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THE EFFECT OF STORAGE TEMPERATURE ON SOME PHYSICOCHEMICAL PROPERTIES OF FLOWER HONEY

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ABSTRACT

The aim of the study was to determine the pH, Brix, moisture, free acidity, hydroxymethylfurfural (HMF) level, and diastase activity that are effective on the quality of unpasteurized and pasteurized flower honey produced in the South-eastern Anatolia Region of Türkiye after storage. A total of 40 samples brought for analysis were divided into two groups: raw and pasteurized at 70 °C. The honey samples were stored at different temperatures (4–6, 21–24, and 35–37 °C) and time periods (3, 6, and 9 months) for analysis. The data were statistically analyzed in a three-factor experimental design with repeated measurements at the levels of one of the factors. Considering the storage periods, it was found that HMF levels were 2.79 ± 3.08, 4.36 ± 3.08, and 184.17 ± 3.08 mg kg^{-1}, while the diastase values were 9.48 ± 0.39, 8.51 ± 0.39, and 1.46 ± 0.39, respectively, and statistically significant ($p \leq 0.01$). Considering temperatures, it was determined that HMF and diastase values at 4–6 and 20–22 °C and 3-month storage periods were in accordance with the Turkish Food Codex Honey Communiqué, while the storage periods of the samples at 35–37 °C were above the legal limits. As a result, it can be recommended that flower honeys should be stored between 4–6 and 20–22 °C since the shelf life and honey quality will be negatively affected when the storage temperature exceeds 35–37 °C.

Key Words: honey quality, hydroxymethylfurfural, diastase activity, storage temperature.

INTRODUCTION

Bees produce honey from various floral nectars and insect secretions (Erbakan *et al.*, 2020). In general, honey is composed of sugar (glucose and fructose) (80 %), water (17 %), and proteins, enzymes, mineral substances, organic acids, and vitamins (3 %) (Khan *et al.*, 2015). Treatments, storage conditions, and the composition of honey have a significant effect on its quality. Some technological processes can be used to maintain the quality criteria of honey during the storage period. One of these processes is the application of heat to improve the its microbiological and physicochemical properties. Heat treatments are performed in double-walled tanks or using technological applications including microwaves and infrared. These processes result in both positive impacts and undesirable effects such as an increased amount of hydroxymethylfurfural (HMF) and heat-induced alterations of the diastase enzyme on the quality of honey (Erbakan *et al*., 2020).

HMF is formed by the reaction of sugars and amino acids during heat treatments. It can be produced by either the breakdown of hexose in an acidic environment or the Maillard reaction (Güzel and Bahçeci, 2020). It is formed by the dehydration of monosaccharides, i.e., boiling in highly acidic environments, and the formation of furfural from pentoses and 5-hydroxymethyl furfural from hexoses as a result of monosaccharide molecules losing three molecules of water (Das *et al*., 2022). HMF can be produced in honey over time as a result of heating, long-term storage, or other processes such as filtering, transporting, and packaging. While HMF is naturally present in very low concentrations even in freshly harvested honey, its formation is dependent on pH, ambient temperature, heat treatment time, and sugar concentration, which is regarded as one of the most important quality criteria for honey. Since the amount of HMF in honey is known to have a negative impact on health, HMF is a critical public health criterion.

The dominant climate group in South-eastern Anatolia, where the study was conducted, is continental, with very hot summers and rarely cold winters. The long-term average temperature of the region in August was 30.7 °C, and it was 32.1 °C in August 2024. The lowest temperature recorded in the region was 16.4 °C, while the highest was 46.1 °C (MGM, 2024). If the storage temperatures of the honeys are not taken into consideration due to the high temperature of the region, it may have negative effects on the quality of the honeys obtained in the region. While the amount of HMF increases at high temperatures, the diastase activity decreases. In other words, there is a negative correlation between HMF and diastase activity (Güzel and Bahçeci, 2020).

The International Agency for Research on Cancer classifies HMF as a probable carcinogen to human health. Related studies have shown that HMF has a highly toxic effect, and its intake at high doses may cause irritation of the upper respiratory tract, eyes, skin, and mucous membranes, as well as genotoxicity and tumor formation. Furthermore, the reaction of HMF with reactive oxygen disrupts antioxidant structures, causing oxidative stress or pathological disorders. Oxidative stress has a negative impact on DNA and damages cells and tissues by forming lesions (Batu *et al*., 2014). The structure of HMF includes aromatic alcohol, aromatic aldehyde, and furan rings. In Türkiye, the maximum authorized concentration in honey is 40 mg kg^{-1}. Although heating processes and their duration affect the formation of HMF, its value can also rise under appropriate conditions when storage time and temperatures are not taken into consideration (Das *et al*., 2022).

The diastase enzyme is another important factor in honey quality. Enzymes in the structure of honey are considered to be its most valuable components. It has been reported that the bee adds enzymes from its own glands while the rest come from the nectar source (Güzel and Bahçeci, 2020). If natural and freshly harvested honey has not been subjected to any other process (such as heating, filtration, pasteurization, or packaging), it is considered to have a high diastase value (enzyme content) and to be of high quality.

The diastase enzyme is associated with honey maturation and has a direct effect on its quality. The diastase value is the volume of a 1 % starch solution that can be completely hydrolyzed by the diastase enzyme found in 1 g of honey in 1 h at 40 °C. Therefore, the amount of this enzyme is determined by the diastase value, and a count of more than eight indicates high quality. This is because the diastase enzyme degrades at high temperatures, poor storage conditions, and long storage times. As a result, the minimum diastase value in honey offered for consumption is eight according to legal regulations (Bell and Grainger, 2023).

The Turkish Food Codex, Honey Communiqué No. 2012/58 stipulates the parameters related to the consumption and quality of honey (TFC, 2020). Its chemical composition and quality vary based on the current conditions inside and outside the hive (after harvest), packaging, and storage conditions, as well as geographical and botanical origin. It has become necessary to carry out a series of chemical analyses to determine the changes in the chemical composition and dynamic structure of honey during the processing, packaging, and preservation processes (Amariei, 2020). Therefore, the aim of this study was to determine the pH, Brix, moisture, free acidity, HMF levels, and diastase activity that are effective on the quality of unpasteurized and pasteurized flower honey produced in the South-eastern Anatolia region of Türkiye after storage at different temperatures and time periods.

MATERIALS AND METHODS

Raw honey samples were delivered to the facility in March, numbered, and separated into two groups: unpasteurized and pasteurized at 70 °C for 5 min. The samples were stored at different temperatures (4–6, 20–22, and 35–37 °C) for nine months and then analyzed every three months (June, September, and December). The analysis included pH, moisture, Brix value, free acidity, hydroxymethylfurfural (HMF), and diastase activity.

Moisture analyses were carried out according to the TS 13365 method (Martin, 1979). The pH and free acidity of honey were determined using the TS 1728 method (ISO, 1970). Brix values were determined by placing 5 g of honey between the prisms of a refractometer (ATC model BX-90, China). The device was properly closed, and the dry matter content of the sample was determined using the indicator (AOAC, 2006).

The diastase activity was measured with a T80+ UV/VIS spectrometer according to the TS 13364 method (Schade *et al*., 1958). The absorbance values of solutions prepared for HMF analysis were measured using a spectrometer (T80+ UV/VIS, United Kingdom) at 550 nm. The wavelength and absorbance values from the control group (HMF content, the solution in the tube with water was considered as the control group) were subtracted, multiplied by 192 (A factor found by taking into account the extinction coefficient of the colored substance formed in the experiment and the concentration unit chosen for the result) coefficients, and recorded as HMF (AOAC, 1995).

Statistical Analysis

Statistical analyses of changes in each parameter were carried out using a three-factor experimental design with repeated measures at one of the factor levels, followed by Tukey's test for multiple comparisons, using SPSS Statistics 21.0 (IBM Statistics). The following model was used:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \pi m(ij) + \gamma k + (\alpha\gamma)ik + (\beta\gamma)jk + (\pi\gamma)\, km(ij) + (\alpha\beta\gamma)ijk + \varepsilon_{ijkm}$$

where μ is the general mean; αi represents the effect of honey group i (1 and 2, unpasteurized and pasteurized); and βj represents the effect of temperature j (4–6, 20–22, and 35–37 °C). $(\alpha\beta)ij$ represents the interaction between honey group and temperature; $\pi m(ij)$ represents the effect of the experimental unit (at honey group i and temperature level j); and γk represents the effect of moisture level k. $(\alpha\gamma)ij$ is the effect of the honey group x moisture interaction; $(\beta\gamma)ij$ is the effect of the moisture x temperature interaction; $(\pi\gamma)km(ij)$ is the effect of the experimental unit x moisture interaction; $(\alpha\beta\gamma)ijk$ is the effect of the honey group x temperature x moisture interaction; and ε_{ijkm} is the error.

RESULTS AND DISCUSSION

From the descriptive values of the properties analyzed in the study (Table 1), it was observed that the HMF value increased in both honey groups as the storage temperatures increased.

While the highest HMF value was determined at 35–37 °C, the lowest diastase value was recorded at the same temperatures during the 3-month storage period among all replicates (Table 1).

The diastase values in the samples decreased as the storage temperature increased, as opposed to the HMF values. The diastase value was zero when the storage temperature rose to 36 °C in the second and third replicates.

No significant differences were found between the values of the other properties (pH, Brix values, moisture content, and free acidity). In all replicates, the pH ranged between 3.89 and 3.92, the brix values ranged between 81.24 and 81.42 %, the moisture content ranged between 16.89 and 17.01 %, and the free acidity values ranged between 17.7 and 18 meq kg^{-1} at the different storage temperatures evaluated for both honey groups (Table 2). These results are similar to the required values in the literature (Czipa *et al.*, 2019; Güzel and Bahçeci, 2020; Balos *et al.*, 2023.

The HMF and diastase values were statistically significant ($p \leq 0.05$) at the storage temperatures evaluated (Table 2). There was no statistically significant difference between the means for both groups of honey. The difference between the means of

Table 1. Descriptive values of the physio-chemical properties evaluated in unpasteurized and pasteurized honey at different temperatures and storage time periods.

Replicate	Temperature	N	Unpasteurized		Pasteurized	
			HMF $\bar{x} \pm S_{\bar{x}}$	DV $\bar{x} \pm S_{\bar{x}}$	HMF $\bar{x} \pm S_{\bar{x}}$	DV $\bar{x} \pm S_{\bar{x}}$
June	4–6 °C	20	1.65 ± 0.62b	10.10 ± 3.50a	2.43 ± 0.83b	9.83 ± 3.53a
	20–22 °C		2.33 ± 2.07b	9.37 ± 3.14a	2.77 ± 1.23b	9.05 ± 3.10a
	35–37 °C		94.87 ± 28.34a	4.60 ± 1.45b	122.38 ± 31.62a	4.19 ± 1.24b
September	4–6 °C	20	2.06 ± 0.64b	9.37 ± 2.92	2.79 ± 0.85b	9.29 ± 2.61
	20–22 °C		4.50 ± 1.91b	8.78 ± 3.04	5.33 ± 1.70b	8.59 ± 3.02
	35–37 °C		150.38 ± 31.61a	0	175.31 ± 32.89a	0
December	4–6 °C	20	3.52 ± 1.31b	9.21 ± 2.83	4.31 ± 1.37b	9.11 ± 2.76
	20–22 °C		5.38 ± 1.62b	7.77 ± 3.03	5.89 ± 1.68b	7.52 ± 3.01
	35–37 °C		269.56 ± 54.89a	0	292.56 ± 55.89a	0

N: numbers of observation; $\bar{x}$: mean; $S_{\bar{x}}$: standard error; HMF: hydroxymethylfurfural; DV: diastase value. Means with different letters are statistically significant ($p \leq 0.01$).

other properties measured in the study (pH value, Brix values, moisture content, and free acidity) was statistically insignificant at different storage temperatures for both honey groups.

Sajid *et al*. (2019) stored fresh honey samples from *Acacia modesta* (Wall.) P.J.H. Hurter, *Justicia adhatoda* L., *Brassica rapa* L., *Ziziphus jojoba* Mill., *Citrus x sinensis* (L.) Osbeck, *Trifolium repens* L., and *Dalbergia sissoo* Roxb. at room temperature (29 °C) and reported HMF values of 33.53 ± 3.46, 25.24 ± 4.32, 31.67 ± 4.32, 24.45 ± 2.82, 31.83 ± 3.75, 40.68 ±

Table 2. Physicochemical properties evaluated in unpasteurized and pasteurized honey at different temperatures and storage time periods.

Factors		N	pH	BV	FA	DV	MC	HMF
Temperature	4–6 °C	40	3.91 ± 0.02	81.33 ± 0.16	17.79 ± 0.90	9.48 ± 0.39a	16.95 ± 0.15	2.79 ± 3.08b
	20–22 °C	40	3.90 ± 0.02	81.33 ± 0.16	17.84 ± 0.90	8.51 ± 0.39a	16.95 ± 0.15	4.36 ± 3.08b
	35–37 °C	40	3.91 ± 0.02	81.33 ± 0.16	17.80 ± 0.90	1.46 ± 0.39b	16.95 ± 0.15	184.17 ± 3.08a
Honey group	Unpasteurized	40	3.91 ± 0.01	81.23 ± 0.13	17.75 ± 0.73	6.57 ± 0.31	17.01 ± 0.12	59.36 ± 2.51
	Pasteurized	40	3.91 ± 0.01	81.42 ± 0.13	17.87 ± 0.73	6.39 ± 0.31	16.89 ± 0.12	68.19 ± 2.51

BV: Brix value; FA: free acidity; DV: diastase value; MC: moisture content; HMF: hydroxymethylfurfural. Means with different letters are statistically significant ($p \leq 0.01$).

4.24 and 28.55 ± 3.6 mg kg^{-1}, respectively. They also reported HMF values in packaged honeys (Quershi Honey, Marhaba Honey, Sulman Honey, Saudi Honey, Young Honey, and Alshifa Honey) of 389.18 ± 296.95, 316.86 ± 205.54, 319.63 ± 268.43, 331.26 ± 263.38, 318.78 ± 214.42, and 516.26 ± 273.2 mg kg^{-1}, respectively.

Balos *et al*. (2023) reported that the HMF content in honey stored at 22 °C was 2.08 ± 0.91 mg kg^{-1}. Kedziersk-Matysek *et al*. (2016) stated in their study on rapeseed honey that the HMF content of newly harvested honey samples was 3.07 ± 1.77 %, which significantly increased (19.74 ± 2.84) while storing the honey at room temperature (20–26 °C). Diafat *et al*. (2017) also reported that the HMF value of honey stored at 4 °C was 11.4 mg kg^{-1}, while Moloudian *et al*. (2018) reported HMF values between 30.7 ± 15.22 and 97.24 ± 129.1 in honey samples of different origins stored at room temperature (20 °C). The values obtained from these studies are higher than the values obtained in the present study. The HMF values obtained in Czipa *et al*. (2019) and Seraglio *et al*. (2021) showed similar values with the results obtained in the present study.

The freshness and quality of foods containing carbohydrates are strongly correlated with the HMF content, which is a crucial criterion used to assess the quality of honey and assess the processing and storage temperatures and conditions. HMF is formed when sugar in honey is broken down due to heat treatment and longer storage periods (Balos *et al*., 2023). In general terms, the HMF value of honey increases to a certain extent even at temperatures such as 30–35 °C. Consequently, honey from areas with high temperatures has higher HMF values.

Karabournioti and Zervalaki (2001) evaluated honey from five different botanical origins at temperatures of 35, 45, 55, 65, and 75 °C for 24 h, and reported that the initial HMF levels in pine, citrus, sunflower, cotton, and thyme honey were 1.20, 2.25, 26.80, 9.70, and 8.78, respectively, whereas these values rose to 1.95, 3.45, 29.20, 9.90, and 10.78 at 35 °C and 43.40, 63.30, 226.35, 173.4, and 191.35 at 75 °C, respectively. Given that the honey was stored for 24 h in their study and a year in the current study, it can be argued that the lower HMF rates of the honey may be the result of different storage times.

It was also observed that the levels of HMF in honey samples varied depending on the source from which they were harvested, but they changed significantly with temperature and time.

Diastase loss in honey is one of the undesirable quality criteria, but the presence of very high levels of diastase is also not desirable. A high level of diastase increases fermentation (Korkmaz and Küplülü, 2017). In another study, Şahinler *et al*. (2007) stored honey samples at 55, 65, and 75 °C for 15, 30, 45, and 60 min, then stored them at room temperature for one year and examined the diastase activity. At room temperature at the end of the ninth month was 13.6. In this study, diastase activity was lower.

In their study, Khan *et al*. (2015) heat treated flower honey samples at 40, 50, 60, 70, and 80 °C for 5, 10, 15, 20, and 25 min each and found that the diastase values were 13.6, 13.9, 14.0, 14.1, and 14.0 at 40 °C (5, 10, 15, 20, and 25 min); 14.0, 14.3, 14.7, 15.7, and 15.2 at 50 °C; 10.2, 9.8, 8.5, 5.3, and 5.6 at 60 °C; 8.2, 7.1, 5.7, 4.9, and 3.2 at 70 °C; and 6.2, 5.1, 3.1, 2.9, and 2.1 at 80 °C, respectively. These values were higher than those obtained in the present study despite the higher temperature of the heat treatments, and this may be attributed to the considerably shorter storage periods.

Korkmaz and Küplülü (2017) stored the extracted flower honey samples collected from different facilities at certain temperatures (10, 22, and 35 °C) in three-month periods (3, 6, 9, and 12 months) and examined the diastase activity in honey. The study found that the mean diastase values at 10 and 22 °C did not fall below 8 (the threshold value established by the Turkish Food Codex Honey Communiqué), but fell below 8 for honey stored at 35 °C after the sixth month. The results obtained are similar to those obtained herein.

Balos *et al*. (2023) determined the diastase activity of honey samples stored at 22 °C as 17.35 ± 3.97. Moloudian *et al*. (2018) stated that diastase activity values ranged between 11.88 ± 0.05 and 28.68 ± 17.75 in honeys of different origins at room temperature (20 °C). Diafat *et al*. (2017) reported that the diastase activity of honeys stored at 4 °C was 129.49 ± 43.67, which is considerably higher than the diastase value found in this study. In their study on raw rapeseed honey, Kedziersk-Matysek *et al*. (2016) reported that diastase activity in freshly harvested honey was 28.37 ± 6.53, while its value for honey stored at room temperature (20–26 °C) decreased to 21.44 ± 4.37.

Sajid *et al*. (2019) reported that the diastase activity of fresh honey samples *Acacia modesta* (Wall.) P.J.H. Hurter, *Justicia adhatoda* L., *Brassica rapa* L., *Ziziphus jojoba* Mill., *Citrus x sinensis* (L.) Osbeck, *Trifolium repens* L., and *Dalbergia sissoo* Roxb. stored at room temperature (29 °C) were 41.47 ± 5.70, 29.57 ± 14.52, 29.27 ± 7.75, 26.97 ± 22.23, 43.46 ± 10.46, 35.55 ± 12.64, and 34.5 ± 14.02, respectively, which is higher than the values in the present study. Sajid *et al*. (2019) reported the diastase activity of packaged honeys (Quershi Honey, Marhaba Honey, Sulman Honey, Saudi Honey, Young Honey, and Alshifa Honey) stored at room temperature (29 °C) as 9.45 ± 1.48, 5.95 ± 1.20, 8.61 ± 1. 99, 6.9 ± 0.55, 10.35 ± 1.48, and 6.15 ± 0.72. The diastase activity values obtained by Boussaid *et al*. (2018), Erbakan *et al*. (2020), and Bell and Grainger (2023) were similar to those obtained in this study.

Yılmaz and Eskici (2017) determined the diastase activities of 45 honey samples by using the spectrophotometric method. They stored the honey samples at 20 °C for one year and found that the mean diastase activity dropped from 14.6 to 10.7. The diastase activities found in their study were similar to those herein. The differences observed between previous studies and the current study were due to the values resulting from long-term storage in inappropriate storage conditions (high storage temperature) following honey harvesting as a result of the high temperatures to which the honey is exposed during factory processing.

Brix value and sugar rates in adulterated honey differ from those in natural honey. It is reported that the Brix value in natural honey ranges between 78.8 and 84 %. The moisture content of honey correlates with its sugar content (Kanbur *et al*., 2021). The brix values obtained here ranged between 81.2 and 81.42 %, which were comparable to those found in natural honey and other studies (Geana *et al*., 2020). Natural honey is an acidic product, and pH is one of the most important properties used to determine honey quality. The concentration of amino acids, peptides, carbohydrates, organic acids, and minerals is important in determining pH values and, thus, acidity (Mohammed Hassan *et al*. 2021). Escuredo and Seijo (2019) found that the enzymes in honey had a significant impact on acid formation, with honey with high enzyme levels being more acidic. Natural honey should have a pH of 3.7 to 6.4 (Mutlu *et al*., 2017). The pH of the honey obtained herein was found to be within the range (3.89–3.92) expected in natural honey.

The pH values detected in numerous studies are lower than those obtained in the present study (Aydın *et al*., 2008; Yılmaz and Küfrevioğlu, 1999). Batu *et al*. (2013) found that Sivrice and Erzurum region honey had similar pH levels as this study, while Malatya, Ovacık, Muş, Artvin, Rize, and Bayburt region honey had higher pH levels than this study. The pH values obtained by Akyüz *et al*. (1995) and El Sohaimy *et al*. (2015) were higher than the present study. The variations in results can be attributed to differences in honey sources, regions, and climatic and ecological conditions.

The moisture content of honey is important for granulation, and it has been reported that an increase in moisture content may not only lead to microbial degradation but also to the crystallization of honey (Chen, 2019). The moisture content of honey should be approximately 17 % in order to attain harvest maturity (TFC, 2020). The moisture content obtained in the present study ranged between 16.89 and 18 %, falling within the values of the Turkish Food Codex Honey Communiqué. While the moisture values recorded in previous studies (Batu *et al*., 2013) were lower than those recorded here, the moisture rates recorded in the studies of Akyüz *et al*. (1995), Serin *et al*. (2018), and Amariei *et al*. (2020) were similar to those detected in the current study. The differences between the studies can be attributed to the honey's different sources and regions of harvest.

CONCLUSION

The two most important criteria for determining the quality and freshness of honey are the hydroxymethylfurfural (HMF) content and diastase activity. The recommended limit values for honey storage conditions are a maximum of 40 mg HMF and a minimum of 8 for the diastase value. The HMF and diastase values of honey stored in June, September, and December deviated from the specified limit values when the temperature exceeded 21 °C. As a result, honey is best stored at room temperature.

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