**GIDA**
***THE JOURNAL OF FOOD***
E-ISSN 1309-6273, ISSN 1300-3070

*Research* / Araştırma
GIDA (2019) 44 (1): 86-92
doi: 10.15237/gida.GD18056

# COMPARISION OF THE ANTIOXIDANT ACTIVITY AND HYDROXYMETHYLFURFURAL (HMF) LEVELS IN HONEY TAKEN FROM HIVES AND MARKETS

**Serap Ünübol Aypak[1*], Aslıhan İnci[1], Sema Bakırcı[1], Evrim Dereli Fidan[2], Mert Soysal[3]**
[1]Department of Biochemistry, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey
[2]Department of Animal Science, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey
[3]Aydın Vocational School of Health Services. Adnan Menderes University, Aydın, Turkey

*Received* / Geliş: 17.05.2018; *Accepted* /Kabul: 28.12.2018 *Publishedonline* / Online baskı: 14.02.2019

*Unubol Aypak, S.,Inci, A., Bakirci, S., Dereli Fidan, E., Soysal, M. (2019). Comparision of the antioxidant activity and hydroxymethylfurfural (HMF) levels in honey taken from hives and markets. GIDA (2019) 44 (1): 86-92 doi: 10.15237/gida.GD18056*

Ünübol Aypak, S., İnci, A., Bakırcı, S., Dereli Fidan, E., Soysal, M. (2019). Kovanlardan ve marketlerden alınan ballardaki antioksidan aktivite ve hidroksimetilfurfural (HMF) düzeylerinin karşılaştırılması GIDA (2019) 44 (1): 86-92 doi: 10.15237/gida.GD18056

**ABSTRACT**
In this study, the antioxidant activity and HMF levels of 20 honey samples directly taken from hives and 20 of those offered for sale in the stores in the province of Aydın, which were total of 40 honey samples. The antioxidant activity in the honey samples directly taken from hives was found as 16.54±1.52, HMF content was found as 26.94±2.07 mg/kg. The antioxidant activity was found as 22.04±0.59, HMF content was found as 56.70±3.83 mg/kg. The difference between the antioxidant activity ($P$ <0.01) and the HMF values ($P$ <0.001) of the honey samples was found statistically significant. The HMF level in the honey collected from the hives were found to comply with the specifications set by the Turkish Food Codex Communique on Honey, whereas the HMF level of the market honey was higher than the level specified in the communiqué.
**Keywords:** Antioxidant activity, Aydın province, diastase number, honey, hydroxymethylfurfural (HMF).

# KOVANLARDAN VE MARKETLERDEN ALINAN BALLARDAKİ ANTİOKSİDAN AKTİVİTE VE HİDROKSİMETİLFURFURAL (HMF) DÜZEYLERİNİN KARŞILAŞTIRILMASI

**ÖZ**
Bu çalışmada; Aydın İlinde üretilen, doğrudan kovandan alınmış 20 adet çiçek balı örneği ile marketlerde satılan 20 adet çiçek balı örneği olmak üzere toplam 40 adet bal örneğinde antioksidan aktivite ve HMF düzeyleri belirlenmiştir. Kovanlardan doğrudan alınan bal örneklerinde antioksidan aktivite 16.54±1.52, HMF düzeyi 26.94±2.07 mg/kg olarak, market ballarında ise antioksidan aktivite 22.04±0.59, HMF düzeyi 56.70±3.83 mg/kg olarak tespit edilmiştir. Balların antioksidan aktivitesi ($P$ <0.01) ve HMF düzeyi için ($P$ <0.001) gruplar arasındaki fark istatistiksel olarak önemli bulunmuştur. Kovan ballarının HMF düzeyinin Türk Gıda Kodeksi Bal Tebliğine uygun olduğu, market balların HMF düzeyinin ise tebliğde belirtilen düzeyden yüksek olduğu görülmüştür.
**Anahtar kelimeler:** Aydın İli, antioksidan aktivite, bal, diastaz sayısı, hidroksimetilfurfural (HMF).

[*]*Correspondingauthor* /Yazışmalardan sorumlu yazar;
✉sunubol@adu.edu.tr ✆ (+90) 256 247 0700/264 🖷 (+90) 256 247 0720

## INTRODUCTION

Honey is a highly viscous product produced by honey bees (*Apis mellifera*). This production of honey involves, in brief, the bees collecting nectar from flowering plants, from the sugary substances secreted by the living parts of plants or from the bugs of the Homoptera genus and then modifying the composition of the substances in their bodies before depositing the honey into hive cells (Anon., 2002). The chemical composition of honey includes a number of compounds, such as sugar (70-80 %), water (10- 20 %), organic acid, minerals, vitamins, protein, phenolic compounds, and free amino acids (Aydın et al., 2008). The well-known antioxidant, antimicrobial, anti-inflammatory, antitumoral, and antimutagenic properties of honey stem from these compounds. Honey bee breeding, which is quite prevalent in Turkey, results in the production of physically and chemically different honeys, depending on the flora constituting the honey bees surrounding environment. In addition, various other factors, such as the processing, heating, and storage applied during harvest also affect the quality of honey (Karkacıer et al., 2000). The substances that give honey its antioxidant properties are flavonoids, polyphenols, vitamins, salicylic acid, sulfhydryl groups, carotenoid derivatives, enzymes, organic acids, amino acids, proteins, and end products of the Maillard reaction (Gheldof et al., 2002; Mandal and Mandal, 2011). Among the end products of the Maillard reaction, melanoidins have radical scavenging properties. The antioxidant property of honey depends on the vegetable origin of the nectar, as well as seasonal and environmental factors. Most of the molecules that give honey its antioxidant properties may be lost during the processing, transport, and storage of honey (Wang et al., 2004; Türkmen et al., 2006; Bertoncelj et al., 2007; Khalil et al., 2011).

Hydroxymethylfurfural (HMF) is a cytotoxic, genotoxic and organotoxic compound (Shapla et al., 2018). Furthermore, HMF is a byproduct formed during hexose decomposition in an acidic environment or during the Maillard reaction (Batu et al., 2013). HMF formation is used as a chemical index to determine the storage time of honey and to confirm whether the appropriate heat treatment method was employed (Küplülü and Kahraman, 2011).

The primary enzymes in honey are diastase, invertase, and beta-glucosidase. Fresh honey contains diastase, which is an enzyme responsible for starch decomposition and is degraded due to heating and storage. Diastase number is defined as the starch amount hydrolyzed by amylase in 100 g of honey under test conditions at 38-40°C until reaching a prespecified finishing point in an hour. Diastase number measurements are used to evaluate honey freshness. The optimum pH of diastase is accepted to be 5.3 (Aydın et al., 2008).

Heat treatment is a mandatory process that should be applied prior to putting the honey onto the market. Through heat treatment, the growth of spoilage microorganisms, especially sugar-resistant osmophilic yeast, is prevented, and the water in honey is reduced to levels that can delay fermentation. In addition, heat treatment enables the viscosity of honey to be reduced and prevents crystallization. However, the improper application of heat treatment can negatively affect the quality of honey. The storage time and storage conditions of honey are also of great importance in terms of maintaining the quality of the honey. Honey that is stored for an extended period of time and exposed to heat and light, results in an increase of the HMF level and a decrease in the diastase number (Aydın et al., 2008; Silva et al., 2009; Karadal and Yıldırım, 2012)
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Honey has acidic properties derived from the gluconic, butyric, acetic, formic, lactic, succinic, malic, citric, and oxalic acids of the vegetable and animal components it contains. The most common organic acid in honey is gluconic acid, and organic acids are partially responsible for the characteristic taste of honey (D'arcy, 2007). The pH value of honey is within the range of 3.5-5.5. The acetic acid formed through the fermentation activated by microorganisms during storage results in a decrease in the pH of honey (Aydın et al., 2008; Kahraman et al., 2010).

The aim of this study is to compare directly taken hive honey and market honey in Aydın province, in terms of antioxidant activity, HMF levels, diastase number and pH values and to evaluate it in terms of compliance with The Turkish Food Codex Honey Comminiqué (Resmi Gazete, 2012).

## MATERIALS AND METHODS

### Materials

In the study, the antioxidant activity, HMF levels, diastase number, and pH of a total of 40 honey samples, which included 20 blossom honey samples that were produced in Aydın and directly collected from the hives, and 20 blossom honey samples collected from the honeys sold on the market, were determined, and the results from the samples were compared. HMF levels and diastase number were evaluated for their compliance to the specifications set by the Turkish Food Codex Communique on Honey.

### Methods

#### Antioxidant activity measurement

Antioxidant activity was measured by applying the DPPH method (1,1-diphenyl-2- picrylhydrazyl radical scavenging capacity method). DPPH is stable organic nitrogen radical. Proton transfer by the antioxidant to the DPPH free radical results in decreased absorbance at 517 nm (Okan et al., 2013). A honey sample of 2 gram was diluted to 10 mL with distilled water. To the 0.1 mL honey solution, 1.9 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl) and 1 mL of sodium acetate buffer solution were added, and the mixture was kept in darkness for 90 minutes at room temperature. Measurements were made with a spectrophotometer at 517 nm. The standard curve for antioxidant activity was prepared using Trolox (Ferreira et al., 2009).

#### HMF analysis

HMF analysis was performed using the White method approved by the International Honey Commission (White, 1979). Following completion of the White method, 5 grams of honey were dissolved in 25 mL of pure water, adding 0.5 mL Carrez - I and 0.5 mL Carrez - II solutions before being diluted to 50 mL with pure water. After filtration with a filter paper, 5 mL of the filtrate was put in two separate tubes. The sample in one of the tubes was mixed with 5 mL of pure water, while the sample in the other tube was mixed with 5 mL of % 0.2 sodium bisulfite; the absorbances of the samples were measured at 284 nm and 336 nm with a spectrophotometer.
HMF was calculated using the following formula:
HMF (mg/kg) = $A_{284}$ - $A_{336}$ x 149.7
$A_{284}$: Absorbance at 284 nm
$A_{336}$: Absorbance at 336 nm

#### Diastase measurement

The diastase measurement was carried out according to the writing by Bogdanov (2009). A honey solution of 10 mL was prepared with the acetate buffer, mixed with a 5 mL starch solution, and kept for 15 minutes at 40°C. The diastase number was measured by adding 1 drop of 0.1 N iodine solution every 5 minutes to the solution and calculated by the absorbance at 600 nm at the $5^{th}$, $10^{th}$, $15^{th}$, and $20^{th}$ minute.

#### pH measurement

A honey sample of 10 grams was dissolved in 100 mL of $CO_2$ free distilled water, and the pH of the sample was measured with a pH meter.

## RESULTS AND DISCUSSION

Statistical calculations for the antioxidant activity, HMF level, diastase number, and pH values of the honey samples were performed with the SPSS 22.0 package program. In the statistical evaluation, to reveal the differences among the groups with respect to the investigated properties, the significance test (t-test) for the difference between two averages was applied. The significance levels for the antioxidant activity, HMF levels, diastase number, and pH of the honey samples were $P < 0.01$, $P < 0.001$, $P < 0.01$, and $P < 0.01$, respectively, and the differences among the groups were found to be statistically significant.

The antioxidant activity of the fresh honey samples collected from districts in Aydın was 16.54 ± 1.52 mg/kg, while the antioxidant activity of the honey samples from the market was 22.04 ± 0.59 mg/kg. In the relevant scientific literature,

although there are a great number of studies on determining the antioxidant content of honeys of different origin (Bertoncelj et al., 2007; Ferreira et al., 2009; Korkmaz and Küplülü, 2017; Meda et al., 2005; Lachman et al., 2010; Kishorea et al., 2011; Flores et al., 2015; Dzugan et al., 2017), no studies for which honey from hives were compared with honey from the market were found. Türkmen et al. (2006) reported increased antioxidant activity in honey samples on which heat treatments at 50°C, 60°C, and 70°C were applied. Brudzynski and Miotto (2011) applied a 30-minute heat treatment at 121°C to honey samples and reported that the melanoidin content and antioxidant activity in dark-colored honey samples increased 2 fold. Wang et al. (2004), who applied heat treatment to honey samples at 60°C for 12-16 hours and at 82°C for 10-12 minutes, which are the optimum temperatures for the heat treatment of honey, reported that the antioxidant activity of the honeys decreased after removing the foam of the samples, re-heating, filtering, and storing for 6 months. Kowalski (2013) found in his study that heat treatment to honey samples of different origins increased the antioxidant activity in lemon honey and buckwheat honey, did not affect the activity in acacia honey, and reduced the activity in secreted honey. The antioxidant levels, both in the honey samples from the hives and in the honey samples from the market, were higher than those reported by Meda et al. (2005), Bertoncelj et al. (2007), and Kishore et al. (2011), while they were lower than those reported by Ferreira et al. (2009), Lachman et al. (2010), Flores et al. (2015), and Dzugan et al. (2017). The results obtained in the study were similar to those obtained by Jimenez et al. (2016). The higher level of antioxidant activity in the market honeys compared to that in fresh honeys was attributed to the end products of the Maillard reaction, which occurred as a result of subjecting the honeys to improper heat treatment; but HMF, which is a byproduct of this reaction, is considerably harmful to human health.

Table 1. Antioxidant activitiy, HMF, diastase number and pH values in honey samples from hives and markets

| | Groups of honey samples | | | | |
|---|---|---|---|---|---|
| | Hive honey | | Market honey | | |
| | n | $\overline{X} \pm S_{\overline{x}}$ | n | $\overline{X} \pm S_{\overline{x}}$ | t |
| Antioxidant activity (mg/kg) | 20 | 16.64 ± 1.52 | 20 | 22.05 ± 0.59 | -3.371 [a] |
| HMF (mg/kg) | 20 | 26.94 ± 2.07 | 20 | 56.70 ± 3.83 | -6.836 [b] |
| Diastase number | 20 | 9.68 ± 0.71 | 20 | 7.43 ± 0.24 | 3.003 [a] |
| pH | 20 | 3.85 ± 0.07 | 20 | 4.22 ± 0.12 | -2.723 [b] |

a: $P<0.01$, *b:* $P<0.001$

HMF levels and the presence of the diastase enzyme are among the chemical quality criteria which have been used for honey for 75 years. Given that long-term storage of honey and exposure to extreme temperatures change the HMF and diastase amounts, HMF analysis and determination of the diastase number are recognized as being the most practical ways to determine the freshness of honey. There are a large number of studies focusing on this subject, both from Turkey and from other regions of the world. Table 2 presents a summary of some of these studies.

The results obtained in the study revealed that the fresh blossom honey samples directly collected from the hives were in compliance with the specifications of the Turkish Food Codex Communique on Honey. However, the HMF levels of the market honeys were higher than the standard level of 40 mg/kg specified in the standards put forth by the Turkish Food Codex

Communique on Honey. The diastase number of the fresh honey samples was higher than the diastase number specified by the standards in the Turkish Food Codex Communique on Honey, which states that the minimum diastase number for honey should be 8. The diastase numbers of the fresh honey were therefore in compliance with the communique. The diastase number in market honeys was; however, lower than the communique-specified levels. The results suggest that the honey samples sold in the market were subjected to improper heat treatment during packaging after harvest or were stored for a prolonged period. Although the acceptable pH range for honey has not yet been specified by the Turkish Food Codex, the results obtained in the study are within the range (3.2-4.5) determined by the International Honey Commission.

Table 2. Literature summary

| Literature | HMF (mg/kg) | Diastase number | pH |
|---|---|---|---|
| Pasias et al. (2017) [a] | 2.4-51 | 7-22 | - |
| Abdulkhaliq and Swaileh (2017) [a] | 2.1-34.2 | - | 3.03-5.98 |
| Boussaid et al. (2014) [a] | 12.07–27.43 | - | - |
| Manzanares et al. (2014) [a] | 5.36–15.00 | 11.50–45.80 | - |
| Juan-Borrás et al. (2014) [a] | 3.30–23.40 | 8.70–19.10 | - |
| Tornuk et al. (2013)[a/b] | 0.09-3.01[a]<br>0.05-4.12[b] | - | 3.68 -4.65[a]<br>3.90-6.42[b] |
| Batu et al. (2013) [a] | 0.15-24.39 | 8.3-17.9 | 3.76-4.90 |
| Yücel and Sultanoğlu (2013) [a] | 5.22-7.49 | 5.33-13.77 | 3.37-3.89 |
| Chakir et al. (2011) [a] | 7.16–30.43 | 6.05–19.10 | - |
| Aydın et al. (2008) [b] | 2.496-205.152 | 0-13.9 | 2.21- 3.54 |
| Kayacıer and Karaman (2008) [b] | - | - | 3.67- 4.57 |
| Moreira et al.(2007) [a] | 2.80–7.40 | 10.55–12.40 | - |

a: Hive honey, b: Market honey

To analyze the HMF levels of the honeys in the market, increasing the number of the samples collected by the Republic of Turkey Ministry of Food, Agriculture and Livestock will prove beneficial. Raising awareness among the commercial enterprises that are responsible for the sale and distribution of honey will increase the quality of honey and thus, improve its compliance with the international standards and contribute to the export potential of Turkey. To raise their awareness of this issue, beekeepers and honey suppliers should receive education on honey quality. Furthermore, as alternatives to heat treatment, high-pressure and ultrasonic processes should be promoted, and their applications should be initiated.

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