ANTIOXIDANT AND SENSORIAL PROPERTIES OF ACACIA HONEY SUPPLEMENTED WITH PRUNES

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The changes in total phenol and flavonoid content, as well as antioxidant activity was monitored in acacia honey supplemented with prunes in 20, 30 and 40% mass concentrations. The total phenolic content increased by 2.5 times (from 16.18 to 41.64 mg GAE/100 g) with increasing concentration of prunes in honey, while the increase in flavonoid content was even higher, approximately 11.5-fold (from 2.65 to 30.86 mg RE/100 g). The addition of prunes also improved the antioxidant activity of acacia honey. The honey samples with highest content of prunes, 40%, exhibited the best antioxidant activity measured by hydroxyl radical scavenging assay \( EC_{50}^{•\text{OH}}=4.56 \text{ mg/ml} \), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay \( EC_{50}^{\text{DPPH}}=16.48 \text{ mg/ml} \), and reducing power \( EC_{50}^{\text{RP}}=81.17 \text{ mg/ml} \). Judging from the high correlation coefficients, ranging from 0.771 to 0.947 for total phenolics, and from 0.862 to 0.993 for total flavonoids, it is obvious that these compounds were associated with the antioxidant mechanisms. On the other hand, sensorial properties of supplemented honeys were lower than that of pure acacia honey, where flavor of supplemented honey was the least affected. Our results indicate that the supplementation of honey with prunes improves antioxidant activity of honey by enriching the phenolic composition, with slight modifications in sensorial characteristics.

KEY WORDS: Acacia honey, prunes, phenolics and flavonoids, antioxidant activity, sensorial properties

INTRODUCTION

Honey is a natural sweet substance that bees produce by transforming flower nectar or other sweet secretions of plants (1). As an easily assimilable food, honey makes a valuable nutritive product for children, athletes and convalescents (2).

It was reported that honey contains about 200 substances (3). It is essentially a concentrated aqueous solution of inverted sugar, but it also contains a very complex mixture
of other saccharides, enzymes, amino and organic acids, polyphenols, carotenoid-like substances, Maillard reaction products, vitamins and minerals (4).

Honey and other bee products have health-promoting properties which make them usable in pharmacy and medicine, both as drug components, prophylactic agents and diet supplements. Honey activity in gastrointestinal disorders has been reported, providing gastric protection against acute and chronic lesions (5). Also, honey antimicrobial properties have been known for thousands of years and have been attributed to phenolic compounds derived directly from the honey (6).

It was reported that the composition and antioxidant activity of honey depend on the floral source used to collect nectar by honeybee, seasonal and climatic factors. Also, processing may have an effect on honey composition and antioxidant activity (7, 8).

The components in honey responsible for its antioxidative effect are flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, and products of the Maillard reaction. The content of these components varies widely according to the floral and geographical origin of honey (8, 9).

Serbia has a very long tradition of beekeeping. Its favourable climate, good geographical conditions and a variety of botanical species provide a great potential for the development of apiculture (10). The most common unifloral honeys are the acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annuus*) and linden (*Tilia cordata*) honey.

In order to expand the range of bee products taking into account the interest and satisfaction of the consumers, new technological solutions are constantly being sought. One method is to enrich the honey with some fruits which contain high-valuable organic compounds such as phenolics.

In view of this the purpose of the present study was to determine the total phenolic and flavonoid content, as well as antioxidant activity of acacia honey supplemented with prunes, by three different assays, hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays and reducing power. Besides, a sensory analysis of the supplemented honey was also performed.

**EXPERIMENTAL**

**Chemicals and instruments**

The chemicals used for these investigations were Folin-Ciocalteu reagent (Fluka Chemical Co., Buchs, Switzerland), trichloroacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrolone-N-oxide (DMPO), rutin and gallic acid (Sigma Chemical Co., St. Louis, Mo, USA). All other chemicals and reagents were of the highest analytical grade, obtained from J.T. Baker (Deventer, Holland).

The total phenolic, flavonoid, DPPH free radical scavenging assay and reducing power were determined using a UV-1800 spectrophotometer (Schimadzu, Kyoto, Japan), while the antioxidant activity against reactive hydroxyl radicals was evaluated by electron spin resonance (ESR) spectroscopy (Bruker 300E ESR spectrometer, Rheinstetten, Germany).
Honey and prunes samples

The honey sample (AH), monofloral honey form of acacia (Rudnik region) (obtained during 2009 from the honeybee farm, Simonović, Beograd) was supplemented with Stanley variety prunes (Blace region) (obtained from the producer Tehno-Božići, Šabac). Prunes were cut into four pieces and added to the acacia honey in mass concentrations of 20% (AH20), 30% (AH30) and 40% (AH40).

Total phenolic content

The total phenolics were determined spectrophotometrically by the Folin-Ciocalteu method (11). The content of total phenolics was expressed as mg of gallic acid equivalents per 100 g of honey sample (mg GAE/100 g).

Total flavonoid content

Total flavonoids were measured by the aluminium chloride spectrophotometric assay (12). Total flavonoid content was expressed as mg of rutin equivalents per 100 g of honey sample (mg RE/100 g).

Hydroxyl radical scavenging activity

Hydroxyl radicals (•OH) were generated in the Fenton reaction system obtained by mixing 0.2 ml of 112 mM DMPO, 0.2 ml of H₂O, 0.2 ml of 2 mM H₂O₂, and 0.2 ml of 0.3 mM Fe²⁺ (control) (13). The influence of honey samples, at the range of concentrations 5.0-25.0 mg/ml, on the formation and stabilization of hydroxyl radicals was investigated by ESR spin trapping method. The ESR spectra were recorded after 5 min, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5 x10⁵, time constant 80.72 ms, conversion time 327.68 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW and temperature 23°C. The SA•OH value of the honey samples was defined as:

\[ \text{SA}^{•}\text{OH} (%) = 100 \times \frac{(h₀ - hₓ)}{h₀} \]

where \( h₀ \) and \( hₓ \) are the heights of the second peak in the ESR spectrum of DMPO-OH spin adduct of the control and the samples, respectively.

DPPH free radical scavenging assay

The scavenging activity of honey samples was determined spectrophotometrically using the modified DPPH method (14). Briefly, honey samples were dissolved in methanol, and 1.5 ml of each sample or 1.5 ml of methanol (blank) was mixed with 3 ml of DPPH in methanol (0.02 mg/ml). The range of investigated concentrations was 0.33-166.67 mg/ml. The mixtures were left for 15 min at room temperature and then the absorbances was measured at 517 nm against reference mixtures that were prepared in the
similar manner, by replacing the DPPH solution with methanol. The capability to scavenge the DPPH radicals, DPPH scavenging activity (SA), was calculated using the following equation:

\[
SA_{DPPH}(\%) = \frac{(A_0 - A_x)}{A_0} \times 100
\]

where \(A_0\) is the absorbance of the blank and \(A_x\) is the absorbance of the sample.

**Reducing power**

The reducing power of honey samples was determined by the method of Oyaizu (15). For this purpose, the solution of honey samples (10–120 mg) in 1 ml of distilled water or 1 ml of distilled water (blank) was mixed with 1 ml of phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide \(K_3[Fe(CN)_6]\). The mixture was incubated at 50 °C for 20 min and then rapidly cooled. Following this, 1 ml of trichloroacetic acid (10%) was added and the mixture was then centrifuged at 3000 rpm for 10 min. An aliquot (2 ml) of the upper layer, mixed with 2 ml of distilled water and 0.4 ml of 0.1% \(FeCl_3\), was left to stand for 10 min. The absorbance of the mixture was measured at 700 nm against the blank.

**Sensory analysis**

The main sensory attributes evaluated in the analysis were: color, viscosity, aroma, and flavor of honey samples, according to the Regulation on quality evaluation of bee products in Novi Sad fair (16). The sensory analysis were carried out by means of analytical descriptive analysis method, based on points (grades) from 0 (unacceptable product) to 3 (optimal quality level). The panel consisted of 5 expert descriptors.

**Statistical analysis**

All analyses were run in triplicate and the results were expressed as means ± standard deviation (SD). Statistical analyses were done by using Origin 7.0 SRO software package (OriginLab Corporation, Northampton, MA, USA, 1991–2002) and Microsoft Office Excel 2007 software. Significant differences were calculated by ANOVA test followed by the least significant difference (LSD) test \((p \leq 0.05)\).

**RESULTS AND DISCUSSION**

Supplemented acacia honey samples, AH20, AH30 and AH40, were subjected to spectrophotometric analysis of total phenolic and flavonoid contents. The results were compared to the contents in pure acacia honey, AH, obtained in our previous study (17) and presented in Figure 1.
The concentrations of both phenolics and flavonoids in supplemented acacia honey increased significantly (p ≤ 0.05) with rising the share of prunes compared to pure acacia honey. In comparison to raw honey, the addition of 40% of prunes increased the contents of total phenolic substances by 2.5 times (from 16.18 to 41.64 mg GAE/100 g), and the flavonoid level was approximately 11.5-fold higher (from 2.65 to 30.86 mg RE/100 g). The flavonoids contribution to the total phenols increased with increase of prune content (16.38%, 35.84%, 49.63% and 74.11% for AH, AH20, AH30 and AH40, respectively). Our results of total flavonoids in acacia honey are in agreement with the reports of Amiot et al. (18) (0.5-1 mg quercetin equivalent/100 g of European acacia honey), but slightly lower than the results of Meda et al. (19) (6.14 mg quercetin equivalent/100 g of Burkina Faso acacia honey). However, the results of total phenolic contents are significantly higher than that of Beretta et al. (20) (5.52 mg GAE/100 g of commercial acacia honey) and Bertoncelj et al. (21) (4.48 mg GAE/100 g of Slovenian acacia honey).

Ramanauskiene et al. (22) have identified p-coumaric and ferulic acids as the main components in acacia honey. Yao et al. (23) reported that myricetin, tricetin, quercetin and luteolin, are dominant flavonoid compounds in Australian honeys. Another study on European honeys showed that in lime tree (Tilia europaea) honey, the propolis flavonoids, pinobanksin and pinocembrin are the most abundant flavonoids and the highest level of pinobanksin was detected in the European acacia (Robinia pseudoacacia) honey sample (2.31 mg QE/100 g) (24). The main compounds in fresh plum (Prunus domestica) and dried plum (prune) are caffeoylquinic acids isomers (3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid) (25). Plums also contain anthocyanins (cyanidin 3-O-rutinoside, cyanidin 3-O-glucoside, and peonidin 3-O-rutinoside are predominant), flavonols (quercetin 3-O-rutinoside is predominant) and proanthocyanidins, which represent 70% of total polyphenols (25). Fang et al. (26) identified 42 compounds in commercial dried plums, among which the hydroxycinnamic acids are essential. The polyphenols contents are halved in commercial prune compared to fresh plum due to the degradation during drying (25). According to our results, flavonoids have been transfered to honey more rapidly than other polyphenols.
In the recent years, there has been an increasing interest in the determination of the antioxidant activity of honey. Beretta et al. (20) have reported that it is necessary to use a combination of antioxidant tests, comparative analyses and statistical evaluation to determine the antioxidant behavior of honey. Therefore, we used three different antioxidant tests to assess the activity of acacia honey samples. In our experiments, the decrease of \('\text{OH}\) radical concentration, generated via Fenton reaction, was monitored using ESR spectroscopy, and the decrease in the DPPH radical concentration was monitored spectrophotometrically. Reducing power, another spectrophotometrical test, was used as a significant indicator of honey potential antioxidant activity. Antioxidant activities were expressed as EC\(_{50}^{\text{•OH}}\) and EC\(_{50}^{\text{DPPH•}}\) value (the amount of antioxidant necessary to decrease the initial concentration of hydroxyl or DPPH radicals by 50%) and EC\(_{50}^{\text{RP}}\) value (the effective concentration assigned at 0.5 value of absorption) and presented in Table 1.

**Table 1.** EC\(_{50}\) values of pure (AH) and supplemented acacia honeys (AH20, AH30 and AH40) measured by three antioxidant assays

<table>
<thead>
<tr>
<th>Honey sample</th>
<th>EC(_{50}^{\text{•OH}}) (mg/ml)</th>
<th>EC(_{50}^{\text{DPPH•}}) (mg/ml)</th>
<th>EC(_{50}^{\text{RP}}) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH</td>
<td>19.14 ± 0.76</td>
<td>164.09 ± 7.38(^*)</td>
<td>100.80 ± 4.54(^*)</td>
</tr>
<tr>
<td>AH20</td>
<td>16.89 ± 0.54</td>
<td>24.48 ± 1.05</td>
<td>96.75 ± 3.82</td>
</tr>
<tr>
<td>AH30</td>
<td>15.89 ± 0.63</td>
<td>18.65 ± 0.84</td>
<td>92.13 ± 4.25</td>
</tr>
<tr>
<td>AH40</td>
<td>4.56 ± 0.18</td>
<td>16.48 ± 0.69</td>
<td>81.17 ± 3.95</td>
</tr>
</tbody>
</table>

\(^*\) Taken from Savatović et al. (17)

The results of all three antioxidant tests presented the same trend. The supplemented honeys, AH20, AH30 and AH40, exhibited higher antioxidant activity than pure honey, AH. The antioxidant activity increased with increasing the concentration of prunes in the honey. The greatest increase of the antioxidant activity was noted for the DPPH free radicals, where EC\(_{50}^{\text{DPPH•}}\) value decreased 10 times compared to pure honey. The result of the AH antioxidant activity was in agreement with Meda et al. (19). It has been shown that dried plums have one of the highest ORAC values (5770) out of a group of 22 fruits and vegetables studied, and that phenolic compounds appear to be the main contributors to their antioxidant capacity (27).

Many studies have shown that the antioxidant activity is strongly correlated with the content of total phenolics (7, 20, 8, 19). Gheldof et al. (4) stated that phenolic compounds significantly contribute to the antioxidant activity of honey. Also, the authors suggested that the antioxidant activity appeared to be a result of the combined activity of the honey phenolics, peptides, organic acids, enzymes and Maillard reaction products.

For the correlation analysis, the EC\(_{50}\) values were transformed into their reciprocal values \((1/\text{EC50})\). Table 2 shows Pearson’s correlation coefficients between the analyzed antioxidant activities of acacia honeys and composition variables.

Our results confirm that phenolic compounds in general, and especially flavonoids, seem to be dominant compounds in honey-participating antioxidant reactions. The highest correlation coefficient of 0.993 reveals a very good correlation between the flavonoid contents and reducing power of analyzed honey samples.
Table 2. Correlation matrix (Pearson’s correlation coefficients) between the composition variables and the antioxidant activities of pure and supplemented acacia honeys

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total flavonoids</th>
<th>Total phenolics</th>
<th>1/EC$_{50}^{RP}$</th>
<th>1/EC$_{50}^{DPHH}$</th>
<th>1/EC$_{50}^{•OH}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/EC$_{50}^{•OH}$</td>
<td>0.895</td>
<td>0.771</td>
<td>0.928</td>
<td>0.605</td>
<td>-</td>
</tr>
<tr>
<td>1/EC$_{50}^{DPHH}$</td>
<td>0.862</td>
<td>0.921</td>
<td>0.851</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1/EC$_{50}^{RP}$</td>
<td>0.993</td>
<td>0.947</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>0.974</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The relation between the three methods for the determination of antioxidant activity, and between the results of total phenolics and total flavonoids, was also good (r was from 0.605 to 0.974). Cimpoiu et al. (28) found a significant correlation (r = 0.9928) between the antioxidant activities determined by the DPPH and ABTS assays, suggesting that these two assays are almost comparable and interchangeable in the case of honey, when an evaluation of antioxidant activity is required. In our study, the antioxidant assays correlated very well except for the correlation between the DPPH and hydroxyl radical assays, which was lower, but still good enough (Table 2). It is assumed that honeys contain compounds that can quench hydroxyl radicals also by additional reactions. Ghel dof and Engeseth (8) found that the antioxidant capacities of honeys, evaluated by the oxygen radical absorbance capacity (ORAC) assay, showed a linear dependence on the total phenolics content. Zalibera et al. (29) investigated radical-scavenging capacities of 15 Slovak honeys using ESR and cation radical of ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt), DPPH and hydroxyl radicals generated by the photochemical decomposition of hydrogen peroxide. They found good ABTS$^{+}$ and DPPH free radical scavenging capacity of tested honeys, and that this capacity correlated well with the phenolics content. However, no correlation was found between the monitored ability to scavenge hydroxyl radicals and either phenolics content. Beretta et al. (20) found that the close interdependence between the phenolics content, FRAP, DPPH, and ORAC indicates that the antioxidant capacity of the honeys is due to their phenolic constituents, which are able to interact with Mo(VI) and Fe(III) with a H/e$^{-}$ transferring mechanism, and less to other chemical entities.

Honey samples with and without prunes were evaluated by a 5-member trained expert descriptive attribute sensory panel. The samples were evaluated for color, viscosity, aroma and flavor. Samples were scored using the 0 to 3 intensity scale and the results are presented in Figure 2.

The consumer evaluations of acacia honey samples indicated that pure honey, AH, exhibited best sensorial properties, while the addition of prunes affected viscosity at the highest level, and only slightly affected the flavor (3.0, 2.6 and 2.5 for AH20, AH30 and AH40, respectively) and aroma (2.0, 2.6 and 2.0 for AH20, AH30 and AH40, respectively) of the honey. However, the color of the supplemented honeys was rated lower, related to the changes in pale yellow color of pure acacia honey due to the dark brown pigmentation of the dried plum (2.0, 2.6 and 2.0 for AH20, AH30 and AH40, respectively).
The color of honey is related to the content of phenolics, besides minerals and pollen (30, 31). In this regard, this result is in agreement with a higher content of phenolic compounds in the supplemented honeys. Núñez de González et al. (27) investigated the effect of prunes inclusion in pork sausages on their sensorial characteristics. The levels of dried plum puree treatments had no effect on flavor intensity, texture, or level of juiciness, but did influence perceptions for overall like/dislike and overall flavor of the product.

The correlation analysis was also employed to analyze the relationships between the sensorial properties and composition variables evaluated, i.e. the total phenolic and flavonoid contents (Table 3).

**Table 3.** Correlation matrix (Pearson’s correlation coefficients) between composition variables and sensorial characteristics of pure and supplemented acacia honeys

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total flavonoids</th>
<th>Total phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>-0.871</td>
<td>-0.738</td>
</tr>
<tr>
<td>Viscosity</td>
<td>-0.582</td>
<td>-0.535</td>
</tr>
<tr>
<td>Aroma</td>
<td>-0.582</td>
<td>-0.535</td>
</tr>
<tr>
<td>Flavor</td>
<td>-0.934</td>
<td>-0.968</td>
</tr>
</tbody>
</table>

Close relationships between the total phenolics and flavonoids of the honeys and their color and flavor were confirmed by high correlation coefficients between these variables. The negative values of the correlation coefficients mean that the rate of sensory characteristic decreased with increasing total phenolic/flavonoid value. The relationship between color and phenolic contents in honeys was also confirmed by some other authors (21, 29, 32).

**CONCLUSION**

This study shows that acacia honey supplemented with prunes can add many healthgiving antioxidants to the diet. The supplemented honeys contain substantial amounts of...
bioactive compounds, originating from honey itself and also from the added prunes. Experimental results and statistical analysis show that these phytochemicals increase the honey’s antioxidant activity but also slightly affect sensorial properties. Considering these findings, the content of prunes could be optimized to obtain the best combination of the antioxidant and sensorial properties of the product. Also, the future studies can be focused on the identification and quantification of phenolics and other compounds present in the supplemented honeys, and revealing other biological activities of these products in respect of health benefits that make these honeys a consumer-valuable product. This product can be recommended to complete other polyphenol sources such as vegetables and fruits.

Acknowledgement

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REFERENCES


АНТИОКСИДАТИВНЕ И СЕНЗОРНЕ КАРАКТЕРИСТИКЕ БАГРЕМОВОГ МЕДА СА ДОДАТКОМ СУВИХ ШЉИВА

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У багремов мед су додате суве шљиве у масењим концентрацијама 20, 30 и 40%. Утицај сувих шљива на особине меда испитан је мерењем садржаја укупних фенолних јединиња и флавоноида, као и антиоксидативне активности. Са повећањем концентрације сувих шљива у меду укупан садржај фенолних јединиња повећао се 2,5 пута (од 16,18 до 41,64 mgGAE/100g), док је повећање садржаја флавонида још веће, око 11,5 пута (од 2,65 до 30,86 mgRE/100g). Додатак сувих шљива утицао је и на повећање антиоксидативне активности меда. Узорци меда са 40% сувих шљива показали су највећу антиоксидативну активност, која је одређена на слободне хидроксил радикале (EC50 =4,56 mg/ml) и 2,2-дифенил-1-пикрилхидразил (DPPH) радикале (EC50 =16,48 mg/ml), као и тестом редукционе способности (EC50 =81,17 mg/ml). Судећи по утврђеним високим коефицијентима корелације, у опсегу од
0,771 до 0,947 за фенолна јединења, односно у опсегу од 0,862 до 0,993 за флавоноиде, може се закључити да ова јединења учествују у механизмах антиоксидативног деловања узорака меда. Са друге стране, сензорне карактеристике меда са додатком сувих шљива оцењене су нижом оценом од чистог багремовог меда, при чему је додатак шљива имао најмањи утицај на укус. Резултати испитивања су показали да се додатком сувих шљива у мед побољшава антиоксидативна активност меда повећањем садржаја полифенолних јединења у њему, уз мале модификације сензорних карактеристика.

Кључне речи: багремов мед, суве шљиве, фенолна јединења и флавоноиде, антиоксидативна активност, сензорне карактеристике

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