Effect of heat treatment for liquefaction and pasteurization on antioxidant activity and phenolic compounds of Astragalus and sunflower-cornflower honeys

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Abstract

In this study, effect of heat treatments (liquefaction at 55 °C for 12 h and pasteurisation at 90 °C 15 sec) on total phenolic content, antioxidant activity and phenolic compounds of astragalus and sunflower-cornflower honeys was investigated. Total phenol content and antioxidant activity of honeys were assessed by Folin-Ciocalteu and ABTS methods, respectively. Phenolic profile was determined by HPLC-DAD system. Only pasteurisation process had a significant impact on sunflower-cornflower honey with regard to total phenolic content and antioxidant activity. Protocatechusic, 4-hydroxybenzoic, caffeic, p-coumaric, and ferulic acids, rutin, kaempferol were detected in astragalus honey, while 2,5-dihydroxybenzoic, chlorogenic, caffeic, p-coumaric, and ferulic acids, apigenin, rutin, kaempferol 3-glucoside, isorhamnetin 3-glucoside, quercetin, luteolin, kaempferol were detected in sunflower-cornflower honey. Pasteurisation significantly decreased caffeic acid in astragalus honey and other detected phenolics showed no significant difference after heat treatments. The impact of liquefaction process is lower than the pasteurisation process in terms of quantitatively phenolic compounds changes.

Keywords: astragalus; sunflower-cornflower; phenolic compounds; antioxidant activity; honey liquefaction; pasteurization.

Practical application: This study provides useful information for determining the appropriate method which will have the least effect on the components of honey, from different heat treatments applied to flower honey for crystallization prevention and pasteurization.

1 Introduction

Honey is a stuff used as food and medical from ancient times to the present day. It has more than 100 useful pharmacological effects as antimicrobial, anti-inflammatory, antimutagenic, antioxidant and prebiotic properties. Flavonoids, phenolic acids, peptides, organic acids, enzymes, Maillard reaction products and other minor components provide antioxidant property for honey, notably phenolics (Bogdanov, 1997; Ghelof et al., 2002). All plants produce phenolic compounds as secondary metabolites in their metabolisms. These compounds are found in different compositions and amounts in all plant-based foods. The analysis of phenolic profiles of honeys guides researchers through their floral and geographical origin studies. Hesperetin, quercetin and ellagic acid have been suggested as chemotaxonomic markers for citrus, sunflower and heather honeys (Bogdanov et al., 2004; Naczk & Shahidi, 2004; Yao et al., 2004; Küçük et al., 2007).

The composition and antioxidant activity of honey depend on the floral sources, seasonal and environmental factors, processing methods and storage conditions (Mendes et al., 1998; Al-Mamary et al., 2002; Ghelof et al., 2002; Aljadi & Kamaruddin, 2004; Akhmazillah et al., 2013).

Crystallization in honey is a natural event. In some cases, honey may become a semi-solid, also known as crystallized or granulated honey. This is a natural phenomenon and occurs as a result of the precipitation of glucose, one of the three main sugars of honey, from the oversaturated honey solution. Glucose loses water, converts to the form of glucose monohydrate, and then takes a crystalline form. However, as for consumers solid appearance and grainy texture resulted from crystallization are unwanted (Tosi et al., 2004; Bradbear, 2009; Escriche et al., 2014). Heat treatment should be applied to honeys due to the averseness of consumers in conjunction with the yeasts causing fermentation. With heating the substances that cause crystallization are dissolved so the shelf life lengthens out and the tendency of crystallization decreases (Gonnet et al., 1964; Tosi et al., 2002; Fallico et al., 2004; Escriche et al., 2014). The thermal processing of honey is carried out with two stages in industry. First, honey is heated at approximately 55 °C to ensure easiness for handling (liquefaction process); and, secondly liquefied honey is subjected to more higher temperature at approximately 80 °C to destroy yeasts and dissolve crystallization nuclei (pasteurization process) (Escriche et al., 2008; Escriche et al., 2014).

The phenolic profiles of heat treated foods can show an alteration. The phenolics in fruits and vegetables are generally bound to insoluble polymers with covalent bonds. It was observed that the antioxidant activity increased due to releasing of bound phenolic compounds in citrus fruits by heating (Choi et al., 2011; Lou et al., 2014). Bornšek et al. (2015) demonstrated that heating has increasing and decreasing effects on phenolic acids, flavonoids and anthocyanins during bilberry jam production. Additionally, biological compounds absent in food matrix can be occurred by thermal processing (Kim et al., 2013). There are limited numbers of studies on determining the phenolic profile.
changes in honeys. Concerning this issue, Wang et al. (2004) and Escriche et al. (2014) observed how different heat treatments resulted alterations in phenolic profiles of honeys. However, industrial processing affects the antioxidant activity and the total phenolics of different honey types differently (Howell et al., 2001; Kowalski, 2013; Chaikham & Prangthip, 2015).

The main aim of this study was to investigate the influence of different heat treatment parameters on total phenolics, antioxidant activity and phenolic compounds of astragalus and sunflower-cornflower mix honeys as compared to untreated honey.

2 Materials and methods

2.1 Honey samples

Two types of crystallized blossom honey samples (astragalus honey and sunflower-cornflower mix honey) were obtained from a firm in Turkey Konya province. Each honey sample was divided into three groups: unheated (raw), liquefied and liquefied plus pasteurized. Samples were subjected to heat treatments which had temperature-time parameters as 55 °C - 12 h (liquefaction process) and 90 °C - 15 sec (pasteurization process) in the plant of Cebel Dairy and Food Products Trading Co. Ltd. (Konya, Turkey). Heating boilers were used for industrial liquefaction and pasteurization processes, respectively. After thermal treatments, all samples were quickly cooled to room temperature (25 ± 1 °C). The honeys were stored at 4 °C in dark before the analysis. In results and discussion section, the shorthands of raw, liquefaction and liquefaction + pasteurization processes are given as the codes 1, 2 and 3.

2.2 Chemicals

Protocatechuic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, chlorogenic acid, cafffeic acid, p-coumaric acid, ferulic acid, apigenin, rutin, kaempferol-3-glucoside,isorhamnetin-3-glucoside, quercetin, luteolin and kaempferol, were purchased from Extrasynthese (Genay, France). Folin-Ciocalteu’s reagent, potassium peroxodisulfate, sodium carbonate, hydrochloric acid, ethanol, methanol, acetic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were from Sigma-Aldrich (Steinheim, Germany).

2.3 Determination of total phenolic content

Total phenolic contents of honeys were determined according to the method of Akbulut et al. (2009). One gram of honey was dissolved in 19.0 ml distilled water and then 0.5 ml of this solution was mixed with 2.5 ml of Folin-Ciocalteu’s reagent solution (0.2 N) and 2.0 ml sodium carbonate solution (75 g/l). After 2 h dark incubation at room temperature (25 ± 1 °C), the absorbance was determined using a spectrophotometer (Hitachi, UV 1800, Japan) at a wavelength of 765 nm. Total phenolic content was expressed as mg gallic acid equivalent/100 g of honey sample.

2.4 Determination of antioxidant activity

The antioxidant activity of honey samples was determined with ABTS method according to Re et al. (1999). To generate the ABTS+ radical, 2.5 ml of potassium persulfate solution (2.45 Mm) were added to 5 ml of ABTS solution (7 mM). The mixture was incubated at room temperature (25 ± 1 °C) for approximately 16 h. The stock solution was diluted with ethanol until an absorbance of 0.700 ± 0.02 at 734 nm. 4 ml ABTS+ solution was mixed with 40 μl of honey solution (0.05 g/ml). The absorbance was measured spectrophotometrically (Hitachi, UV 1800, Japan) at room temperature (25 ± 1 °C) after 6 min. The antioxidant activity was expressed as mmol TE (Trolox Equivalent)/kg of honey.

2.5 Determination of phenolic profile

For HPLC analysis, phenolics in samples were purified by using C18 cartridge (Waters, Milford, MA). Firstly, the SPE cartridges were conditioned by methanol and acidified water. Secondly, adequately diluted honey sample was loaded to cartridge. Sugars and organic acids in honey were removed from the material via acidified water. The phenolics were eluted with methanol. Methanol eluent was evaporated at 40 °C and then resuspended in methanol. The samples were transferred into vials.

Phenolic acids and flavonoids were analyzed by high-performance liquid chromatography (HPLC) (Agilent 1260, Waldbronn, Germany), using a reversed phase column (5 μm, 250 × 4.6 mm i.d.) with a diode array detector at 280, 320 and 360 nm. The mobile phase consisted of water:acetic acid and water:acetonitrile:acetic acid at a flow rate of 0.75 ml/min. The injection volume was 50 μl. Identification of phenolic compounds was carried out by comparing retention time and UV spectra. The data were analyzed using Chemstation software (Coklar & Akbulut, 2017).

2.6. Statistical analysis

Results were presented as means ± standard deviation (SD) and with a level of significance of 95%. One way analysis of variance (ANOVA) was applied to study the effect of the temperature parameters on the total phenolics, the antioxidant activity and the phenolic compounds of honey using the Minitab (Minitab Inc., MINITAB® Release 14.12.0). Duncan's multiple range test in "MSTAT C" packaged software was used for significance levels between the means of results.

3 Results and discussion

3.1 Total phenolic content and antioxidant activity of honeys

Two crystallized blossom honey samples were studied to determine the effects of heat treatments on total phenolic compound amount, antioxidant activity and phenolic profile. The results of the total phenolic content of honey samples are presented in Figure 1. Total phenolic contents of astragalus honey were found as 51.48 ± 2.34, 53.47 ± 1.97 and 51.82 ± 1.39 mg GAE/100 g, while those of sunflower-cornflower honey were 39.26 ± 0.27, 39.65 ± 0.74 and 46.13 ± 0.56 mg GAE/100 g (raw, liquefied and pasteurized honey, respectively). Total phenolic result of unprocessed astragalus honey was similarly obtained by Can et al. (2015), which reported that the mean value of five different astragalus honeys were 43.63 mg GAE/100 g. Since there have been no studies on sunflower and cornflower mix honeys, the comparison of analysis results was made and found
similar when mixing was considered. Sarı & Ayyıldız (2012) demonstrated that total phenolics of fifty sunflower honey were in between 6.896-23.201 mg GAE/100 g. Results for six cornflower honey samples was determined by Kuś et al. (2014) as 260.0 mg GAE/100 g. However, it has been determined that astragalus honey has more total phenolic content and antioxidant activity than sunflower-cornflower honey.

It was found that liquefaction process slightly increased total phenol content of astragalus honey sample as compared to unheated sample, with no significant difference (p>0.05). Although liquefied sunflower-cornflower honey statistically showed no significant difference between unheated samples regarding aforementioned subject, pasteurized honey samples showed significantly increase (p<0.01).

The heated and unheated astragalus honey showed non-significance difference on antioxidant activity (p>0.05). Just like in total phenol content changes, the statistical differences among the antioxidant activity of liquefied and unheated honeys were not important (p>0.05), but among pasteurised and unheated honeys were important (p<0.01).

Wang et al. (2004) reported that processing clover honey did not significantly impact total phenolic content and antioxidant activity as with liquefied astragalus and sunflower-cornflower honeys and pasteurized astragalus honey in our study results. The increase in antioxidant activity and total phenolic content values in sunflower-cornflower honey resulted from pasteurization showed a similar result in the study of Kowalski (2013) regarding thermal treated lime honey.

The results demonstrated that there is a relationship between antioxidant activity and total phenol of honeys. The correlation coefficients (r) were as 0.8878 for astragalus honey and 0.9987 for mix honey. We can say that total phenol of sunflower-cornflower honey is very impactful on antioxidant activity. These findings are in agreement with those reported by Akbulut et al. (2009) and Al-Mamary et al. (2002), who found a high correlation between the total antioxidant activities of various honey and their total phenol contents.

### 3.2 Phenolic profiles of honeys

Chromatograms of phenolics in raw astragalus and sunflower-cornflower honeys are seen in Figure 3 and Figure 4. Table 1 shows the phenolic acids and flavonoids quantified in astragalus and sunflower-cornflower honey, before (raw) and after heat treatment (liquefaction and pasteurization). There are more phenolic compounds in sunflower-cornflower honey even if their amount is only a few.

Ferulic acid, p-coumaric acid, protocatechueic acid, caffeic acid and 4-hydroxybenzoic acid were present from less to more in raw astragalus honey investigated, while 2,5-dyhydroxybenzoic acid and chlorogenic acid were not detected also in heated honeys. Among the flavonoids were rutin and kaempferol detected only in astragalus honey. Apigenin, kaempferol-3-glucoside, isorhamnetin-3-glucoside, quercetin and luteolin were not detected in heated honey, either. Can et al. (2015) analyzed five astragalus honeys supplied from Turkey and reported that gallic acid (0.29 μg/g), protocatechueic acid (5.082 μg/g), 4-hydroxybenzoic acid (33.19 μg/g), vanillic acid (1.67 μg/g), caffeic acid (5.14 μg/g), p-coumaric acid (5.08 μg/g), ferulic acid (0.94 μg/g), rutin (4.61 μg/g) and apigenin (0.61 μg/g) were determined. There are many similarities in the way of the...
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phenolic profile (especially 4-hydroxybenzoic acid) of astragalus honey in the present study. We can say that 4-hydroxybenzoic acid is the dominant phenolic compound of astragalus honey.

All phenolic compounds except for protocatechuic acid and 4-hydroxybenzoic acid were present in raw sunflower-cornflower honey. Tomas-Barberan et al. (2001) analyzed four sunflower

Table 1. Phenolic profile of raw, liquefied and pasteurised astragalus and sunflower-cornflower honeys.

<table>
<thead>
<tr>
<th>Phenolic Compounds</th>
<th>Astragalus honey</th>
<th>Sunflower-cornflower honey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Liquefaction</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>5.86 ± 0.56</td>
<td>7.07 ± 1.07</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>15.01 ± 2.52</td>
<td>14.99 ± 0.60</td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>6.40 ± 0.07</td>
<td>5.86 ± 0.25</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>1.94 ± 0.18</td>
<td>1.90 ± 0.15</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.47 ± 0.04</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>9.36 ± 5.74</td>
<td>9.39 ± 5.25</td>
</tr>
<tr>
<td>Kaempferol-3-glucoside</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Isorhamnetin-3-glucoside</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Luteolin</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>1.61 ± 0.60</td>
<td>1.56 ± 0.75</td>
</tr>
</tbody>
</table>

nd: Not detected; Results were given as mean ± SD (mg/kg).

Figure 3. Chromatogram of phenolics in raw astragalus honey (1: protocatechuic acid, 2: 4-hydroxybenzoic acid, 3: caffeic acid, 4: p-coumaric acid, 5: ferulic acid, 6: rutin, 7: kaempferol).

Figure 4. Chromatogram of phenolics in raw sunflower-cornflower honey (1: 2,5 dihydroxybenzoic acid, 2: chlorogenic acid, 3: caffeic acid, 4: p-coumaric acid, 5: ferulic acid, 6: apigenin, 7: rutin, 8: kaempferol-3-glucoside, 9: isorhamnetin-3-glucoside, 10: quercetin, 11: luteolin, 12: kaempferol).
honey and indicated that this honey type included quercetin (125.2-286.2 μg/100 g), caffeic acid (90.0-158.8 μg/100 g), p-coumaric acid (14.4-180.8 μg/100 g) and ferulic acid (25.5-113.0 μg/100 g). Luteolin was present in all samples except for one sample as 32.6-85.2 μg/100 g. Phenolics common in both studies seemed like similar. We would also like to point out that present study shows that kaempferol-3-glucoside in sunflower-cornflower honey is found much more amount than the others. It is not known that whether this phenolic comes from sunflower honey although it has never been analyzed or it comes from cornflower honey which has never been through phenolic profile study.

For astragalus honey both heat treatments quantitatively reduced 4-hydroxybenzoic acid, caffeic acid, p-coumaric acid and kaempferol, and enhanced protocatechuic acid. Ferulic acid and rutin amounts in the honey were increased by liquefaction and then decreased by pasteurization compared to the profile of unheated sample. The differences created by heat treatments of the amounts of all phenolics except for caffeic acid (p<0.01) were found insignificant (p>0.05). Caffeic acid in astragalus honey was impressed with only pasteurization process. Liquefied and pasteurised sunflower-cornflower honeys did not include protocatechuic acid and 4-hydroxybenzoic acid as in the raw honey. Both treatments quantitatively decreased 2,5-dihydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, rutin, kaempferol-3-glucoside, isorhamnetin-3-glucoside, quercetin and luteolin, and increased caffeic acid. Apigenin and kaempferol were reduced with liquefaction and then enhanced with pasteurization compared to the profile of raw sample. No significant difference between the phenolics of unheated and heated samples were statistically found (p>0.05). Up to our knowledge, this is the first time the influence of heat treatments on phenol compounds and antioxidant activity of astragalus and sunflower-cornflower honeys has been studied.

Wang et al. (2004) investigated the alteration in phenolic profiles of clover honey and buckwheat honey by thermal processing. Heated at 130 °F (54.4 °C) until the granules in honeys melted were called raw samples, while processed samples were called pasteurized at 180 °F (82.2 °C) for 10-12 sec after heated at 140 °F (60 °C) for 12-16 h until granules melted. It was said that processing significantly increased quercetin and galangin concentrations, which, unlike clover honey, decreased after processing. In our study caffeic acid amounts in astragalus and sunflower-cornflower honeys changed like galangin in clover and buckwheat honeys in the case study. Escriche et al. (2014) studied the influence of heat treatments on phenolic profiles of citrus, rosemary, polyfloral and honeydew honeys. Each type of honey samples was divided into three groups: first group included unheated (raw) samples, second group was liquefied at 45 ± 1 °C for 48 h and third group was pasteurised at 80 ± 0.05 °C for 4 min of liquefied honeys. When the researchers surveyed the effect of heat treatments on phenolics, they statistically determined a difference which is that galangin and myricetin were very significant (p<0.001) and p-coumaric acid and kaempferol were significant (p<0.05) (Escriche et al., 2014).

The effect of heat treatments on phenolic compounds has been investigated for various foods by researchers. Bornšek et al. (2015) studied the behavior of phenolics of bilberries and reported that quercetin and myricetin amounts almost doubled after making bilberry jam. They based the increase of the quantity of quercetin on the hydrolysis of epicatechin and quercetin derivatives (glucosides and gallates). Some of the simple phenolics can increase as a result of breakdown of supramolecular structures containing phenolic groups (Bunea et al., 2008). It seems that in the present work, pasteurization process treated to sunflower-cornflower honey increased the amount of kaempferol by decreasing the amount of kaempferol-3-glucoside. Perez-Jimenez et al. (2014) commented on the absence of phenolics after cooking process as probably due to both heat degradation and contribution of the formation of Maillard reaction products, like melanoindis.

4 Conclusions

The effect of variation of processing on the total phenol content and antioxidant activity of honey was based on the type of honey. There were no significant differences in antioxidant activity and total phenolic content of astragalus honey after both heat treatments and of sunflower-cornflower honey after liquefaction, while pasteurization significantly affected sunflower-cornflower honey. The results indicated that both heat treatment methods had no significant effect on phenolics. Only caffeic acid in astragalus honey showed a significant decrease due to pasteurization. It seems that pasteurisation process quantitatively impacted honey phenolics more than liquefaction process. However, further research is required to investigate the phenolic profile of raw and processed of cornflower honey.

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References


