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The influence of storage conditions and heating on diastase, invertase activity and hydroxymethylfurfural in Iraqi honey

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Abstract

The purpose of this research was to evaluate the effect of heating treatments and storage on diastase number, invertase activity and hydroxymethylfurfural (HMF) in several types of Iraqi honey (represented in most of Iraq's governorates). Average values for the quality characteristics of fresh honey ranged between (8-21) units of diastase number, 107.5-284.3 U/kg invertase activity and 7.6-40.3 mg/kg HMF. The honey samples were subjected to heating at 50, 65, 75 Cº for 30 minutes. Then the honey samples were left at room temperature 25-30 °C and stored for 6 months for evaluation. Changes in diastase number, invertase activity and HMF content were observed in 3 and 6 month of storage. The values of these indicators decreased from the basic values and ranged between (1.1-3.6 units), (26.8-70.8 U/kg) and (21.3-112.8 mg/kg) respectively after being subjected to heating at a temperature of 75°C for 30 minutes after being stored for 6 months.

Keywords: Honey, diastase, invertase, hydroxymethylfurfural

Introduction

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (Codex, 2001).

The importance of honey has been known for thousands of years, it is considered the favourite food by all people in different eras and times. Since it is considered a food and medicine at the same time because it contains bioactive compounds that have important effects on human health, honey varies according to the diversity of the nectar source, whether it is from flowers, plant secretions, or substances excreted from insects, and accordingly, its biochemical components vary, even if by a small percentage

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(Bogdanov *et al*., 1999). Honey is consumed as a portion of healthy food and is also widely used in folk and clinical medicine as a treatment. It must contain the standard parameters of natural honey that are accurately diagnosed including sugar, moisture, electrical conductivity, invertase, diastase, and hydroxymethylfurfural content.

The enzymes are the most important and interesting components of it, which are responsible for converting the nectar and live plant secretions into honey, they serve as a sensitive indicator for use of it as a treatment. Enzyme specifications are a legally binding indicator in some countries (Bogdanov *et al*., 1999). The enzymes that are transformed into honey by bees are diastase (amylases) which digests starch and converts it into maltose, which is relatively stable at storage temperature, and invertase, whose role is to convert sucrose into glucose and fructose (Čeksterytė *et al*., 2020).

Since honey is a vital natural product and is used for therapeutic purposes; It is necessary to find unconventional ways to determine the quality of honey and not to cheat or treat honey with industrial materials. Therefore, the study aimed to study different types of local honey, determine the indicators that are considered a criterion for assessing the purity of honey and study the effect of different heating treatments and storage on its quality.

Materials and Methods

Defferent varieties of Iraqi honey were collected in a field manner from beekeeping apiaries for several Iraqi beekeepers, representing most of the governorates of Iraq during the year 2021 for scientific research. The samples were labeled and stored in glass bottles at laboratory temperature until use.

Table (1): Regional distribution of honey samples used in the study.

Regional distribution of honey	No. of honey sample	Type of honey	Code of sample
Areas of Northern	1	Mountain	H1
	2	Eucalyptus	H2
	3	Jujube	H3
	4	Thistle	H4
	5	Multiflower	H5
Areas of Central	6	Clover	H6
	7	Eucalyptus	H7
	8	Jujube	H8

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	9	Multiflower	H9
	10	Bitter honey	H10
Areas of southern	11	Clover	H11
	12	Multiflower	H12
	13	Jujube	H13
	14	Eucalyptus	H14
	15	Thistle	H15

Diastase Number (DN) assay

Followed the method presented in I.H.C (2009) and described in Almeida-Muradian *et al*. (2020) in determinenation DN .

Invertase Activity Assay

Invertase activity was determined according to the officially prescribed method (IHC, 2009). and p-Nitrophenyl-α-D-glucopyranoside (pNPG) was used as substrate.

Determination of Hydroxymethylfurfural (HMF)

Determinenation was carried out by the Spectrophotometer (UV) method based on what was reported in I.H.C (2009) and described in Tesfaye *et al*. (2016) and Sereia *et al*. (2017).

Effect of Heating and Storage on Honey Quality.

The effect of heat treatments and storage on the quality characteristics of the studied varieties of Iraqi honey was followed up and evaluated by following the same procedures in each of the test steps every three months.

Statistical Analysis

Data was analyzed using Special Program for Statistical System (SPSS).

Results & Discussion

Diastase (DN)

Figure (1) shows the results of the diastase enzyme activity values, which are expressed by DN in the studied honey samples. The statistical analysis showed significant differences between the means ($P< 0.05$). The highest Diastase Number in sample H12 was 21 units, with a significant difference from the rest of the samples, but it did not differ significantly with the H2 sample, as it reached 20 units, followed by the samples H5 and H6, where the DN reached 19 and 18 units respectively, with a significant difference from the other samples, then the honey samples: H1, H15, H13. H10, H14 and H7 as the DN reached: 16, 15, 13, 12, 10 and 10 units, respectively, while the lowest value was 8 units in samples H4 and H8, with a significant difference from the rest of the samples, but they did not differ significantly with samples H3 and H11, as the DN reached 9 units.

The hypopharyngeal glands of worker honey bees specialize in collecting nectar

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have a key role in increasing the activity of the diastase enzyme, as they become active in relation to the age of worker bees. bees and others in the surrounding environmental nature. The effectiveness of honey enzymes, including diastase, is affected by several factors, such as food source, the techniques used in the process, as well as storage conditions. the activity of enzymes is a criterion for honey ripening processes after its conversion from the nectar phase (Taha, 2020; Salman *et al*., 2019a).

The results obtained from the study are within the range of AL-Farsi *et al*. (2018) who found that the DN values in Omani honey ranged 1.46-18.4 units in 29 samples of Sidr honey, while the values ranged 1.22-27.1 units in 21 samples of Sumar honey.

Some of the results converged with those of Xagoraris *et al*. (2021) results who found that the highest value obtained was 51.1 units, while the lowest value was 11.1 units with an average of 27.3 units when studying eight samples of Greek honey (thyme honey) volatile compounds using SPME-GC-MS technology.

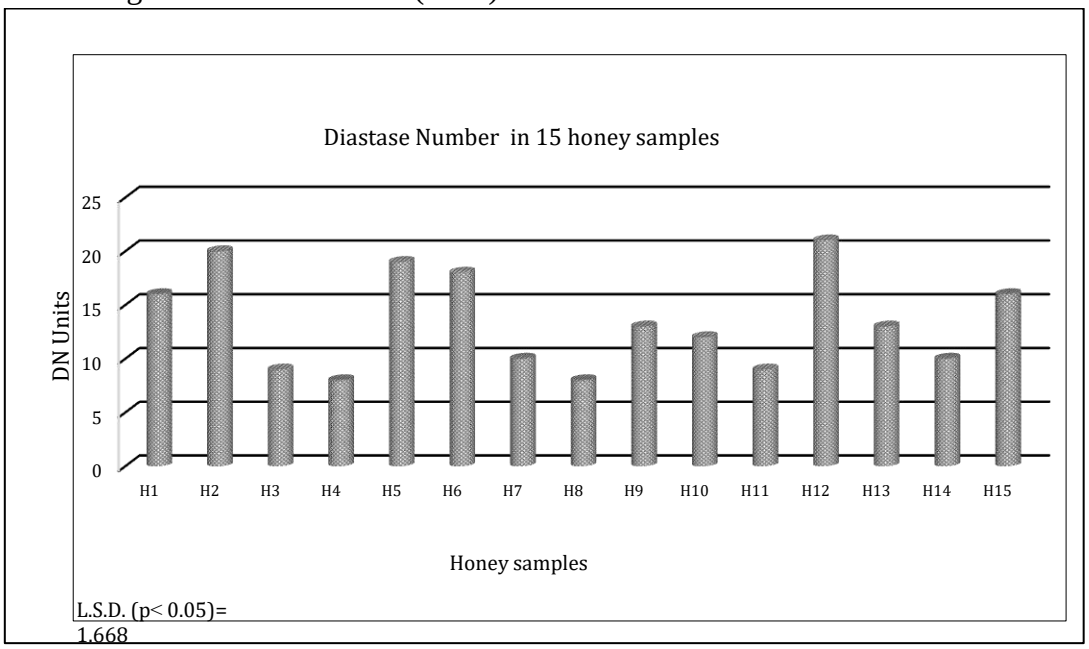

Figure 1: Diastase Number in honey samples.

As for Saeed and Jayashankar (2020), it was found that there were significant differences in the rate of diastase activity in honey when conducting a comparative study the chemical and physical properties of some Indian and Yemeni, as the DN reached 9.6 ± 0.25 and 11.2 ± 0.15 units in Indian honey (Coorg and Kashmiri) respectively, while the activity rate in each of yemeni honey (Sidr and clover) 11.9 ± 0.60 and 10.5 ± 1.12 units respectively, which it is within the standard specifications defined by codex. While Salman *et al*. (2019b) found that the DN

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reached the highest 14.36 in the Basrah region and the lowest in the Babylon region 8.67.

Invertase

Figure (2) shows the values of the invertase activity (IA) in honey varieties. The results of the statistical analysis showed that there were significant differences at ($p<0.05$) between the average values of IA in the studied samples and the highest activity was recorded in the H7 sample, which amounted to 284.3 U/kg followed by the samples H12, H14 and H8 where the activity reached 279.6, 278.5 and 278.1 U/kg respectively, with no significant difference between them, then honey samples H13, H15, H10, H5, H2 , H1, H9 and H6, which amounted to 242.5, 238.4, 234.3, 201.2, 198.1, 190.4, 178.4, 140.2 U/kg respectively, while the lowest activity of IA in sample H11 was 107.5 U/kg with a significant difference with the other samples, but it did not differ significantly with samples H3 and H4 which the IA was 122.1 and 120.2 U/kg respectively.

Invertase activity is more sensitive to heat than diastase. The evaluation of its effectiveness is very important to determine the quality and freshness of honey, while the HMF compound comes in the second degree of importance, and according to international standards, the level of invertase enzyme activity in honey must not be less than 40 U/kg (Bogdanov *et al*., 1999). The activity of diastase and invertase in bee honey varies from one type to another and depends on factors including the type of sample, the quality and composition of the nectar, the season, also the type and age of the bees.

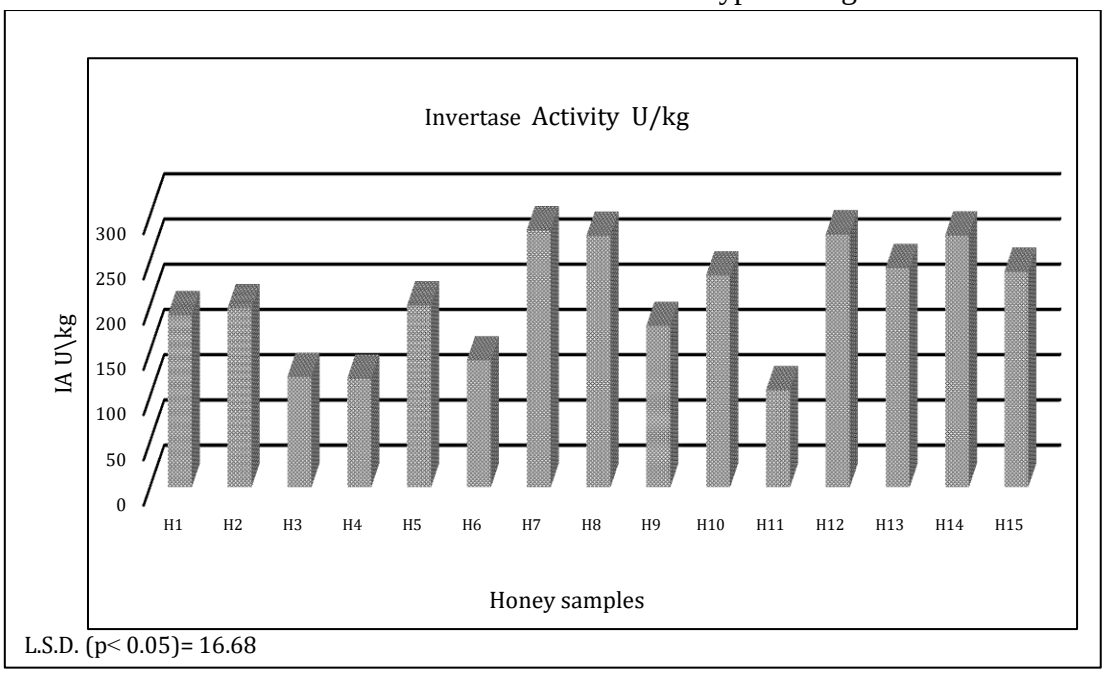

Figure 2: Invertase Activity in honey samples .

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The results came within the range reached by Beykaya (2021) who reported that the invertase activity in Croatian honey that was subjected to the test was between 103.3 - 378.1 U/kg. Some of them were similar to the findings of Flanjak *et al*. (2016) in a study conducted on samples of single-source Croatian honey, including buckwheat honey, chestnut, sage, and honeydew. The average invertase activity for each of them was 52.1, 155.2, 94.7 and 176.1 U/kg respectively. Whereas Boussaid *et al*. (2018) reported that the average values of invertase activity: 184.68, 52.29, 46.25, 92.66, 73.74 and 82.01 U/kg for each of thyme honey, eucalyptus, rosemary, sidr, orange and mint respectively, in six honey samples of different botanical and geographical sources in Tunisia. The geographical location and type of plant the variation in the values of enzymatic activity from one species to another. It was close in part with the control sample of Kanelis *et al*. (2022) who found during his study that honey samples that represent the control and the sample produced after feeding on Candy paste gave values for IA that were 153.7 and 129.9 U/kg, while samples which were produced after feeding with inverted syrup and sugar syrup gave low IA values of 32.4 and 68.9 U/kg respectively (classified as adulterated), as for invertase activity, supplementary feeding during the production led to lower invertase values in the collected samples. This may be attributed to the fact that bees, when they are fed with syrup, collect it quickly and have no time to enrich the product with enzymes, resulting in honey samples with low enzymatic content. According to the International Honey Commission, honey with invertase values greater than 10 IN (invertase number) or 73.43 U/kg is characterized as fresh (Bogdanov *et al*., 1999).

Hydroxymethylfurfural (HMF)

From Figure (3) we note that the concentration of HMF in the studied honey samples ranged between 7.6-40.3 mg/kg and an average of 21.21 mg/kg, as the maximum value of HMF was in the honey sample H2 while the lowest was in the sample H5, and based on the statistical analysis, it was found that the variance in the average concentration of HMF between honey samples of different types and sources was significantly different at ($p<0.05$). The variation in the content of honey varieties is mainly due to the nature of the conditions surrounding honey during the periods of picking, storage and handling. Studying its levels is one of the important indicators of honey quality (Shapla *et al*., 2018).

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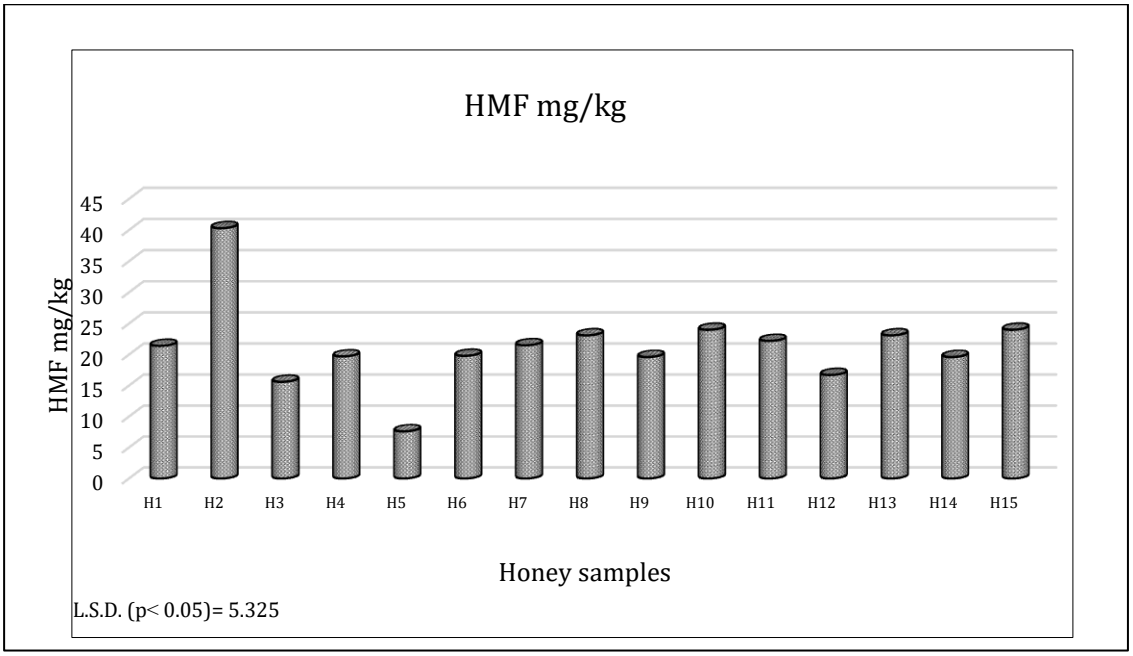

Figure 3: HMF concentration mg/kg in honey samples .

The results came within the international standard for honey based on the Codex for the year 2001, as most of the samples conform to international standards. Also, within the range reached by Polak-Sliwinska and Tanska (2021), who found that the content of HMF compound in five types of Polish honey ranged between 6.05-54.25 mg/kg. It also came close to the findings of Tesfaye *et al*. (2016) who found that the content of HMF ranged between 27.10-40.80 mg/kg in his study evaluating the chemical and physical properties of Ethiopian honey. While Salman *et al* (2019b) found the content of HMF between 12.35-41.54 mg/kg according to producing location.

Effect of Heating Treatments and Storage on Honey Quality

Effect of temperature and storage on diastase number (DN)

The results in table (2) show the effect of different thermal treatments and storage at laboratory temperature (25-30°C) on the DN of the studied samples of Iraqi honey. The results of the statistical analysis showed that the DN values decreased significantly at the probability level of $p<0.05$ for all studied samples, as it was noted that there was a gradual decrease in DN values during the storage periods, but the values of the decrease varied in varying proportions with the heat treatment of honey and at different degrees for 30 minutes. In sample H12 which value of HMF before thermal treatments and storage 21 units, the values reached: 21, 16.7 and 15.8 units at thermal treatments: 50, 65 and 75°C, respectively, then this decrease increased to: 16.5, 16.2 and 8.6 units, respectively, after 3 months of storage, then the values reached: 11.2, 6 and 3.3 units respectively after 6 months, and so on for all honey samples, as it was

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noted that the decrease in DN had reached below the internationally permissible limits of 8 units when honey was heated at 65 and 75 °C after a storage period of 6 months. The values in the rest of the samples at the end of the storage period reached: 3, 3.6, 1.5, 1.6, 2.6, 2.6, 1.8, 1.1, 1.8, 1.9, 1.8, 1.2, 1.5 and 2.8 units for each of samples H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H13, H14 and H15 respectively, upon heat treatment of 75°C.

Table (2): Effect of heat and storage on DN in honey samples.

honey samples	DN units	DN units before storage			DN units after 3 months			DN units after 6 months		
		50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min
H1	16	16	12.7	11	12	12	6.5	8	5.5	3
H2	20	20	15.9	14.9	15.8	15.2	8.2	11	7	3.6
H3	9	9	7.1	6	7	6.7	3.7	6	3.2	1.5
H4	8	8	6.3	5.1	6.3	5.5	3.3	4.3	2.8	1.6
H5	19	19	15.1	13.7	15	14	7.8	10.1	5.8	2.6
H6	18	18	14.3	13	14.3	13.3	7.3	9.5	5.5	2.6
H7	10	10	7.9	6.9	7.9	7.4	4.1	5.2	2.4	1.8
H8	8	8	6.3	5.3	6.3	5.8	3.3	4	2.1	1.1
H9	13	13	10.3	9.1	10	9.7	5.3	7	3.1	1.8
H10	12	12	9.5	8.3	9.5	9	4.9	6.9	3.3	1.9
H11	9	9	7.1	6	7.1	6.3	3.7	4.8	2.2	1.8
H12	21	21	16.7	15.8	16.5	16.2	8.6	11.2	6	3.3
H13	13	13	10.3	9.1	10.2	9.8	5.3	6.9	3.8	1.2
H14	10	10	8.0	7	7.9	7.4	4.1	5.1	2.4	1.5
H15	16	16	12.7	11.7	12.7	12.2	6.5	8	4.2	2.8

The results were close to what Samira (2016) indicated that the DN is severely reduced during the heating treatment and this in turn affects the quality of the honey. The decrease in diastase activity begins at 55 °C, and this decrease in activity continues below the internationally permissible limits when the temperature is raised to 75 °C. Blidi *et al*. (2017) showed

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this in their study on Greek honey to evaluate the effect of heat treatment on the quality of honey, and the average values for the diastase number were 14.93 units at 0 hours, and it was noticed that the values decreased by the effect of temperature, reaching: 13.67, 12.90, 13.17 and 12.17 units respectively.

Effect of temperature and storage on invertase activity (IA)

The results in Table (3) indicate the effect of different heat treatments and storage at laboratory temperature (25-30°C) on the invertase activity (IA) in the studied samples of Iraqi honey, as the results of the statistical analysis showed that the activity values of the enzyme had decreased significantly at ($p<0.05$) for all samples, as it was observed that there was a gradual decrease in the IA values during the storage periods, but the values of the decrease in the enzymatic activity differed in varying proportions with honey heat treatment at different degrees for 30 minutes, the H7 sample which gave the highest value of IA before heat treatment and storage, it reached 284.3 U/kg, while this value decreased to 283.2, 265.8 and 254.7 U/kg when honey was exposed to different temperatures 50, 65 and 75° C respectively before storage, then this decrease continued to 253.3, 237.6 and 228.1 U/kg respectively, after 3 months of storage, and then reached 122.8, 91.3 and 70.8 U/kg, respectively, after 6 months, so on for all honey samples, as it was noted that the decrease in IA had reached in some honey samples below the permissible limits it has internationally 40 U/kg when the honey is heat treated at 65 and 75 ºC and then stored for a period of 6 months. The values were: 30.4, 29.9, 34.9 and 26.8 U/kg for each of the samples: H3, H4, H6, H7 and H11, respectively at the heat treatment of 75 °C. The activity of invertase is more sensitive to heat than other compounds, as the evaluation of its effectiveness is very important to determine the quality and freshness of honey, while HMF comes in the second degree of importance.

Table (3): Effect of heat and storage on IA in honey samples.

honey samples	IA U/kg	IA U/kg before storage			IA U/kg after 3 months			IA U/kg after 6 months		
		50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min
H1	190.4	189.6	178.0	170.6	169.6	159.1	152.8	82.3	61.1	47.4
H2	198.1	197.3	185.2	177.5	176.5	165.5	159.0	85.6	63.6	49.3
H3	122.1	121.6	114.2	109.4	108.8	102.0	98.0	52.7	39.2	30.4
H4	120.2	119.7	112.4	107.7	107.1	100.4	96.4	51.9	38.6	29.9
H5	201.2	200.4	188.1	180.3	179.3	168.1	161.4	86.9	64.6	50.1
H6	140.2	139.6	131.1	125.6	124.9	117.2	112.5	60.6	45.0	34.9

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H7	284.3	283.2	265.8	254.7	253.3	237.6	228.1	122.8	91.3	70.8
H8	278.1	277.0	260.0	249.2	247.8	232.4	223.1	120.1	89.3	69.2
H9	178.4	177.7	166.8	159.8	159.0	149.1	143.1	77.1	57.3	44.4
H10	234.3	233.4	219.1	209.9	208.8	195.8	188.0	101.2	75.2	58.3
H11	107.5	107.1	100.5	96.3	95.8	89.8	86.3	46.4	34.5	26.8
H12	279.6	278.5	261.4	250.5	249.1	233.6	224.4	120.8	89.8	69.6
H13	242.5	241.5	226.7	217.3	216.1	202.6	194.6	104.8	77.8	60.4
H14	278.5	277.4	260.4	249.5	248.1	232.7	223.5	120.3	89.4	69.3
H15	238.4	237.4	222.9	213.6	212.4	199.2	191.3	103.0	76.5	59.4

The results are in line with those of Flanjak *et al*. (2016) the invertase activity in samples of Croatian honey that were subjected to the test was between 103.3 - 378.1 U/kg, the highest IA was found in raw honey, while the effectiveness was lower in heat-treated honey. Honey and its exposure to heating processes, it approximated what found Hasan (2013) when studying the effect of heating on different types of honey from different floral sources, as the average values of IA decreased from 131.6 to 104.9 U/kg when heat treatment was 75° C for 25 minutes and a three-months storage period, whereas, IA values reached 20.6 U/kg after 6 months of storage.

Effect of heat and storage on HMF

The results in table (4) show the effect of different thermal treatments and storage at laboratory temperature (25-30°C) on the content of HMF in the studied samples of Iraqi honey, as the results of the statistical analysis showed that the content of this compound increased significantly at ($p< 0.05$) for all samples, as it was noted that there was a gradual rise in HMF values when heat treated honey at different temperature for 30 minutes, but the percentages differed in the content of this compound during storage periods in the H5 sample which gave the lowest HMF content before heat treatment and storage, as it reached 7.6 mg/kg, while this amount increased to: 8.8, 12.2 and 14.7 mg/kg when honey was exposed to different temperatures: 50, 65 and 75°C respectively before storage, then this increase continued to: 13.5, 15.6 and 17.2 mg \ kg respectively after 3 months of storage, then the concentration reached: 18.6, 19.7 and 21.3 mg/kg respectively after 6 months of storage, so on for all honey samples, as it was noted that the increase in HMF content reached the internationally permissible limits of 40 mg \ kg according to Codex recommendations when treating honey at a temperature of 50, 65 and 75 ºC during the storage period.

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Table (4): Effect of heating and storage on HMF content in honey samples.

honey samples	HMF mg/kg	HMF mg/kg before storage			HMF mg/kg after 3 months			HMF mg/kg after 6 months		
		50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min	50 Cº/ 30min	65 Cº/ 30min	75 Cº/ 30min
H1	21.4	24.8	34.2	41.5	37.9	43.9	48.4	52.4	55.4	59.9
H2	40.3	46.7	64.5	78.2	71.3	82.6	91.1	98.7	104.4	112.8
H3	15.6	18.1	25.0	30.3	27.6	32.0	35.3	38.2	40.4	43.7
H4	19.7	22.9	31.5	38.2	34.9	40.4	44.5	48.3	51.0	55.2
H5	7.6	8.8	12.2	14.7	13.5	15.6	17.2	18.6	19.7	21.3
H6	19.8	23.0	31.7	38.4	35.0	40.6	44.7	48.5	51.3	55.4
H7	21.5	24.9	34.4	41.7	38.1	44.1	48.6	52.7	55.7	60.2
H8	23.1	26.8	37.0	44.8	40.9	47.4	52.2	56.6	59.8	64.7
H9	19.6	22.7	31.4	38.0	34.7	40.2	44.3	48.0	50.8	54.9
H10	24.0	27.8	38.4	46.6	42.5	49.2	54.2	58.8	62.2	67.2
H11	22.2	25.8	35.5	43.1	39.3	45.5	50.2	54.4	57.5	62.2
H12	16.7	19.4	26.7	32.4	29.6	34.2	37.7	40.9	43.3	46.8
H13	23.1	26.8	37.0	44.8	40.9	47.4	52.2	56.6	59.8	64.7
H14	19.6	22.7	31.4	38.0	34.7	40.2	44.3	48.0	50.8	54.9
H15	24.0	27.8	38.4	46.6	42.5	49.2	54.2	58.8	62.2	67.2

The results were in line with what Samira (2016) who observed that there was an increase of 65.4% HMF production resulting from heating at 55-75°C and stated that HMF formation is a slow natural phenomenon at room temperature, but heat treatment of honey to high temperatures can cause a significant increase in the content of this compound. It agreed with the study by Bodor *et al*. (2022) on three types of Hungarian honey to evaluate the effect of heat on the HMF compound in honey, and the average values of this compound in fresh honey were as follows: 18.5, 14.7 and 7.0 mg/kg respectively, the three heated samples were: 40, 80 and 100 ° C for 60 and 120 minutes. He noticed that the HMF content was greatly affected by the temperature. It reached: (16.2, 17.6, 20.2 and 40.3) and

(17.3, 20.5, 31.8 and 155.1) mg/kg respectively in sunflower honey, and it reached: (14.1, 18.0, 11.9 and 16.7) and (15.1, 15.8, 14.3 and 81.4) mg/kg, respectively, in Indigo honey also reached: (7.7, 8.0, 9.1 and 16.1) and (8.0, 8.8, 13.3 and 44.7) mg/kg respectively in Acacia honey.

Conclusion

On the results obtained in the current study, the results of tests to determine the values of diastase and invertase activity showed that there are significant differences in enzyme activity according to types and sources of honey. The honey samples varied significantly in hydroxymethylfurfural content as the criteria determining the purity of honey. Decrease in the values of indicators that depend on the diagnosis of honey purity under the influence of thermal treatments.

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References

Al-Farsi, M.; Al-Belushi, S.; Al-Amri, A.; Al-Hadhrami, A.; Al-Rusheidi, M. and Al-Alawi, A. (2018). Quality evaluation of Omani honey. Food Chemistry, 262, 162–167.

Almeida-Muradian, L. B.; Barth, O. M.; Dietemann, V.; Eyer, M.; Freitas, A. S.; Martel, A.; Marcazzan, G. L.; Marchese, C. M.; Mucignat-Caretta, C.; Pascual-Maté, A.; Reybroeck, W.; Sancho, M. T. and Sattler, J. A. G. (2020). Standard methods for Apis mellifera honey research. J. of Apicultural Research, 59(3): 1–62, https://doi.org/10.1080/00218839.2020.1738135

Beykaya, M. (2021). Determination of physicochemical properties of raw honey samples. Progress in Nutrition, 23 (1): 1-7.

Blidi, S.; Gotsiou1, P.; Loupassaki1, S.; Grigorakis, S. and Calokerinos, A. C. (2017). Effect of Thermal Treatment on the Quality of Honey Samples from Crete. Advances in Food Science and Engineering, 1(1): 1-9.

Bodor, Z.; Benedek, C.; Aouadi, B.; Zsom-Muha, V. and Kovacs, Z. (2022). Revealing the Effect of Heat Treatment on the Spectral Pattern of Unifloral Honeys Using Aquaphotomics. Molecules, 27, 1-16.

Bogdanov, S.; Lüllmann, C.; Martin, P.; von der Ohe, W.; Russmann, H.; Vorwohl, G.; Flamini, C. (1999). Honey quality and international regulatory standards: Review by the International Honey Commission. Bee World, 80, 61–69. [CrossRef].

Boussaid, A.; Chouaibi, M.; Rezig, L.; Hellal, R.; Donsı, F.; Ferrari, G. and Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. Arabian Journal of Chemistry, 11, 265–274.

Čeksterytė, V.; Kurtinaitienė, B.; Jaškūnė, K. and Kretavičius, J. (2020). The influence of storage conditions on invertase, glucose oxidase activity and free acidity of bee bread and bee-collected

pollen mixed with honey and vegetable oils. Journal of Apicultural Research, 59(5): 862-875 .

Codex Alimentarius Commission (2019). Draft amended standard for standard for honey. CXS 12 – 1981, Amended in pp. 1–9.

Codex. (2001). Codex Alimentarius standard for honey 12-1981. Revised

Flanjak, I.; Strelec, I.; Kenjerić, D. And Primorac L. (2016). Croatian produced unifloral honeys characterised according to the protein and proline content and enzyme activities. J. Apic. Sci. 60 (1) 39-48.

Harmonised methods of the international honey commission (2009). http://www.bee-hexagon.net/en/network.htm

Hasan, S. H. (2013). Effect of Storage and Processing Temperatures on Honey Quality. Journal of Babylon University / Pure and Applied Sciences. 21 (6): 2244 - 2253.

Kanelis, D.; Liolios, V.; Tananaki, C.; Rodopoulou, M.-A. (2022). Determination of the Carbohydrate Profile and Invertase Activity of Adulterated Honeys after Bee Feeding. Appl. Sci., 12, 3661. https://doi.org/10.3390/app12073661.

Polak-Sliwinska, M. and Tanska, M. (2021). Conventional and Organic Honeys as a Source of Waterand Ethanol-Soluble Molecules with Nutritional and Antioxidant Characteristics. Molecules, 26, 3746. https://doi.org/10.3390/ molecules26123746.

Saeed, M. A. and Jayashankar, M. (2020). Physico-chemical characteristics of some Indian and Yemeni Honey, Journal of Bioenergy and Food Science, 7(2), e2832019JBFS. doi: 10.18067/jbfs.v7i2.283.

Samira, N. (2016). The effect of heat treatment on the quality of Algerian honey. Researcher,8 (9): 1-6.

Salman, A. A.; AL-Mossawi, A. H. and Al-fekaiki, D. F. (2019a). Characterizations of raqi types of Honey and Identification of Organic Volatile Compounds and Study The Antioxidant Activity**.** Thesis of Philosophy in food Science, Agriculture-University of Basrah in Iraq.

Salman, A. A.; AL-Mossawi, A. H. and Al-fekaiki, D. F. (2019b). Determination of quality compounds in Some of Iraqi honey types. J. of Kerbala for Agr. Sci. 17(1): 49- 55.

Sereia, M. J.; Março P. H.; Perdoncini, M. R. G.; Parpinelli, R. S.; De Lima, E. G. and Anjo, F. A. (2017). Techniques for the Evaluation of Physicochemical Quality and Bioactive Compounds in Honey. In Honey analysis Chapter, 9 P 194-214.

Shapla, U. M.; Solayman, M. ; Alam, N.; Khalil1, M. I. and Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Review, Chemistry Central Journal, 12:35 https://doi.org/10.1186/s13065-018-0408-3

Taha, A. A. (2020). Profile Analysis of Major and Minor Honey Contents from Different African Countries. Egypt. Acad. J. Biolog. Sci., 13(2): 269-281.

Tesfaye, B.; Begna, D. and Eshetu, M. (2016). Article Evaluation of Physico-Chemical Properties of Honey Produced in Bale Natural Forest, Southeastern Ethiopia. International J. of Agricultural Sci. and Food Technology, 2(1): 021-027.

Xagoraris, M.; Skouria, A.; Revelou, P.-K.; Alissandrakis, E.; Tarantilis, P.A.; Pappas, C.S. (2021). Response Surface Methodology to Optimize the Isolation of Dominant Volatile Compounds from Monofloral Greek Thyme Honey Using SPME-GC-MS. Molecules, 26, 3612. https:// doi.org/10.3390/molecules26123612