JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2009,** *57,* 3895–3900 **3895**
DOI:10.1021/jf803991j

Methyl Syringate: A Chemical Marker of Asphodel (*Asphodelus microcarpus* Salzm. et Viv.) Monofloral Honey

Carlo I. G. Tuberoso,*,† Ersilia Bifulco,† Igor Jerković,# Pierluigi Caboni,† Paolo Cabras,† and Ignazio Floris§

†Dipartimento di Tossicologia, Università di Cagliari, via Ospedale 72, 09124 Cagliari, Italy, #Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia and §Istituto di Entomologia Agraria, Università degli Studi di Sassari, Via E. De Nicola, 07100 Sassari, Italy

During the liquid chromatographic study of the phenolic fraction of monofloral honeys was detected in the asphodel honey (*Asphodelus microcarpus* Salzm. et Viv.) chromatogram a distinctive peak not detected in other monofloral honeys such as *Arbutus unedo* L., *Hedysarum coronarium*, *Eucalyptus* spp., and *Galactites tomentosa*. After thin layer chromatography (TLC) purification and characterization by NMR and LC-MS/MS, the compound was identified as methyl syringate (MSYR) and confirmed against an original standard. Levels of MSYR were measured in honeys of 2005, 2006, and 2007 by HPLC-DAD analysis. Level determination of MSYR was repeated in 2008 for 2006 and 2007 honeys to evaluate chemical stability of this phenolic compound. Levels of MSYR measured 1 year after the sampling did not show significant statistical differences ($p < 0.05$). The stability of MSYR was also confirmed by 12 asphodel honey samples collected in 2005 that showed amounts of methyl syringate comparable with those found in fresh honey. For the evaluation of MSYR origin, samples of nectars were collected from flowers and the content of MSYR was measured. Levels of MSYR in honeys are originated from the nectar with an average contribution of the nectar to the honey of 80%. Melissopalinological analysis did not allow the attribution of the honey monofloral origin because levels of asphodel pollen were <6% for all analyzed samples. Previously reported levels of MSYR for robinia, rape, chestnut, clover, linden blossom, dandelion, sunflower, thyme, manuka, and fir honeys were <5 mg/kg. For this reason, a minimum level of 122.6 mg/kg for MSYR in asphodel honeys can be considered as a chemical marker and, unlike the melissopalynological analysis, can be used for the origin attribution and to evaluate the percent of asphodel nectar in the honey.

KEYWORDS: Methyl syringate; honey; phenolic compounds; asphodel; HPLC-DAD; HPLC-MS/MS

Published on Web 3/23/2009

DOI:10.1021/jf9024147

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Floral Markers of Strawberry Tree (*Arbutus unedo* L.) Honey

Carlo I. G. Tuberoso,*,† Ersilia Bifulco,† Pierluigi Caboni,†
Filippo Cottiglia,§ Paolo Cabras,† and Ignazio Floris#

†Dipartimento di Tossicologia, and §Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy, and #Dipartimento Protezione Piante, Sez. Entomologia Agraria, University of Sassari, Via E. De Nicola, 07100 Sassari, Italy

Strawberry tree honey, due to its characteristic bitter taste, is one of the most typical Mediterranean honeys, with Sardinia being one of the largest producers. According to specific chemical studies, homogentisic acid was identified as a possible marker of this honey. This work, based on HPLC-DAD-MS/MS analysis of strawberry tree (*Arbutus unedo* L.) honeys, previously selected by sensory evaluation and melissopalynological analysis, showed that, in addition to the above-mentioned acid, there were other high levels of substances useful for the botanical classification of this unifloral honey. Two of these compounds were isolated and identified as (±)-2-*cis*,4-*trans*-abscisic acid (*c*,*t*-ABA) and (±)-2-*trans*,4-*trans*-abscisic acid (*t*,*t*-ABA). A third compound, a new natural product named unedone, was characterized as an epoxidic derivative of the above-mentioned acids. Structures of *c*,*t*-ABA, *t*,*t*-ABA, and unedone were elucidated on the basis of extensive 1D and 2D NMR experiments, as well as HPLC-MS/MS and Q-TOF analysis. In selected honeys the average amounts of *c*,*t*-ABA, *t*,*t*-ABA, and unedone were 176.2 ± 25.4, 162.3 ± 21.1, and 32.9 ± 7.1 mg/kg, respectively. Analysis of the *A. unedo* nectar confirmed the floral origin of these compounds found in the honey. Abscisic acids were found in other unifloral honeys but not in such high amount and with a constant ratio of about 1:1. For this reason, besides homogentisic acid, these compounds could be used as complementary markers of strawberry tree honey.

KEYWORDS: *Arbutus unedo* L. honey; botanical characterization; abscisic acids; unedone; HPLC-DAD; HPLC-MS/MS; Q-TOF; NMR

 Published on Web 11/18/2009

Molecules **2010**, *15*, 6375-6385; doi:10.3390/molecules15096375

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Composition of Sulla (*Hedysarum coronarium* L.) Honey Solvent Extractives Determined by GC/MS: Norisoprenoids and Other Volatile Organic Compounds

Igor Jerković [1,*], Carlo I.G. Tuberso [2], Mirko Gugić [3] and Dragan Bubalo [4]

[1] Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia

[2] Dipartimento di Tossicologia, Università di Cagliari, via Ospedale 72, 09124 Cagliari, Italy

[3] Marko Marulić Polytechnic in Knin, P. Krešimira IV 30, 22300 Knin, Croatia

[4] Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia

* Author to whom correspondence should be addressed; E-Mail: igor@ktf-split.hr.

Received: 1 July 2010; in revised form: 7 September 2010 / Accepted: 9 September 2010 / Published: 9 September 2010

Abstract: Samples of unifloral sulla (*Hedysarum coronarum* L.) honey from Sardinia (Italy) were analysed. To investigate the chemical composition of the honey volatiles two solvent systems were used for ultrasonic solvent extraction (USE): 1) a 1:2 (v/v) pentane and diethyl ether mixture and 2) dichloromethane. All the extracts were analysed by GC and GC/MS. These procedures have permitted the identification of 56 compounds that include norisoprenoids, benzene derivatives, aliphatic compounds and Maillard reaction products. Norisoprenoids were the major compounds in both extracts, dominated by vomifoliol (5.3-11.2%; 9.6-14.0%) followed by minor percentages of other norisoprenoids such as α-isophorone, 4-ketoisophorone, 3-oxo-α-ionol or 3-oxo-α-ionone. Other abundant single compounds in the extracts were 3-hydroxy-4-phenylbutan-2-one (0.8-5.4%; 0.6-5.7%) and methyl syringate (3.0-5.7%; 2.2-4.1%). The composition of the volatiles and semi-volatiles in the obtained extracts suggests that sulla honey is quite distinctive relative to the other honeys that have been chemically studied by GC/MS, but no specific markers of the honey botanical origin were found.

Keywords: sulla (*Hedysarum coronarium* L.) honey; ultrasonic solvent extraction (USE); gas chromatography and mass spectrometry (GC and GC/MS); norisoprenoids

Food Chemistry 124 (2011) 401–410

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Contribution to the characterisation of honey-based Sardinian product *abbamele*: Volatile aroma composition, honey marker compounds and antioxidant activity

I. Jerković [a,*], A. Kasum [a], Z. Marijanović [b], C.I.G. Tuberoso [c]

[a] Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, 21000 Split, Croatia
[b] Department of Food Technology, Marko Marulić Polytechnic in Knin, Petra Krešimira IV 30, 22300 Knin, Croatia
[c] Department of Toxicology, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

ARTICLE INFO

Article history:
Received 17 December 2009
Received in revised form 11 May 2010
Accepted 12 June 2010

Keywords:
Abbamele
Headspace solid-phase microextraction (HS-SPME)
Ultrasonic solvent extraction (USE)
GC and GC–MS
DPPH and FRAP assay
HPLC-DAD

ABSTRACT

Sardinian *abbamele* is a typical product obtained from the honey recuperation from combs (traditional procedure) or by concentration of the honey diluted in water (industrial procedure). Seven *abbamele* samples were obtained to study the volatiles' composition, the presence of honey marker compounds and their relationship with the production procedures. The long thermal treatment applied in *abbamele* production caused very high (1007.0–4405.8 mg/kg) HMF content (HPLC-DAD), while glucose and fructose amounts were quite similar to the honey ones (HPLC-RI). Total antioxidant activity (FRAP assay) of the samples ranged between 13.3 and 71.2 mmol Fe^{2+}/kg, while antiradical activity (DPPH assay) ranged between 3.8 and 23.3 mmol TEAC/kg. Such high antioxidant values were linearly correlated with total phenol amount (1297.8–4469.5 mg GAE/kg) determined by Folin–Ciocalteau method. Thermally derived furan derivatives and terpenes were abundant among the headspace volatiles (HS-SPME), particularly limonene (0.5–76.0%) that probably originated from citrus rinds' addition during *abbamele* production. GC and GC–MS analyses of USE isolates revealed HMF predominance as well as the honey marker compounds (if/when existing) such as methyl syringate (up to 49.2%), marker of *Asphodelus microcarpus* honey. High isophorone percentage (up to 30.9%) determined by HS-SPME followed by minor percentage of 4-ketoisophorone and norisoprenoids in one sample indicated *Arbutus unedo* L. honey use in the production. HPLC-DAD analysis confirmed the presence of specific honey markers: two samples showed high methyl syringate concentrations (150.4–120.1 mg/kg) while homogentisic acid and other specific markers of *A. unedo* honey were found in one sample. The compared GC–MS and HPLC-DAD data proved to be useful to obtain information about the use of specific honey in the production and to verify citrus addition.

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doi:10.1016/j.foodchem.2010.06.047

Volatile Compounds of *Asphodelus microcarpus* SALZM. et VIV. Honey Obtained by HS-SPME and USE Analyzed by GC/MS

by **Igor Jerković***[a]), **Carlo I. G. Tuberoso**[b]), **Ana Kasum**[a]), and **Zvonimir Marijanović**[c])

[a]) Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, N. Tesle 10/V, HR-21000 Split (phone: +385-21-329434; fax: +385-21-329461; e-mail: igor@ktf-split.hr)

[b]) Department of Toxicology, University of Cagliari, via Ospedale 72, IT-09124 Cagliari

[c]) Department of Food Technology, Marko Marulić Polytechnic in Knin, Petra Krešimira IV 30, HR-22300 Knin

Chemical analysis of *Asphodelus microcarpus* SALZM. et VIV. honey is of great importance, since melissopalynology does not allow the unambiguous determination of its botanical origin. Therefore, the volatile compounds of eight unifloral asphodel honeys have been investigated for the first time. The honey extracts were obtained by headspace solid-phase microextraction (HS-SPME) and ultrasonic solvent extraction (USE) and analyzed by GC and GC/MS. In the honey headspace, 31 volatile compounds were identified with high percentages of 2-phenylacetaldehyde (**2**; 14.8–34.7%), followed by somewhat lower percentages of methyl syringate (**1**; 10.5–11.5%). Compound **2** is not a specific marker of the botanical origin of the honey, but its high percentage can be emphasized as headspace characteristic of asphodel honey. The extraction solvent for all the samples was selected after extracting a representative sample with pentane, Et_2O, pentane/Et_2O 1:2 (*v*/*v*), and CH_2Cl_2. Compound **1** was the major constituent of all the USE extracts (46.8–87.0%). According to these preliminary results, all the honey samples were extracted by USE with the solvent pentane/Et_2O 1:2. A total of 60 volatile compounds were identified with **1** as predominant compound (69.4–87.0%), pointing out **1** as *Asphodelus* honey volatile marker.

Food Chemistry 129 (2011) 1045–1053

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress

Antonella Rosa [a,*], Carlo Ignazio Giovanni Tuberoso [b], Angela Atzeri [a], Maria Paola Melis [a], Ersilia Bifulco [b], Maria Assunta Dessì [a]

[a] Dipartimento di Biologia Sperimentale, Sez. Patologia Sperimentale, Università degli Studi di Cagliari, Cittadella Universitaria, SS 554, km 4.5, 09042 Monserrato, Cagliari, Italy
[b] Department of Toxicology, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

ARTICLE INFO

Article history:
Received 7 January 2011
Received in revised form 18 March 2011
Accepted 18 May 2011
Available online 25 May 2011

Keywords:
Arbutus unedo L. honey
Homogentisic acid
Antioxidant activity
Antiradical activity
Caco-2

ABSTRACT

The antioxidant activity of several honeys was evaluated considering the different contribution of entire samples. The strawberry tree honey emerged as the richest in total phenols and the most active honey in the DPPH and FRAP tests, and could protect cholesterol against oxidative degradation (140 °C). Homogentisic acid (2,5-dihydroxyphenylacetic acid, HGA), the main phenolic compound from strawberry tree honey, showed interesting antioxidant and antiradical activities, and protective effect against thermal-cholesterol degradation, comparable to those of well known antioxidants. Moreover, the pre-treatment with HGA significantly preserved liposomes and LDL from Cu^{2+}-induced oxidative damage at 37 °C for 2 h, inhibiting the reduction of polyunsaturated fatty acids and cholesterol and the increase of their oxidative products. This phenol had no toxic effect in human intestinal epithelial Caco-2 cells within the concentration range tested (5–1000 μM). HGA was able to pass through the Caco-2 monolayers, the apparent permeability coefficients (P_{app}) in the apical-to-basolateral and basolateral-to-apical direction were $3.48 \pm 1.22 \times 10^{-6}$ and $2.18 \pm 0.34 \times 10^{-6}$ cm/s, respectively, suggesting a passive diffusion pathway as the dominating process. The results of the work qualify HGA as natural antioxidant, able to exert a significant *in vitro* protective effect and to contribute to the strawberry tree honey antioxidant activity.

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doi:10.1016/j.foodchem.2011.05.072

DOI:10.1021/jf1039074

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Lumichrome and Phenyllactic Acid as Chemical Markers of Thistle (*Galactites tomentosa* Moench) Honey

Carlo I. G. Tuberoso,*,† Ersilia Bifulco,† Pierluigi Caboni,† Giorgia Sarais,† Filippo Cottiglia,# and Ignazio Floris§

†Dipartimento di Tossicologia, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy, §Dipartimento Protezione Piante, Sez. Entomologia Agraria, University of Sassari, via E. De Nicola, 07100 Sassari, Italy, and #Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

HPLC-DAD-MS/MS chromatograms of thistle (*Galactites tomentosa* Moench) unifloral honeys, previously selected by sensory evaluation and melissopalynological analysis, showed high levels of two compounds. One was characterized as phenyllactic acid, a common acid found in honeys, but the other compound was very unusual for honeys. This compound was extracted from honey with ethyl acetate and purified by SPE using C_{18}, SiOH, and NH_2 phases. Its structure was elucidated on the basis of extensive 1D and 2D NMR experiments as well as HPLC-MS/MS and Q-TOF analysis, and it was identified as lumichrome (7,8-dimethylalloxazine). Lumichrome is known to be the main product of degradation obtained in acid medium from riboflavin (vitamin B_2), and this is the first report of the presence of lumichrome in honeys. Analysis of the *G. tomentosa* raw honey and flowers extracts confirmed the floral origin of this compound. The average amount of lumichrome in thistle honey was 29.4 ± 14.9 mg/kg, while phenyllactic acid was 418.6 ± 168.9 mg/kg. Lumichrome, along with the unusual high level of phenyllactic acid, could be used as a marker for the botanical classification of unifloral thistle (*G. tomentosa*) honey.

KEYWORDS: Lumichrome; *Galactites tomentosa* Moench; honey; botanical characterization; HPLC-DAD; HPLC-MS/MS; Q-TOF; NMR

Published on Web 12/02/2010