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RESEARCH ARTICLE

Differential volatile organic compounds in royal jelly associated with different nectar plants

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Abstract

The aim of this work was to distinguish volatile organic compound (VOC) profiles of royal jelly (RJ) from different nectar plants. Headspace solid-phase microextraction (HS-SPME) was used to extract VOCs from raw RJ harvested from 10 nectar plants in flowering seasons. Qualitative and semi-quantitative analysis of VOCs extracts were performed by gas chromatography-mass spectrometry (GC-MS). Results showed that VOC profiles of RJ from the samples were rich in acid, ester and aldehyde compound classes, however, contents of them were differential, exemplified by the data from acetic acid, benzoic acid methyl ester, hexanoic acid and octanoic acid. As a conclusion, these four VOCs can be used for distinguishing RJ harvested in the seasons of different nectar plants.

Keywords: royal jelly, volatile organic compounds, nectar plant, headspace solid-phase microextraction, gas chromatography-mass spectrometry

1. Introduction

Flavor or aroma qualities of natural food are greatly dependent upon the volatile organic compounds (VOCs) presented both in the matrix and the headspace (Overton and Manura 1995). The VOC profile is an important feature of food, for both quality and authenticity (Careri *et al*. 1993). Owing to the large number of VOCs, the VOC profile represents a typical character of food, which can be used for determining the origins of food (Anklam 1998; Anklam and Radovic 2001). It has been demonstrated that sound analysis of the VOCs in royal jelly (RJ) could be an advisable way to determine its origin, quality and even freshness (Isidorov *et al*. 2009; Isidorov *et al*. 2012). Researches on composition of RJ have been reported extensively (Isidorov *et al*. 2011; Daniele and Casabianca 2012; Ferioli *et al*. 2014). However, little information about the VOC profiles of RJ is available up to now. RJ, secreted by the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera* L.), is a pasty and slightly acidic (pH 3.5–4.5) substance and is used to feed the queen and larvae (Zhou *et al*. 2012). Raw RJ has a special aroma, and tastes tart, acrid, and slightly sweet (Ramadan and Al-Ghamdi 2012). Since the ancient times, RJ has been utilized broadly for its high nutritional value. As the main source of flavor, VOCs in RJ are generally influenced by honeybee species, harvesting time, regions, storage methods and processing technologies, etc. There-

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fore, different origins of RJ might make a great difference to VOCs in RJ (Boselli *et al*. 2003; Isidorov *et al*. 2009; Wu *et al*. 2009; Isidorov *et al*. 2012).

The flowering seasons of nectar plants refer to the flowering and nectar-secreting periods of the nectar plants when RJ is produced massively by bee colonies. For example, the flowering season of rapeseeds, a widely cultivated crop in China, is in late spring. The period is just after spring propagation of honeybee colonies, thus high activity level of honeybee colonies results in large amounts of pollen, honey and high yield of RJ as well (Stocker *et al*. 2005). Since RJ is secreted by worker bees, which feed on bee bread made by pollens and honey (Sereia *et al*. 2013). Therefore, compounds of RJ (both volatile and non-volatile) are influenced by bee bread and nutrition of which depends on the pollen. As a result, the changing pattern of VOCs in RJ associates with flowering seasons of nectar plant, when work bees collect pollen intensively. Analysis of VOCs is also influenced by extraction and detection technologies (Boselli *et al*. 2003; Isidorov *et al*. 2009). Extraction technologies of VOCs include simultaneous distillation-extraction (SDE), dynamic headspace extraction, static headspace extraction, ultrasonic-assisted extraction, steam distillation, solvent extraction, solid-phase microextraction, ect. Some of these technologies may be suitable for extraction of VOCs in RJ (Boselli *et al*. 2003; Isidorov *et al*. 2009; Zhou *et al*. 2009), however, some technologies still need to be improved. For example, steam distillation is time-consuming, especially when the sample size is big. Heating process of SDE may promote the formation of artificial by-products. Solvent elimination process of solvent extraction may lead to residues. Besides, low concentrations of VOCs in RJ and interferences of protein and sugar that are main components of RJ should be concerned. With the development of technologies (Snow and Bullock 2010), headspace extraction appears to be the optimal method for extracting VOCs in RJ.

In this study, VOC profiles of RJ samples harvested in different flowering seasons in China were established using gas chromatography-mass spectrometry (GC-MS) preceded by headspace solid-phase microextraction (HS-SPME) to extract VOCs in RJ. This quick, economical and efficient method circumvented the issues associated with solvent, protein and sugar. The final results of VOC profiles of RJ will provide a theoretical basis for assessing the RJ quality.

2. Results

2.1. Water content of raw RJ samples

Isomerization of monosaccharide, e.g., fructose, and formation rate of Amadori compounds in RJ are known to be influenced by water content. What follows next is composition change of aldehydes (Ferrer *et al*. 2002), some of which are volatile; we checked water contents of every type of RJ samples. As shown in Fig. 1, the water contents of all samples are almost equal. Samples harvested in flowering season of *Citrullus lanatus* have water contents at (62.98±0.22)%, which is significant lower than all the other samples (P<0.05, n=3). Whereas samples harvested in flowering season of *Lichi chinensis* have water contents at (68.40±0.37)%, which is significant higher than all the other samples (P<0.05, n=3). The water contents of the other 8 types of samples show even smaller differences ranging from (64.81±0.06)% to (65.94±0.13)%.

2.2. Total ion chromatogram of VOCs

The total ion chromatograms (TICs) of all samples were similar. Fig. 2 shows an example of one sample harvested in flowering season of *Tilia tuan*. The VOC profiles of different types of RJ only differ in the contents regarding a few rare compounds which exist in low concentration in one or two samples, suggesting the similar flavor profiles of all RJ samples. The chromatographic conditions are well suitable to VOCs absorbed by HS-SPME, making the chromatographic peaks fully separated and overlapped rarely. Except for solvent (n-hexane) peaks, impure peaks and ghost peaks, the first VOC peak was reached at about the 4th min and the last one was reached at about the 36th min. The whole analysis process lasted for 40 min.

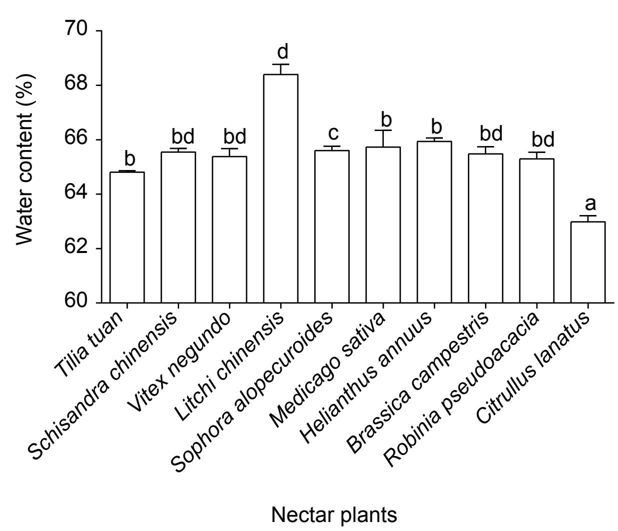

Fig. 1 Water contents of royal jelly samples harvested in flowering seasons of different nectar plants. Different letters (a, b, c, d, e) above the bars represented significant different levels (P<0.05, n=3).