

Enzymes Activity Study Of Multiple Types 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 and included the following: The protein content study of the different honey varieties was studied and it was noted that there were significant differences at the $p < 0.05$ level in the percentages of protein. It was noted that the protein percentage ranged between 0.14-0.73% and the highest percentage was in the sample H12 and the lowest in the sample H15.

The results of tests to determine the values of the enzyme activity of honey enzymes (diastase, invertase and glucose-oxidase) showed that there were significant differences for these values between types of honey, 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, while the activity of glucose-oxidase was the highest in the H12 sample amounting to 133.5 micrograms H₂O₂/g/h and the least effective in the H11 sample reaching 72.2 micrograms H₂O₂/g/h.

Keywords: Honey, protein content, enzyme activity

INTRODUCTION

The importance of honey has been known for thousands of years, as it is one of the important nutrients in human life because of its many benefits to his health. It is an important source of sugars, which are the main source of energy, in addition to containing important enzymes that humans need to sustain their life (Bogdanov, 2009). Honey varies according to the diversity of the nectar source, whether it is from flowers, plant secretions or substances from insects, and its biochemical components vary, even if in a small percentage and it is on several types, including: mountain honey, nigella, Jujube, citrus, eucalyptus and clover. Enzymes are the most important and interesting components of honey, which are responsible for converting nectar and live plant secretions into honey and it serves as a sensitive indication for the use of honey as a treatment. Enzyme specifications are a legally binding indicator in some countries (Bogdanov *et al.*, 1999).

The activity of enzymes is involved in the biological value of honey. Other elements also participate in the nutritional value of honey and are one of the factors that affect the color of honey. As natural bee honey must contain some enzymes within its components, including glucose oxidase, which is effective in the case of immature honey or in honey added to water. This enzyme converts glucose sugar into gluconic acid and hydrogen peroxide (Szweda, 2017). The enzymes that are transformed into honey by bees are diastase which digests starch and turns it into maltose which is relatively stable at storage temperature and invertase, whose role lies in the conversion of sucrose to glucose and fructose (Čeksterytė *et al.*, 2020). A source Flowers and environmental conditions affect the nectar content of sugars, including sucrose and this is accompanied by the effect of the invertase enzyme activity, which is produced by the workers, as it was found that the relationship between sucrose concentration and invertase activity is highly symmetric in the honey ripening process (Lichtenberg-Kraag, 2014).

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.

MATERIALS AND METHODS

Varieties of Iraqi honey were collected in a field manner from beekeeping apiaries for a number of Iraqi beekeepers,

representing most of the governorates of Iraq during the year 2021 for the purpose of 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 south	11	Clover	H11
	12	Multiflower	H12
	13	Jujube	H13
	14	Eucalyptus	H14
	15	Thistle	H15

Determination of protein content

Protein content was determined by the Semi-Micro-Kjeldahl method (A.O.A.C, 2012).

Diastase activity Assay

Diastase activity is defined as the amount of enzyme that convert 1% starch to the determined endpoint in one hour at 40°C under experimental conditions. Diastase number (DN) was determined according to (IHC, 2009).

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.

Glucose oxidase activity Assay

Glucose oxidase activity was determined according to the officially prescribed method (White and Subers, 1963). and O-Dianisidine [3,3- Dimethoxybenzidine] was used as substrate.

RESULTS & DISCUSSION

Protein

Figure (1) shows the results of the percentages of protein content in honey, as it was noted that there are significant differences between the average protein content of the studied samples at ($p < 0.05$). The sample H12 recorded the highest protein content 0.73% with a highly significant difference from the rest of the samples. What was found by Kaid (2021) where the average protein content in Jujube honey was 0.7%, followed by samples H14 and H7, with their ratios reaching 0.52% and 0.54% respectively, with a significant difference from the other samples, then honey samples: H5, H9, H8, H10, H13, H4, H11, H6, H1, H2 and H3 where the protein percentages were: 0.42, 0.42, 0.41, 0.39, 0.39, 0.36, 0.33, 0.32, 0.30, 0.19 and 0.16% respectively, while the lowest protein content was in the sample H15 was 0.14% .

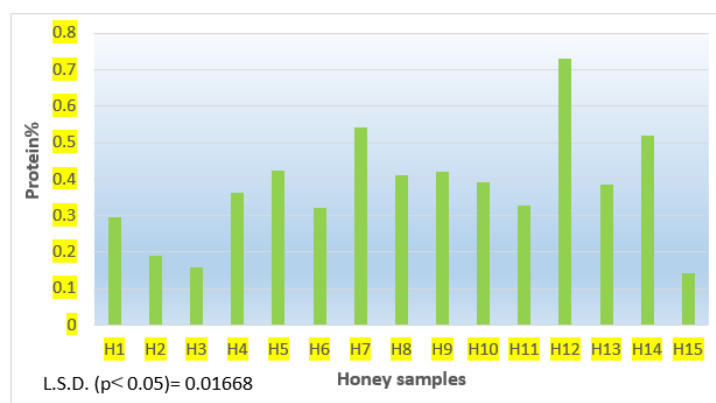


Fig. (1): protein values of honey samples.

The protein content of honey varies depending on different nectar types and geographical regions that bees collect (Alvarez-Suarez *et al.*, 2018). And that the difference in results is due to the difference in the type of plants as well as the methods

used by beekeepers in feeding bees (El-Sohaimy *et al.*, 2015). The results agreed with that of De-Melo *et al.* (2018) found that the percentage of protein in honey ranged between 0.1 - 0.7%. It also came within the range recorded by Da Silva *et al.* (2016) when conducting an analytical study of honey samples with the aim of diagnosing some of the natural components of honey. It was found that the total protein content was in the range of 0.1-1.5%. Whereas, the protein content ranged from 1.73-2.61% in samples of Thai honey of diverse plant and geographical origins (Sommano *et al.*, 2020).

ENZYMES ACTIVITY OF HONEY

Diastase

Figure (2) shows the results of the diastase enzyme activity values, which are expressed by the diastase number (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.

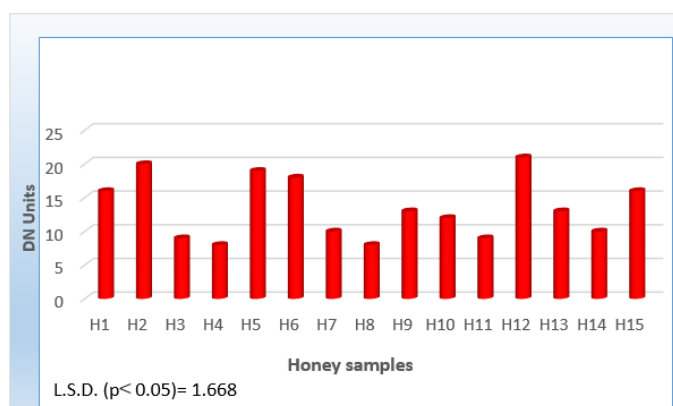


Fig. (2): Diastase Number in honey samples.

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.

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 $DN 9.6 \pm 0.25$ 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 (3) 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.

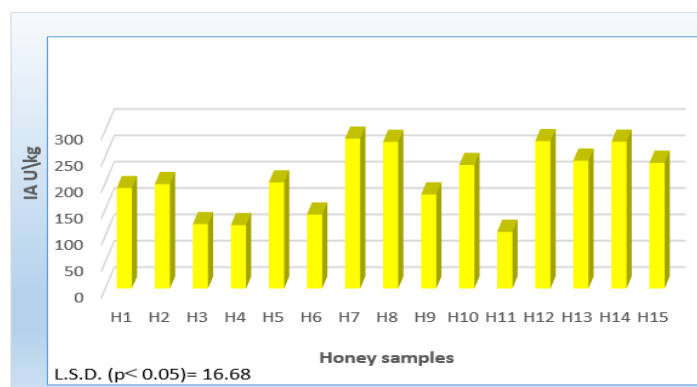


Fig. (3): Invertase Activity in honey samples.

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).

Glucose Oxidase

Figure (4) shows the activity values of glucose oxidase (GOX) enzyme in honey varieties. According to the results of the statistical analysis that there are significant differences at ($p < 0.05$) between the mean values of GOX activity in the studied samples, as the average values for them range between 55.5 - 133.5 $\mu\text{g H}_2\text{O}_2/\text{g honey/h}$, and the H12 gave the highest activity of GOX without significant difference with samples: H10, H9, H7 and H6, where the activity reached: 131.2, 127.1, 127.1 and 122 $\mu\text{g H}_2\text{O}_2/\text{g/h}$. Followed by the samples: H3, H5, H2, H1, H13 and H15 which amounted to: 107, 104.1, 102.6, 98, 95.4 and 92.3 $\mu\text{g H}_2\text{O}_2/\text{g/h}$, while the lowest activity was in the H11 which did not differ significantly. With samples: H14, H8 and H4 whose activity reached: 72.2, 72 and 61.9 $\mu\text{g H}_2\text{O}_2/\text{g/h}$ respectively.

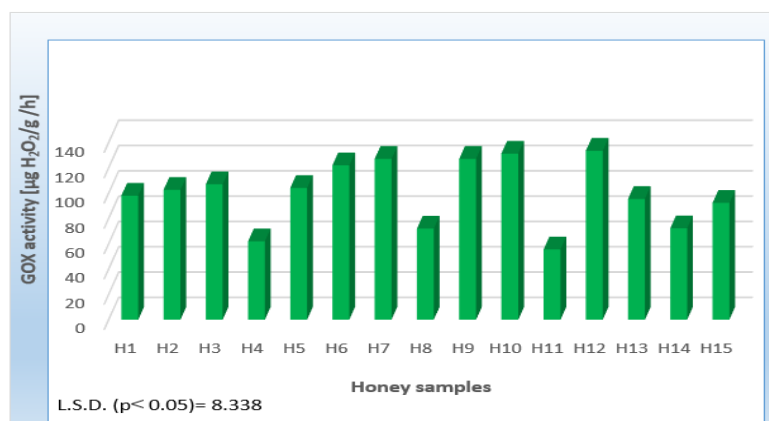


Fig. (4): Glucose Oxidase Activity in honey samples.

The discrepancy in the results of glucose oxidase activity may be due to the fact that the samples may be exposed to heat or sunlight for a long time while extracting honey from bee colonies. Bees are the main source of this enzyme, as it is

secreted from the lingual appendage of the bee and added during pollen collection and formation, which could also be responsible for part of the antibacterial effect of this food. Denisow and Denisow-Pietrzyk (2016). The results are within the range of that of Flanjak *et al.* (2016) when estimating the activity of the GOX enzyme in samples of Croatian honey from various plant sources, and the average activity for each type was 28.2–354.7 $\mu\text{g H}_2\text{O}_2/\text{g/h}$. Also within the range that Strelec (2018) was found in four different samples in order to estimate GOX activity and the extent of H_2O_2 accumulation in Croatian honey. The average activity was: 25.58, 341.26, 350.16, 376.82 and 402.47 $\mu\text{g H}_2\text{O}_2/\text{g/h}$ for each of the following honeys : Buckwheat, chestnut, citrus, honeydew and mint respectively. The mint honey has a significant difference in the enzyme activity compared to the rest of honeys. The results converged in some of them with Badawy *et al.* (2004) who was found that the rate of the enzyme activity It was 0.00 - 95.50 $\mu\text{g H}_2\text{O}_2/\text{g/h}$ in samples of Egyptian honey.

The researcher Sahin *et al.* (2020) found that the enzyme activity ranged between 0.25–70.0 $\mu\text{g H}_2\text{O}_2/\text{g/h}$ for each of the Turkish honeys, which included: flowers, pines and oaks, which were picked at a temperature 45 °C. Invertase and glucose oxidase are secreted by the hypopharyngeal glands of honeybees for the hydrolysis of sucrose and the preservation from microbial effects, respectively. It is also prominent to understand how the levels of invertase and glucose oxidase of raw honey samples are being influenced by different conditions because the behavior of these specific enzymes in raw honeys could be a marker for the quality parameters.

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