one	1740	0.03	0.09	0.11	RI
selina-3, 11-dien-14-ol	1750	0.00	0.00	0.00	RI
guaia-1(10), 11-dien-9-one	1752	0.02	0.02	0.02	RI
selina-4, 11-dien-14-al	1758	0.00	0.02	0.03	RI
guaia-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
sinenofuranol	1776	0.01	0.01	0.01	RI
dihydrokaran one	1799	0.00	0.00	0.00	RI
guaia-1(10), 11-dien-15-oic acid	1811	0.00	0.00	0.00	RI
pentad ecanoic 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
palmitic acid	1912	0.00	0.00	0.04	RI
2-hydroxyguaia-1(10), 11-dien-15-oic	1932	0.31	0.05	0.09	RI
guaia-1(10), 11-dien-15, 2-olide	2019	0.00	0.02	0.02	RI

Monoterpene Hydrocarbon	**2.53**	**1.63**	**1.05**
Sesquiterpernes Hydrocarbon	**13.28**	**13.89**	**11.99**
Oxygenated Sesquiterpernes	**62.02**	**61.89**	**66.60**
Total	**77.83**	**77.41**	**79.64**

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

In addition, the result also reveals that the capability of different kinds of SPME fibres to absorb the volatile compounds of agarwood vapour was varied. Apparently, the DVB-CAR-PDMS fibre is considered as the best absorption fibre because of its capability to extract more compounds and have 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 the 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 finding in Table 1 is summarized in Figure 2 for graphical observation. It can be seen that there are five peaks significantly exist in the graph. They belong to β-selinene, α-elemol, nor-ketoagarofuran, caryophellene oxide and 10-epi-ϒ-eudesmol. All these compounds are from sesquiterpenes group and all of them were extracted the highest percentage area using DVB-CAR-PDMS fibre except for β-selinene. The highest of percentage area for β-selinene is extracted by PDMS-DVB.

Figure 3 shows the GC-FID chromatogram of high quality agarwood extracted by using three kinds of SPME fibres at 30 minute extraction. Ten compounds were chosen due to high percentage are and their chromatogram pattern are assessed. The compounds were β-maaliene, α-guaiene, β-selinene, γ-guaiene, α-elemol, nor- ketoagarofuran, caryophellene oxide, 1, 5-epoxy-nor-ketoguaiene, 10-epi-γ- 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. Therefore, the marker compounds in high quality agarwood are consistently present even though different fibres were applied during the extraction

Figure 4 shows the chemical structure of marker compounds in agarwood incense by headspace volatile; β-maaliene, α-elemol, β-selinene, 10-epi-γ-eudesmol, agarospirol and caryophellene oxide. It shows that every compound has different and unique carbon-hydrogen bond molecule. Among all, it is noticed that caryophellene oxide has a complex structure, complimented with three hydrogen and one oxigen separately in its bond, make it to have the highest percentage area during the extraction.

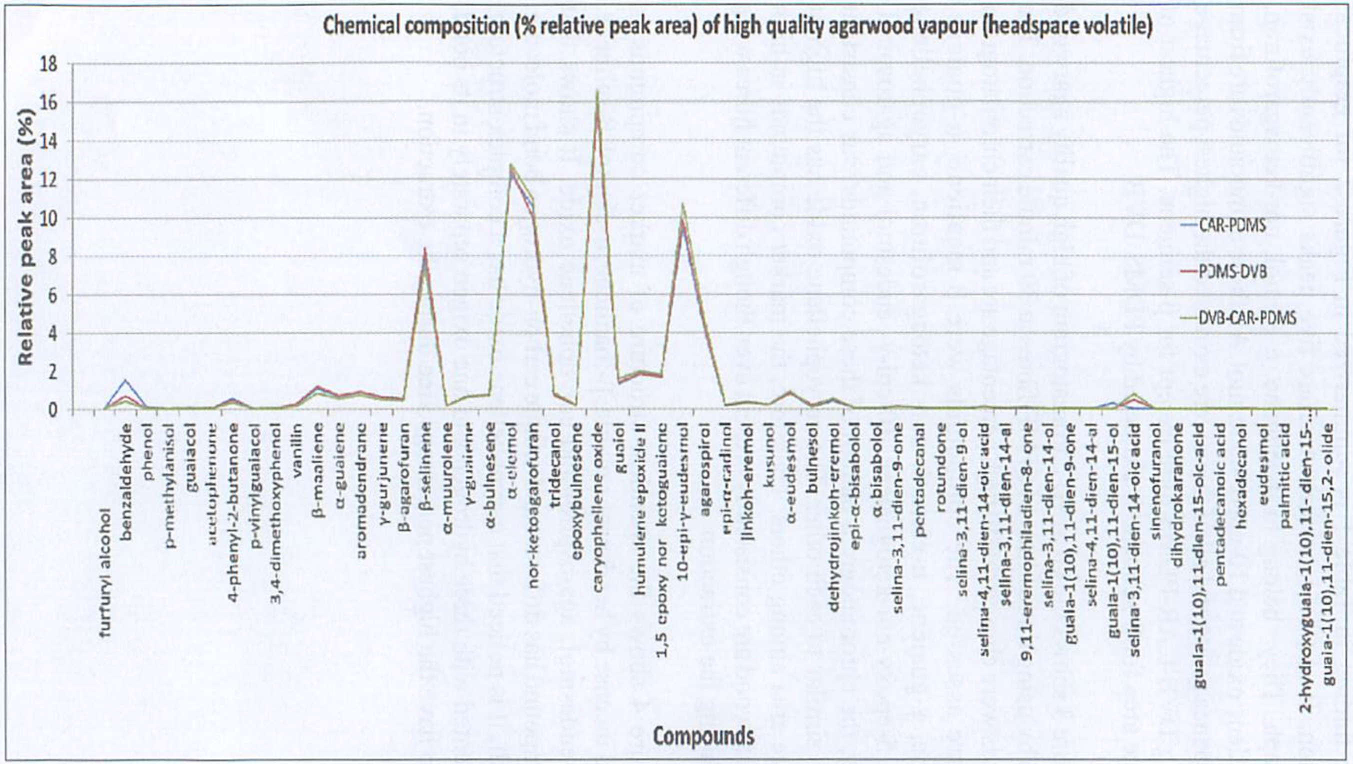
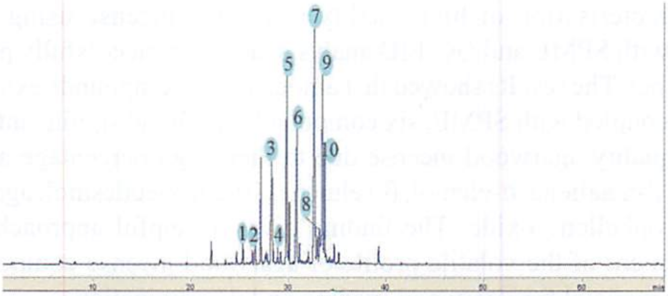
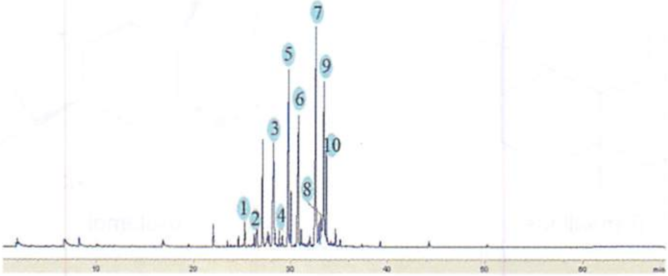


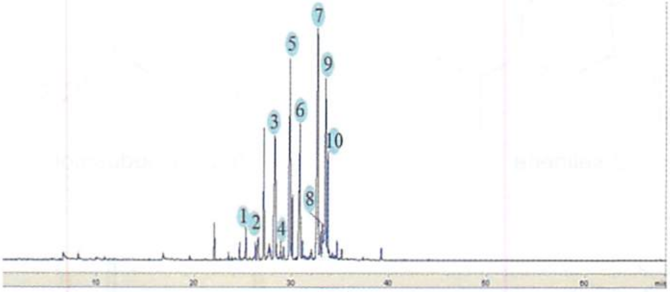
Figure 2: Chemical Composition (% Relative Peak Area) of High Quality Agarwood Vapours (Headspace Volatile)



(a)



(a)



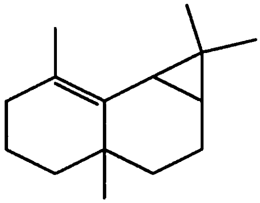
(a)

Note: (1) β-maaliene; **(2)** α-guaiene; **(3)** β-selinene; **(4)** γ-guaiene; **(5)** α-elemol; **(6)** nor- ketoagarofuran; **(7)** caryophellene oxide; **(8)** 1,5-epoxy-nor-ketoguaiene; **(9)** 10-epi-γ- eudesmol; **(10)** agarospirol

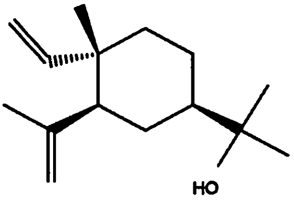
Figure 3: GC-FID Chromatogram of High Quality Agarwood Vapour Using (a) DVB- CAR-PDMS, (b) CAR-PDMS, (c) PDMS-DVB Fibres

CONCLUSION

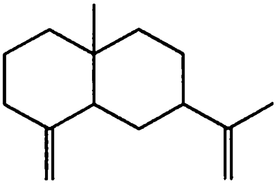
The characterisation of high quality agarwood incense using GC-MS coupled with SPME and GC-FID analysis has been successfully presented in this paper. The results showed that among many compounds extracted by GC-MS coupled with SPME, six compounds are found significantly exists in high quality agarwood incense due to their high percentage area (%). They are β-maaliene, α-elemol, β-selinene, 10-epi-γ-eudesmol, agarospirol and caryophellene oxide. The finding gives a helpful approach for the establishment of the volatile profile of agarwood incense components as well as beneficial for agarwood grading and discrimination.



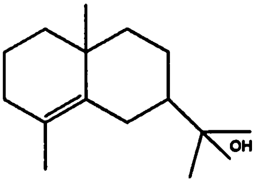
β-maaliene



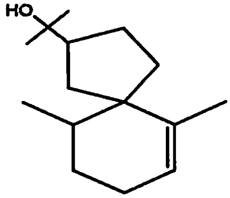
α-elemol



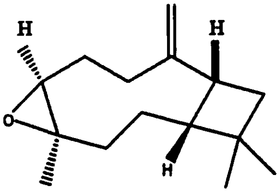
β-selinene



10-epi-ϒ-eudesmol



agarospirol



Caryophyellene oxide

Figure 4: Chemical Structure of Major Compounds in Agarwood Incense by Headspace Volatile

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