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# Effect of filtration on colour, antioxidant activity and total phenolics of honey

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ABSTRACT

82 Honey samples harvested in 2009 and 2010 flowering seasons were investigated in order to examine the influence of filtration on colour, antioxidant activity and total phenolics content. In unfiltered honey samples the total phenolics content varied between 40.5 and 177 mgGAE/100 g. The average antioxidant activity ranged from 47.2 to 83.4% when measured with DPPH$^{\bullet}$ and from 6 to 79% once measured with ABTS$^{+}$. The results indicated that filtration had no significant effect on the above-mentioned parameters, however they were found to depend on the types of honey.



## 1. Introduction

EC Honey Directive No 2001/110/EC stipulates that: "honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" (Annex I, point 1). According to this definition, honey is a natural product in which nothing is added or taken away. On the other hand, with the same regulation it is now possible to market filtered honey – "honey obtained by removing foreign inorganic or organic matter in such a way as to result in the significant removal of pollen" (Annex I, point 2 (b) (viii)).

The main reason of filtration is to stop the crystallization process and remove small impurities, i.e. yeast cells. In Poland filtered honey does not meet the approval of consumers. The criticism has been made that the filtration, beside air bubbles, sugar crystals and pollen, would eliminate the essential elements of honey, i.e. vitamins, colour and flavour, and that the quality of filtered honey would be lower than that of unfiltered one. It is thought that filtered honey is usually more clear and light, but observations of colour readings of samples processed using the modern filtering methods indicated that there were no significant differences in colour of filtered honeys and the same unfiltered samples (Root & Root, 2005). It was found that after filtration the amounts of HMF were higher and enzyme activities suppressed, resulting from the honey being heated prior to the filtration process (Beckmann, Beckh, Luellmann, & Speer, 2010). There is no data about other honey components, which exhibit antioxidant, antimicrobial and wound healing properties. Honey is known as a source of enzymatic and non-enzymatic antioxidants including glucose oxidase, ascorbic acid, catalase, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino-acids and proteins (Aljadi & Kamaruddin, 2004; Al-Mamary, Al-Meeri, & Al-Habori, 2002; Gheldof & Engeseth, 2002; Gheldof, Wang, & Engeseth, 2002; Weston, 2000). The main components responsible for the antioxidant activity of honey are phenolics (Aljadi & Kamaruddin, 2004; Küçük et al., 2007). The antioxidant activity depends on the floral source, as well as on seasonal and environmental factors. The processing may also have affect honey composition and antioxidant activity. In general, a higher antioxidant activity was found in dark honeys as well as in honeys with a higher content of water (Aljadi & Kamaruddin, 2004; Al-Mamary et al., 2002; Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000; Frankel, Robinson, & Berenbaum, 1998). The antioxidant activity and browning have been reported to increase during prolonged heating (Brudzynski & Miotto, 2011a; Turkmen, Sari, Poyrazoglu, & Velioglu, 2006). Until now there has been no research that would examine the influence of filtration on the antioxidant activity and total phenolic contents.

In view of the above, the aim of the present study was to compare filtered and unfiltered honeys (82 samples) in regard to

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their colour, antioxidant activity and phenolics content in order to evaluate the effect of filtration on the above-mentioned parameters.

## 2. Materials and method

### 2.1. Chemicals and instruments

All the chemicals and reagents used were of analytical grade. DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl), ABTS$^{+}$ (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) Folin-Ciocalteu reagent, gallic acid and methanol were obtained from Sigma–Aldrich, Steinheim, Germany; potassium persulfate and sodium carbonate from POCH, Gliwice, Poland; and phosphate-buffered saline from AppliChem, Darmstadt, Germany. For absorbance measurements a UV–VIS spectrophotometer Unicam UV2-100 (ATI Unicam, Cambridge, UK) was used.

### 2.2. Samples

82 Honey samples of different botanical and geographical origin (Table 1) were analyzed to evaluate the effect of filtration on selected quality parameters. The unprocessed samples, bought directly from beekeepers, were harvested during 2009–2010 flowering season. Each sample was available in unfiltered and originally filtered form to compare the honeys directly. 50–100 g of each sample were rapidly warmed to 45 °C (5–10 min), mixed thoroughly and filtered out with Schott filters (Duran, Mainz, Germany), pore size $< 40$ μm, under the pressure of 0.3–0.4 MPa. Afterwards, the honey was cooled down. Sample extraction was carried out directly before and after the filtration. The result of filtration was confirmed by pollen analysis.

### 2.3. Comparison of filtered and unfiltered honeys

Pollen analysis was determined by microscopy using the method by Loveaux, Maurizio, and Worwohl (1978). Monofloral honeys were considered as such whenever the dominant pollen was found at over 45% of the total pollen.

Colour parameters ($L^*$, $a^*$, $b^*$) were established in the CIE system using a Minolta CR-400 Chroma-meter (Konica-Minolta, Osaka, Japan) with illuminant $D_{65}$ and 2° standard observer. The instrument was calibrated with a white reference tile. In order to evaluate the colorimetric changes occurring in the samples due to the filtration process, colour differences $\Delta E^* = ((L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2)^{1/2}$ was calculated (CIE DS 014-4.3/E:2007).

To determine the total phenolics content of honeys, the method of Meda, Lamien, Romito, Millogo, and Nacoulma (2005) was employed. Honey solutions with the concentration of 1 g/10 mL were centrifuged and filtered by a paper filter. Afterwards, 0.5 mL of the resultant solution were mixed with 2.5 mL of 0.2 mol/L solution of Folin–Ciocalteu reagent and 2 mL of sodium carbonate solution (75 g/L) was added. After incubation in dark and at room temperature for 2 h, absorbance of the reaction mixture was measured at $\lambda = 760$ nm using a UV–VIS spectrophotometer. The standard curve was produced for gallic acid within the concentration range from 0 to 200 mg/L. The total phenolic content was expressed as gallic acid equivalents in mg/100 g of honey sample (mgGAE/100 g).

The scavenging activity against 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH$^{\bullet}$) radical of honey was estimated according to procedure described by Turkmen et al. (2006) with some modifications. In brief, 2 g of honey sample were dissolved in 10 mL of distilled water, centrifuged (200 × g) and filtered by a paper filter. Then 0.75 mL of the solution were mixed with 2.25 mL of 0.1 mmol/L methanol solution of DPPH$^{\bullet}$ 1,1-diphenyl-2-picrylhydrazyl. The control test was made with distilled water in place of the honey solution. The reaction mixtures were vortex-mixed well and left at a room temperature in the dark for incubation for 60 min. Absorbance was measured at $\lambda = 517$ nm against methanol, using a UV–VIS spectrophotometer Unicam. The antioxidant activity was expressed as a percentage of inhibition of DPPH$^{\bullet}$ radical and calculated from the equation:

$$\mathrm{AA}\,[\%] = \left(\mathrm{Abs}_{\mathrm{contr}} - \mathrm{Abs}_{\mathrm{sample}}\right) \Big/ \mathrm{Abs}_{\mathrm{contr}} \cdot 100\%$$

The antioxidant activity of honey samples in the reaction with stable ABTS$^{+}$ radical cation was determined according to Baltrušaitytė, Venskutonis, and Čeksterytė (2007). ABTS$^{+}$ was obtained in the reaction of a 2 mmol/L stock solution of 2,2′-azino-bis-(3-ethylobenzothiazoline-6-sulfonic acid) diammonium salt in phosphate-buffered saline with potassium persulphate. The mixture was left to stand for 24 h. Prior to analysis the ABTS$^{+}$ solution was diluted with phosphate buffer saline to produce a solution with absorbance of $0.80 \pm 0.03$ at $\lambda = 734$ nm. The 2-g honey sample was dissolved in 10 mL of distilled water, centrifuged (200 × g) and filtered by a paper filter. Then 0.1 mL of the solution were mixed with 6 mL of ABTS$^{+}$ solution, vortex-mixed well and after 15 min absorbance was measured at a wave length of 734 nm. The control test was made with distilled water in place of the honey solution. The antioxidant activity was expressed as percent inhibition of ABTS$^{+}$, calculated from the same equation as for DPPH$^{\bullet}$. Additionally, Trolox was used as a reference compound, the antioxidant activity was expressed in μmol Trolox equivalent (TE) per 100 g of honey.

### 2.4. Statistical analysis

Each parameter was tested with two replication and then averaged, the results are shown as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the colour parameters, antioxidant activity and phenolics content of filtered and unfiltered honey samples, as well as different types of honey. Correlation coefficients ($r$) were calculated in order to determine the correlation between the particular parameters. Calculations were performed with statistical software package Statistica 10.0 (StatSoft Inc., Tulsa, USA).

## 3. Results and discussion

Using melissopalynology, only a small amount of pollen was found in the filtered honeys, as expected. About 90% of pollen amount were lost after filtration.

Colour is an important quality parameter of honey. It results from the desirable yellow component and the undesirable brown component. The tristimulus colour system CIELab was used to record the colour parameters of the unfiltered and filtered honey samples. The $L^*$ is the measure of the brightness (lightness) from black (0) to white (100). The $a^*$ is the function of the red-green difference. Positive $a^*$ indicates redness, whereas negative $a^*$ indicates greenness. The $b^*$ is the function of the green–blue difference. Positive $b^*$ indicates yellowness, whilst negative $b^*$ indicates blueness. The units within the $L^*$, $a^*$, $b^*$ system give equal perception of the colour difference to a human observer. The average $L^*$, $a^*$, $b^*$ values of the honey samples of different types are presented in Table 2.

Considering the colour parameters of the unfiltered honey samples, the highest $L^*$ values (brightness) were found with the acacia honeys, while buckwheat honeys had the lowest $L^*$ values (higher brownness), which confirmed visual observations. In turn, $a^*$ values (redness) were the highest in buckwheat honeys and the lowest (negative values – green direction) in acacia honeys. The highest $b^*$ values were observed in acacia honeys. The buckwheat