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Investigating the Changes in the Antioxidant Activity of Honey in Different Storage Conditions

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Abstract Honey is a naturally sweet substance that the bee collects from the nectar of flowers and sap of plants, after adding various enzymes, processing and evaporating excess moisture, it stores it in the hive. The aim of this study is investigating the changes in the antioxidant activity of the 10 honey samples under different storage conditions [The samples were heated in water bath (48 Degree Celsius for 1 day and 80 Degree Celsius for 4 minutes), or kept at room temperature (25 Degree Celsius) for 3 and 6 months. Then they were re- evaluated for antioxidant activity]. Antioxidant activity by three methods: DPPH, beta-carotene-linoleic acid and reduction power method in honey samples evaluated**.** Measurements for all methods used in this research were done in three replications for each sample. Data were analyzed by SPSS software. Kolmogorov-Smirnov test was used to check the normality of the distribution of variables. The one-way ANOVA was used to compare the mean between groups. According to the results, antioxidant activity of honeys were increased under thermal condition and were decreased during storage. Storage and processing condition (thermal treatment) can cause changes in antioxidant activity as well as the quality of honey.

Keywords: *honey, antioxidant activity, DPPH, reduction power, storage*

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1. Introduction

Honey is a naturally sweet substance that the bee collects from the nectar of flowers, sap of plants. [1] After adding various enzymes, processing and evaporating excess moisture, it stores it in the hive. Honey because of its useful ingredients not only have high nutritional value, but also can protect human health and cure some disorders and diseases. [2,3]

It has long been a human interest and nutrient as an energetic and nutritious food. [4] In addition to its nutritional properties, honey has other properties such as antimicrobial properties. The therapeutic effect of this substance has been proven in the treatment of heart disease, strengthening the immune system, treating digestive diseases, healing wounds, treating eye diseases, treating arthritis, diabetes and many other disorders. [3]

Honey contains at least 181 compounds. Chemically, honey contains sugar (80-70%), water (10-20%) and other minor components such as organic acids, mineral salts, vitamins, proteins, phenolic compounds and free amino acids. [5,6,7]

Honey can prevent the development of oxidation reactions in foods such as browning of fruits, vegetables and fat oxidation in meat and inhibits the growth of foodborne pathogens and the factors that cause food spoilage. [8]

These changes usually limit the shelf life of food products. The above illustrates the growing need for a natural substance that can inhibit these reactions without any harmful effects on the food. [9]

Honey is generally due to compounds containing phenolic acids and flavonoids, enzymes such as glucose oxidase, catalase, ascorbic acid, carotenoid-like substances, organic acids, Millard reaction compounds, amino acids and proteins, causing antioxidant activity. [4,10]

Antioxidants are additives that enhance the shelf life of foods by protecting them against oxidation-induced corruption such as fatty acids, color changes and loss of nutritional value. Antioxidant properties have been reported today for hundreds of natural and synthetic compounds, but their use in nutrients is limited by their safety. Since food additives undergo very rigorous toxicological tests, only a small number of synthetic antioxidants have been used in foods at any one time. [11]

With the discovery of the harmful and carcinogenic effects of synthetic antioxidants such as BHT and BHA, the use of surrogate natural antioxidants, including plant-derived compounds that pose the least risk to human health and the environment, has received much attention. This illustrates the growing need for the identification and study of natural antioxidants. [12,13]

The physicochemical properties as well as the honey compounds involved in inhibiting such adverse reactions depend on various factors such as plant origin, seasonal factors, environmental factors and honey processing. Socha et al. (2011) examined the antioxidant activity and phenolic acids profile of eight different Polish honey samples. The results of the study showed a positive correlation between antioxidant activity and phenolic content of honey. Antioxidant activity reported in eight Polish honey samples ranged from 18.21 to 46.4%. [14]

Iritie et al. (2014) examined the physicochemical properties of fresh and aged honey (after one year of storage). The results of the study showed that there was a significant difference between ash content and insoluble matter in the 2011 and 2012 samples. Ash content was higher in 2011 than in 2012 while insoluble matter was lower in 2011 than in 2012, all properties of honey samples studied were within the legal range. [15]

The antioxidant activity varies widely depending on flower sources and environmental factors. Honey's plant origin has the greatest effect on its antioxidant activity, while honey processing and storage are less effective in this respect. [16,17]

Moniruzzaman et al. (2013) monitored changes in phenolic, flavonoid and antioxidant activity of Malaysian acacia honey collected in different months over a two-year period. Based on the results, there was a gradual increase in the phenolic content of honey samples collected between September and December. Honey collected at the beginning of January showed the highest color intensity, antioxidant properties and the amount of phenolic compounds. [18]

Saric et al. (2012) investigated the antioxidant and phenolic content of honey during the two-year storage, over six-month periods. After one year of storage the phenolic and flavonoid contents of honey samples decreased by 91.8% and 45.6%, respectively, and antioxidant activity decreased by 30% after two years. [19]

The aim of this study is investigating the changes in the antioxidant activity of the 30 honey samples under different storage conditions.

2. Material & Methods

2.1. Materials

In this study, 10 samples of honey from different regions of Iran were collected from reputable production and distribution centers in that region by simple and random sampling method and transferred to Food Laboratory in optimum conditions. The samples were stored at ambient temperature (25 Degree Celsius) until tests.

2.2. Methods

2.2.1. Determination of Antioxidant Activity of Honey in the Presence of DPPH Radical

Antioxidant activity of honey in the presence of free radicals 2 and 2-diphenyl-1-picryl hydrazyl is performed by spectrophotometric method. 1.25 ml of honey solution dissolved in distilled water is mixed with 1.5 ml of DPPH methanol solution and kept at room temperature for 90 minutes in the dark. The absorbance was read at 517 nm against 1: 1 methanol-water as a control. The control sample is 1.25 cc of methanol with 1.5 cc of methanol solution of DPPH. The amount of antioxidant activity is expressed as a percentage. [10,20]

2.2.2. Determination of Antioxidant Activity of Honey by Beta-carotene-linoleic acid Method

Baseline solution is prepared from beta-carotene-linoleic acid. (0.025 g of beta-carotene dissolved in 50 ml of chloroform, extract two cc of this solution and add 25 ml of linoleic acid and 200 mg of tween 40 and mix thoroughly. After separation and evaporation of chloroform, add 100 ml of distilled water. Add 2.5 cc of the base solution above to the test tube and add 350 ml of honey solution (0.1 g / ml).

All of these steps are performed with the BHT solution as standard (positive control) and control (containing only 350 µl of ethanol). The samples were placed in a 50°C hot water bath and the absorption of the samples was read at time 0 and 120 at 470 nm.

The amount of antioxidant activity was evaluated as a percentage by comparing the absorption of the samples with time zero and by [21]

2.2.3. Determination of Reduction Power

Mix 1 ml of honey solution with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide 1% and place in a 50°C water bath for half an hour. After adding 2.5 ml of trichloroacetic acid and 10 min of centrifugation, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride then read absorption at 700 nm. BHT solution is considered as standard (positive control). High absorption rate indicates high reduction power. [21]

The samples were heated in water bath (48 Degree Celsius for 1 day and 80 Degree Celsius for 4 minutes), or kept at room temperature (25 Degree Celsius) for 3 and 6 months. Then they were re-evaluated for antioxidant activity. [19]

2.3. Statistical Analysis of Results

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

Data were analyzed by SPSS software. Kolmogorov-Smirnov test was used to check the normality of the distribution of variables. The one-way ANOVA was used to compare the mean between groups, with the significance level 0.05 considered. [22]

3. Results and Discussion

Our results suggest that honey samples have great antioxidant properties as determined by DPPH, Reduction power and β-carotene as shown in Table 1. It should be noted that there was a wide range of 22.6-68.49% for DPHH, 9.02-29.97% for β-carotene and 0.84-1.78 AU for Reduction power. However when we tested the samples after 3 and 6 months of storage at normal room temperature

we found reduced antioxidant activity in DPPH method in all samples (~-2.7%) in 3 months and (~-5.6%) in 6 months. The ability to inhibition of beta-carotene bleaching by honey samples decreased by (~-2.6%) and (~-4.3%) and the ability of honey samples to reduction of Fe3+ decreased by (~-0.08 AU) and (~-0.15 AU) respectively, after 3 and 6 months of storage (Table 1, Figure 1)

Table 1. Changes in the antioxidant activity of honey samples after 3 and 6 months of storage

Test	Time 0	After 3 months	After 6 months
DPPH (%)	30.89	29.66	27.53
	68.49	64.16	61.23
	45.37	40.34	33.80
	60.95	55.35	51.17
	62.67	58.60	55.02
	22.60	21.60	19.31
	53.93	50.73	48.91
	47.94	46.97	45.13
	38.18	37.91	35.29
	60.10	58.35	55.12
β-carotene (%)	22.81	20.42	18.38
	25.65	20.98	19.06
	29.97	23.46	21.15
	12.89	11.15	9.54
	18.92	17.03	16.26
	9.02	8.12	6.94
	16.55	13.11	11.74
	14.27	11.58	9.63
	15.85	12.98	11.04
	21.04	20.01	18.58
Reduction power (AU)	0.84	0.79	0.75
	0.85	0.81	0.8
	1.01	0.97	0.93
	1.17	1.15	1.11
	1.25	1.14	1.1
	1.54	1.39	1.28
	1.32	1.3	1.24
	1.31	1.22	1.17
	1.78	1.52	1.41
	1.40	1.31	1.26

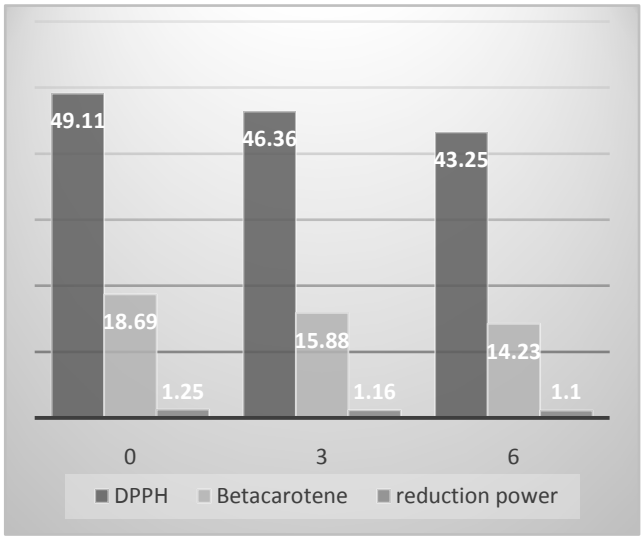

Figure 1. Changes in the average of antioxidant activity of honey samples after 3 and 6 months of storage

Perna et al. (2013) and Pichichero et al. (2009) attribute antioxidant properties in honey to enzymes, Millard reaction products, organic, amino, ascorbic and phenolic acids, flavonoids, peptides and carotenoid-like substances. [23,24]

After 3 and 6 months storage at normal room temperature, the samples were re-evaluated for antioxidant activity. Saric et al. (2012) Examined changes in antioxidant activity and phenolic content of honey during storage for two years and for 6 months. After one year of storage, the phenolic and flavonoid content of honey samples decreased by 91.8% and 45.6%, respectively, compared to the initial value, and the antioxidant activity decreased by 30% after two years [19].

Table 2. Changes in the antioxidant activity of honey samples after heating

Test	Without heating	80 °C for 4 minutes	48°C for 1 day
DPPH (%)	30.89	66.6	63.69
	68.49	74.62	68.92
	45.37	65.23	62.67
	60.95	66.6	61.81
	62.67	74.65	69.34
	22.60	52.91	56.67
	53.93	59.21	57.06
	47.94	62.67	64.16
	38.18	61.64	60.44
	60.10	70.89	66.95
β-carotene (%)	22.81	48.31	41.68
	25.65	56.94	52.33
	29.97	64.83	63.56
	12.89	28.13	22.88
	18.92	36.54	35.93
	9.02	20.32	18.13
	16.55	30.98	30.01
	14.27	48.15	43.12
	15.85	41.73	40.51
	21.04	36.11	35.6
Reduction power (AU)	0.84	0.91	0.90
	0.85	1.02	0.97
	1.01	1.09	1.07
	1.17	1.23	1.22
	1.25	1.41	1.38
	1.54	1.73	1.65
	1.32	1.38	1.37
	1.31	1.34	1.33
	1.78	1.82	1.80
	1.40	1.67	1.63

After being heated to 48 ° C for 24 hours and 80°C for 4 minutes to re-evaluate the antioxidant activity. The results showed that all the samples after the heat of the antioxidant activity increased, this increasing was greater at 80°C than at 48°C. After heating the honey samples to 80°C for 4 minutes, radical scavenging ability of DPPH (~+16.4%), inhibition of beta-carotene bleaching (~+22.6%) and the ability of honey samples to reduction of Fe3+ (~+0.11 AU) increased. We found less increasing in antioxidant activity in heating to 48°C for 24 hours (than heating 80°C) that was as following: DPPH (~+13.15%), beta-carotene-linoleic acid (~+19.7%) and reduction power (~+0.08 AU) (Table 2, Figure 2).

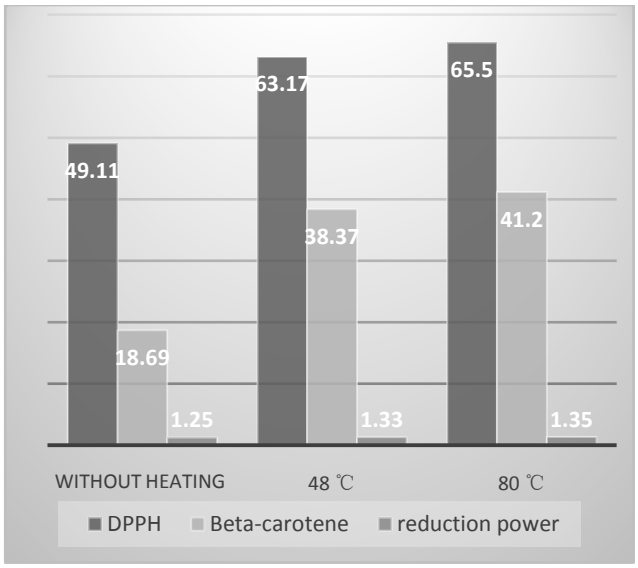

Figure 2. Changes in the average of antioxidant activity of honey samples after heating

Socha et al. (2011) reported antioxidant activity of 7 Polish honey samples ranging from 18.21 to 46.4%. [14]

The antioxidant activity of natural honey may be attributed to the presence of many different substances such as enzymes, Millard reaction products, organic acids, phenolic acids, flavonoids, amino acids, peptides, ascorbic acid and carotenoid-like substances. The antioxidant activity of honey, like other properties, depends on flower sources, which are often dependent on seasonal and environmental factors as well as the method of processing. [24]

The reduction power of the samples was in the range of 0.840-1.78 AU. Based on this test, the amount of high absorption each sample indicates its high reducing power.

The results of β-carotene test were evaluated by comparing the absorption of the samples between time zero and two hours and the percentage of beta-carotene yellow color stability. The basis of this method is the reaction of unsaturated beta-carotene with free radicals produced by the formation of hydroperoxide from linoleic acid, which results in the modified beta-carotene structure losing its orange color. The presence of antioxidants can prevent the expansion of beta-carotene discoloration by neutralizing the linoleate radical and other radicals in the system. [25]

Turkmen et al. (2006) suggested that heat treatment increases antioxidant power due to the formation of melanoid polymeric compounds following the Millard reaction. [26]

In a similar study, the effect of heat treatment on antioxidant activity varied according to the type of honey. According to the results of the study, the antioxidant activity after heat treatment in acacia honey was unchanged, in honeydew decreased and in lemon and Wheat honey increased. [6]

4. Conclusion

According to the results, increased antioxidant activity under thermal condition and decreased antioxidant activity were observed during storage.

In addition, honey can be used as a natural antioxidant as well as a substance to reduce the effects of browning on the processing of fruits and vegetables. Based on this research and comparing it with similar studies, it can be stated that honey in different regions of Iran has a good quality in term of antioxidant properties, which can be the result of improving management techniques and beekeeping in recent years.

At the end, Storage and processing conditions, thermal treatment can cause changes in antioxidant activity as well as the quality of honey.

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Conflict of Interest

There are no conflicts of interest to declare by authors

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