Journal of Analytical and Applied Pyrolysis xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Analytical and Applied Pyrolysis

journal homepage: www.elsevier.com/locate/jaap

Characterization of Chinese crude propolis by pyrolysis–gas chromatography/mass spectrometry

Hongli Yang[a,b], Zhongping Huang[a], Yilei Huang[a], Wenxia Dong[a], Zaifa Pan[a], Lili Wang[a,*]

[a] College of Chemical Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, PR China
[b] College of Food and Medicine, Zhejiang Ocean University, Zhoushan 316022, PR China

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Pyrolysis–gas chromatography/mass spectrometry
Crude propolis
Chemical composition
Flavonoids
Terpenoids

ABSTRACT

A method for analysis of Chinese crude propolis was developed by pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) with a vertical microfurnace pyrolyzer. Propolis sample powder about 0.1 mg was pyrolyzed directly at the optimum pyrolytic temperature of 550 °C without any time-consuming pretreatments. 76 compounds were identified on the resulting pyrogram after the pyrolysis of crude propolis, and all the compounds were grouped into 8 categories such as flavonoids, terpenoids, acids, esters, hydrocarbons, phenols and alcohols, aldehydes and ketones, and others. On the basis of peak areas, the relative intensities of 76 peaks were precisely determined with the relative standard deviations (RSDs (%), $n = 3$) between 1.1 and 14.0%. Furthermore, the compositions of crude propolis samples from different geographical regions in China were analyzed and compared. The contents of 8 categories of compounds in crude propolis samples were different, the predominant categories being flavonoids, esters or hydrocarbons. And also the contents of main bioactivity compounds such as flavonoids and terpenoids were extremely different, and varied largely with 0–54.9% and 0.6–21.6%, respectively. In addition, abundant triterpenoids were identified in Chinese propolis for the first time. It is proved that Py–GC/MS is a simple, rapid and sensitive method for the characterization of the crude propolis.

1. Introduction

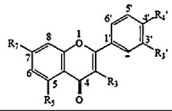
Crude propolis, as a balmy and resinous substance, is produced by honeybees from resinous substance of certain plants mixed with their saliva and beeswax, and used to seal holes in honeycombs, smooth the internal walls, and protect the entrance against intruder [1]. It has also been used in folk medicines in many regions of the world due to its extensive biological activities including antioxidative [2,3], antibacterial [4], anti-inflammatory [5], anticancer [6,7] and anti-diabetic [8] properties. More than 200 compounds with a large range of boiling points have been found in propolis such as flavonoids, terpenoids, aromatic acids, fatty acids, esters, phenols, aldehydes, ketones and others [9–13]. The flavonoids, terpenoids, aromatic acids and their esters are responsible for the biological activities of propolis. Main constituents most often found in Chinese propolis are shown in Fig. 1.

The chemical compositions of crude propolis often vary largely due to their raw material coming from resinous substance, usually depending on geographical regions, floristics and seasons [9] [10,11]. Similarly, the main components in Chinese crude propolis are also very complex and different, because bee farmers always migrate from the south of China to the north, tracing the flower source to increase the honey production. Therefore, it is necessary to develop a method for analysis of the chemical composition in crude propolis for their quality control.

So far, several methods including spectrographic and chromatographic methods have been developed to analyze the chemical composition of propolis. Thin layer chromatography (TLC) [13] and high performance liquid chromatography (HPLC) [14] were used to investigate the fingerprints of propolis and discriminate their geographical origins. HPLC and high performance liquid chromatography–mass spectrometry (HPLC–MS) [15,16] could be used to conveniently quantify the flavonoids, aromatic acids and their esters in propolis. Gas chromatography/mass spectrometry (GC/MS) [17,18] was preferred method to study the volatile components of propolis. With derivatization, GC/MS can also be performed for analysis the isoflavonoids, triterpenoids, and flavonoids [19,20]. Besides, high temperature high resolution gas chromatography(/mass spectrometry) (HTHRGC(/MS)) was applied to analyze several classes of compounds including flavonoids, fatty acid esters in propolis extract without derivatiza-

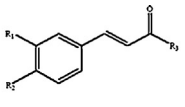
* Corresponding author. Tel.: +86 571 88320797/13958071016; fax: +86 571 88320797.
E-mail address: lili_wang@zjut.edu.cn (L. Wang).

http://dx.doi.org/10.1016/j.jaap.2014.12.006
0165-2370/



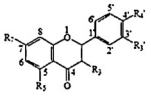
flavones and flavonols

Peak * Number	Compounds	R_3	R_5	R_7	$R_{3'}$	$R_{4'}$	Fragment Ions
62	5-Hydroxy-7-methoxyflavone(Tectochrysin)	H	OH	OCH3	H	H	268(M+), 239, 225
63	4',5-Dihydroxy-7-methoxyflavone(Genkwanin)	H	OH	OCH3	H	OH	284(M+), 255, 241
66	5,7-Dyhydroxy flavone (Chrysin)	H	OH	OH	H	H	254(M+), 226, 152, 124
67	3,4'-Dihydroxy-3'-methoxyflavone	OH	H	H	OCH3	OH	284(M+), 283
68	3,5,7-Trihydroxy flavone (Galangin)	OH	OH	OH	H	H	270(M+), 269, 242, 213



Aromatic acids and their esters

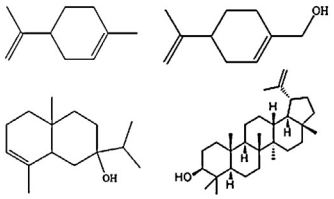
Peak * Number	Compounds	R_1	R_2	R_3
30	Cinnamic acid	H	H	OH
47	3,4-Dimethoxy-cinnamic acid	OCH3	OCH3	OH
54	Ferulic acid	OCH3	OH	OH
61	Caffeic acid phenylethyl etster (CAPE)	OH	OH	



flavanones and dihydroflavonols

Peak * Number	Compounds	R_3	R_5	R_7	$R_{3'}$	$R_{4'}$	Fragment Ions
59	5,7-Dihydroxyflavanone (Pinocembrin)	H	OH	OH	H	H	256(M+), 255, 179, 152, 124
64	4',5,7-Trihydroxyflavanone (Naringenin)	H	OH	OH	H	OH	272(M+), 153, 120

Terpenoids



Wax ester

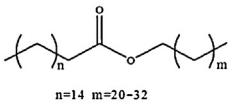


Fig. 1. Main constituents most often found in Chinese propolis.

*peak numbers consistent with those in Fig. 3 and Table 1.

tion [21,22], and highly polar compounds, such as aromatic acids, fatty acids, polysaccharides in propolis with derivatization [23]. Although more compounds with higher molecular weight in propolis can be analyzed by HTHRGC (/MS), it is harmful to GC system for final column temperatures reaching up to 370 °C or even higher.

However, troublesome pretreatments are necessary for all the above methods prior to the final analysis such as solvent extraction, purification, and derivatization. Most of the studies were limited to some components of interest, particularly the flavonoids, lacking of a whole chemical composition analysis. However, only the total content of flavonoids in crude propolis was utilized as the criterion to classify crude propolis in China [24]. Therefore, it is meaningful to develop a simple, rapid and sensitive method for characterization of the whole chemical composition in crude propolis.

Recently, pyrolysis–GC(/MS) (Py–GC(/MS)) has been used extensively in the characterization of both low and high molecular components in various natural products. This technique yields a pyrogram consisting of the characteristic peaks of constituents in the given natural product without any pretreatment. Py–GC(/MS) has been performed for accurate determination of terpenoids, alcohols in volatile constituents in *Houttuynia cordata Thunb* [25], phenolic compounds in *origanum heracleoticum* [26], and terpenic acids, aleuritic acid, fatty acids in natural resin shellac [27]. Furthermore, it has been successfully applied for characterization of the whole components of beeswax, including fatty acids, fatty alcohols, aromatic acids and high molecular weight esters [28,29].

In this study, Py–GC/MS was first applied to analysis of chemical composition in crude propolis without any cumbersome pretreatment. Furthermore, samples obtained from different regions of China were also compared, based on the chemical composition of crude propolis.

2. Experimental

2.1. Propolis samples

Crude propolis samples were collected from hives by bee farmers from Shandong province (sample Taian (TA) and Linyi (LY)), Zhejiang province (sample Zhoushan (ZS) and Quzhou (QZ)), and Hubei province (sample HB) from September 2011 to February 2012. Bees of five apiaries belong to *Apis mellifera ligustica*. Propolis samples were frozen at −18 °C for 24 h, and then milled into fine powders with the particle size of 120 μm as quickly as possible prior to Py–GC/MS measurements, to improve the efficiency of the pyrolysis.

2.2. Py–GC/MS analysis

A vertical microfurnace pyrolyzer (PY2020iD, Frontier Lab Ltd., Fukushima, Japan) was directly attached to a GC/MS system (Thermo Finnigan (Austin, TX, USA), trace DSQ). The Py–GC interface was kept at 350 °C. Propolis powder about 0.1 mg, taken in a sample cup, was first mounted at the waiting position of the pyrolyzer at around room temperature, and then dropped into the heated center of the pyrolyzer under the flow of nitrogen carrier gas. In order to obtain high peak intensity and appropriate peak number, the particle size of 120 μm of propolis was used after studying four different particle size (D_p), namely 120, 180, 350 and 830 μm. The optimum pyrolysis temperature was determined by evolved gas analysis (EGA). When EGA was performed, capillary column was replaced by inert stainless steel column without stationary phase (length 2.5 m, inner diameter 0.15 mm). The column oven temperature was set at 300 °C. The pyrolyzer temperature was initially set at 70 °C, and then heated from 70 °C to 700 °C at a rate of 20 °C min^{-1}, the final temperature was held for 3.5 min.

A capillary column (30 m × 0.25 mm i.d, 0.25 μm film thickness) from Frontier Lab., Ltd. (Ultra ALLOY-5, Fukushima, Japan) was used to separate pyrolyzate of crude propolis. The flow-rate of carrier gas (He ≥ 99.999%) was at 1.0 mL/min. A split injection with a ratio of 1:30 was used, with the temperature of injector at 300 °C. The column temperature was initially set at 40 °C, and then heated from 40 °C to 300 °C at a rate of 10 °C min^{-1}. The final temperature was held for 10 min.

Identification of peaks on the pyrograms was carried out by a Thermo Trace DSQII GC–MS, supplemented with comparison of the

Please cite this article in press as: H. Yang, et al., Characterization of Chinese crude propolis by pyrolysis–gas chromatography/mass spectrometry, J. Anal. Appl. Pyrol. (2014), http://dx.doi.org/10.1016/j.jaap.2014.12.006