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Discrimination of honeys using colorimetric sensor arrays, sensory analysis and gas chromatography techniques

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ABSTRACT

Aroma profiles of six honey varieties of different botanical origins were investigated using colorimetric sensor array, gas chromatography–mass spectrometry (GC–MS) and descriptive sensory analysis. Fifty-eight aroma compounds were identified, including 2 norisoprenoids, 5 hydrocarbons, 4 terpenes, 6 phenols, 7 ketones, 9 acids, 12 aldehydes and 13 alcohols. Twenty abundant or active compounds were chosen as key compounds to characterize honey aroma. Discrimination of the honeys was subsequently implemented using multivariate analysis, including hierarchical clustering analysis (HCA) and principal component analysis (PCA). Honeys of the same botanical origin were grouped together in the PCA score plot and HCA dendrogram. SPME-GC/MS and colorimetric sensor array were able to discriminate the honeys effectively with the advantages of being rapid, simple and low-cost. Moreover, partial least squares regression (PLSR) was applied to indicate the relationship between sensory descriptors and aroma compounds.

1. Introduction

More than 600 volatile compounds have been identified in honey samples. These compounds belong to different chemical families, including aldehyde, ketone, acid, alcohol, hydrocarbon, norisoprenoids, terpenes, benzene compounds, esters, furan and pyran derivatives (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014a; Plutowska, Chmiel, Dymerski, & Wardencki, 2011).

Traditionally, the aroma of honey is determined by sensory analysis, where 10 or 12 assessors recognise and score well-defined descriptors. However, this method is expensive and time-consuming (Vaclavik & Christian, 2014). As a substitute, instrumental techniques, such as solid-phase microextraction couple to gas chromatography–mass spectrometry (SPME-GC–MS), can be used to recognise and measure singular aroma components. Although SPME-GC–MS shows good reproducibility, sensitivity and provides qualitative and quantitative data for these compounds (Cuevas-Glory, Pino, Santiago & Sauri-Duch, 2007), it is relatively high-cost and time-consuming (Špánik, Pažitná, Šiška, & Szolcsányi, 2014). Investigation of volatile compounds of honey up to date has given slight importance to the relationship between instrumental and sensory analysis (Manyi-Loh, Ndip, & Clarke, 2011). The association of sensory investigation with instrumental analysis has increased in recent years, as it permits the characterisation of food such as honey according to definite standards.

In the last 20 years, there has been expanding study in achieving a speedy system for surveying food flavours, which has led to the advancement of gas sensor array systems. These systems, known as electronic noses, have been applied for the identification of the botanical origin of Chinese honeys (Huang, Liu, Zhang, & Wu, 2015). Electronic noses based on mass spectrometry, piezoelectric effects and electrical resistance have been tested on the volatile compounds of some American (Lammertyn, Veraverbeke, & Irudayaraj, 2004) and European honeys (Ampuero, Bogdanov, & Bosset, 2004). These methods need the preceding removal of sugar and water, which are the major honey constituents (Kaškonienė, Venskutonis, & Čeksterytė, 2008). Hence, it remains a challenge to identify volatile compounds rapidly and to remove interferences resulting from changes in humidity (Ouyang, Zhao, Chen, & Lin, 2013). The colorimetric sensor array has stood out as an effective means to address this problem (Janzen, Ponder, Bailey, Ingison, & Suslick, 2006). An advantage of this type of sensor is its ability to sense compounds that cannot be detected by an electrochemical sensor (Borrás-Linares et al., 2015). Recently, this type of colorimetric sensor array has been used to investigate wine (Ouyang et al., 2013), monitoring pork sausage spoilage (Salinas et al.,

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2014) as well as discrimination of Chinese liquors (Li et al., 2014). Based on the aforementioned studies we can conclude that the colorimetric sensor array has vast potential in the investigation of honey quality. However, there are no data about the application of colorimetric sensor arrays for honey discrimination. Additionally, there are few studies on the relationship between sensory descriptors and volatile compounds identified by SPME-GC–MS in honey.

Multivariate statistical techniques tools, including principal component analysis (PCA), hierarchical clustering analysis (HCA) and partial least-squares regression (PLSR) have been specifically designed for the analysis and visualisation of complex sets of different samples. Several authors have used multivariate analysis tools to correlate sensory analysis with GC–MS data (Aznar, Lopez, Cacho, & Ferreira, 2003; Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009; Vilanova, Genisheva, Masa, & Oliveira, 2010). The aims of this study were: (1) to discriminate Sudanese honeys using multivariate data analysis coupled with colorimetric sensor array and SPME-GC–MS; (2) to examine the sensory profile of Sudanese honey in order to simplify its discrimination and; (3) to explain the correlation between aroma compounds and sensory analysis using PLSR.

2. Materials and methods

2.1. Honey samples and chemicals

Six varieties of honey based on floral type, which consist of *Acacia nilotica* ($n = 10$), *Acacia seyal* ($n = 20$), *Ziziphus spina-christi* ($n = 20$), *Amaranthus graecizan* ($n = 10$), *Eucalyptus spp* ($n = 10$) and multifloral ($n = 30$) honeys were collected from different sources across Sudan. Table S1 showed the botanical, geographical origin and samples codes. The pollen types were placed into four percentage classes, as determined by Louveaux, Maurizio and Vorwohl (1978): predominant pollen (>45%); secondary pollen (⩽45% to >15%); important minor pollen (⩽15% to ⩾3%); and minor pollen (<3%). The botanical origin of honeys was based on the pollen spectrum (45% and above), which is the ratio of the frequency of each pollen type in the honey.

5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese(III) chloride; 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine; 5,10,15,20-tetrakis (4-sulfonatophenyl)-21*H*,23*H*-porphine manganese(III) chloride; 5, 10,15,20-tetraphenyl-21*H*,23*H*-porphine; zinc 29*H*,31*H*-tetrabenzo [b,g,l,q]porphine; zinc 5,10,15,20-tetra(4-pyridyl)-21*H*, 23*H*-porphine tetrakis(methochloride) were purchased from Sigma–Aldrich (St. Louis, MO). Hydrophobic nanoporous film was obtained from (Millipore Co, Billerica, MA, USA). Gentian violet (methyl violet 10B); tetrasodium [29*H*,31*H*-phthalocyanine-tetrasulfonato(6-)-*N*29,*N*30,*N*31,*N*32] cuprate (4-); bromocresol green; methyl yellow; nickel(ii) phthalocyanine-tetrasulfonic acid tetrasodium salt; congo red; methyl orange; screened methyl orange (first transition); sodium chloride and chloroform were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). A series *n*-alkanes (C5–C25) was purchased from Sigma–Aldrich, St. Louis, MO.

2.2. Colorimetric sensor array preparation

The pattern of the colorimetric sensor array is often grounded on two essential fundamentals: (1) each chemical responsive dye contains a centre to interact with analytes, and (2) the interaction centre must be strongly coupled to a deep chromophore. The prerequisite dyes comprise: (1) Lewis acid/base dyes (i.e., metal ion containing dyes), (2) Brønsted acidic or basic dyes (i.e., pH indicators), and (3) dyes with large enduring dipoles (i.e., zwitterionic solvatochromic dyes) (Janzen et al., 2006). Porphyrins and their metal composites are accepted for identification of analytes with Lewis acid/base abilities. Metalloporphyrins are ideal for the detection of metal-ligating vapours due to their open coordination sites for axial ligation, their great spectral changes upon ligand binding, and their strong coloration. Ordinary pH indicator pigments change in response to changes in the proton (Brønsted) acidity or basicity of their environment (Feng, Musto, Kemling, Lim, & Suslick, 2010; Suslick, Rakow, & Sen, 2004). In this work, we investigated numerous commercially available sensing materials. Finally, we decided that the abovementioned six metalloporphyrins and eight pH indicators were the best option in this experiment. For developing the colorimetric sensor, every chemically responsive dye (20 mg) was diluted with 10 mL of chloroform and sonicated for 2 h at room temperature. A fresh 4×4 colorimetric sensor array was prepared by spotting approximately 0.1 μL of solution on the surface of hydrophobic nanoporous film membranes. After spotting, the arrays were kept at room temperature for 15 min prior to use.

2.3. Data acquisition

A flatbed scanner (Epson Perfection 1200S, Seiko Epson Corporation, Nagano-ken, Japan) was used to obtain the pictures of the colorimetric sensor array. The initial image was first taken before exposure to the honey samples, and then the array was exposed to honey samples. For each type of honey 1.5 g of honey were placed into a 100 mL beaker and dissolved with 40 mL of sodium chloride solution (30%). The array was attached to a preservative film, which was used to seal the beaker containing the honey solutions. Then the sample solutions were partially submerged in an ultrasonic bath (Wuxi Fanbo Biological Engineering, Wuxi, China) for 35 min at 45 °C for extraction (Karabagias et al., 2014a). After extraction, the sensor arrays were removed from the preservative film and dried in the fume hood for one hour at room temperature. Then the sensor was scanned to get the final image. Based on our previous work (Huang et al., 2015) colour change plots were obtained from the RGB images by digitally deducting the "initial" image from "final" image using a 314-pixel average from the centre of each dye spot as follows:

$$\Delta R = |R_a - R_b| \tag{1}$$

$$\Delta G = |G_a - G_b| \tag{2}$$

$$\Delta B = |B_a - B_b| \tag{3}$$

Here, a, correspond to "final"; b, correspond to "initial". $\Delta R, \Delta G, \Delta B$ are the colour differences.

The colour change profile is, afterward, simply a $3N$-distance vector (where N = number of dyes) which can be merely studied by multivariate statistics. It is furthermore appropriate to visually denote these vectors as colour difference maps which represent every spot as the absolute value of its colour difference in RGB. The response of each dye is expressed as the relative difference of the RGB as follows:

$$S = \frac{\Delta R + \Delta G + \Delta B}{Rb + Gb + Bb} \tag{4}$$

2.4. GC–MS analysis

The identification and verification of the isolated compounds was conducted following the method reported by Tahir, Xiaobo, Zhihua and Yaodi (2015).