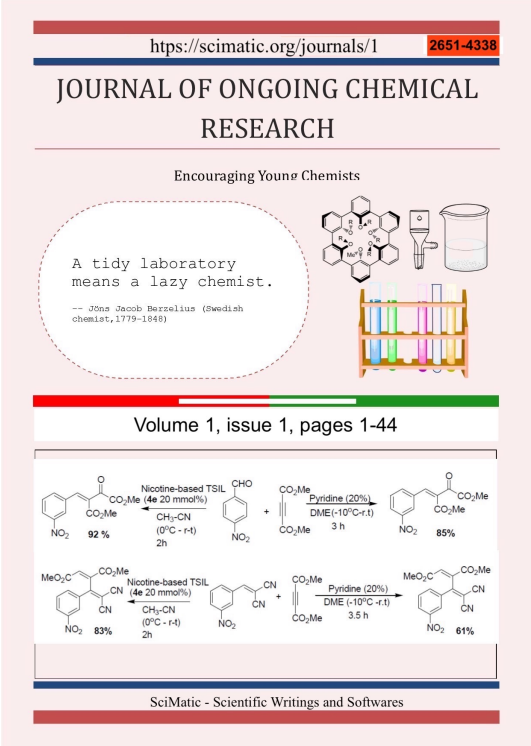
SUNFLOWER HONEY PRODUCED IN TURKEY SCAVENGES SINGLET OXYGEN AND INHIBITS LIPID PEROXIDATION VIA ANTIRADICAL ACTIVITY



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Research Article

Sunflower Honey Produced in Turkey Scavenges Singlet Oxygen and Inhibits Lipid Peroxidation Via Antiradical Activity

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Abstract

Honey is a spectacular food due to its great ingredients and bioactivity. Since antic times, it has been known and used by the people as food and medicine. Herein, the antiradical and lipid peroxidation inhibitory activities of sunflower honey produced in four regions of Adana city and various wholesalers through Turkey were studied. The antiradical activity was performed using ABTS and DPPH assays, while lipid peroxidation inhibitory activity was carried out using the □-carotene-linoleic acid test. All sunflower honey samples exhibited excellent antioxidant and lipid peroxidation inhibitory activity. The honey samples scavenged DPPH and ABTS radicals in the range of 27-30 and 15-18 µg/mL, IC_{50} values, respectively. The sunflower honey samples from various wholesalers served 46-60 and 31-45 µg/mL IC_{50} values, respectively. The sunflower honey inhibited lipid peroxidation dose-dependently. The IC_{50} values were calculated in the range of 5-7 µg/mL. On the other hand, the sunflower honey purchased from various wholesalers displayed 11-14 µg/mL inhibitory activity. The results of antiradical activities supported those results of lipid peroxidation inhibitory activity. The activities of processed sunflower honey samples exhibited less activity than those of produced ones. It was concluded that the filtering process and honey sample's shelf life might affect the lesser antiradical and lipid peroxidation inhibitory activity. Moreover, the possibilities such as storage and processing of honey are among the factors that affect the composition of honey. Consuming or using honey freshly, therefore, advised to be benefited from the medicinal properties of honey.

Keywords: Honey, Singlet Oxygen, Sunflower Honey, Antiradical Activity, Lipid Peroxidation Inhibitory Activity

INTRODUCTION

Honey is a sweet substance obtained from flower nectar and secretes through transformations by honeybees. Honey is a complex mixture that contains many different ingredients. Honey is a valuable ingredient with excellent properties nutritional value (Boutoub et al., 2021). These properties vary depending on the botanical origin of honey (Erban et al., 2021). Honey is composed of sugars, water, and a myriad of minor components, including minerals, vitamins, proteins, and polyphenols (Chen et al., 2021). Honey is not only an essential and precious foodstuff found in kitchens worldwide but is also used as a multipurpose natural medicine.

There are many different substances whose antioxidant properties have been discovered. Antioxidants such as carotenoids, flavonoids, hydroxycinnamic acids, phenolic acids, and vitamins are natural bioactive compounds available in several sources (e.g., fruits, vegetables, etc.) which could protect essential biomolecules from oxidative damage (Pisoschi et al., 2018; Rostami et al., 2019; Parın et al., 2021).

The positive effect of various kinds of honey has been determined as antioxidant activity (Gul and Pehlivan, 2018; Sarhan and Azzazy, 2015; Bonta et al., 2020; Maric et al., 2021), antidepressant effects (Filho et al., 2016), the improvement of morphology in memory-related brain areas, reduction of brain oxidative stress (Arshad et al., 2020), increase of the brain-derived neurotrophic factor (Goes et al., 2018) and acetylcholine concentrations, reduction of acetylcholinesterase activity in brain homogenates (Baranowska-Wójcik et al., 2020).

Sunflower honey is in the color of gold and looks like a cream and a unique taste and aroma. It can be produced where the *Helianthus* species are cultured. The local people used it against fever and to strengthen the immune system. Every year, it is thought that more than 8.000 tons are produced in Turkey. It crystallizes quickly. Therefore, it is not preferred in Turkey due to false belief. So, it is not sold in markets in Turkey.

In contrast, according to science, natural honey crystallization is a natural process. The medicinal usage of sunflower honey and to promote the natural sunflower honey to the people, we have studied its antiradical and lipid peroxidation inhibitory activities. The goal of the study is to compare the freshly produced sunflower honey with those processed in wholesalers.

RESULTS AND DISCUSSION

There are several methods to determine antiradical activities. The chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity, and chemical behavior, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays for evaluating the activity potential of honey samples would be even necessary. In this study, mainly two methods, DPPH free radical scavenging activity, ABTS cation radical scavenging activity, were used to determine the antiradical activity of the honey samples. In both tests, electrons are transferred to the media by the antioxidant. Some bulky antioxidants were unable to approach DPPH radical, which makes the ABTS assay superior.

In the DPPH assay, the sunflower honey samples produced in Adana showed higher radical scavenging activity than the processed wholesalers' samples (Table 1). The IC_{50} of freshly produced sunflower honey samples were calculated in the range of 27.75 and 29.79 µg/mL value. This range belongs to the freshly produced honey. Among them, **the sample produced in Kozan (East) and Yumurtalık (South) regions** exhibited (IC_{50}: 27.75 ± 0.88 and 28.91 ± 0.92 µg/mL) the higher activity. On the other hand, **the sample produced in Kozan (North) and Yumurtalık (East) regions** (IC_{50}: 29.10 ± 0.86 and 29.79 ± 0.90 µg/mL) demonstrated close activity with the others. There was no more statistical difference between produced sunflower honey samples. At the same condition, the BHT and *a*-tocopherol displayed 54.97 ± 1.01 and 12.26 ± 0.05 µg/mL IC_{50} values. These results indicate that the sunflower honey has potent antiradical capacity than BHT used as a standard compound. The samples provided from wholesalers possessed IC_{50} values in the range of 46.35 ± 1.08 and 59.46 ± 1.19 µg/mL. The DPPH free radical scavenging activity was lesser than the freshly produced ones.

In the ABTS assay, The IC_{50} of freshly produced sunflower honey samples ranged 15.25 and 18.90 µg/mL values. Among them, **the sample produced in Kozan (North) and Yumurtalık (South) regions** exhibited (IC_{50}: 15.25 ± 0.66 and 16.67 ± 0.60 µg/mL) the higher activity. On the other hand, **the sample produced in Kozan (East) and Yumurtalık (East) regions** (IC_{50}: 17.68 ± 0.68 and 18.90 ± 0.72 µg/mL) demonstrated close activity with the others. There was no more statistical difference between produced sunflower honey samples. At the same condition, the BHT and a-tocopherol displayed 2.91 ± 0.55 and 4.87 ± 0.45 µg/mL IC_{50} values. These results also indicate that the sunflower honey has significant antiradical capacity. However, the samples provided from wholesalers possessed IC_{50} values in the range of 31.77 ± 0.66 and 44.11±1.10 µg/mL. The ABTS cation radical scavenging activity was lesser than the freshly produced ones (Table 1).

The media of β-carotene-linoleic acid assay consists of oxygen-centered free radicals. Antioxidants scavenge reactive radicals by giving H radical to the medium. This method is also essential to elucidate the antioxidants scavenging singlet oxygen. It is well known that singlet oxygen propagates chain reactions when exposed to double bonds during radical reactions.

Table 1. Antiradical activity and lipid peroxidation inhibitory activity results of Sunflower honey

[a] Sunflower honey collection places/regions		Honey Samples	ABTS assay	DPPH assay	Lipid peroxidation inhibitory assay
			IC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)
The honey get produced freshly	ADANA	Kozan (North)	15.25±0.66	29.10±0.86	6.25±0.88
		Kozan (East)	17.68±0.68	27.75±0.88	5.63±0.97
		Yumurtalık (South)	16.67±0.60	28.91±0.92	5.06±0.69
		Yumurtalık (East)	18.90±0.72	29.79±0.90	6.58±1.01
The honey provided from wholesalers	KONYA	Honey 1	31.77±0.66	49.05±0.99	11.61±1.14
		Honey 2	34.73±0.76	50.80±1.12	13.78±1.13
	İZMİR	Honey 3	35.43±2.18	49.91±1.15	13.99±1.16
		Honey 4	32.95±1.12	46.35±1.08	12.45±1.01
	ANKARA	Honey 5	40.78±2.12	55.13±1.01	11.66±1.11
		Honey 6	42.29±1.08	51.78±1.20	13.11±0.97
		Honey 7	43.55±1.23	59.46±1.19	13.55±1.05
	ADANA	Honey 8	41.40±1.15	49.95±1.11	12.01±1.08
		Honey 9	43.37±1.03	48.89±1.23	11.98±1.04
		Honey 10	44.11±1.10	51.43±1.02	12.11±1.03
	Standard compounds	*a*-tocopherol	4.87±0.45	12.26±0.05	2.10±0.08
		BHT	2.91±0.55	54.97±1.01	1.34±0.04

a: The results presented herein are mean ± Standard error meaning (S.E.M.) of three parallel measurements (p>0.05)

Table 1 also shows the lipid peroxidation inhibitory activity of the honey samples compared with a-tocopherol and BHT, by the β-carotene-linoleic acid assay. The inhibitory activity increased with the increasing amount of the samples. All the tested extracts showed lesser activity than BHT and a-tocopherol. In this assay, The IC_{50} of freshly produced sunflower honey samples ranged from 5.06 and 6.58

µg/mL values. Among them, **the sample produced in Yumurtalık (South) and Kozan (East) regions** exhibited (IC_{50}: 5.06 ± 0.69 and 5.63 ± 0.97 µg/mL) higher activity. On the other hand, **the sample produced in Kozan (North) and Yumurtalık (East) regions** (IC_{50}: 6.25 ± 0.88 and 6.58 ± 1.01 µg/mL) demonstrated close activity with the others. There was no more statistical difference between produced sunflower honey samples. At the same condition, the BHT and a-tocopherol displayed 1.34 ± 0.04 and 2.10 ± 0.08 µg/mL IC_{50} values. These results also indicate that the sunflower honey has significant lipid peroxidation inhibitory activity. However, the samples provided from wholesalers possessed IC_{50} values in the range of 11.61 ± 1.14 and 13.99 ±1.16 µg/mL. Herein the activity of wholesalers' sunflower honey samples was also lesser than the freshly produced ones. Typically, according to science, the IC_{50} value lower than 50% is very remarkable. All sunflower samples can also be considered powerful inhibitors against lipid peroxidation.

Among the sunflower honey samples, the honey produced in Adana at four regions exhibited the higher DPPH free radical scavenging activity, ABTS cation radical scavenging activity, and lipid peroxidation inhibitory activity than the sunflower honey samples purchased from wholesalers. Different factors may be affected this result. One of these is that the wholesalers filter the honey with less than 0.2 mm sieve. Thus, honey does not crystallize since the pollens of honey are removed from the honey. During this process, most of the antioxidants may also be removed from the honey. Another reason is the holding period after filtration. In this case, the shelf life of the honey samples becomes essential. It is seen from the study that the freshly produced sunflower honey samples, therefore, contain more antioxidants. It can be said herein that the concentration of antioxidants decreases as time passes.

EXPERIMENTAL

2.1. *Chemicals and reagents*

1,1-diphenyl-2-picrylhydrazyl (DPPH), α-tocopherol, β-carotene, polyoxyethylene sorbitan mono palmitate (Tween-40), linoleic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Optical densities for bioassays were read on a 96-well microplate reader, SpectraMax340 PC^{384} (Molecular Devices, Silicon Valley, CA). Solvents and chemicals were of analytical grade.

2.2. Collection of Honey Samples

The Sunflower honey samples were get produced by the beekeepers in Adana in four locations in June 2020. The locations were in **Kozan (North), Kozan (East), Yumurtalık (East), and Yumurtalık (South) districts.** The produced honey samples were used for the comparison. The processed sunflower honey samples were purchased from different wholesalers in Konya, Ankara, İzmir, and Adana. Ten sunflower honey samples were purchased from wholesalers.

2.3. Preparation of samples for the bioactivity

Honey samples (2 g of each honey sample) was diluted with 5 mL (40%), 10 mL (20%), 20 mL (10%), 10 mL (5%), and 40 mL (2.5%) distilled water.

2.4. Antioxidant activity

2.4.1. Free radical-scavenging activity (DPPH assay)

The free radical scavenging activity of honey samples was determined by the DPPH assay described by Blois (1958) with slight modification (Blois, 1958; Sabudak et al., 2009). DPPH absorbs at 517 nm in its radical form, but on reduction by an antioxidant or a radical species, its absorption decreases. Briefly, a 0.1 mmol/L solution of DPPH in methanol was prepared, and 4 mL of this solution was added to 1 mL of the sample solution in methanol at different concentrations and incubated for 30 minutes. The absorbance of each sample was measured at 517 nm. The capability to scavenging of DPPH radical of an antioxidant was calculated using the following equation:

$$DPPH\ radical\ scavenging\ activity(\%) = (\frac{A_{control} - A_{sample}}{A_{control}}) \times 100$$

The sample concentration providing 50% free radical scavenging activity (IC_{50}) was calculated from the graph of DPPH scavenging percentage against sample concentration. BHT and α-tocopherol were used as antioxidant standards for comparison.

2.4.2. ABTS cation-radical scavenging assay

The $ABTS^{\bullet+}$ scavenging activity was evaluated according to the literature (Re et al., 1999) with minor modifications. The $ABTS^{\bullet+}$ solution was prepared by mixing ABTS (7 mM) and potassium persulfate (2.45 mM) and diluted with ethanol to get an absorbance of 0.350±0.025 at 734 nm in 200 µL well. To each well

containing 40 µL of the sample solution in methanol at different concentrations, 160 µL of prepared $ABTS^{\bullet+}$ solution was added. After incubation for ten minutes, the absorbance was measured at 734 nm using a 96-well microplate reader. Each assay was carried out in triplicate, quercetin, BHT, and *α*-tocopherol were used as standards for comparison. The capability of the sample to scavenge $ABTS^{\bullet+}$ was calculated using the following formula:

$$ABTS\ cation\ radical\ scavenging\ activity(\%) = (\frac{A_{control} - A_{sample}}{A_{control}}) \times 100$$

The results were presented as $IC_{50,}$ which is the concentration that scavenges 50% of the radical.

2.4.3. β-*carotene-linoleic acid assay*

The lipid peroxidation inhibitory activity of honey samples was evaluated using the β-carotene-linoleic acid test (Miller, 1971; Sabudak et al., 2009) with slight modifications. β-carotene (0.5 mg) in 1 mL chloroform was added to a mixture of 25 µL of linoleic acid and 200 mg of Tween-40 emulsifier. The chloroform was evaporated under vacuum, and 100 mL distilled water saturated with oxygen was added by vigorous shaking. 4 mL of this mixture was transferred into test tubes containing different extract concentrations. The absorbance of each sample was recorded at 470 nm as soon as after adding emulsion at zero-time using a 96-well microplate reader (SpectraMax 340PC, Molecular Devices, USA). The emulsion system was incubated for 2 hours at 50 ºC. A blank, devoid of β-carotene, was prepared for background subtraction. BHT and *α*-tocopherol were used as standards. The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \frac{ln\frac{a}{b}}{t}$$

where: ln = natural logarithm, a = absorbance at time zero, b = absorbance at time t (120 min) The lipid peroxidation inhibitory activity (AA) was calculated in terms of percent inhibition relative to the control, using the following equation below:

$$AA(lipid\ peroxidation\ inhibitory\ activity\ \%) = (\frac{R_{control} - R_{sample}}{R_{control}}) \times 100$$

The concentration (IC_{50}), which provides 50% inhibition of lipid peroxidation, was calculated from the graph of lipid peroxidation inhibition versus sample concentration.

2.5. Statistical analysis

All data on bioactivity tests were averages of triplicate analyses. Activities assays were carried out at five concentrations, and the results are given as IC_{50} values. Data were recorded as mean ± SEM (standard error of the mean). *p* values <0.05 were regarded as significant.

CONCLUSION

The honey is a significant antiradical and lipid peroxidation inhibitory capacity. The radicals by various diseases or by the environmental factors can be easily eliminated by the sunflower honey. The crystallization, which could be formed naturally, does not show the honey's poor quality. Therefore, as mentioned in the Turkish honey codex (No. 2020/7), the crystallization of honey is natural. The study results indicate that the sunflower honey, which could crystalize easily, should be placed in markets. To overcome the prejudices of people, it is necessary to reveal all the properties of sunflower honey and share these medicinal properties with people. Moreover, the sunflower honey should not be filtered by the wholesalers to protect its nutrition and bioactivity.

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