Food Chemistry 138 (2013) 851–856

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Nutritional value and antioxidant activity of honeys produced in a European Atlantic area

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ARTICLE INFO

Article history:
Received 19 June 2012
Received in revised form 10 October 2012
Accepted 7 November 2012
Available online 17 November 2012

Keywords:
Honey
Nutritional value
Phenolic content
Flavonoid content
Antioxidant activity

ABSTRACT

One hundred eighty-seven honey samples from an Atlantic European area were studied to determine their nutritional compositions and antioxidant capacities, as well as the relationships between them. The results showed that heather, polyfloral, blackberry, and eucalyptus honeys had the highest carbohydrate contents, whereas honeydew and chestnut honeys had the lowest. There were some important differences among the honey types, which were related to the presence of minor components. The protein contents were significantly higher in honeydew and chestnut honeys, and the same results were obtained for mineral contents. Related to the presence of several antioxidant compounds, heather honey had the highest phenolic content, whereas honeydew and chestnut honeys had the highest flavonoid contents. Multivariate analysis showed that some variables, such as the amounts of flavonoids, minerals, proteins, and phenols, were significantly correlated with antioxidant activity. The regression analysis produced a significant model ($R^2 = 0.716$; $F = 154.680$; $P < 0.001$) that related the antioxidant activity and the flavonoids, K, and P contents.

1. Introduction

Honey, one of the oldest and most traditional sweetening foods, has been reported to contain about 200 substances (Ferreira, Aires, Barreira, & Estevinho, 2009; Gheldof, Wang, & Engeseth, 2002). This natural product is essentially a concentrated aqueous solution of different carbohydrates, including fructose, glucose, maltose, sucrose, and other oligo- and polysaccharides. These carbohydrates comprise about 95% of a honey's dry weight (Bogdanov, Jurendic, Sieber, & Gallmann, 2008); fructose and glucose are the primary sugars. In the process of digestion after honey intake, the principal carbohydrates are transported into the blood and can be utilised for energy requirements by the human body. Besides these components, honey contains certain minor constituents, such as proteins, enzymes (alpha-glucosidase, beta-glucosidase, catalase, and phosphatases), amino and organic acids (gluconic acid), lipids, vitamins, minerals, and phytochemical substances, mainly flavonoids and phenolic compounds (Alvarez-Suárez, Tulipani, Romandini, Bertoli, & Battino, 2010). The dietary contributions of these minor components to the recommended daily intake are marginal. However, their importance with respect to nutrition resides in their physiological effects, since they display several health-related properties (Alvarez-Suárez et al., 2012; Sant'Ana, Sousa, Salgueiro, Affonso, & Castro, 2012). Minerals and other trace elements play key roles in the biomedical activities associated with honey. These elements have known biological functions, such as the maintenance of intracellular oxidative balance (Shoham & Youdim, 2000).

Over the past decade, the use of honey for therapeutic purposes has been re-evaluated in a more scientific setting, and several properties have been identified (Alvarez-Suárez et al., 2010; Bogdanov et al., 2008; The National Honey Board, 2002). Fresh honey displays significant antioxidant activity, similar to many fruits and vegetables (Gheldof et al., 2002). Plants contain a variety of polyphenolic derivatives with high structural diversity and complexity, and consequently, when the bees collect nectar or honeydew, these bioactive compounds may be transferred from plants to honey (Silici, Sagdic, & Ekici, 2010). As natural antioxidants, phenolic compounds in honeys (especially flavonoids) constitute an important group for their functional properties and their scientific and therapeutic significance (Yao et al., 2004). Several studies have demonstrated a strong correlation between the contents of phenolic compounds in honeys from various floral sources and their antioxidant capacities and antibacterial activities (Alvarez-Suárez et al., 2012; Escuredo, Silva, Valentao, Seijo, & Andrade, 2012; Gheldof et al., 2002; Meda, Lamien, Romito, Millogo, & Nacoulma, 2005).

The aim of this study was to determine the nutritional composition and antioxidant activities of honeys from different botanical origins produced in an Atlantic area. Multivariate analysis was

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http://dx.doi.org/10.1016/j.foodchem.2012.11.015

carried out to find the relationship between the studied parameters and the antioxidant activity.

2. Materials and methods

2.1. Honey samples

The present study was carried out on 187 honeys collected directly from beekeepers in the Northwest of Spain. The samples were chestnut (*Castanea sativa*), eucalyptus (*Eucalyptus*), blackberry (*Rubus*), heather (*Erica*), honeydew, and polyfloral honeys. The botanical origins of the honeys were tested by melissopalynological analysis. The quality of the honeys was checked before the quantification of the different nutritional compounds.

2.2. Determination of nutritional compounds

The moisture in the samples was measured using a refractometer (Abbe 325). All measurements were performed at 20 °C.

The sugar contents were determined using an ion chromatography system (Dionex ICS-3000 SP) incorporating an analytical column (Carbopac PA1), a guard column, and a pulse amperometric detector (PAD). A honey solution (10 μl) in water (10 mg/l) was injected into the loop of the chromatograph. Two mobile phases (A and B) were used for the separation of the sugars. Phase A was ultrapure water and phase B was 200 mM NaOH. The identified sugars were the monosaccharides, fructose and glucose; the disaccharides, sucrose, maltose, and trehalose; and the trisaccharide, melecitose.

The total protein content was measured using the Kjeldahl method for proteins (AOAC, 2005), based on the conversion of the organic nitrogen present in the sample to $(NH_4)_2SO_4$. Dried sample (1 g) was subjected to two processes: digestion and distillation. The sample was mixed with a selenium catalyst and H_2SO_4 (15 ml, 95–98%). The resulting solution was distilled after adding NaOH, and the distillate was collected in a flask with H_3BO_3 (4%) and mixed indicator. Finally, the mixture was titrated with HCl (0.1 N). The percentage of nitrogen quantified was transformed into protein content by multiplying by a conversion factor of 6.25.

Lipid content was assessed by the Soxhlet method (AOAC, 2006), quantifying the dry matter using an automatic extractor (Model SX-6 Raypa) with petroleum ether as solvent. Dried honey (5 g) was introduced into an extraction cartridge and extracted for about 1 h at 100 °C. Then, the vessel was heated until the solvent was completely evaporated, cooled, and finally, weighed. The difference in the weight of the vessel before and after the extraction was used to calculate the lipid content.

The minerals K, Ca, Fe, Mg, Na, P, Zn, and Cu were quantified using an atomic absorption spectrophotometer (Varian SpectrAA-600; Agilent Technologies, Santa Clara, CA, USA). In a preliminary step, samples were heated and sonicated to facilitate honey homogenisation. Aliquots of honey (0.5 g) were transferred into Teflon®-coated vessels and digested in a microwave oven (CEM MARSXpress) after addition of a mixture of HNO_3–H_2O_2 (9:2, 5 ml) (Caroli, Forte, Iamiceli, & Galoppi, 1999).

The energy values of the samples were computed in kilocalories per gramme of honey using the Atwater numbers.

2.3. Determination of the antioxidant activities and the phenolic and flavonoid contents in the honeys

The antioxidant activities of the honey samples were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Brand-Williams, Culivier, & Berset, 1995). The DPPH· assay is considered a valid and simple assay for evaluating the scavenging activity of antioxidants because of the stability of the radical compound (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009). Honey samples were dissolved in methanol at a concentration of 0.1 g/ml. A sample of this solution (0.3 ml) was mixed with DPPH· solution (2.7 ml, 6×10^{-5} M). The sample mixture and a DPPH· solution (blank) were maintained in the dark at room temperature for 30 min. Then, the absorbance of the solutions was measured at 517 nm with a UV–vis spectrophotometer (Jenway 6505). The antioxidant activity of each sample was calculated as the percentage of RSA (radical scavenging activity) using the formula: $RSA = [(A_B - A_A)/A_B] \times 100$, where A_B is the absorbance of the DPPH· solution and A_A is the absorbance of the honey sample solution.

The concentration of honey required to scavenge 50% DPPH· (IC_{50}) was calculated using a standard curve for ascorbic acid, and was expressed in mg/ml.

The total phenolic content was measured according to the Folin–Ciocalteu method (Singleton & Rossi, 1965). Honey was diluted with bidistilled water to a concentration of 0.1 g/ml. The diluted honey sample (1 ml) was added to bidistilled water (10 ml) and Folin–Ciocalteu reagent (1 ml). The mixture was gently agitated and left to rest for 2 min; then, Na_2CO_3 (4 ml) was added to the mixture. The solution was incubated for 1 h at room temperature in the dark, and the absorbance was read at 765 nm against a blank solution. Gallic acid standard solutions were used as references (Meda et al., 2005) to construct the calibration curve. The results were expressed as mg gallic acid per 100 g honey (mg GAE/100 g).

The total flavonoid content was measured using the Dowd method, as adapted by Arvouet-Grand, Vennat, Pourrat, and Legret (1994). The honey sample was diluted in bidistilled water to a concentration of 0.3 g/ml. The honey solution (2 ml) was mixed with $AlCl_3$ (0.5 ml) and bidistilled water. The solution was kept in the dark for 30 min and the absorbance at 425 nm was measured (Meda et al., 2005). Quercetin was used as reference for the calibration curve. Finally, the results were expressed as mg quercetin per 100 g honey (mg QE/100 g).

2.4. Statistical analysis

All analyses were carried out in duplicate and the data were presented as means ± standard deviations. Analysis of variance (ANOVA) according to the Bonferroni test was used to compare the quantified variables in the samples of honey. The relationships between the nutritional composition and several of the antioxidant compounds studied in the honey were investigated by Spearman's correlation coefficient in bivariate linear correlations. Besides the correlation analysis, a factorial analysis was used to simplify the structure of the data by transforming a set of interdependent variables into another independent set or a set of smaller dimension. The factor analysis shows similarities between samples projected on a plane and makes it possible to identify which variables determine these similarities and in what way. Finally, to investigate the parameters most related to the antioxidant capacity of honey, a stepwise regression analysis was performed. The significance was calculated for $P < 0.05$. The statistical analyses were performed with the SPSS Statistic 19.0 software for Windows and STATGRAPHICS Centurion 16.0.

3. Results and discussion

3.1. Nutritional composition of the honeys

The nutritional composition of the collected honeys is summarized in Table 1. The moisture content of each honey depended principally on the production season and the meteorological conditions in the area where the honey was produced. This content