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Assessment of Quality Parameters in Samples of Iraqi Honey

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Abstract

This study was carried out on samples of honey that represented most of the governorates of Iraq from different plant sources. The estimation was made for the values of indicators or criteria on which to determine the purity of honey, as the electrical conductivity values ranged from 1205-322 µS/cm. The highest value was recorded in the H8 sample, while the lowest value was in the H1 sample, the proline content ranged within the limits of 181.7 - 398.3 mg/kg, as the H12 sample recorded the highest content of proline and the lowest content in the sample H8. as the highest value of diastase in sample H12 was 21 unit and the lowest value reached 8 units in samples H4 and H8, in While the highest activity of invertase was recorded in the H7 sample which amounted to 284.3 U/kg and the lowest activity in the H11 sample, which amounted to 107.5 U/kg. Also, the values of HMF ranged between 7.6-40.3 mg/kg, as the maximum value of HMF was in northern eucalyptus honey (H2), while it was the lowest In northern floral honey (H5), the content of ascorbic acid was between 138.83-305.5 mg/kg, as it reached its maximum concentration in sample H15, while it was lower in sample H5.

Keywords: Honey, diastase, invertase, proline, electrical conductivity, HMF.

I. 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 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 (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.

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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).

It is a natural, vital product that is used for therapeutic purposes. Unconventional methods must be found to determine the quality of honey and Knowing the enzymes in honey, which are the important substances in giving honey qualities as antibiotics and therapeutics at the same time. Due to the lack of local research that dealt with the quality characteristics of local honey, the study aimed to study different types of local honey, and study honey enzymes that give a standard for honey quality.

II. MATERIALS AND METHODS

Varieties of Iraqi honey were collected in a field manner from beekeeping apiaries for several of 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
	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

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Determination of Electrical Conductivity (EC)

Followed the method presented in I.H.C (2009) in determinenation EC. Dissolve an amount of honey, equivalent to 20.0 g anhydrous honey, in distilled water. Transfer the solution quantitatively to a 100 ml volumetric flask and make up to volume with distilled water. The determination of the electrical conductivity is based on the measurement of the electrical resistance.

Determination of proline

Followed the method presented in I.H.C (2009) in determinenation proline content in study samples.

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).

Determination of Vitamin C (Ascorbic acid)

Followed the method presented in Ferreira *et al*. (2009) and described in Moniruzzaman *et al*. (2013) in determinenation ascorbic acid content.

III.RESULTS & DISCUSSION

Electrical Conductivity (EC)

Figure (1) shows the results of the EC values in the studied honey samples. It is noted from the figure that there are significant differences between the average EC values of the studied honey varieties at a significant level $p<0.05$, as the highest EC value was recorded in the H8 sample. It was 1205 µS/cm, followed by samples H2, H12 and H3 the values were: 888, 800 and 794 µS/cm respectively with a significant difference from the rest of the samples, then the honey samples: H7, H15 and H11 where the EC values reached: 676 and 600 and 595 µS/cm, respectively, while the lowest value of EC in sample H1 was 322 µS/cm with a significant difference with other samples, but it did not differ significantly with samples H9 and H4 as it reached 361 and 362 µS/cm.

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The results show that the electrical conductivity values for most of the studied samples of Iraqi honey of different types and sources are in line with the internationally required standards. And that the variance in the EC values is the product of the difference in honey content of minerals and organic acids. The electrical conductivity test is the most important and fastest analytical indicator in determining quality! It can be used as a test to distinguish between flower honey and honeydew honey (Saxena *et al*., 2010).

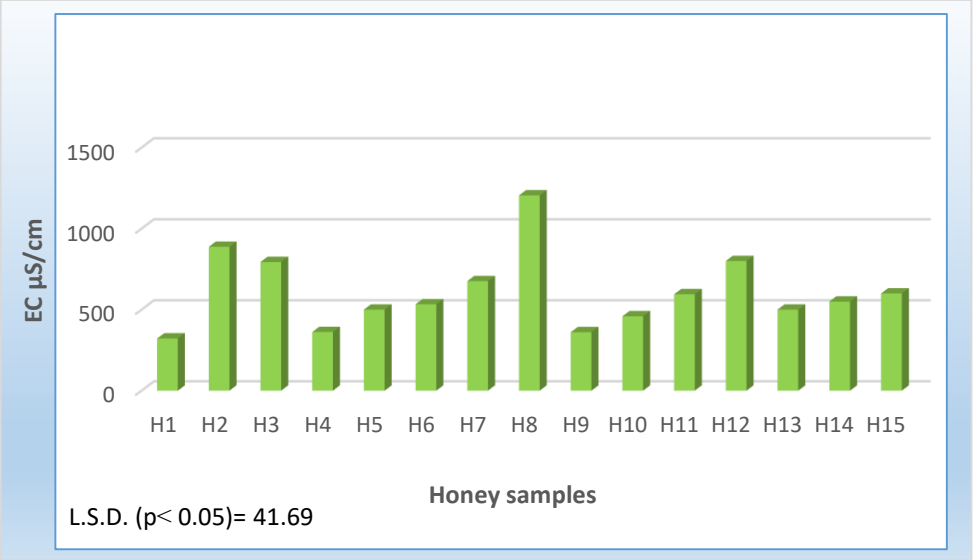

Fig. (1): Electrical conductivity values in honey samples .

The results came within the range of Lewoyehu and Amare (2019) in their study on a variety of Ethiopian honey from Tigray regions with various plant sources, as they noticed that EC values ranged between 0.19 - 0.89 µS/cm and converged with Galhardo *et al*. (2021) in his study on a variety of Brazilian honey, it was found that EC values range between 181.20 - 565.80 µS/cm. In some of them, they converged with what was found by Xagoraris *et al*. (2021) as it was found that the highest value of EC is 600 µS/cm,While the lowest value was obtained by 251 µS/cm and an average of 457 µS/cm in eight samples of Greek thyme honey, these values are considered a good criterion to determine whether the honey was collected from nectar.

Prolein

Figure (2) shows the results of concentration of the amino acid proline in the studied honey varieties, as significant differences appeared, according to the results of the statistical analysis at probability level $p<0.05$ between average content of the honey samples of proline, as the highest concentration reached 398.3 mg/kg in the honey sample. H12 with a significant difference with the rest of the samples, followed by the two samples: H5 and H2, as concentration in both of them reached 338.3 and 325 mg/kg, respectively without a significant difference between them, while they differed significantly with other samples, while no significant differences appeared between three samples: H9, H1 and H15. The concentrations in it reached: 285, 271.7 and 261.5 mg/kg respectively, while lowest concentration was in sample H8, as the proline content in it

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reached 181.7 mg/kg, it did not differ significantly with the samples: H14, H3, H10, H6 and H4, as it reached : 198.3, 191.6, 188.3, 185 and 185 mg/kg, respectively.

Most of the active compounds contained in honey including proline, vary in content based on nectar and geographical origin. The source of proline as well as the other amino acids in honey is derived from bee secretions. The content of proline rises after the enzymatic treatment of nectar that bees collect from nectar plants, which explains why it is more abundant than other amino acids in honey; It is used in feeding bee workers to help them as aviation fuel, as well as its effective effect on increasing the ability of queen bees to lay eggs for reproductive purposes (Von der ohe, 1994).

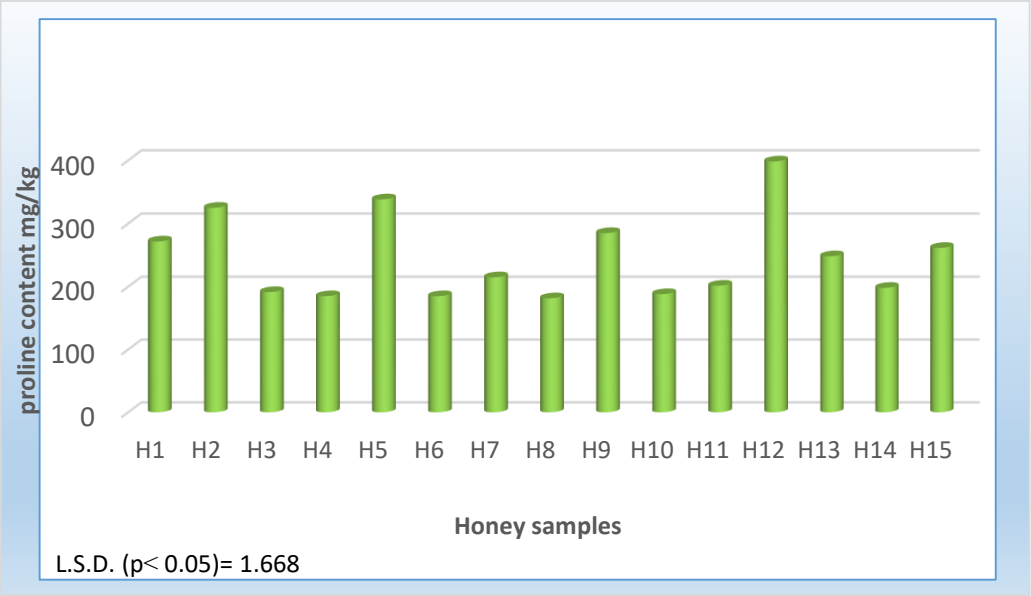

Fig. (2): proline content in honey samples .

The results are consistent with Tarapatskyy *et al*. (2021) as it was found that proline content of four types of Polish honey, which are: multi-flowered honey, linden, buckwheat and honeydew, reached: 184.44, 214.65, 251.57 and 277.52 mg/kg respectively, it was the lowest value in multi-flowered honey at a rate of 35 % for the highest value in honeydew honey. As for Beykaya (2021) he conducted tests on 11 types of Turkish raw honey, found that their proline content ranged between 300.0 - 881.7 mg/kg. The total proline content was estimated by Taha (2020) when conducting a comprehensive study for the purpose of

analyzing major and minor components of honey in different African countries with the aim of evaluating the quality of some types of African honey and judging their suitability for export. All honey samples from Cameroon, Algeria, Libya, Zimbabwe and Egypt contained proline content above standard limit. Its general average was: 838.3, 528.5, 390.2, 223.3 and 200.3 mg/kg respectively. He stated that measuring levels of flavonoids and proline can be used to study the floral and geographical origin of honey.

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Diastase (DN)

Figure (3) 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 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 was They have 9 units.

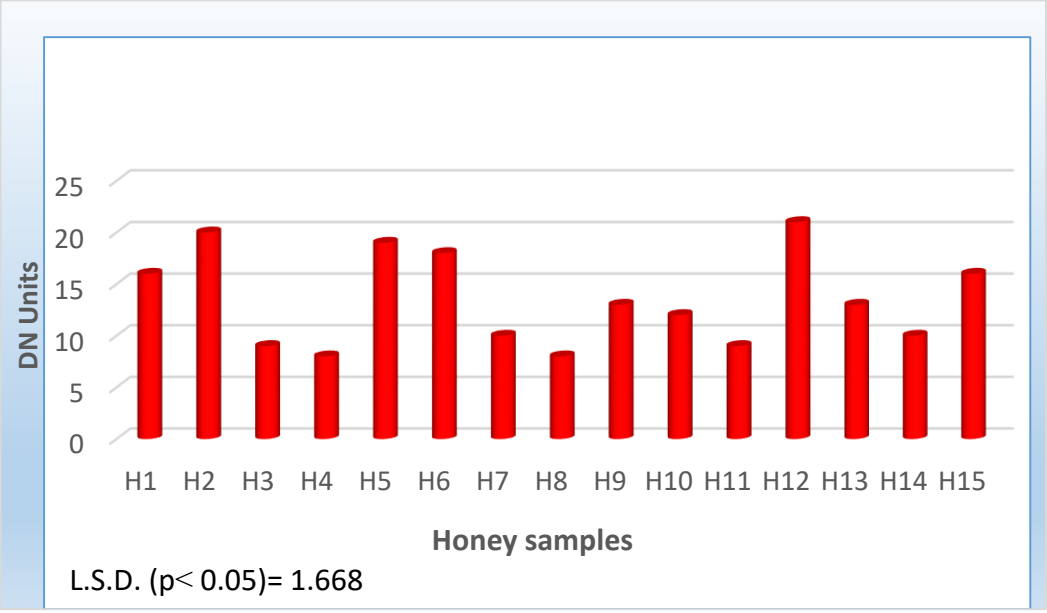

Fig. (3): Diastase Number in honey samples .

The hypopharyngeal glands of worker honey bees who specialize in collecting nectar have a key role in increasing the activity of the diastase enzyme, as they become active with the passage of time 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 honey picking process, as well as the 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 are within the range of AL-Farsi *et al*. (2018) found that the DN values in Omani honey ranged in the range of 1.46-18.4 units in 29 samples of Sidr honey, while the values ranged in the range of 1.22-27.1 units in 21 samples of Sumar honey.

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Some of the results converged with those of Xagoraris *et al*. (2021) 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 single source Greek honey (thyme honey) for the purpose of diagnosing volatile compounds using SPME-GC-MS technology. 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 in the chemical and physical properties of some Indian and Yemeni honeys, as it reached DN 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, It is within the standard specifications defined by codex. While Salman *et al*. (2019b) found diastase number the highest was 14.36 unit In Basrah region and the lowest diastase number in Babylon region 8.67 unit

Invertase

Figure (4) 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, without significant difference, 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 and the activity was 122.1 and 120.2 U/kg respectively.

Invertase activity is more sensitive to heat than diastase. As 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, the type and age of the bees.

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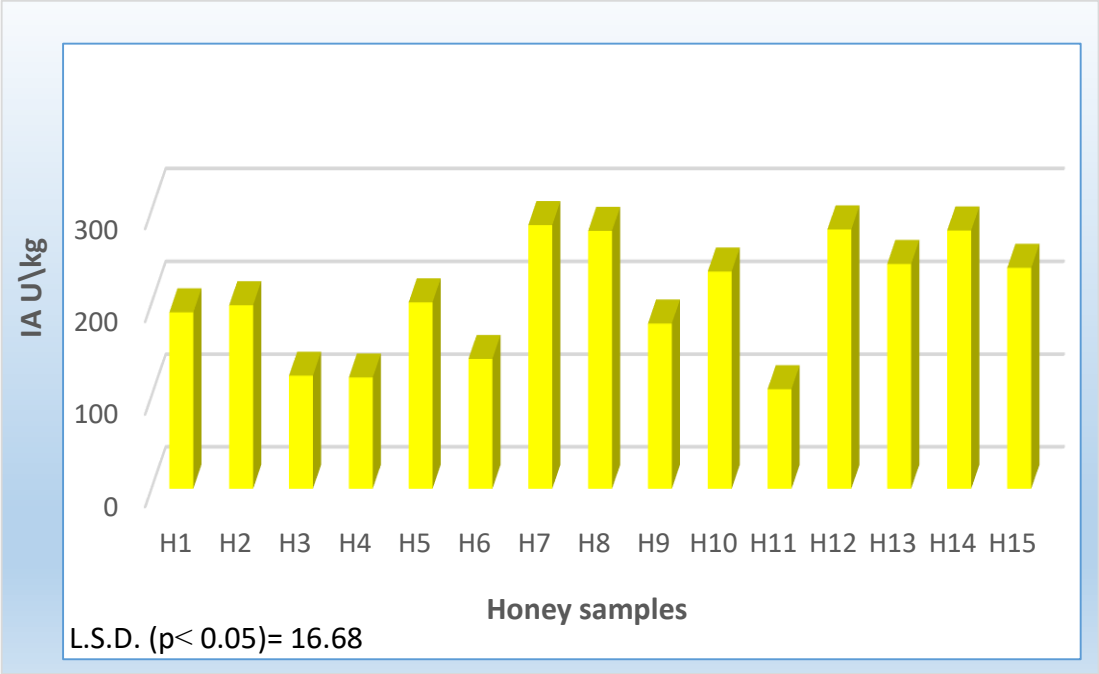

Fig. (4): Invertase Activity in honey samples .

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. In some of them, were similar to with 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) 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 had an effect on 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), reported: 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).

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Hydroxymethylfurfural (HMF)

From Figure (5) 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 it was the lowest in the sample H5 and based on the analysis Statistical: 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).

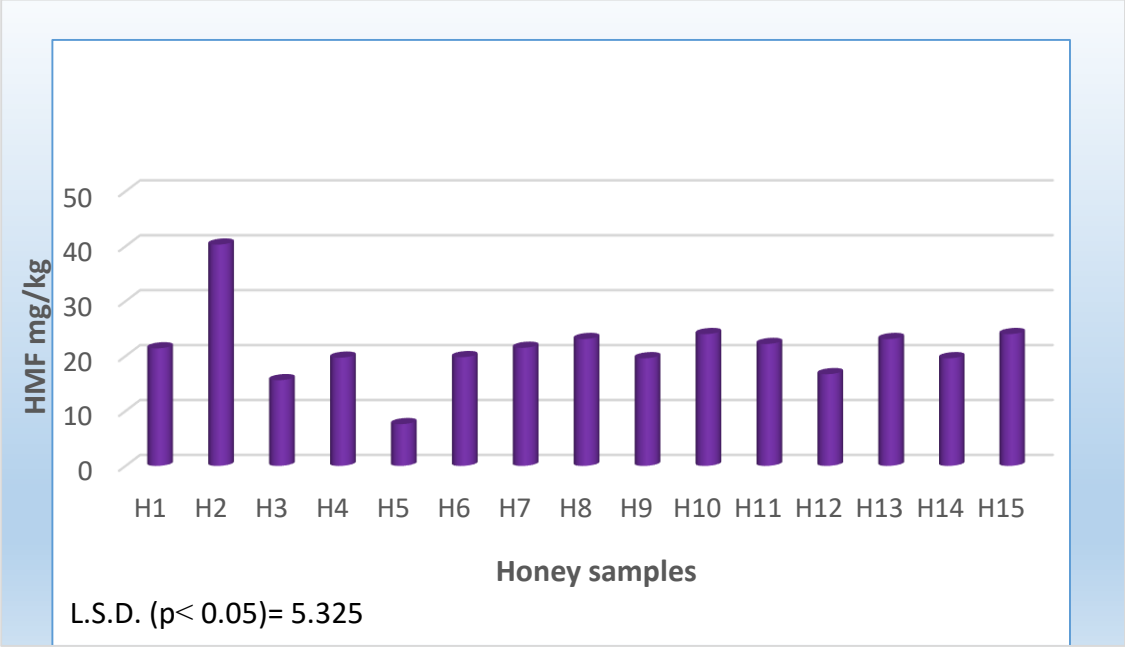

Fig. (5): HMF concentration mg/kg in honey samples .

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), it was 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) found that the content of HMF ranged between 27.10-40.80 mg/kg in his study of evaluating the chemical and physical properties of Ethiopian honey. While Salman *et al* (2019b) found content of HMF was ranage between 12.35-41.54 mg/kg according to producing location.

Vitamin C

Figure (6) shows the results of concentration of vitamin C in studied honey varieties, as significant differences appeared, according to results of statistical analysis at probability level $p<0.05$ between the average content of this vitamin in honey samples, as the maximum concentration reached 305.5 mg/kg in honey sample. H15

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with a significant difference with the rest of the samples, but it did not differ significantly with two samples: H8 and H12, as concentration in both of them reached 303.83 and 300.5 mg/kg, respectively, followed by samples: H6, H7, H11, H10, H5, H3 and H9, as concentrations in them reached: 290.50, 253.83, 248.83, 230.50, 227.17, 218.83, 213.83, 198.83 and 182.17 mg/kg respectively, while the lowest concentration in sample was H2, as vitamin C content in it was 138.83 mg/kg and it did not differ significantly with the samples: H14, H13 and H4: 198.83, 182.17 and 158.83 mg/kg respectively.

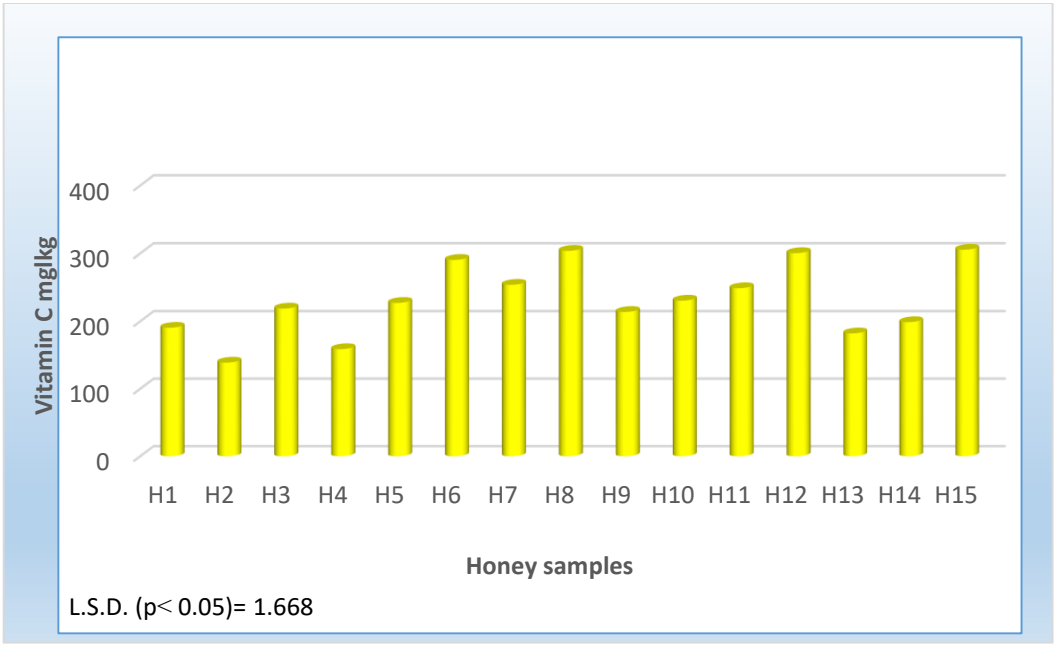

Fig. (6): Vitamin C concentration in honey samples .

The amount of vitamin C in honey can be affected by multiplicity of bee food sources (nectar and pollen), as well as Propolis, which is one of the products of bee colony, as its concentration varies from one species to another, according to the floral and geographical origin, as well as storage periods.

The results are consistent with finding of Yayinie *et al*. (2022) in his study, which included determination of the total antioxidant content in types of Ethiopian honey collected from different geographical areas, as the vitamin C content ranged between 16.23 - 26.59 mg/100 gm of honey. It also converged in part, with what Salman (2019) found, as he estimated vitamin C content in samples of Iraqi honey with different production sites with a range ranging from 190.54 - 350.23 mg/kg and indicated that vitamin C content could give an indication of extent of its relationship to the plant source. The differences in its concentration show extent of the diversity of those sources, the age of honey can be judged by the concentration of the vitamin in it, as it decreases with the passage of time. As for findings of Karimah *et al*. (2020) that honey content of vitamin C varies according to the bee breed, as it reached 326 mg / kg in honey produced from the wild giant bee *Apis dorsata*, while it reached 183 mg / kg in honey produced from *Apis cerana*.

IV. CONCLUSION

Depend on the results were 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 values of hydroxymethylfurfural, proline, electrical conductivity and vitamin C as the indicators depends in determining the purity of honey.

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