Food Chemistry 229 (2017) 527–533

Contents lists available at ScienceDirect

Food Chemistry

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

Synephrine – A potential biomarker for orange honey authenticity

Patrícia A.S. Tette [a], Letícia R. Guidi [a], Esther M.A.F. Bastos [b], Christian Fernandes [a,c], Maria Beatriz A. Gloria [a,*]

[a] LBqA – Laboratório de Bioquímica de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, bloco 3, sala 2091, Pampulha, CEP 31210-901 Belo Horizonte, MG, Brazil
[b] Serviço de Recursos Vegetais e Opoterápicos, Fundação Ezequiel Dias, Rua Conde Pereira Carneiro, 80, Gameleira, CEP 30510-010 Belo Horizonte, MG, Brazil
[c] Laboratório de Controle de Qualidade de Medicamentos e Cosméticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, bloco 2, sala 4029, Pampulha, CEP 31210-901 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form 2 February 2017
Accepted 21 February 2017
Available online 23 February 2017

Chemical compound studied in this manuscript:
Synephrine (PubChem CID: 7172)

Keywords:
Synephrine
Orange honey
Citrus honey
Honey
Amines
Botanical origin
Biomarker

ABSTRACT

A LC-MS/MS method for synephrine as a biomarker for orange honey authenticity was developed and validated. The sample was extracted with 5% TCA and cleaned up with Florisil providing 83.7% recoveries. Ions transitions for quantification and identification were 168 → 135.0 and 168 → 107.0, respectively. The limits of detection and quantification were 0.66 and 1.0 ng/g, respectively. Synephrine was detected in orange honey at levels from 79.2 to 432.2 ng/g, but not in other monofloral honeys. It was also present in some wildflower honeys (9.4–236.5 ng/g), showing contribution of citrus to this polyfloral honey. Results were confirmed by qualitative pollen analysis. No citrus pollen was detected in honey containing synephrine levels ≤43.8 ng/g, suggesting that synephrine in honey is more sensitive compared to pollen analysis. Synephrine was found in citrus but not in other apiculture flowers. Therefore, synephrine is a botanical marker to differentiate and attest authenticity of orange honey.

1. Introduction

Honey is a natural food known for its nutritional and medicinal value. It is often used as a sugar substitute due to its sweetness, desirable color and flavor characteristics. It is also utilized as ingredient or natural preservative in many foods (Pyrzynska & Biesaga, 2009). Chemically, honey is composed of a mixture of sugars, including monosaccharides (75%), disaccharides (10–15%) and small amounts of other sugars (Silva, Gauche, Gonzaga, Costa, & Fett, 2016). Other components are also present in minor proportions, such as minerals (calcium, copper, iron, magnesium, phosphorus, and potassium), proteins, amino acids, vitamins, flavonoids, pigments, and several organic acids. Among components, several show antioxidant properties including chrysin, pinobanksin, vitamin C, catalase and pinocembrine (Blasco, Vazquez-Roig, Onghena, Masia, & Picó, 2011; Downey, Hussey, Kelly, Walshe, & Martin, 2005; Fallico, Zappala, Arena, & Verzera, 2004; Finola, Lasagno, & Marioli, 2007; Silva et al., 2016).

The quality of honey can be affected by several factors including types of bees, presence of sucking insects, botanical origin, geographical location, climatic conditions, ripening stage, as well as processing and storage conditions. For this reason, honey may show different consistency, color, flavor and aroma (Downey et al., 2005; Komatsu, Marchini, & Moreti, 2002; Silva et al., 2016).

The most common types of plants used for honey production are eucalyptus, citrus and wildflowers (Komatsu et al., 2002). Honey can be produced from the nectar of a single botanical species - monofloral - or more than one species - polyfloral (Bastos, Franco, Silva, Janzantti, & Marques, 2002). Generally, a monofloral honey has defined aroma and taste which makes it especially appreciated by consumers (Fallico et al., 2004).

Citrus honey is considered one of the best monofloral honeys. In addition to the appreciated flavor, the floral fragrance is exclusive of this type of honey. It is also quite popular. It is characterized by a light color, intense odor, mild flavor and fine crystallization

* Corresponding author.
E-mail address: mbeatriz@ufmg.br (M.B.A. Gloria).

http://dx.doi.org/10.1016/j.foodchem.2017.02.108
0308-8146/

(Komatsu et al., 2002; Sesta, Piana, Oddo, Lusco, & Belligoli, 2008; Terrab, Díez, & Heredia, 2003).

Evaluation of honey authenticity is important in the context of consumer protection, quality control and trade purposes (Verzera, Tripodi, Condurso, Dima, & Marra, 2014). The most commonly used approach is melissopalynological analysis, which consists in the botanical classification of honey by identification and quantification of the percentage of pollen under microscopic examination. Although this method is widely used, it has shown limitations for citrus honey, because its pollen is considered 'under represented' (Escriche, Kadar, Juan-Borrás, & Domenech, 2011; Kus & Ruth, 2015; Rodriguez, Salud, Hortensia, Luis, & Jodral, 2010). When compared to other honeys, the amount of pollen present in citrus honey is lower, considering the strongly present characteristics of flavor and taste (Escriche et al., 2011; Rodriguez et al., 2010). Chemical components can also be used to discriminate honeys from different botanical origins. Several classes of compounds can be botanical markers, among them, phenolics, flavonoids, organic acids, terpenes, sugars, amino acids, among others (Boffo, Tavares, Tobias, Ferreira, & Ferreira, 2012; Escriche et al., 2011; Ferreres, Viguera, Lorente, & Barberán, 1993; Liang, Cao, Chen, Xiao, & Zheng, 2009; Schievano, Morelato, Facchin, & Mammi, 2013; Serrano, Villarejo, Espejo, & Jodral, 2004; Verzera et al., 2014). Some compounds have been suggested as markers of orange honey authenticity, such as caffeine, (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (Schievano et al., 2013), hesperetin (Escriche et al., 2011; Ferreres et al., 1993; Liang et al., 2009); naringenin and caffeic acid (Escriche et al., 2011). Although these compounds are important for characterization of citrus honeys, they are complex, may require long analysis time, and are not specific of orange honey, and could be present in other monofloral honeys. Furthermore, they require confirmation by other techniques.

Synephrine is an aromatic amine, characteristic of citrus. It has been successfully used as a biomarker for authenticity of orange juice and orange soft drink (Stewart & Wheaton, 1964; Vieira, Silva, & Gloria, 2010). Synephrine is a sympathomimetic amine. It can cause vasoconstriction, increased blood pressure and relaxation of the bronchial muscle. It is also useful in reducing fat mass in obese humans as it stimulates lipolysis and raises metabolic rate and oxidation of fat through increased thermogenesis (Kusu, Matsumoto, Arai, & Takamura, 1996; Stewart, Newhall, & Edwards, 1964; Tsujita & Takaku, 2007; Vieira, Theodoro, & Gloria, 2007; Vieira et al., 2010). Because of beneficial properties associated with this compound, the identification of synephrine in honey would be a value-added feature. Therefore, the aim of this study was to develop and validate a LC-MS/MS method for the analysis of synephrine in orange honey and to evaluate its use as an authenticity index for this monofloral honey.

2. Experimental

2.1. Honey samples

Monofloral honey samples from *Apis mellifera* bees, including citrus (*Citrus* sp., eight different brands, n = 8), 'assa-peixe' or vernonia (*Vernonia* sp., n = 2), and eucalyptus (*Eucalyptus* sp., n = 3) were purchased from the consumer's market and 'aroeira' honey (*Myracrodruon urundeuva*, n = 5) was provided by Serviço de Recursos Vegetais e Opoterápicos (SRVO, FUNED). Wildflower (polyfloral) honeys (16 different brands) were also purchased from the consumer's market of São Paulo and Minas Gerais, Brazil, eight different brands from each state. Eucalyptus honey was used as blank during method development. The samples were stored at room temperature (20 °C) until analysis.

2.2. Chemicals and reagents

Trichloroacetic acid (Neon, Vila Prudente, SP, Brasil), hydrochloric acid (Quimica Moderna, Barueri, SP, Brasil), glycerin (Furlab, Campinas, SP, Brasil), polymerically bonded, ethylenediamine-*N*-propyl phase (PSA) (Agilent Technologies, Lake Forest, CA, USA) and Florisil (Sigma-Aldrich, Saint Louis, MO, USA) were of analytical grade. Synephrine and L-norvaline were both from Sigma-Aldrich (Saint Louis, MO, USA). Ultrapure water was obtained from Milli-Q Plus system (Millipore Corp., Milford, MA, USA).

2.3. Liquid chromatography coupled to mass spectrometry

Chromatography was performed on an Agilent (Santa Clara, CA, USA) 1200 HPLC coupled to a 5500 Triple Quadrupole mass spectrometer (Applied Biosystems, MDS SCIEX, Ontario, Canada). Chromatography was carried out using a Luna C18 column (150 × 2.0 mm, 3 μm) and a mobile phase consisting of water acidified with 0.1% formic acid at a flow rate of 0.2 mL/min. The injection volume was 10 μL and the column temperature was set at 20 °C. The chromatographic run was 8 min.

The mass spectrometer was operated using electrospray ionization (ESI) in the positive ion mode. Instrument settings, data acquisition and processing were controlled by Analyst software (Version 1.6, Applied Biosystems). Source parameters were optimized as follows: ion spray voltage 5.500 kV for ESI (+), curtain gas at 20 psi, collision gas at 4 psi, nebulizer gas and auxiliary gas at 20 psi and ion source temperature of 500 °C. Retention time, precursor ion, transitions, optimal declustering potential (DP), collision energy potentials (CE) and collision exit potentials (CXP) for synephrine and norvaline are shown in Table 1.

2.4. Sample preparation

The study was undertaken at Laboratório de Bioquímica de Alimentos – LBqA, UFMG, which is accredited by INMETRO (National Institute of Metrology, Quality and Technology) according to ISO 17025:2005 for the analysis of histamine in fish. The methods developed at LBqA for analysis of amines in food were used as a starting point in this study. Individual stock solutions were prepared at 100 μg/mL and 10 μg/mL in 0.1 M HCl for synephrine and L-norvaline (internal standard), respectively. Honey samples (1 g) were weighed in 50 mL tubes and spiked with proper amounts of working standard solution of synephrine and of the internal standard norvaline (10 ng/mL). Afterwards, 9 mL of 5% trichloroacetic acid (TCA) was added and the sample was vortexed (Velp Scientifica, Wizard, Usmate, Italy), sonicated (LS Logen Scientific, Diadema, São Paulo, Brazil) and centrifuged (Jouan MR23I, Saint Herblain, France). The supernatant was filtered through qualitative paper. Filtrates were collected and the volume was brought up to 10 mL in calibrated volumetric flasks. An aliquot (1 mL) was transferred to 2 mL tube containing Florisil and PSA for clean-up, and submitted to vortex and centrifugation (Eppendorf 5424R, Hauppauge, New York, NY, USA). Finally, the extracts were filtered through qualitative filter paper and 0.45 μm membrane filter (Millipore Corp., Milford, MA, USA) prior to injection into the HPLC-MS/MS system.

2.4.1. Optimization of parameters affecting synephrine extraction from honey

Initially, a Plackett-Burman design was used to screen the main factors that could affect synephrine recovery from honey. The design included 12 tests and three repetitions at the central point. The variables investigated were vortexing time (X_1 = 30, 90 and 150 s), vortexing speed (X_2 = 100, 200 and 300 × *g*), relative centrifugal force (X_3 = 1000, 11,000 and 21,000 × *g*), centrifugation