Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1349150

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 6 - Issue 4: 522-526 / October 2023

DETERMINATION OF HMF VALUE AND DIASTASE ACTIVITIES IN STRAINED HONEYS SOLD IN MARKETS

Seda Dicle KORKMAZ[1]*

[1]*Giresun University, Espiye Vocational School, Food Processing Department, 28600, Giresun, Türkiye*

Abstract: This study aimed to determine HMF values and diastase activities of the strained honeys collected from various markets in Türkiye and to evaluate their suitability to Turkish Food Codex, Directive on Honey. The samples were obtained with original package and their shelf lifes were remarked by companies. A total of 90 honey samples, 45 were flower honey and 45 were honeydew honey, were analysed for HMF using High Performance Liquid Chromatography and for diastase activity using UV spectrophotometer. According to the results, it was observed that the 35.5% of flower honey samples and the 20% of honeydew honey samples were not fulfilled the HMF value and/or diastase activity standards of the Turkish Food Codex. The highest HMF value of the samples was 119.8 mg/kg, while the lowest diastase activity of samples was 0.9. Furthermore, 15 of 45 flower honey and 8 of 45 honeydew honey samples were in critical limits for the mentioned standards. In conclusion, in strained honey offered for consumption, either heat treatment that is applied during the production or increasing HMF value and decreasing diastase activity depending on storage temperature limit the product's shelf life. HMF in honey is known to be a potential risk for food safety and public health. Appropriate production and storage conditions for honey should be ensured until it reaches the consumer, and all quality criteria, especially HMF and diastase, should be targeted to comply with the Turkish Food Codex Directive on Honey, until the end of the shelf life. Thus, it will be possible to ensure food safety by protecting both public health and producer rights. In addition, raising consumers' awareness on the subject will enable the development of internal control at both manufacturers and markets.

Keywords: Honey, HMF, Shelf life, Diastase activity, Storage temperature, HPLC

***Corresponding author:** Giresun University, Espiye Vocational School, Food Processing Department, 28600, Giresun, Türkiye
E mail: diclekahraman@gmail.com (S. D. KORKMAZ)
Seda Dicle KORKMAZ https://orcid.org/0000-0002-4272-300X

Received: August 24, 2023
Accepted: September 28, 2023
Published: October 15, 2023

Cite as: Korkmaz SD. 2023. Determination of HMF value and diastase activities in strained honeys sold in markets. BSJ Eng Sci, 6(4): 522-526.

1. Introduction

Türkiye has a very rich structure in term of geography, climate, and flora in honey production and bee breeding. Türkiye takes first place in world rankings with its increasing number of bee hives (4.734.938) and its 118.297 tons of honey production. It is very important to increase quality standarts in honey in order to increase this potential in exportation. (TUIK, 2022).

The amount of hydroxymethyl furfural (HMF) and diastase activity are two important parameters in evaluating the quality and freshness of honey (An Ajlouni and Sujirapinyokul, 2009; Castro-Vazquez et al., 2008). The source of diastase, which is the most important enzymes that gives honey the quality of easy digestibility, is the salivary gland secretion of bees. The heat treatment applied to honey and time dependent disintegration of this enzyme are important indicators in identifying the freshness of honey. However, enzyme activity varies within hide limit even in fresh honey (Sancho et al., 1992; Belitz and Grosch, 1999). According to the standarts of Turkish Food Codex (TGK), Directive on Honey diastase activity is determined as at least 8 for flower honey and honeydew honey and at least 3 for citrus honey. Diastase activity which is used in the measurement of enzyme is defined as ml of starch solution (% 1) which is hidrolized by enzyme in 1 g honey in one hour at 38-40 °C (TGK, 2012).

Hydroxymethyl furfural is an important quality criteria which occurs as a result of the dehidration of hexoses in honey in acidic ambient and which changes according to chemical properties of honey (sugar, pH, total acidity), honey processing or storage temperature (Sahinler, 2007; Fallico et al. 2008). HMF is a cytotoxic, genotoxic, and organotoxic compound. Also, it is a byproduct formed during hexose decomposition in an acidic environment or during to Maillard reaction (Islam et al., 2014; Pasias et al., 2017; Shapla et al., 2018). According to TGK, Directive on Honey, HMF value is determined as maximum 40 mg/kg in flower honey and honeydew honey (TGK, 2012).

It is known that as a result of heat treatment applied to honey during the production stage in order not to undergo fermentation and to prevent it from crystallization (Zappala et al., 2005; Morales et al., 2009; Korkmaz and Küplülü; 2017). Honey is preserved from crystallization and it increases marketing quality of honey. However HMF is found in very small amounts in fresh honey (in harvest period) and it increases with high and long term heat treatment. This increases the HMF in the beginning on the shelf, depending on inappropriate storage temperature increasing effect of HMF shelf life is shorten (Ramirez et

al., 2000; Sanz et al., 2003; Tosi et al., 2022). The quality criteria reported in the TGK Honey communiqué (no. 2005/49) were revised in 2012 without making any changes, and it is stated that honey should be kept under 25°C by protecting it from direct sunlight at all stages until it is delivered to the consumer (TGK, 2005; TGK 2012). Also it causes the reduction of nutritional value by the disintegration of diastase enzyme which has an important role in honey digestion and quality lost by creating changes in taste, flavor and color (Cocco et al., 1996; Kalabova et al., 2003).

In this study, strained honey the shelf life of which is determined by firms in their original package and marketed in the markets was used. The suitability were evaluated according to Turkish Food Codex Honey Commission by identifying the diastase activities and HMF values, storage time determined concerning the dates of analysis.

2. Materials and Methods

2.1. Honey Samples

In this study a total of 90 honey samples, 45 were flower honey and 45 were honeydew honey bought from various markets in several cities of Türkiye (Ankara, Antalya, Muğla, Hatay, İzmir, Konya, Adana) in their original package and shelf lifes remarked by companies and which had different expiraton date were used as material. Also, all honey samples used in the study were collected in 2020.

2.2. HMF Analysis

HMF determination of samples were analyzed by using HPLC-UV sensor according to Harmonised Methods of the International Honey Commission (Bogdanov, 2009). For the instrument calibration, the standard of 99% pure 5-hydroxymethyl-) furan-2-carbaldehyde (HMF) (Merck 820 678) standard was used. The working standards for 1, 2, 5 and 10 mg/L were prepared daily. After their preliminary preparations, the samples were taken into 2 ml vials and introduced to HPLC device with UV detector capable of measuring at a wavelength of 285 nm. The analyses were made by using C18-reversed phase (Hypersil ODS 5µm, 125x4 mm), under the instrument conditions with 90% distilled water- 10% methanol at mobile phase, with a flow rate of 1.0 ml/minute and injection amount of 20 µl.

2.3. Diastase Analysis

Diastase numbers of honey samples were determined by using UV -Spectrophotometer (Rayleigh, VIS- 723G, England), according to the Harmonised Methods of The International Honey Commission (Bogdanov, 2009). Calibration of the starch solution to be used during the analysis was provided in the UV-Spectrophotometer with an absorbency value of 600 nm; and the solution of sodium chloride-acetate buffer required for analysis was prepared. Honey solution was prepared by taking 10 g from the samples, and adding acetate buffer solution. 10 ml honey solution and 5 ml starch solution taken into tubes were kept waiting in water for 15 minutes, at 40 °C. At the end of the period, they were mixed again and taken to the water bath. 5 ml diluted iodine solution was added to the 0,5 ml honey- starch solution taken into the tubes containing 11 ml distilled water, after being kept with 5-minute intervals; and the absorbance value at the 5th, 10th, 15th and 20th minutes were read through 600 nm UV-Spectrophotometer for determining its diastase activity.

3. Results

In the study, totally 90 honey samples consisting of 45 flower honey and 45 honeydew samples were analyzed. 4, 2 and 10 of the 45 flower honey samples analyzed were not found to be in accordance with the T.F.C. Directive on Honey, in terms of their HMF values, diastase activities, and both HMF values and diastase activities, respectively. Accordingly, the HMF values of the 14 honey samples analyzed, which were higher than the limit values, were found to be 119.8, 114.6, 102.4, 100.3, 98.5, 87.6, 87.4, 73.3, 53.6, 49.2, 48.2, 46.7, 42.5 and 40.2 mg/kg, respectively; and the diastase activities of the 12 honey samples, which were determined to be lower than the limit value, were 0.9, 1.5, 5.4, 5.6, 5.9, 6.0, 6.2, 6.4, 7.2, 7.5, 7.6, 7.7, respectively. 45 honeydew honey samples were examined, and HMF value of 6 of totally 9 samples were not suitable for T.G.K. Honey Communiqué, while diastase activity of 3 samples were not found in accordance with T.G.K. Honey Communique. Accordingly, the amount of HMF exceeding the limit value in 9 of the pine honey samples was 82.5, 54.6, 52.4, 50.2, 46.4, 45.5, 44.7, 42.5, 40.6 mg/kg, respectively, while the diastase activity of 3 samples was found to be 7.5, below the limit value (Table 1 and Table 2).

This study found that among 90 honey samples, 16 flower honey and 9 honeydew honey were not suitable for the limit value specified in the Turkish Food Codex Directive on Honey. Furthermore, as a result of the analysis, ıt was determined that 10 flower honey and 8 honeydew honey samples were in proximity to the HMF limit value , while 5 flower honey samples were found close to the limit value with diastase activity. Given the samples' expiration dates, there is concern that this situation might pose a potential public health risk.

In addition, closed to the limit value HMF amounts were found to be between 35-40 mg/kg (35.3, 35.6, 36.0, 37.2, 38.0, 38.6, 38.9, 39.1, 39.4, 39.8 mg/kg) in 10 flower honey and 8 honeydew honey samples, while closed to the limit value of diastase activities in 5 flower honey samples were determined as (8.8, 8.5, 8.5, 8.3, 8) respectively. The samples were evaluated considering the packaging dates and the analysis date. According to the findings, the minimum and maximum HMF values and diastase activities of honey samples are shown in Table 3.

Table 1. HMF value and diastase activities of flower honey samples

Sample no	*Analysis time (month)	**Shelf life (month)	HMF(mg/kg)	Diastase activity
1	6th	24	119.8	0.9
2	16th	24	114.6	5.9
3	22nd	24	102.4	1.5
4	28th	36	100.3	6.0
5	19th	24	98.5	5.6,
6	24th	36	87.6	7.2
7	20th	24	87.4	10
8	20th	24	73.3	7.6
9	12th	18	53.6	5.4
10	7th	18	49.2	7.5
11	10th	18	48.2	6.2
12	18th	24	46.7	11.5
13	12th	24	42.5	10.6
14	8th	18	40.2	14.2
15	11th	18	30.8	7.7
16	8th	18	32.2	6.4

*Analysis time= time from packaging date to analysis date, **Shelf Life= the period between the packaging date and the expiration date on the labels of honey samples.

Table 2. HMF value and diastase activities of honeydew honey samples

Sample no	*Analysis time (month)	**Shelf life	HMF(mg/kg)	Diastase activity
1	6th	24	82.5	7.5
2	6th	18	54.6	9.2
3	9th	18	52.4	7.5
4	17th	24	50.2	12.8
5	8th	18	46.4	7.5
6	18th	24	45.5	11.4
7	4th	24	44.7	13.2
8	6th	18	42.5	15.6
9	14th	18	40.6	10.5

*Analysis time= time from packaging date to analysis date, **Shelf Life= the period between the packaging date and the expiration date on the labels of honey samples.

Table 3. Maximum and minumum values of HMF and diastase activities of honey samples

Parametres	Type	Number of samples	Min. value	Max. value
HMF (mg/kg)	Flower	45	19.8 mg/kg	119.8 mg/kg
	Honeydew	45	11.5 mg/kg	82.5 mg/kg
Diastase activity	Flower	45	0.9	15.8
	Honeydew	45	7.5	19.6

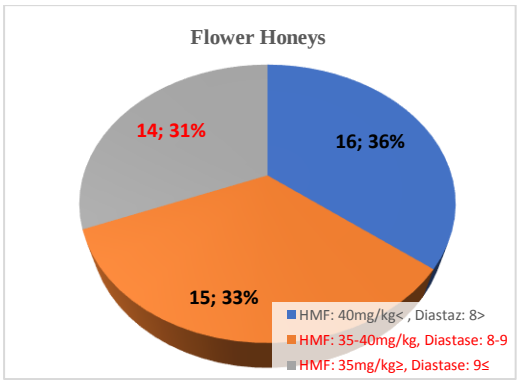

Figure 1. HMF values and diastase activity rates of flower honeys (%).

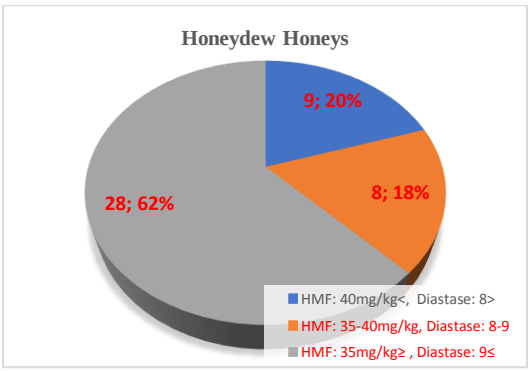

Figure 2. HMF values and diastase activity rates of honeydew honeys (%).

4. Discussion and Conclusion

In this study HMF values of 45 flower honey samples of 90 honey samples collected from various markets in their original package were determined as 19.8-119.8 mg/kg and diastase of them were determined as 0.9- 15.8. According to the data, in terms of HMF value 4 of them, in terms of diastase activity 2 of them, in terms of both HMF value and diastase activity 10 of them are not suitable for TGK Directive on Honey. Moreover, HMF values of 45 honeydew honey were found as 10.6-82.5 mg/kg and their diastase activities were found as 7.5-19.6. With reference to this in terms of HMF value 6 of the samples, in terms of both HMF value and diastase activity 3 of the samples were not suitable for TGK Directive on Honey. When retention periods and markets with their expiry dates and analysis date were taken into consideration, it's thought that the reason of improper HMF values and diastase activities were because of the adverse storage conditions. Also initial HMF values and diastase activities were important facts. When storage periods, expiration dates, and analysis dates are taken into consideration, the combination of high HMF values and low diastase activities indicates that the honeys might be subjected to unfavorable storage conditions within the markets. As it is specified in the transportation and storage section of the revised TGK (NO:2012/58) communiqué, ensuring the healthiness of the shelf life and the food safety of honeys that meet the quality standards set by the honey regulations when they reach the retail stage is crucial. This involves maintaining suitable storage conditions consistently from the point of sale to the end consumer. Thus, it will become feasible to safeguard both the rights of producers and the health of consumers, thereby guaranteeing food safety.

Study by Guler (2005) suggests HMF values of 30 honey samples collected from Eastern Black Sea region producers were determined as 3.83-11.0 mg/kg and they were found quite lower than other HMF values in the study. Furhermore in their study, Sahinler and Gul (2004) found avarage HMF values of untreated flower and sunflower honey quite lower than other values in the study. They were 5.73, 2.17 mg/kg and their diastase activities were higher than other with the 17.9, 18. Turgay (2009) reported that 50 honey samples (different floral feature), HMF values were found to be 31.46-40.70 mg/kg and diastase activities as 8-25.4. Bölükbaşı (2007) determined the HMF values as 0-38.6 mg/kg in 47 honey samples collected from different parts of Türkiye. It is thought that the reasons why HMF values were found higher and diastase activities lower were high and long-term temperature treatment and improper storage conditions. In another study conducted by Al-Diab et al. (2015), various types of honey obtained from markets exhibited an increase in HMF levels following a 6-month storage period at 25°C. The most significant rise, amounting to 14.7%, was observed in a polyfloral honey sample that initially contained 39.7±3.3 mg/kg of HMF. The samples in our study were analyzed directly without being kept in a controlled storage temperature. Nevertheless, the substantial elevation in HMF values indicates that the samples have likely been exposed to increased temperatures both during the production and storage processes. In another study carried out within Saudi Arabia, the highest HMF value among 14 honey samples collected from local markets was found to be 28.97 mg/kg. It was concluded that the honeys were not heat-treated and were fresh since the HMF values were at relatively low level (Alghamdi et al., 2020). Although the HMF limit value in countries with tropical ambient temperatures is 80 mg/kg according to Codex Alimentarius (2019), the HMF values of the honey samples in our study, which exceeded the limit value (40 mg/kg) in Türkiye, indicate that the samples were exposed to inappropriate storage conditions in markets after being distributed from factories under controlled conditions. Similarly, a study conducted in the Aydın province involved a total of 40 honey samples, including 20 honey samples taken directly from the beehives and 20 honey samples taken from the market. In the study, it was reported that the average HMF values of the market samples were 56.70±3.83 mg/kg, and the HMF values of all market samples exceeded the limit value. Diastase activities were found to be lower than the values of the samples in our study, with an average of 7.4±0.24 (Aypak et al., 2019).

In this study 16 flower honeys and 9 honeydew honeys of 90 honey samples were improper according to the limit values of T.G.K. Directive on Honey. Also in terms of HMF value on 10 flower honey and 8 honeydew honey and in terms of diastase activities 5 flower honey were found close to limit value. Therefore, it is thought that when expiry dates are taken into consideration they are potential risks for human health.

In conclusion, HMF forming in honey is known to be a potential risk in terms of food safety and public health. Suitability of the all quality criteria- HMF and diastase criteria in particular- to the Turkish Food Codex Directive on Honey should be ensured throughout the shelf life determined by companies for strained honeys offered for consumption. As revised with this aim, as stated in TGK (NO: 2012/58), it is crucial to store honey under appropriate conditions (especially <25 °C. from markets to consumers to maintain quality criteria. This will ensure food safety while protecting both public health and producer rights. Moreover, raising consumer awareness on this issue will facilitate the development of internal controls at both the production and markets. Suitability of the all quality criteria- HMF and diastase criteria in particular- to the Turkish Food Codex Directive on Honey should be ensured throughout the shelf life determined by companies for strained honeys offered for consumption.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	S.D.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

References

Al-Diab D, Jarkos B. 2015. Effect of storage and thermal treatment on the quality of some local brands of honey from Latakia markets. J Ento Zoo Stud, 3: 328-334.

Alghamdi BA, Alshumrani ES, Bin Saeed MS, Ghufran MR, Alharthi NA, Baeshen MN, Helmi NM, Alam MZ, Suhail M. 2020. Analysis of sugar composition and pesticides using HPLC and GC–MS techniques in honey samples collected from Saudi Arabian markets. Saudi J Biol Sci, 27(12): 3720-3726.

An Ajlouni S, Sujirapinyokul P. 2009. Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. Food Chem, 119: 1000-1005.

Aypak SÜ, İnci A, Bakırcı S, Fidan ED, Soysal M. 2019. Comparision of the antioksidant activity and HMF levels in honey taken from hives and markets. Gıda, 44(1): 86-92.

Belitz HD, Grosch W. 1999. Food chemistry, 2th. Edition. Springer, Berlin, Germany, pp: 145-157.

Bogdanov S. 2009. Harmonised Methods of the international honey commission. IHC responsible for the methods. Bee Product Sci, 2009: 9-54.

Bölükbaşı DN. 2007. Ambalajlı balların melitopalinolojik, kimyasal ve organoleptik analizleri. MSc Thesis, Hacettepe University, Institute of Science, Ankara, Türkiye, pp: 27-28.

Castro-Vazquez L, Diaz-Maroto MC, Gonzalez-Vinas MA, De La Fuente E, Perez- Coello MS. 2008. influence of storage conditions on chemical composition and sensory properties of citrus honey. J Agricult Food Chem, 56: 1999-2006.

Cocco LF, Valentini C, Novelli V, Ceccon L. 1996; High-performance liquid chromatography determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in honey. J Cromatogr A, 749: 95-102.

Fallico B, Arena E, Zappala M. 2008. Degradation of 5-Hydroxymethylfurfural in honey. J Food Sci, 73: 625-631.

Güler Z. 2005. Doğu Karadeniz Bölgesi'nde üretilen balların kimyasal ve duyusal nitelikleri. Gıda, 30: 379-384.

Islam N, Khalil L, Islam A, Gan SH. 2014. Toxic compounds in honey. J Appl Toxicol, 34: 733-742.

Kalabova K, Vorlova L, Borkovcova I, Smutna M, Vecerek V. 2003. Hydroxymethylfurfural in Czech honeys. Czech J Anim Sci, 48: 551-557.

Korkmaz SD, Küplülü Ö. 2017. Effects of storage temperature on HMF and diastase activity of strained honeys. Ankara Üniv Vet Fak Derg, 64: 281-287.

Morales V, Sanz ML, Martin-Alvarez PJ, Corzo N. 2009. Combined use of HMF and furosine to assess fresh honey quality. J Sci Food Agric, 89: 1332-1338.

Pasias IN, Kiriakou IK, Proestos C. 2017. HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chem, 229: 425-431.

Ramirez MA, Gonzales SA, Sauri E. 2000. Effect of the temporary thermic treatment of honey on variation of the quality of the same during storage. Apiacta, 35: 162-170.

Sahinler N, Gül A. 2004. Chemical composition and physical properties of honey produced in Turkey from sunflower, cotton, orange and pine. First European Conference of Apidology, September 19-23, 2004, Udine İtaly, pp: 136-137.

Sahinler N. 2007. Effects of heating and storage on hydroxy methylfurfural and diastase activity of different Turkish honeys. J Apicult Res, 46: 34-39.

Sancho TM, Muniata S, Huidobro FJ, Lozano SJ. 1992. Aging of Honey. J Agricult Food Chem, 40: 134-138.

Sanz ML, Castillo MD, Corzo N, Olano A, 2003. 2-Furoylmethyl amino acids and Hydroxymethylfurfural as indicators of honey quality. J Agric Food Chem, 51: 4278-4283.

Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 2018. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem Cent J, 12: 2-18.

Tosi E, Ciappini M, Re E, Lucero, H. 2022. Honey thermal treatment effects on hydroxymethylfurfural content. Food Chem, 77(1): 71-74.

TUIK. 2022. İstatistik Veri Portalı; Arıcılık. URL: https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=18 (accessed date: June 8, 2022).

Turgay O. 2009. Characteristic properties of Kahramanmaraş honey samples. KSU Doğa Bil Derg, 12: 21-24.

Türk Gıda Kodeksi Bal Tebliği, No: 2005/49, 17/12/2005 Türkiye. URL: https://www.resmigazete.gov.tr/eskiler/2020/04/20200422-13.htm (accessed date: Agust 13, 2020).

Türk Gıda Kodeksi Bal Tebliği, No: 2012/58, 27/07/2012 Türkiye. URL:http://mevzuat.basbakanlik.gov.tr/Metin.Aspx?MevzuatKod=9.5.16425&MevzuatIliski=0&sourceXmlSearch=BAL (accessed date: Agust 12, 2020).

Zappalà M, Fallico B, Arena E, Verzera A. 2005. Methods for the determination of HMF in honey: a comparison. Food Control, 16(3): 273-277.