LWT - Food Science and Technology 64 (2015) 547–553

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Antimicrobial an antioxidant activity of selected Polish herbhoneys

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form
8 June 2015
Accepted 11 June 2015
Available online 19 June 2015

Keywords:
Herbhoneys
Enzymatic activity
Antimicrobial effect
Antioxidants
Total phenolic content

ABSTRACT

Herbhoneys are bee products obtained by feeding bees with a sugar medium enriched with plant extracts. Five types of such products including: nettle, chokeberry, pine, hawthorn and aloe as well as three types of natural honeys: buckwheat (*Fagopyrum* spp.), acacia (*Robinia pseudoacacia*) and honeydew honey were analyzed. The studies focused on the determination of the major enzymes activities, antioxidant capacity and identification of antimicrobial effects. It was found, that among others nettle herbhoney possessed the highest antimicrobial ability. This ability was evident especially against *Escherichia coli* and *Proteus myxofaciens*. All samples tested had no detectable antimicrobial activity against *Bacillus subtilis*. The investigated herbhoneys were also characterized by higher antioxidant capacity compared to natural honey. In herbhoneys samples high concentration of citric acid (particularly nettle and chokeberry herbhoneys – 572 and 508 mg/100 g respectively) were found. It was noted that all herbhoney samples utilized in this study fulfill the quality parameters established for natural honeys by the EC directive.

1. Introduction

Honey is one of the oldest food products known to mankind. Since the start of recorded human history, honey has been used not only for nutrition but also in therapeutic applications.

In order to extend the range of bee products, with a focus on consumer satisfaction and desire, researchers are constantly looking for new technological developments, as exemplified by the production of herbhoneys. The beginning of herbhoney production dates back to the first half of the 20th century. The real development of interest and production of herbhoneys took place in Poland in 1980s. Despite some controversy surrounding the term "herbhoney", it is still in use (Kędzia & Kostrzewski, 2013a).

Herbhoneys are obtained via the process of feeding bees by a sugar medium enriched with plant extracts. Initially, this sugar medium was only enriched with herbal extracts. The addition of plant juices began to occur much later. The different types of herbhoneys have different functional effects depending on the types of raw materials used in their production. In Poland they are used in phytotherapy as prophylactic agents and nutritional supplements (Juszczak, Socha, Rożnowski, Fortuna, & Nalepka, 2009; Kędzia & Kostrzewski, 2013a).

Herbhoneys exhibit similar properties to "natural" honeys. In herbhoneys most of the biological properties are more pronounced compared to the natural types. This is due to the presence of a higher content of biologically active substances of plant origin. An example of this can be found in higher antibiotic and/or antioxidant activity of herbhoneys compared to the varietal honeys (Kędzia & Hołderna-Kędzia, 2013; Kędzia & Kostrzewski, 2013b). The presence of flavonoids in herbhoneys has been recently confirmed by Socha, Juszczak, Pietrzyk, and Fortuna (2009). The principal herbhoneys component *p*-coumaric acid, and, in smaller amounts, gentisic acid were identify. Other phenolic acids (gallic, chlorogenic, caffeic, sinapinic, ferulic and syringic) occurred as minor components (Socha et al., 2009).

One of the factors affecting the health benefits of honeys is the presence of glucose oxidase (GOX). This enzyme is employed in the production of gluconic acid from glucose. As a by-product of the reaction hydrogen peroxide is g. GOX is a light and temperature sensitive enzyme that is active in nectar or dew but inactive in viscous honey. After dilution, however enzyme becomes active again and may produce hydrogen peroxide as a by-product of this action. This phenomenon is the milestone of the traditional application of honey solutions used to prevent bacteria growth which may occur as secondary infection in diseases such as influenza and colds. Additionally worth pointing out that H_2O_2 produced as a

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http://dx.doi.org/10.1016/j.lwt.2015.06.029
0023-6438/

result of GOX action may be destroyed by the action of catalase, another important honey enzyme. Until recently, few research was done on glucose oxidase activity in honey and thus its activity is not a normalized parameter (Kretavičius, Kurtinaitiene, Račys, & Čeksteryte, 2010; Mahmoud & Owayss, 2006).

Although nowadays the importance of herbhoneys has a rather local character, due to the globalization of the market, the interest in these products has increased significantly. In addition to this, from the scientific point of view, there is increasing reports of the beneficial composition and health properties of these products (Isidorov, Bagan, Bakier, & Swiecicka, 2015; Juszczak et al., 2009; Socha et al., 2009).

2. Materials and methods

2.1. Materials

Herbhoney samples (nettle – *Urtica dioica*, chokeberry – *Aronia melanocarpa*, pine – *Pinus* sp., hawthorn – *Crataegus monogyna* and aloe – *Aloe vera*) as well as natural honey samples (buckwheat – *Fagopyrum* spp., acacia – *Robinia pseudoacacia* and honeydew honey) were purchased from local beekeeping cooperatives (Bartnik Sadecki – Poland). The botanical origin of each honey was confirmed via the producer's statement.

2.2. Methods

2.2.1. Electrical conductivity, water, acidity, specific rotation

Determination of electrical conductivity, moisture, acidity and specific rotation, were carried out according to Harmonized Methods of the International Honey Commission (Bogdanov, 2002).

2.2.2. Chromatographic analysis of HMF, monosaccharides and organic acids

Chromatographic analysis was carried out using Knauer HPLC system (Knauer, Germany) as indicated in Table 1.

2.2.3. Enzymes activity determination

Diastase and invertase activity were expressed as diastase (DN) and invertase (IN) number respectively. The applied methods followed the Harmonized Methods of the International Honey Commission (Bogdanov, 2002). Diastase number, in Schade scale, is defined as the amount of starch (g) hydrolyzed during 1 h at 40 °C per 100 g of honey. Invertase number indicates the amount of sucrose in grams, hydrolyzed in 1 h by the enzymes in 100 g of honey under test conditions (Bogdanov, 2002). The glucose oxidase (GOX) activity was determined based on the H_2O_2 detection method developed by Mottola, Simpson, and Gorin (1970). Activity of GOX was indicated as milligrams of H_2O_2 produced in 1 kg of honey over 24 h. The detailed procedure involves the oxidation of leucocrystal violet (LCV) by H_2O_2 from honey using horseradish peroxidase (HRP) added to the sample. According to this procedure LCV solution was prepared by dissolving 25 mg of LCV in 50 mL of a 0.5% HCl solution. HRP solution was obtained by dissolving 10 mg of HRP (activity 156 U/mg) in 10 mL of water. Honey/herbhoney samples were prepared by 1:4 (w/v) dilution with distilled water. Subsequently 1 mL of LCV solution was added to 4 mL of honey solution placed in a 10-mL volumetric flask. 0.5 mL of HRP solution and 4 mL of buffer (acetic buffer; pH 4.5) were then added. The mixture was diluted to 10 mL with distilled water. After a24 h period of incubation the absorbance of the sample was measured at 596 nm against a reference prepared in the same manner but with no honey present. The concentration of H_2O_2 was determined by comparing to a calibration curve. The measurements were done in duplicate.

2.2.4. Total phenolic content

Total phenolic content was measured using Folin–Ciocalteu reagent (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). All analyzed samples were prepared at a concentration of 0.5 g/mL. Results were expressed as mg of gallic acid equivalent (GAE) per 100 g of sample.

2.2.5. Antioxidant activity

The antioxidant activity with stable $ABTS^{\bullet+}$ radical cation was analyzed by means of spectrophotometric method according to Baltrusaityte, Venskutonis, and Ceksteryte (2007) based on work of Re et al. (1999). The antioxidant activity against the $DPPH^{\bullet}$ radical was evaluated according to Turkmen, Sari, Poyrazoglu, and Velioglu (2006). Samples were diluted with distilled water to a concentration of 0.2 g/mL and then filtered. The scavenging activity (S_A) (against both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$) was expressed as percent of suppression of $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ radical and calculated as follows:

$$S_A[\%] = \frac{ABS_{blank} - ABS_{sample}}{ABS_{blank}} \cdot 100\%$$

where S_A = $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ inhibition; ABS_{blank} = absorption of a blank sample; ABS_{sample} = absorption of a tested sample. Measurements were done in duplicate.

2.2.6. Antimicrobial activity

For antimicrobial activity measurements, five different microorganisms were used. These included: *Escherichia coli* (DSMZ 20030), *Micrococcus luteus* (DSMZ 4261), *Proteus myxofaciens* (DSMZ 4482), *Bacillus subtilis* (DSMZ 10) and *Pseudomonas putida* (DSMZ 291). Microbial strains were purchased from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). Tested microorganisms were grown in nutrient broth (Biocorp PS 90) at 35 °C for 24 h. All bacterial samples were enumerated using the McFarland densitometer method (Silici, Sagdic, & Ekici, 2010). The final cell concentrations were in the range of 10^8–10^9 cfu/mL. 250 μL of each bacterial suspension was added to a flask containing 25 mL sterile nutrient agar (Biocorp PS 85) at 45 °C and poured into

Table 1
Chromatographic analysis conditions.

Analyte	Column	Detection	Eluent, v/v	Flow, mL/min	Loop, μL	Reference
HMF	RP-18 Lichrosphere (250 × 4 mm, 5 μm particle size)	UVVIS, λ = 285 nm	H_2O/MeOH 9:1	1.0	20	(Bogdanov, 2002)
Carbohydrates	Lichrosphere 100–10 NH_2 column (250 × 4 mm, 10 μm particle size)	RI	ACN/H_2O	1.3	10	(Bogdanov, 2002)
Acids	RP-18 Lichrosphere (250 × 4 mm, 5 μm particle size)	UVVIS, λ = 218 nm	5.75 mM NaH_2PO_4 pH adjusted to 2.2 with H_3PO_4	1.0	20	(Marciniak & Lukasiewicz, 2011)