Changes of Antioxidant Activity in Honey after Heat Treatment

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Abstract

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We determined how the antioxidant activity and total phenolic content of honey changed after being subjected to a high temperature. Antioxidant activity was determined using two methods – FRAP (ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Total phenolic content was determined by modified Folin-Ciocalteu method. The research was conducted on 31 samples of acacia honey and 8 samples of chestnut honey. All measurements were done at two temperatures – at 23°C (room temperature) and after 5 min of heating at 95°C. The obtained results show uneven changes of antioxidant activity and total phenolic content among individual samples, i.e. in some samples antioxidant activity decreased after heating, while in others it increased. The same applies to the total phenolic content. Statistical analysis of the results (*t*-test) showed no statistically significant differences between the results measured at two different temperatures ($P > 0.05$) in all three methods used, and in both types of honey. The only statistically significant difference ($P < 0.05$) was observed when using DPPH method in acacia honey.

Keywords: heating; phenolic compounds; ferric reducing antioxidant power (FRAP); 1,1-diphenyl-2-picrylhydrazyl (DPPH)

Heat-treated or otherwise processed food is frequently represented in today's diet. Technological processes used for such treatments cause more or less changes in its nutritional value, chemical composition, and consequently, in the antioxidant activity of food. It is well known that natural nutrients can be lost to a significant degree during thermal processing due to the fact that most of the bioactive compounds are relatively unstable at higher temperatures. However, heat treatment sometimes causes no change or it increases the antioxidant activity of selected food. Moreover, novel compounds having an antioxidant property, such as Maillard reaction products, can be formed as a result of heat treatment (Choi *et al*. 2006). The antioxidant activity of natural honey is mainly caused by phenolic antioxidants originating from the pollen of flowering plants and trees, and especially the dark-coloured honeydew honey types contain them in high amounts (Lachman *et al*. 2010a,b). However, high-molecular-mass melanoidins have also been identified as the main components responsible for radical scavenging capacity of unheated and heat-treated honey (Brudzynski & Miotto 2011a). Principal component analysis revealed the highest correlation between ORAC (oxygen radical absorbance capacity) and Maillard reaction-like products and, in addition, the extremely significant correlations among the antioxidant activity, Maillard reaction-like products, phenolic content and honey colour may suggest that these compounds represent the same chemical entity and exert their antioxidant activity while being part of a higher molecular mass structure (Brudzynski & Miotto 2011b).

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Understanding the consequences of food processing for food composition is one of the important steps to a correct interpretation of study results regarding dietary habits, nutrition, and human health (Nicoli *et al.* 1999). Preservation methods are generally believed to be responsible for depletion of naturally occurring antioxidants in food. However, some reports have shown that processing can cause some or no loss of the content and activity of naturally occurring antioxidants (Hong *et al*. 2004; Amin & Lee 2005; Oszmianski *et al*. 2007). On the other hand, some reports say that antioxidant activity increases after processing (Dewanto *et al*. 2002; Turkmen *et al*. 2005; Durmaz & Alpaslan 2007).

Honey is no exception since it is often used as a sweetener in hot drinks and pastries, biscuits and other confectionery products, which are more or less thermally processed. Turkish scientists demonstrated that the antioxidant activity of honey increased after heat treatment (Turkmen *et al*. 2006), while Wang *et al*. (2004) found that processing did not have a significant impact on the antioxidant capacity of honey samples.

The objective of this study was to determine how the antioxidant activity and total phenolic content change in honey samples after they were subjected to a high temperature in a short period of time.

MATERIAL AND METHODS

Samples. Thirty-one acacia (*Robinia pseudoacacia*) and eight chestnut (*Castanea sativa*) honey samples from Croatia were used. To confirm their botanical origin, all of the samples were subjected to melissopalynological analysis. Unheated samples (23°C), as well as the same honey samples heated at 95°C for 5 min were investigated. The temperature of honey samples after 5 min of heating was 95 ± 1°C and was cooled down to room temperature before measurement. Those parameters of thermal treatment were chosen because they best correspond to the conditions during and after the preparation of hot beverages.

Total phenolic content analysis. Total phenolic content in aqueous honey solutions was determined according to Beretta *et al.* (2005) and Bertoncelj *et al.* (2007). The method is based on the coloured reaction of phenolics with Folin-Ciocalteu reagent. Upon the reaction with phenols, Folin-Ciocalteu reagent is reduced to a blue-coloured oxide. The intensity of the resulting colour was measured using a spectrophotometer (Pye Unicam SP6-500) at 750 nm.

DPPH radical scavenging assay. Radical scavenging activity of honey samples was determined according to Beretta *et al*. (2005) and Brand-Williams *et al*. (1995). Stable DPPH radical reached the absorbance maximum at 517 nm and its colour was purple. The change of this colour into yellow was a result of pairing of an unpaired electron of a DPPH radical with the hydrogen of the antioxidant, thus generating reduced DPPH-H. Adding an antioxidant resulted in the decrease of absorbance, which was proportional to the concentration and antioxidant activity of the compound. The absorbance was measured in a spectrophotometer at 517 nm. Besides that, the absorbance of the blank and control samples was measured. Results are shown as IC_{50} values, i.e. the concentration of an antioxidant (honey concentration) that causes 50% inhibition of DPPH. IC_{50} was calculated from the equation of the curve for each individual sample.

FRAP (ferric reducing antioxidant power) assay. Antioxidant activity of honey was determined according to Bertoncelj *et al.* (2007) and Benzie *et al.* (1999). The method is based on the ability of the honey sample to reduce the ferri form of 2,4,6-tri(2-pyridyl)-1,3,5-triazine complex (Fe^{3+}-TPTZ) to ferro, the coloured form (Fe^{2+}-TPTZ) at acidic pH. The reduction was monitored by measuring the changes of absorbance at 593 nm.

Measurements for all methods used in this research were done in three replications for each sample.

RESULTS AND DISCUSSION

The antioxidant activity of acacia honey measured by FRAP method decreased in 16 samples after thermal treatment. Average reduction in all samples was 31.4% (55.05–37.78 μm Fe(II) in 10% honey solution – HS). FRAP values in the other 14 samples increased 36.9% on average (37.10 to 58.77 μm Fe(II) in 10% HS). FRAP values measured in chestnut honey decreased in all samples 13.1% on average (321.48–279.35 μm Fe(II) in 10% HS). All of the FRAP values are shown in Tables 1 and 2. IC_{50} values increased in 23 samples of acacia honey after heating, with an average reduction of 43.8% (96.81–172.38 mg/ml), where this increase means a decrease in the antioxidant

Table 1. Changes in FRAP values in acacia honey before and after heat treatment

Sample	FRAP (μM Fe(II) in 10% honey solution)		Change (%)
	23°C	95°C	
Decrease			
8A	65.67	55.67	15.2
9A	56.00	0.00	100.0
10A	31.67	25.67	18.9
12A	50.50	44.33	12.2
13A	47.33	0.00	100.0
19A	97.00	61.33	36.8
21A	92.33	91.33	1.1
23A	42.50	6.00	85.9
24A	44.50	33.50	24.7
25A	23.83	13.17	44.8
26A	56.17	53.50	4.7
27A	67.83	60.50	10.8
29A	74.83	44.50	40.5
30A	55.50	49.00	11.7
31A	52.50	45.50	13.3
34A	22.67	20.50	9.6
Average	55.05	37.78	31.4
Increase			
2A	6.00	34.00	82.4
4A	38.83	44.67	13.1
5A	0.00	8.50	100.0
7A	23.33	32.00	27.1
11A	41.67	43.33	3.8
14A	41.50	87.50	52.6
15A	62.67	116.33	46.1
16A	78.83	90.50	12.9
17A	34.50	45.83	24.7
18A	41.50	92.83	55.3
22A	18.50	54.50	66.1
28A	54.67	73.83	26.0
32A	44.83	45.00	0.4
33A	32.50	54.00	39.8
Average	37.10	58.77	36.9

Table 2. Changes in FRAP values in chestnut honey before and after heat treatment

Sample	FRAP (μM Fe(II) in 10 % honey solution)		Change (%)
	23°C	95°C	
Decrease			
6C	335.17	288.17	14.0
7C	242.17	229.17	5.4
20C	336.33	282.00	16.2
21C	363.83	339.33	6.7
22C	410.50	291.50	29.0
25C	264.00	251.17	4.9
28C	225.83	200.00	11.4
30C	394.00	353.50	10.3
Average	321.48	279.35	13.1

activity. Antioxidant activity increased by 30.8% on average (151.35–104.72 mg/ml) in 8 acacia honey samples. In 4 chestnut honey samples antioxidant activity decreased by 21.1%, while in 4 samples it increased by 9.4% on average. IC_{50} values are shown in Tables 3 and 4. Total phenolic content decreased after heating on average by 31.6% in 13 acacia honey samples and increased on average by 39.2% in 16 samples. In chestnut honey, total phenolic content decreased by 20.0% on average in 3 samples and increased by 9.1% on average in 5 samples. Total phenolic content values are shown in Tables 5 and 6.

The results show uneven changes in the antioxidant activity and total phenolic content among individual samples, i.e. in some samples the antioxidant activity decreased after heating, while in others it increased. The same applies to the total phenolic content. In effort to explain these results, a statistical analysis was done. The statistical analysis of the results (*t*-test) showed no statistically significant differences between the results obtained in both types of honey at two different temperatures ($P > 0.05$) using all three methods. The only statistically significant difference ($P < 0.05$) was observed in acacia honey when DPPH method was used. This is due to the fact that the increase in the antioxidant activity was observed in more samples (23) than its decrease (8). Taking into consideration the results of statistical analysis used to interpret the obtained data, it can be concluded that heating honey at 95°C for 5 min does not affect its antioxidant activity and total phenolic content.

There are several reports that show how antioxidant activity increases after thermal processing. Research on how fermentation process and heat treatment (both short-term and prolonged) affect the antioxidant properties of cabbage was done by Polish scientists. They demonstrated that both methods of processing greatly improve the antioxidant capacity of cabbage and that heat processing seemed to compensate for the loss of natural antioxidants by the formation of non-nutritional

Table 3. Changes in IC_{50} values in acacia honey before and after heat treatment

Sample	IC_{50} (mg/ml)		Change (%)
	23°C	95°C	
Decrease			
5A	173.03	270.75	36.1
7A	57.50	125.55	54.2
8A	52.06	151.75	65.7
9A	159.73	179.53	11.0
10A	121.49	283.33	57.1
11A	160.56	210.61	23.8
12A	144.10	345.07	58.2
13A	118.75	143.54	17.3
19A	90.14	125.72	28.3
20A	79.77	105.40	24.3
21A	53.39	131.95	59.5
22A	106.29	162.80	34.7
24A	94.94	158.77	40.2
25A	107.36	158.77	32.4
26A	72.94	131.95	44.7
27A	84.37	206.75	59.2
28A	98.01	176.65	44.5
29A	73.78	162.83	54.7
30A	67.04	158.76	57.8
31A	48.99	106.69	54.1
32A	79.22	81.87	3.2
33A	125.38	182.08	31.1
34A	57.70	203.54	71.7
Average	96.81	172.38	43.8
Increase			
2A	161.36	135.99	15.7
4A	170.79	102.82	39.8
14A	157.23	86.41	45.0
15A	161.68	81.33	49.7
16A	138.25	81.59	41.0
17A	136.62	69.92	48.8
18A	176.57	174.64	1.1
23A	108.30	105.07	3.0
Average	151.35	104.72	30.8

Table 4. Changes in IC_{50} values in chestnut honey before and after heat treatment

Sample	IC_{50} (mg/ml)		Change (%)
	23°C	95°C	
Decrease			
6C	24.56	30.69	20.0
7C	23.04	33.56	31.3
28C	20.69	24.23	14.6
30C	14.24	16.18	12.0
Average	20.63	26.17	21.1
Increase			
20C	23.29	20.69	11.2
21C	23.08	18.84	18.4
22C	17.05	16.74	1.8
25C	23.74	22.73	4.2
Average	21.79	19.75	9.4

antioxidants such as Maillard reaction products (Kusznierewicz *et al.* 2008). Kim *et al.* (2006) heated grape seeds at four different temperatures and then measured the antioxidant activity of the obtained extracts. Their results showed that thermal treatment of grape seeds increased the antioxidant activity of the extracts. This happened because the heat treatment of grape seeds liberated phenolic compounds, and thus increased the amount of active compounds in the extracts. Also, changes in temperature and physical shape (whole vs. powdered grape seeds) of grape seeds affected the antioxidant activity of the extract. Similar results were obtained by Choi *et al.* (2006), who investigated the influence of heat treatment on the antioxidant activity and polyphenolic compounds in shiitake (*Lentinus edodes*) mushroom. They also showed that polyphenolic content and antioxidant activity in the mushroom extracts increases as the heating temperature and time increases. Research done by Serpen *et al.* (2012) demonstrated that upon heating at 180°C, total antioxidant capacity of meat (chicken, pork, beef, and fish) increased to an apparent maximum at 5 min followed by a sudden decrease until 15th min, while the final stage of heating was characterised by slight increases. They explained the obtained results by considering some factors such as denaturation and exposure of reactive protein sites, degradation of endogenous antioxidants and the formation of Maillard reaction products, which all have antioxidant properties.

The influence of different heat treatments on the quality parameters of various types of honey is well investigated, and it is known that it significantly impairs its quality (Hebbar *et al.* 2003; Subramanian *et al.* 2007; Bartáková *et al.* 2011). Microwave heating, infrared heating, ultrasound processing, membrane processing, etc. have been investigated in order to find a rapid method for achieving the desired level of reduction of microorganisms and quality parameters with reduced thermal damage, while the antioxidant

Table 5. Changes in total phenolic content in acacia honey before and after heat treatment

Sample	Total phenolics (mg gallic acid/kg honey)		Change (%)
	23°C	95°C	
Decrease			
8A	66.67	34.44	48.3
9A	48.89	41.11	15.9
10A	56.67	36.67	35.3
12A	142.22	126.67	10.9
13A	78.89	73.33	7.0
19A	38.89	32.22	17.1
24A	74.44	16.67	77.6
25A	28.89	4.44	84.6
26A	31.11	12.22	60.7
29A	31.11	15.56	50.0
30A	42.22	32.22	23.7
31A	56.67	45.56	19.6
34A	58.89	45.56	22.6
Average	58.12	39.74	31.6
Increase			
2A	42.22	73.33	42.4
4A	31.11	110.00	71.7
5A	36.67	52.22	29.8
7A	61.11	92.22	33.7
11A	38.89	42.22	7.9
14A	6.67	17.78	62.5
15A	18.89	26.67	29.2
16A	66.67	132.22	49.6
17A	50.00	75.56	33.8
18A	0.00	20.00	100.0
22A	26.67	35.56	25.0
23A	27.78	32.22	13.8
27A	17.78	32.22	44.8
28A	14.44	32.22	55.2
32A	64.44	86.67	25.6
33A	55.56	57.78	3.8
Average	34.93	57.43	39.2

Table 6. Changes in total phenolic content in chestnut honey before and after heat treatment

Sample	Total phenolics (mg gallic acid/kg honey)		Change (%)
	23°C	95°C	
Decrease			
6C	281.11	237.78	15.4
28C	242.22	183.33	24.3
30C	292.30	231.11	20.9
Average	271.88	217.41	20.0
Increase			
7C	200.00	240.00	16.7
20C	227.78	246.67	7.7
21C	262.22	285.56	8.2
22C	180.00	200.00	10.0
25C	277.78	291.11	4.6
Average	229.56	252.67	9.1

capacity of honey after certain heat treatments has remained unknown. The influence of thermal treatment at various temperatures (50, 60, and 70°C) during a prolonged period of time on the antioxidant activity and colour of honey was investigated by Turkish scientists. They came to a conclusion that the biggest changes occurred at 70°C, while at lower temperatures these changes were less pronounced – the higher the temperature, the bigger the increase in the antioxidant activity (Turkmen *et al.* 2006). In a similar study, researchers thermally treated the samples at 60°C for 12 to 16 hours. The conclusion was that processing did not have any significant impact on the antioxidant capacity as determined by ORAC assay (Wang *et al.* 2004).

As it can be seen from the already published papers, antioxidant activity may or may not change after heat treatment. A considerable amount of research has proved that antioxidant capacity can be increased by thermal treatment. This is probably due to some other compounds occurring at high temperatures than flavonoids or other phenolic compounds, which are known to be susceptible to thermal degradation. Taking into consideration the divergence among the results published by other researchers, it can be concluded that changes in the antioxidant activity generally depend on two important factors: chemical composition of the investigated foodstuff and temperature and duration of heat treatment. This research showed that a short period of heat treatment of honey at a relatively low temperature does not affect its antioxidant activity, so a typical home usage of honey should preserve all of its "good" attributes.

References

Amin I., Lee W.Y. (2005): Effect of different blanching times on antioxidant properties in selected cruciferous

vegetables. Journal of the Science Food and Agriculture, **85**: 2314–2320.

Bartáková K., Dračková M., Borkovcová I., Vorlová L. (2011): Impact of microwave heating on hydroxymethylfurfural content in Czech honeys. Czech Journal of Food Sciences, **29**: 328–336.

Benzie I.F.F., Strain J.J. (1999): Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, **299**: 15–27.

Beretta G., Granata P., Ferrero M., Orioli M., Facino R.M. (2005): Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Analitica Chimica Acta, **533**: 185–191.

Bertoncelj J., Doberšek U., Jamnik M., Golob T. (2007): Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry, **105**: 822–828.

Brand-Williams W., Cuvelier M.E., Berset C. (1995): Use of a free-radical method to evaluate antioxidant activity. LWT-Food Science and Technology, **28**: 25–30.

Brudzynski K., Miotto D. (2011a): The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. Food Chemistry, **125**: 570–575.

Brudzynski K., Miotto D. (2011b): The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. Food Chemistry, **124**: 869–874.

Choi Y., Lee S.M., Chun J., Lee H.B., Lee J. (2006): Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chemistry, **99**: 381–387.

Dewanto V., Adom X.W.K., Liu R. (2002): Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, **50**: 3010–3014.

Durmaz G., Alpaslan M. (2007): Antioxidant properties of roasted apricot (*Prunus armenica* L.) kernel. Food Chemistry, **100**: 1177–1181.

Hebbar H.U., Nandini K.E., Lakshmi M.C., Subramanian R. (2003): Microwave and infrared heat processing of honey and its quality. Food Science and Technology Research, **9**: 49–53.

Hong Y. J., Barrett D. M., Mitchell A. E. (2004): Liquid chromatography/mass spectrometry investigation of the impact of thermal processing and storage on peach procyanidins. Journal of Agricultural and Food Chemistry, **52**: 2366–2371.

Kim S.-Y., Jeong S.-M., Park W.-P., Nam K.C., Ahn D.U., Lee S.-C. (2006): Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry, **97**: 472 –479.

Kusznierewicz B., Smiechowska A., Bartoszek A., Namiesnik J. (2008): The effect of heating and fermenting on antioxidant properties of white cabbage. Food Chemistry, **108**: 853–861.

Lachman J., Hejtmánková A., Sýkora J., Karban J., Orsák M., Rygerová B. (2010a): Content of major phenolic and flavonoid antioxidants in selected Czech honey. Czech Journal of Food Sciences, **28**: 412–426.

Lachman J., Orsák M., Hejtmánková A., Kovářová E. (2010b): Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT-Food Science and Technology, **43**: 52–58.

Nicoli M. C., Anese M., Parpinel M. (1999): Influence of processing on the antioxidant properties of fruit and vegetables. Trends in Food Science and Technology, **10**: 94–100.

Oszmianski J., Wolniak M., Wojdylo A., Wawer I. (2007): Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. Journal of the Food Science and Agriculture, **87**: 573–579.

Serpen A., Gökmen V., Fogliano V. (2012): Total antioxidant capacities of raw and cooked meats. Meat Science, **90**: 60–65.

Subramanian R., Hebbar H.U., Rastogi N.K. (2007): Processing of honey: A review. International Journal of Food Properties, **10**: 127–143.

Turkmen N., Sari F., Velioglu Y. S. (2005): The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry, **93**: 713–718.

Turkmen N., Sari F., Poyrazoglu E.S., Velioglu Y.S. (2006): Effects of prolonged heating on antioxidant activity and colour of honey. Food Chemistry, **95**: 653–657.

Wang X.-H., Gheldof N., Engeseth N.J. (2004): Effect of processing and storage on antioxidant capacity of honey. Journal of Food Science, **69**: 96–101.

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