

# Composition of Honeys Collected from Eastern and South-Eastern Anatolia and Effect of Storage on Hydroxymethylfurfural Content and Diastase Activity

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**Abstract:** The chemical composition of honeys produced in Eastern and South-Eastern Anatolia (Turkey) was studied. For this aim, the composition of 45 honey samples collected from the mentioned regions and the effect of one year storage ( $20 \pm 5^\circ\text{C}$ ) on the diastase activity and hydroxymethylfurfural (HMF) content were determined. Compositional data measured in the fresh honeys were HMF, diastase number, moisture, invert sugar, sucrose, ash, proline, pH, free acid and lactone. Average values were as follows: HMF  $3.3 \text{ mg kg}^{-1}$ , diastase number 14.6, moisture 16.0%, invert sugar 70.3%, sucrose 1.8%, ash 0.1%, proline 53.0 mg/100g; pH 3.8, free acid 22.3 meq  $\text{kg}^{-1}$  and lactone 7.4 meq  $\text{kg}^{-1}$ . The changes in HMF content and diastase number of the samples after one year storage ( $20 \pm 5^\circ\text{C}$ ) were also investigated. The average HMF content ( $\text{mg kg}^{-1}$ ) increased from 3.3 to 19.1, and the average diastase number decreased from 14.6 to 10.7 following one year storage. The results indicate that storage has a significant role in the increase HMF contents and the decrease in diastase numbers, and changes in these two parameters were statistically significant ( $P < 0.001$ ).

**Key Words:** honey, composition, storage, HMF, diastase number

## Doğu ve Güneydoğu Anadolu Bölgelerinden Toplanan Balların Bileşimi ve Depolamanın Hidroksimetilfurfural Miktarı ve Diastaz Aktivitesine Etkisi

**Özet:** Bu çalışmada, Türkiye'nin Doğu ve Güneydoğu Anadolu Bölgelerinde üretilen balların kimyasal analizleri yapıldı. Bu amaçla bu bölgelerden toplanılan 45 bal örneğinin bileşimi ve bir yıl depolamanın ( $20 \pm 5^\circ\text{C}$ ) diastaz aktivitesi ve hidroksimetilfurfural (HMF) miktarı üzerine etkisi belirlendi. Taze bal örneklerinin bileşim verileri; HMF, diastaz sayısı, prolin, nem, invert şeker, sukroz, kül, pH, serbest asit ve lakton içerir. Bulunan ortalama değerler: HMF  $3,3 \text{ mg kg}^{-1}$ ; diastaz sayısı 14,6; nem % 16,0; invert şeker % 70,3; sukroz % 1,8; kül % 0,1; prolin  $53,0 \text{ mg kg}^{-1}$ ; pH 3,8; serbest asit  $22,3 \text{ meq kg}^{-1}$  ve lakton  $7,4 \text{ meq kg}^{-1}$  olarak bulundu. Ayrıca bir yıl depolama ( $20 \pm 5^\circ\text{C}$ ) sonunda bal örneklerinde HMF miktarı ve diastaz sayısındaki değişiklikler incelenmiştir. Depolama sonunda ortalama olarak, HMF miktarı ( $\text{mg kg}^{-1}$ ) 3,3'ten 19,1'e kadar artış göstermiş, diastaz sayısı ise 14,6'dan 10,7'ye kadar düşüş göstermiştir. Bu sonuçlara göre depolamanın HMF miktarlarındaki artış ve diastaz sayılarındaki düşüşte çok etkin bir rol oynadığı gözlenmiştir. Bu iki parametredeki değişiklikler istatistiksel olarak çok anlamlı bulunmuştur ( $P < 0.001$ ).

**Anahtar Sözcükler:** bal, bileşim, depolama, HMF, diastaz sayısı

## Introduction

Turkey has an important place among the honey producing countries, and is suitable for apiculture in terms of nectar sources. Turkey produced 63,000 tons of honey in 1997 (Anonymous, 1999). The chemical composition of Turkish honeys has previously been studied (Balci, 1972; Orak, 1986; Bozkurt and Aydoğan, 1986). In Eastern and South-Eastern Anatolia, the honeys are from blossom (floral source), and the honey production in these regions was 9,000 tons in 1997 (Anonymous, 1999).

According to the Turkish Standards Institute (Anonymous, 1990), blossom honey should contain at most 21% moisture, 5% sucrose, 0.6% ash, 40  $\text{mg kg}^{-1}$  HMF and 40 meq  $\text{kg}^{-1}$  acid, and at least 65% invert sugar and 8 diastase number (Gothe's scale). Diastase number is expressed as ml of 1% starch solution hydrolyzed at  $40^\circ\text{C}$  for one hour by the enzyme present in 1 g of honey (AOAC, 1990).

HMF is a breakdown product of sugars, and diastase is an enzyme in the honey. Diastase is a group designation

for the starch-digesting enzymes  $\alpha$ - and  $\beta$ -amylase. The  $\alpha$ -amylase splits the starch chain randomly, producing dextrins, and the  $\beta$ -amylase splits the reducing sugar maltose from the ends of the starch chain (Crane, 1975). The levels of these two constituents change during storage. Changes in HMF contents and diastase activity during storage have previously been studied (Tharasyvoulou, 1986; Bosch and Serra, 1986; Sanho et al., 1992). In addition HMF content and diastase activity are considered to be the two major parameters for evaluating the freshness and the quality of honey (Bosch and Serra, 1986; Sanho et al., 1992).

In the present study, HMF content, diastase number, moisture, invert sugar, sucrose, ash, proline, pH, free acid and lactone in 45 fresh honey samples collected from Eastern and South-Eastern Turkey were determined. The changes in HMF contents and diastase activity of the samples were also examined after one year storage ( $20 \pm 5^\circ\text{C}$ ).

## Materials and Methods

### Honey samples

The study was carried out on 45 blossom honeys of the Eastern and South-Eastern Anatolia regions, provided by different beekeepers in different areas. The samples were harvested in September, and analyzed immediately.

### Chemical analysis

The samples of honey were analyzed by the standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Moisture in honey was determined in a refractometer (JENA 181282). Ash percentage was measured by calcination at  $600^\circ\text{C}$  to constant mass in a furnace. To measure the acidity, 10 g of the sample were accurately dissolved in 75 mL  $\text{CO}_2$ -free distilled water and titrated with 0.1 N NaOH. The pH of the honey solution was measured by a pH meter (Schoot C6 840). HMF was determined colorimetrically after dilution with distilled water and addition of p-toluidine solution. Absorbance of the solution was determined at 550 nm using a 1 cm cell in an LKB-Biochrom spectrophotometer. To determine the diastase activity, a certain amount of honey was kept at a permanent temperature by mixing with 1% starch solution. The starch was hydrolyzed by the diastase enzyme of the honey. The results are expressed as mL of 1% of starch hydrolyzed by an enzyme in 1 g of honey per hour. Proline reacted with acid ninhydrin solution to form a coloured component, and was colorimetrically

determined by measuring the absorbance at 520 nm using a 1 cm cell in a LKB-Biochrom spectrophotometer.

### Storage

The honey samples were stored at  $20 \pm 5^\circ\text{C}$  sealed in 1 kg vials for one year in the laboratory, and then HMF contents and diastase numbers were determined.

## Results and Discussion

The means and ranges of values for each chemical component in the fresh honey samples are shown in Table 1. The results showed that the mean values of sucrose, ash and HMF in the honeys collected from Eastern and South-Eastern Anatolia were lower than those in other samples collected from different parts of Turkey. For example, Balcı (1972) found that the mean values of sucrose, ash and HMF for 31 honey samples were 3.0%, 0.15% and  $6.9 \text{ mg kg}^{-1}$ , respectively; Şengonca and Temiz (1981) reported similar results for 33 honey samples. In the present study, the levels of average sucrose, ash and HMF were found to be 1.8%, 0.1% and  $3.3 \text{ mg kg}^{-1}$ , respectively, lower than those mentioned above.

The average values of pH, free acid and lactone were found to be 3.8,  $22.3 \text{ meq kg}^{-1}$  and  $7.4 \text{ meq kg}^{-1}$ , respectively. These results did not differ from the previous findings (Orak, 1986; Bozkurt and Aydoğan, 1986). The average proline values were  $53.0 \text{ mg}/100\text{g}$ , very similar to the results reported by Orak (1986). In addition, the results concerning the concentration of invert sugar were in accordance with those reported previously (Orak, 1986; Bozkurt and Aydoğan, 1986).

Table 1. Chemical composition of the 45 fresh honey samples collected in Eastern and South-Eastern Anatolia.

Analytical value	Mean	Range
Moisture (%)	16.0	14.6 - 19.4
Invert sugar (%)	70.3	67.6 - 75.3
Sucrose (%)	1.8	0.4 - 4.5
Ash (%)	0.1	0.02 - 0.43
PH	3.8	3.2 - 4.3
Diastase number	14.6	9.0 - 26.1
HMF ( $\text{mg kg}^{-1}$ )	3.3	0.0 - 11.5
Free acid ( $\text{meq kg}^{-1}$ )	22.3	14.0 - 30.5
Lactone ( $\text{meq kg}^{-1}$ )	7.4	4.0 - 10.5
Proline ( $\text{mg}/100\text{g}$ )	53.0	30.0 - 86.0

The chemical compositions of fresh honey samples collected from the Eastern and South-Eastern regions conform well to the standards (Anonymous, 1990). As shown in Table 1, the analytical values of HMF, diastase number, moisture, invert sugar, sucrose, ash, proline, pH, free acid and lactone are in agreement with the standards values.

The HMF contents and diastase activity of fresh and stored honey samples are shown in Table 2. The mean diastase numbers in the honey samples following one year storage (20±5°C) decreased from 14.6 to 10.7. Thrasyvoulou (1986) found that the average decrease in diastase activity for 20 samples stored for one year at 25±4°C was 40%, while Sancho et al. (1992) reported a decrease of 33% in 115 samples after one year storage at 15-25°C. In the present study, the 27% average decrease in diastase activity found after storage was lower than those found for other honeys in the research above.

On the other hand, the mean HMF contents (mg kg<sup>-1</sup>) increased from 3.3 to 19.1 (Table 2). Thrasyvoulou (1986) found that the average HMF contents (mg kg<sup>-1</sup>)

Table 2. Diastase number and HMF content of fresh and stored honey samples collected in Eastern and South-Eastern Anatolia.

Analytical values	Number of samples	Mean	Min.	Max.
HMF * (mg kg <sup>-1</sup> )	45	3.3	0.0	11.5
HMF** (mg kg <sup>-1</sup> )	45	19.1	8.6	39.0
Diastase number *	45	14.6	8.7	26.1
Diastase number **	45	10.7	6.7	18.2

\* Fresh honey , \*\* Stored honey

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increased from 0.0 to 8.8 after one year storage. Sancho et al. (1992) found that the average amount of HMF (mg kg<sup>-1</sup>) increased from 4.7 to 13.1 after one year storage. The rates of HMF in the honey samples in our study showed similarity to those mentioned above.

The mean diastase numbers and HMF contents of fresh and stored honey samples were compared paired t-test and the results are shown in Table 3. The test show that changes in HMF contents and diastase numbers after one year storage in the honeys were statistically significant (P< 0.001).

Storage has a remarkable influence on the increase in HMF content and the decrease in diastase number of the honeys collected. However, it was found that HMF contents in all honey samples were lower than 40 mg kg<sup>-1</sup>, but five honey samples showed diastase numbers less than 8 after one year storage (Table 2). According to the results presented, the HMF contents and diastase numbers fell within the standard values stated by the Turkish Standards Institute (Anonymous, 1990), except five samples whose diastase numbers were under the standard limits.

Table 3. Statistical analysis of diastase number and HMF content in the 45 honey samples.

Analytical values	Fresh honey		Stored honey		t	P
	X	SD	X	SD		
HMF (mg kg <sup>-1</sup> )	3.3	2.2	19.1	6.4	-17.8	P<0.001
Diastase number	14.6	4.2	10.7	2.7	12.9	P<0.001