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Glucose Oxidase and Catalase Activities in Honey Samples from the Southwestern Region of Saudi Arabia

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Abstract: The activity of honey enzymes are affected by floral and geographical origins, climate conditions, honeybee species, health and nutrition. This article investigated the effect of floral and geographical origins on the activity of glucose oxidase (GOx) and catalase (CAT) enzymes in honey samples from the southwestern region of Saudi Arabia. Moreover, the moisture, total sugars, pH and conductivity were measured as quality parameters. The floral origin of the honey samples was determined microscopically while the quality parameters were measured according to the methods of international honey commission. The activity of the honey enzyme was determined following the instructions of the Megazyme International kits. The obtained results were statistically analyzed by the statistical Package for Social Sciences (SPSS, v.20). The GOx activity of the *Acacia*, *Ziziphus* and polyfloral honey samples of the Asir region were (5.19 ± 2.33 U/g), (4.01 ± 1.17 U/g) and (5.69 ± 1.67 U/g), respectively. The *Acacia*, *Ziziphus* and polyfloral honey samples from the Jazan region had GOx activities of (6.85 ± 0.47 U/g), (10.48 ± 9.22 U/g) and (5.31 ± 2.7 U/g), respectively. The geographical origin significantly affected the GOx activity of *Ziziphus* honey (p-value = 0.005) and the GOx activity of the *Ziziphus* honey was significantly more than that of the polyfloral honey of the Jazan region (p-value = 0.009). With regard to the CAT activity in Asir region honey samples, the mean values of the *Acacia, Ziziphus and* polyfloral honeys were (2.89 ± 1.08 U/g), (3.58 ± 1.59 U/g) and (2.84 ± 1.24 U/g), respectively. The mean values of the CAT activity in the Jazan honey samples were *Acacia* (4.35 ± 1.01 U/g), *Ziziphus* (3.94 ± 0.04 U/g) and polyfloral (3.43 ± 0.67 U/g). The geographical origin significantly affected the CAT activity in *Acacia* honey (p-value = 0.014). The geographical and floral origins had significant effects on the activity of the honey GOx and CAT enzymes.

Keywords: enzymes; floral origin; geographical origin

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1. Introduction

Honeybees (*Apis mellifera*) produce seven products; honey, royal jelly, propolis, bee venom, beeswax, bee bread and pollens. However, honey is the most famous and important among them as it is the only natural sweetener that humans consume without any processing [1–5]. Honey is a unique, sticky and aromatic substance that honeybees make from different sources such as the nectar of plants, from the secretions of the living parts of the plant or from the excretions of plant sucking insects [3,6]. Based on those sources, there are two types of honey named according to the source visited by the bee. The nectar honey comes from the nectar of the flowers and honeydew that is produced when the honeybees feed on the secretions of plants and excretions of plant-sucking insects [6].

The criteria for honey quality according to the international standards for honey are moisture, insoluble solids, sugars (glucose, fructose and sucrose), conductivity, pH, free

acidity and diastase [6]. The quality of honey depends on the botanical and geographical origin. It was proven that the oil composition of the plants depends on its geographical location even if they were from the same species [7]. Because of the effect of environmental conditions on the physiology of plants and chemical composition, there are no two similar types of honey [8,9]. Honey composition depends on several factors such as the type and flow of nectar, the vitality of the bee colony, climate and environmental conditions, seasons, treatment of beekeepers, extraction and storage [10,11].

Honey is well known by its biological and medical activities such as an antiseptic and a natural remedy, antibiotics (antibacterial, antiviral and antifungal) and antioxidants [12–15]. The activity of the honey varies according to the chemical composition of honey, its floral source and geographical location [10,11].

Enzymes are minor components in honey, and they are among the most important and interesting constituents. Being a mark of quality, it gives honey and contributes to its healing and nutritional properties [16–18]. Honey contains four main enzymes: diastase (amylase), invertase (sucrase), glucose oxidase, catalase and acid phosphatase [19]. Enzymes originate from different sources including the honeybees, nectar, pollen grains and microorganisms [20].

GOx is sensitive to light and storage conditions, and it is inactive in mature honey, while it is active in diluted honey due to the easy access of the enzyme to the substrate (glucose). Through the reaction of glucose oxidation, gluconic acid is produced, which is the most representative acid in honey. The hydrogen peroxide is the second product of the GOx. It is the primary and main factor behind the honey antimicrobial activity and has been called "inhibin" because it inhibits microbial growth in honey [16,21].

CAT degrades and hydrolyzes the hydrogen peroxide (H_2O_2) to water and oxygen. The origins of the catalase enzyme in honey are the plants and microbes. The activity of this enzyme varies according to its plant sources and storage conditions [22,23]. CAT detection methods vary, but all depend on the principle of H_2O_2 determination before and during the incubation period [24,25].

The aim of this article was to study and compare honey samples from the Asir and Jazan regions in the southwestern part of Saudi Arabia based on the effect of floral and geographical origins on GOx and CAT activity along with the values of moisture, total sugars, conductivity and pH.

2. Material and Methods

2.1. Honey Samples and Their Floral Origins

Thirty-seven and twenty-one honey samples were collected from the regions of Asir and Jazan, respectively (Figure 1). The Jazan area is approximately at sea level while the samples of the Asir region are from an altitude of 900 m above sea level. Jazan is characterized by the high temperature, high humidity and high barometric pressure compared to the Asir region.

Figure 1. Map of Asir and Jazan regions at the southwestern region of Saudi Arabia. Thirty-seven samples were collected from Asir region and twenty-one from Jazan region.

The floral origin of the honey samples was determined microscopically according to the method of Louveaux et al. [26]. In addition, 3 mL of each honey sample were diluted with 11 mL of distilled water in a 15 mL falcon tube. The content of the falcon tubes was centrifuged at 6000 rpm for 30 min, the pellet was mounted on a light microscope slide, and the pollens were identified and their percentage was determined. The honey sample is considered monofloral if the percentage of one pollen is ≥50%.

2.2. Moisture and Total Sugar Percentages (%)

The procedure of the harmonized methods of the international honey commission (2009) was used to determine the moisture percentage as follows: a small amount of honey was placed to cover the prism of a refractometer (ATC Refractometer for honey; Tiaoyeer, Xindacheng, China), the moisture percentage was read twice, and the mean value was taken as the final result [26].

The total sugar percentage was determined from the ATC refractometer reading screen.

2.3. Electrical Conductivity (mS/cm)

Two grams of each honey sample were dissolved in 10 mL distilled water (20%, W/V), and the conductivity was measured twice at a constant temperature of 20 ± 0.5 °C using conductivity meter (Hanna HI9811-5 Portable pH/EC/TDS/°C Meter, Phoenix, AZ, USA). The conductivity meter was calibrated with a ready-made KCl solution with a conductivity of 1413 µS/cm [26]. The average value of the two conductivities was considered as the final result of the conductivity.

2.4. pH

To the honey solution prepared to measure the conductivity, an extra 5 mL of distilled water were added to reach the dilution of 13.3% (*w*/*v*). The pH of the honey solution was measured twice using a pH meter at a constant temperature of 20 ± 0.5 °C (Hanna HI9811-5 Portable pH/EC/TDS/°C Meter, Phoenix, AZ, USA). The pH meter was calibrated using two ready-made buffer solutions of pH 4 and 7. The mean values of the two pH readings were taken as the sample result [26].

2.5. GOx Activity (U/g)

The GOx activity was determined according to the instructions of the megazyme international company kit (GOx kit number: 200221-8).

The GOx kit principle is the glucose oxidase reaction with the glucose in honey samples and with standard solution of glucose to produce (hydrogen peroxide). The product of the first reaction (hydrogen peroxide) reacts with peroxidase (POD) and p-hydroxybenzoic acid + 4-aminoantipyrine (chromogen) so as to produce a colored dye (quinoneimine). The dye density is measured spectrophotometrically at 500 nm. One gram of each honey sample was dissolved in 2 mL of distilled water (50%), and 0.5 mL of each sample solution was transferred to a test tube. In addition, 0.5 mL of standard glucose solution (90 mg/mL) was pippetted in a second tube and 2 mL of POD were blended with the glucose standard, and their absorbance was determined at 510 nm (A1) against a blank tube with 0.5 mL of water and 2 mL of POD. The contents of the sample tube were poured into the second tube contents, stirred, kept for 20 min at room temperature and their absorbance was measured at the same wavelength (A2). Standard GOx solutions were used to create a standard curve (0.0, 0.38, 0.75, 1.5, 3.0 and 6.0 U/mL). A straight line equation was created, and the GOx activity of the honey samples was determined using the Excel program and multiplied by 2 to obtain the activity in 1 g (U/g).

2.6. CAT Activity

CAT catalyzes the degradation of hydrogen peroxide to water and oxygen. CAT activity was evaluated following the decrease in hydrogen peroxide concentration after incubation of the honey sample with a standard solution of H_2O_2. The CAT activity was

measured following the instructions of the megazyme international company kit (CAT kit number: 200206-6). The kit contains two separate reactions: (1) The CAT of the honey sample is incubated with a standard solution of H_2O_2 for 5 min. Then, the enzyme is strongly inhibited by adding sodium azide solution. (2) The remaining H_2O_2 is degraded by POD in the presence of 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS) and 4-aminoantipyrine (AAP) to produce a complex dye (quinoneimine), which is measured at a wavelength of 520 nm.

One gram of each honey sample was diluted in 2 mL of distilled water (50%). In addition, 0.05 mL from each honey sample was added to 0.05 mL of a standard solution of H_2O_2 (130 mM) in a test tube and incubated for 5 min at 25 °C. After that, 0.9 mL of sodium azide solution (15 mM) was added to stop the reaction. Immediately after stopping the reaction, 0.04 mL of the mentioned tube was transferred to a spectrophotometer cuvette. To the cuvette, 3 mL of peroxidase solution (POD+ DHB+ AAP) were added and the contents were mixed and incubated for 15 min at 25 °C. Finally, the absorbance was read at 520 nm against a blank tube containing 0.05 mL of phosphate buffer (150 mM Potassium phosphate, pH 7.0) instead of the honey sample. For calculating the CAT activity in the different honey samples, a standard curve was created using CAT with different activities (0.0, 0.4, 0.8, 1.6, 3.2 and 6.4 U/mL) and following the same procedure as the honey and blank samples. A straight line equation was created using the Excel program, and the CAT activities were calculated following the line equation and multiplied by 2 (sample dilution factor) to obtain the activity of CAT in U/g of honey.

2.7. Statistical Analysis

The obtained results were statistically analyzed using the *t*-test and ANOVA of the Statistical Package of Social Sciences (SPSS). The difference between the compared results of the studied parameters was considered significant at the level of ≤ 0.05. Moreover, the agglomerative hierarchical clustering analysis for the results was carried out.

3. Results

3.1. Results of Floral Origin

The microscopic analysis of the honey pollens showed that the honey samples were *Acacia* (25), *Ziziphus* (6) and polyfloral (27) (Figure 2). The number of the *Acacia*, *Ziziphus* and polyfloral honey samples of Asir region were 21, 4 and 12, respectively. The numbers of the Jazan honey samples were *Acacia* (4), *Ziziphus* (2) and polyfloral (15).

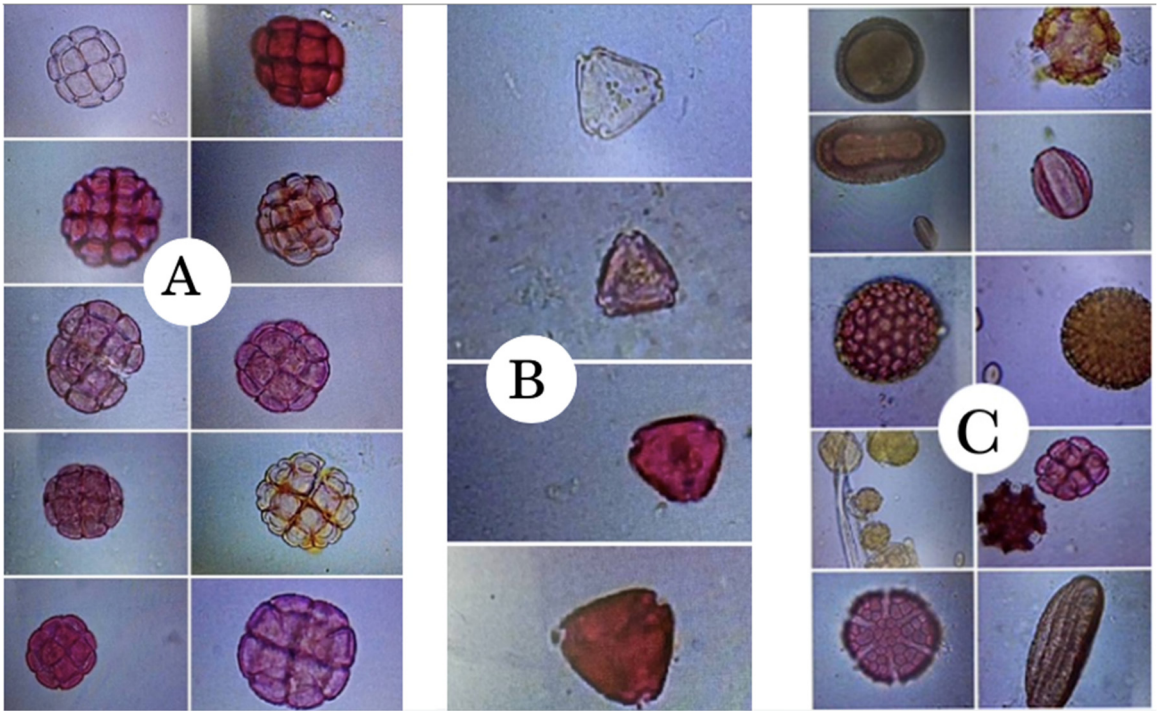

Figure 2. Representative pollens for the floral origins of the studied honey samples. The studied honey samples were of three floral origins: Acacia (**A**), Ziziphus (**B**) and polyfloral (**C**).

3.2. Results of the Studied Parameters

Table 1 presents the results of the studied parameters, and Table 2 summarizes the significant effects of the floral and geographical origins on the studied analytes.

Table 1. The results of the studied parameters.

		N	Mean	Std. Deviation	Minimum	Maximum
Moisture %	Ziziphus Asir	4	17.2500	1.84842	14.50	18.50
	Ziziphus Jazan	2	17.0500	0.07071	17.00	17.10
	Acacia Asir	21	16.5814	1.89471	13.95	20.25
	Acacia Jazan	4	14.7125	0.41105	14.25	15.15
	Polyfloral Asir	12	17.6750	1.50038	15.40	19.60
	polyfloral Jazan	15	17.2400	1.92977	14.20	19.50
	Total	58	16.9114	1.82670	13.95	20.25
Sugars %	Ziziphus Asir	4	80.4375	1.78390	79.00	83.00
	Ziziphus Jazan	2	81.3750	0.17678	81.25	81.50
	Acacia Asir	21	81.9643	2.25450	77.50	84.50
	Acacia Jazan	4	83.7125	0.40285	83.25	84.05
	Polyfloral Asir	12	80.8250	1.99437	78.50	84.00
	polyfloral Jazan	15	81.1633	1.96891	78.60	84.40
	Total	58	81.5164	2.07556	77.50	84.50
Conductivity µS/cm	Ziziphus Asir	4	651.2500	412.88366	35.00	910.00
	Ziziphus Jazan	2	595.0000	304.05592	380.00	810.00
	Acacia Asir	21	622.3810	324.79078	170.00	1390.00
	Acacia Jazan	4	475.0000	233.02360	130.00	630.00
	Polyfloral Asir	12	766.6667	423.52060	210.00	1420.00
	polyfloral Jazan	15	486.0000	136.84193	160.00	720.00
	Total	58	607.8448	316.78361	35.00	1420.00

Table 1. *Cont.*

		N	Mean	Std. Deviation	Minimum	Maximum
pH	Ziziphus Asir	4	4.7750	0.81803	3.80	5.50
	Ziziphus Jazan	2	5.4500	2.47487	3.70	7.20
	Acacia Asir	21	4.5667	0.55528	3.70	5.70
	Acacia Jazan	4	4.6000	0.27080	4.20	4.80
	Polyfloral Asir	12	4.5292	0.76410	3.70	6.00
	polyfloral Jazan	15	3.9400	0.47026	3.60	4.70
	Total	58	4.4440	0.73521	3.60	7.20
GOX U/g	Ziziphus Asir	4	4.0085	1.16655	3.02	5.70
	Ziziphus Jazan	2	10.4800	9.22067	3.96	17.00
	Acacia Asir	21	5.1934	2.33261	2.24	10.47
	Acacia Jazan	4	6.5800	0.46847	5.92	6.96
	Polyfloral Asir	12	5.6886	1.67169	3.44	9.02
	polyfloral Jazan	15	5.3100	2.70043	3.00	11.70
	Total	58	5.5222	2.64076	2.24	17.00
CAT U/g	Ziziphus Asir	4	3.5828	1.58816	1.22	4.68
	Ziziphus Jazan	2	3.9404	0.04295	3.91	3.97
	Acacia Asir	21	2.8909	1.07980	0.91	4.89
	Acacia Jazan	4	4.3531	1.01113	3.52	5.59
	Polyfloral Asir	12	2.8409	1.24159	1.44	5.47
	polyfloral Jazan	15	3.4265	0.67140	2.08	4.63
	Total	58	3.2038	1.09661	0.91	5.59

Table 2. The significant effects of the floral and geographical origins on the studied parameters.

Parameter	Honey Samples		*p*-Value
Moisture	Acacia Jazan	polyfloral Jazan	0.013
Sugars	Acacia Jazan	polyfloral Jazan	0.028
	Polyfloral Asir	polyfloral Jazan	0.025
pH	Ziziphus Jazan	polyfloral Jazan	0.005
	Polyfloral Asir	polyfloral Jazan	0.030
Glucose oxidase	Ziziphus Asir	Ziziphus Jazan	0.005
	Ziziphus Jazan	polyfloral Jazan	0.009
Catalase	Acacia Asir	Acacia Jazan	0.014

The floral origin in the Jazan region significantly affected the moisture, sugars, pH and the glucose oxidase while the geographical origin significantly affected the sugars, pH, GOx and CAT.

3.3. Hierarchical Clustering of the Honey Samples

The agglomerative hierarchical clustering was carried out to classify the honey samples according to the results of the studied parameters. Moreover, the classification according to the results of the studied parameters is compared to the classification of the floral and geographical origins (Figure 3).

The agglomerative clustering showed that there were two second level clusters. The first one clustered five samples containing two *Acacia* from the Asir region, one *Acacia* honey from Jazan, one polyfloral honey from Jazan and one *Ziziphus* honey from Asir region. The first cluster of level 2 grouped *Acacia* and ploy floral honey samples from the Asir and Jazan regions. However, one *zizphus* honey sample from the Asir region was classified with the *Acacia* honey samples, which may be due to the presence of *Acacia* pollens in the

honey. The second cluster of level one contained eleven honey samples containing four *Acacia* from Asir region, one *Acacia* from Jazan region, two polyfloral from Asir region and four polyfloral from Jazan region. The second cluster of level 2 classified some *Acacia* and polyfloral honey samples irrespective of their geographical origin. There is a presence of polyfloral honey samples with the *Acacia* honey samples because they contain *Acacia* pollens. The conclusion of level 2 classification is that the studied parameters can be used for the prediction of the floral origin (Figure 3).

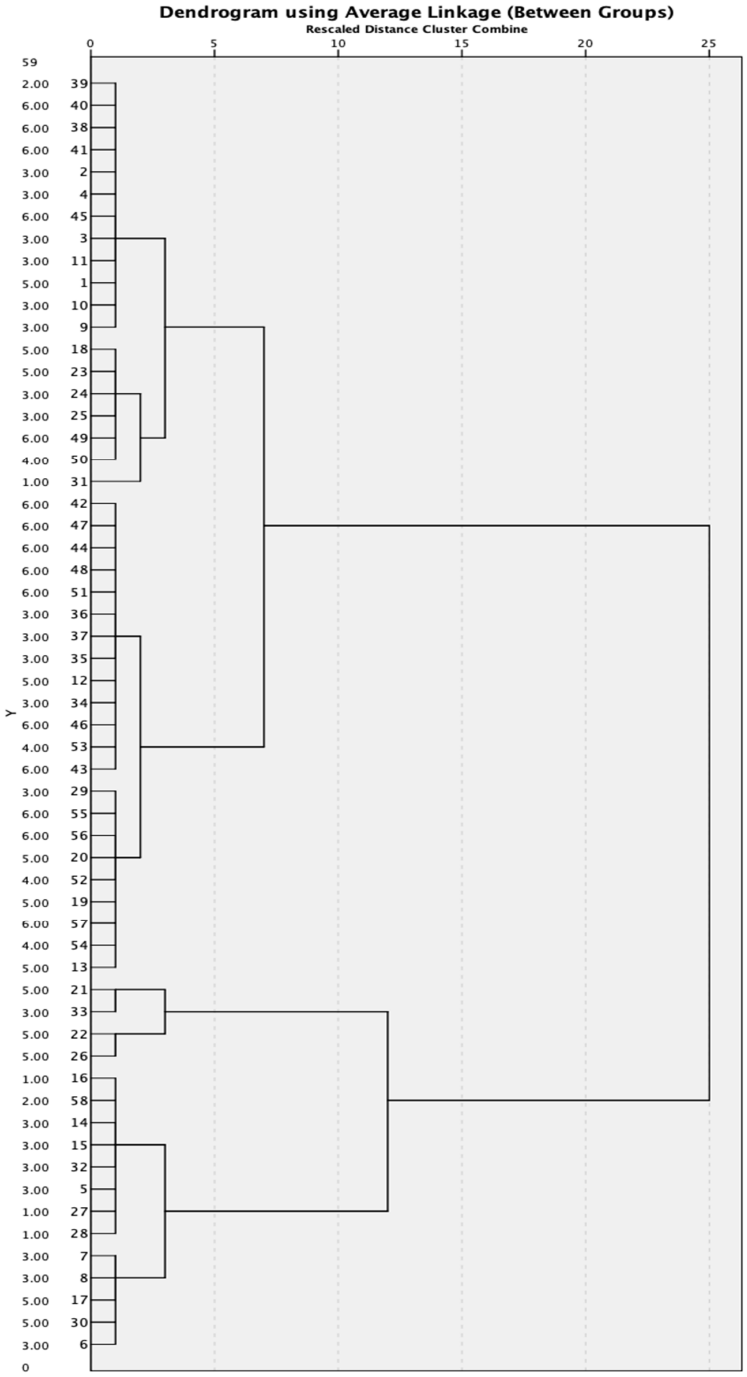

Figure 3. Agglomerative hierarchical clustering of the studied honey samples. The clustering was carried out to investigate the possibility of using the results of the studied parameters to predict the floral and geographical origins of the honey samples.

Level three contained three clusters with different numbers of honey samples. The first cluster was composed of 12 honey samples while the second cluster contained three honey samples. The third cluster was composed of seven honey samples. The samples of the first cluster were six *Acacia* samples from Asir region, one *Acacia* from Jazan region, three poly floral from Asir region, one polyfloral from Jazan and one *Ziziphus* from the Asir region. The first cluster classified the honey samples according to their floral origin except for one sample (*Ziziphus*). The second cluster of level three contained three samples, two ploy floral and one *Acacia* honey from the Asir region. The second cluster classified the honey samples according to their geographical origin. The third cluster of level 3 classified the honey samples according to their geographical origin since it was composed of seven honey samples from the Asir region. However, the seven honey samples were five *Acacia* honey and two *Ziziphus* honey samples (Figure 3).

Level four was composed of one group containing group one and two of level two, and group one of level three besides some other honey samples. The level four cluster contained 29 honey samples. The 29 honey samples were 12 *Acacia* honey from Asir region, five polyfloral from Asir, one *Ziziphus* from Asir, two *Acacia* from Jazan and nine polyfloral from Jazan. Level 4 classified the honey samples according to their floral and geographical origin (Figure 3).

Level five was composed of one cluster containing 14 honey samples. The honey samples were seven *Acacia* from Asir, three *Ziziphus* from Asir region, three polyfloral from the Asir region and one *Ziziphus* from the Jazan region. Level 5 clustered 13 honey samples according to their geographical origin (Asir region (Figure 3)).

Level six involved the clusters of level 4, level 5 and five samples (48 honey samples). Level six classified the honey samples according to their floral and geographical origins (Figure 3).

Ten samples were not involved in the different clusters: three *Acacia* Asir, two polyfloral Asir, four polyfloral Jazan and one *Ziziphus* Jazan (Figure 3).

4. Discussion

The studied quality parameters (moisture, pH and conductivity) were within the ranges of the Codex standards [6]. The moisture was significantly affected by the floral origin (*Acacia* vs. polyfloral) in the honey samples of the Jazan region. The total sugars are affected by the floral origin in the Jazan region (*Acacia* vs. polyfloral) and the geographical origin (polyfloral Jazan versus polyfloral Asir) [27]. The floral origin significantly affected the pH of the honey samples from the Jazan region (*Ziziphus* vs. polyfloral), whereas the geographical origin had a significant effect on the pH of the polyfrloal honey (Jazan vs. Asir). The geographical origin significantly affected the GOx activity of the *Ziziphus* honey samples and CAT activity of the *Acacia* honey samples. The GOX activity of the polyfloral honey samples was significantly different compared to its activity in the *Ziziphus* honey samples of the Jazan region.

The range of the moisture percentage in the honey samples of this study was (13.95–20.25%). The results were within the moisture range of honey according to the Codex standards [6] and according to the guide to the production, trading and import of honey and bee products issued by the Saudi Food and Drug Authority [28]. The previously published articles showed that the moisture percentage of the Saudi honey was ranging from 8.8–18.5% [29–31]. This study found that the floral origin in the Jazan region had a significant effect on the percentage of moisture similar to the findings of Corbella and Cozzolino [32] and Khan et al. [33].

The range of the pH of the honey samples was (3.6–7.2). The upper limit of the pH range was slightly higher than the previously reported pH values in Saudi Arabia (5.7) [31,33]. The US National Honey Board adopted a pH range for honey samples from 3.4 to 6.1 [34]. With regard to the effect of floral and geographical origin on the pH of honey, this study reported that the *Ziziphus* honey of the Jazan region had significantly increased pH value compared to the polyfloral honey of Jazan. The polyfloral honey of Asir region had significantly increased pH value compared to the pH of the polyfloral honey from the

Jazan region. Similar to our findings, Khan et al. [33] and Mohammed et al. [31] reported significant effects of the floral and geographical origins on the value of the honey pH.

Concerning the concentration of total sugars in honey, the range of the total sugars in the study samples was (77.5–84.5%). Similar to our findings, Buba et al. found that the range of the total sugars in Nigerian honey samples was (77.60–86.20) [35]. Other studies reported different total sugar ranges such as (62.85–77.39), (62.24–77.26), (64.99–72.54) and (69.76–79.92) [36]. Baloš et al. [36] concluded that the sugar concentration in honey samples from Serbia can be used to differentiate between honey samples of different plant origins. The total sugars of honey are affected by the geographical origin of the honey samples [37]. However, this study reported significant effects of the floral and geographical origins on the concentration of total sugars in honey.

The conductivity range of the honey samples of this study was (35–1420 µS/cm) and the floral and geographical origins had insignificant effects on the conductivity values. According to the Codex Alimentarius [6], the conductivity of honey depends on the floral origin; some honey samples have conductivity of not more than 800 µS/cm, while others have conductivity of not less than 800 µS/cm. One previous study reported very high conductivity values for honey samples from Egypt and Yemen ranging from 0.53 ± 0.03 to 4.18 ± 0.05 mS/cm [1].

The range of the GOx activity in the studied honey samples was (2.24–17.00 U/g). The unit of enzyme activity (U) is defined as the amount of the enzyme that converts micromoles of substrate to a product per minute (µmol/min) [38]. We were not able to compare our results to the previous findings of the published articles due to the difference in the measurement units. Strelec et al. [16] and Sahin et al. [39] measured the activity of GOx in micrograms of the hydrogen peroxide produced in one hour per gram of honey, while Bucekova et al. [19] measured the amount of the GOx enzyme in micrograms per gram of honey. The GOx activity of the *Ziziphus* honey samples from the Asir and Jazan regions was significantly different and the *Ziziphus* honey of the Jazan region was significantly increased compared to the polyfloral honey of Jazan. The conclusion of this study is that the floral and geographical origin had significant effects on the GOx activity. Similarly, Bucekova et al. [13], Strelec et al. [16], and Belay et al. [18] reported that the floral origin had significant effects on the activity of honey enzymes including the GOx.

This study found that the range of the CAT enzyme activity in the studied honey samples was (0.91–5.59 U/g). Schepartz and Subers [40] studied the CAT activity in different honey samples and found that the enzyme activity depends on the floral origin and ranges from 0–17 g/min [40]. Fourteen Brazilian honey samples from different floral origins were studied and their CAT activity was ranging from 9.97 to 99.07 U/mg [41].

The significant effect of the floral origin on the studied parameters in the Jazan region may be due to its climate conditions such as the high temperature, high humidity and barometric pressure.

The agglomerative hierarchical clustering showed the possibility of using the studied parameters' results to predict the floral and geographical origins of the honey samples. Many previous studies proved that the results of honey analysis are useful for the prediction of the floral and geographical origins of honey samples [42–44].

This study suffers from the small number of samples in the subgroups, but it opens windows to conduct future studies on the activities of the GOx and CAT enzymes. Moreover, measurement of the hydrogen peroxide is highly recommended.

5. Conclusions

Concerning the effect of the geographical origin on the studied parameters, it significantly affected the total sugars, pH and the activity GOx and CAT in the honey samples. Specifically, the polyfloral honey of Asir region was characterized by significantly high pH and low total sugars while its *Ziziphus* honey had significantly low GOx activity. Furthermore, the *Acacia* honey from the Asir region was with significantly low CAT activity compared to the *Acacia* honey from the Jazan region. The effect of the geographical origin

may be due to the fact that the Asir and Jazan regions are characterized by different climate conditions due to the different altitude levels.

The floral origin had significant effects on the studied parameters of the Jazan region while the floral origin in Asir region had no significant effect on the studied parameters. The *Acacia* honey of Jazan was characterized by significantly low moisture and high total sugars compared to the polyfloral honey. The *Ziziphus* honey compared to the polyfloral honey of Jazan was characterized by significantly high pH and GOx activity.

As the floral origin had significant effects on the studied parameters of Jazan honey only, it can be concluded that the effect of the floral origin of honey on the studied parameters is regulated by the geographical origin and its climate conditions.

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References

1. El Sohaimy, S.A.; Masry, S.H.D.; Shehata, M.G. Physicochemical characteristics of honey from different origins. *Ann. Agric. Sci.* **2015**, *60*, 279–287. [CrossRef]
2. De Sousa, J.M.B.; de Souza, E.L.; Marques, G.; de Toledo Benassi, M.; Gullón, B.; Pintado, M.M.; Magnani, M. Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *LWT Food Sci. Technol.* **2016**, *65*, 645–651. [CrossRef]
3. de Almeida-Muradian, B.; Barth, O.M.; Dietemann, V.; Eyer, M.; Freitas, A.D.S.D.; Martel, A.-C.; Marcazzan, G.L.; Marchese, C.M.; Mucignat-Caretta, C.; Pascual-Maté, A.; et al. Standard methods for Apis mellifera honey research. *J. Apic. Res.* **2020**, *59*, 1–62. [CrossRef]
4. Desissa, Y. Detection of the electrical conductivity and acidity of honey from different areas of Tepi. *Food Sci. Technol.* **2014**, *2*, 59–63.
5. Martinello, M.; Mutinelli, F. Antioxidant Activity in Bee Products: A Review. *Antioxidants* **2021**, *10*, 71. [CrossRef] [PubMed]
6. Codex Alimentarius. Standard for Honey CXS 12-1981. Adopted in 1981. Revised in 1987, 2001. Amended in 2019. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B12-1981%252Fcxs_012e.pdf (accessed on 26 July 2022).
7. Monika, T.; Tarapatskyy, M.; Dżugan, M. The influence of geographical origin on honey composition studied by Polish and Slovak honeys. *Czech J. Food Sci.* **2019**, *37*, 232–238.
8. Irish, J.; Blair, S.; Carter, D.A. The antibacterial activity of honey derived from Australian flora. *PLoS ONE* **2011**, *6*, e18229. [CrossRef] [PubMed]
9. Bilandžić, N.; Sedak, M.; Đokić, M.; Bošković, A.G.; Florijančić, T.; Bošković, I.; Kovačić, M.; Puškadija, Z.; Hruškar, M. Element content in ten Croatian honey types from different geographical regions during three seasons. *J. Food Compost. Anal.* **2019**, *84*, 103305. [CrossRef]
10. El Sohaimy, S.A.; Masry, S.H.D.; Mohamed, G.; Rashad, H. Evaluation of functional properties of local and imported honey in Egypt. *Am.-Eurasian J. Agric. Environ. Sci.* **2015**, *15*, 1147–1154.
11. Mohammed, M.E.A. Factors Affecting the Physicochemical Properties and Chemical Composition of Bee's Honey. *Food Rev. Int.* **2022**, *38*, 1330–1341. [CrossRef]
12. Meo, S.A.; Al-Asiri, S.A.; Mahesar, A.L.; Ansari, M.J. Role of honey in modern medicine. *Saudi J. Biol. Sci.* **2017**, *24*, 975–978. [CrossRef]
13. Bucekova, M.; Jardekova, L.; Juricova, V.; Bugarova, V.; Marco, G.D.; Gismondi, A.; Leonardi, D.; Farkasovska, J.; Godocikova, J.; Laho, M.; et al. Antibacterial Activity of Different Blossom Honeys: New Findings. *Molecules* **2019**, *24*, 1573. [CrossRef] [PubMed]

14. Adgaba, N.; Al-Ghamdi, A.; Sharma, D.; Tadess, Y.; Alghanem, S.M.; Khan, A.K.; Mohammed, J.A.; Mohamed, G.K.A. Physico-chemical, antioxidant and anti-microbial properties of some Ethiopian mono-floral honeys. *Saudi J. Biol. Sci.* **2020**, *27*, 2366–2372. [CrossRef] [PubMed]
15. Tafere, D.A. Chemical composition and uses of Honey: A Review. *J. Food Nutr. Res.* **2021**, *4*, 194–201.
16. Strelec, I.; Crevar, B.; Kovač, T.; Rajs, B.B.; Primorac, L.; Flanjak, I. Glucose oxidase activity and hydrogen peroxide accumulation in Croatian honeys. *CJFST* **2018**, *10*, 33–41. [CrossRef]
17. Rossano, R.; Larocca, M.; Polito, T.; Perna, A.M.; Padula, M.C.; Martelli, G.; Riccio, P. What Are the Proteolytic Enzymes of Honey and What They Do Tell Us? A Fingerprint Analysis by 2-D Zymography of Unifloral Honeys. *PLoS ONE* **2012**, *7*, e49164. [CrossRef]
18. Belay, A.; Haki, G.D.; Birringer, M.; Borck, H.; Lee, Y.-C.; Kim, K.-T.; Baye, K.; Melaku, S. Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. *J. Food Sci. Technol.* **2017**, *54*, 2769–2778. [CrossRef] [PubMed]
19. Samarghandian, S.; Farkhondeh, T.; Samini, F. Honey and Health: A Review of Recent Clinical Research. *Pharmacogn. Res.* **2017**, *9*, 121–127.
20. Weston, R.J. The contribution of catalase and other natural products to the antibacterial activity of honey: A review. *Food Chem.* **2000**, *71*, 235–239. [CrossRef]
21. Albaridi, N.A. Antibacterial Potency of Honey. *Int. J. Microbiol.* **2019**, *2019*, 2464507. [CrossRef]
22. Gillette, C.C. Honey catalase. *J. Econ. Entomol.* **1931**, *24*, 605–606. [CrossRef]
23. Brudzynski, K. A current perspective on hydrogen peroxide production in honey. A review. *Food Chem.* **2020**, *332*, 127229. [CrossRef]
24. Huidobro, J.F.; Sánchez, M.P.; Lorenzo, S.M.; Sancho, M.T. Precise method for the measurement of catalase activity in honey. *J. AOAC Int.* **2005**, *88*, 800–804. [CrossRef] [PubMed]
25. Kadhum, M.A.; Hadwan, M.H. A precise and simple method for measuring catalase activity in biological samples. *Chem. Pap.* **2021**, *75*, 1669–1678. [CrossRef]
26. Louveaux, J.; Maurizio, A.; Vorwohl, G. Methods of melissopalynology. *Bee World* **1978**, *59*, 139–157. [CrossRef]
27. International Honey Commission. *Harmonized Methods of the International Honey Commission*; 2009; pp. 1–63. Available online: https://ihc-platform.net/ihcmethods2009.pdf (accessed on 26 July 2022).
28. Saudi Food and Drug Authority. *Guide to the Production, Trading and Import of Honey and Bee Products*; Saudi Food and Drug Authority Publications: Riyadh, Saudi Arabia, 2021; pp. 14–18. Available online: https://sfda.gov.sa/sites/default/files/2021-03/%D8%AF%D9%84%D9%8A%D9%84%20%D8%A7%D9%84%D8%B9%D8%B3%D9%84%202021.pdf (accessed on 26 July 2022).
29. Mesallam, A.S.; EI-Shaarawy, M.I. Quality Attributes of Honey in Saudi Arabia. *Food Chem.* **1987**, *25*, 1–11. [CrossRef]
30. Al-Ghamdi, A.; Mohammed, S.E.A.; Ansari, M.J.; Adgaba, N. Comparison of physicochemical properties and effects of heating regimes on stored Apis mellifera and Apis florea honey. *Saudi J. Biol. Sci.* **2019**, *26*, 845–848. [CrossRef] [PubMed]
31. Mohammed, M.E.A.; Almadawi, A.; Alfifi, A.; Alfaifi, M.Y.; Elbehairi, S.E.I.; Al-bushnaq, H.A. Some physiochemical properties of Acacia honey from different altitudes of the Asir Region in Southern Saudi Arabia. *Czech J. Food Sci.* **2017**, *35*, 321–327. [CrossRef]
32. Corbella, E.; Cozzolino, D. Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics. *LWT Food Sci. Technol.* **2006**, *39*, 534–539. [CrossRef]
33. Khan, K.A.; Ghramh, H.A.; Babiker, M.; Ahmad, Z.; El-Niweiri, M.A.A.; Ibrahim, E.H.; Brima, E.I.; Mohammed, M.E.A. Tolerance of Ziziphus and Acacia honeys to one year storage conditions and altitude. *J. King Saud Univ. Sci.* **2021**, *33*, 101577. [CrossRef]
34. National Honey Board. Honey: A Reference Quide to Nature's Sweetener. Firestone. 2005. Available online: https://honey.com/files/general/refguide.pdf (accessed on 26 July 2022).
35. Buba, F.; Gidado, A.; Shugaba, A. Analysis of Biochemical Composition of Honey Samples from North-East Nigeria. *Biochem. Anal. Biochem.* **2013**, *2*, 139.
36. Baloš, M.M.Ž.; Popov, N.S.; Radulović, J.Z.P.; Stojanov, I.M.; Jakšić, S.M. Sugar profile of different floral origin honeys from Serbia. *J. Apic. Res.* **2020**, *59*, 398–405. [CrossRef]
37. Agus, A.; Umami, N. The sugar content profile of honey produced by the Indonesian Stingless bee, Tetragonula laeviceps, from different regions. *Livest. Res. Rural Dev.* **2019**, *31*, 91.
38. Labuda, J.; Bowater, R.P.; Fojta, M.; Gauglitz, G.; Glatz, Z.; Hapala, I.; Havliš, J.; Kilar, F.; Kilar, A.; Malinovská, L.; et al. Terminology of bioanalytical methods (IUPAC Recommendations 2018). *Chem. Int.* **2018**, *40*, 34. [CrossRef]
39. Sahin, H.; Kolayli, S.; Beykaya, M. Investigation of Variations of Invertase and Glucose Oxidase Degrees against Heating and Timing Options in Raw Honeys. *J. Chem.* **2020**, *2020*, 5398062.
40. Schepartz, A.I.; Subers, M.H. Catalase in Honey. *J. Apic. Res.* **1966**, *5*, 37–43.
41. de Abreu Franchini, R.A.; Costa Matos, M.A.; Matos, R.C. Amperometric Determination of Catalase in Brazilian Commercial Honeys. *Anal. Lett.* **2011**, *44*, 232–240. [CrossRef]
42. Maione, C.; Barbosa, F.; Barbosa, R.M. Predicting the botanical and geographical origin of honey with multivariate data analysis and machine learning techniques: A review. *Comput. Electron. Agric.* **2019**, *157*, 436–446.
43. She, S.; Chen, L.; Song, H.; Lin, G.; Li, Y.; Zhou, J.; Liu, C. Discrimination of geographical origins of Chinese acacia honey using complex 13C/12C, oligosaccharides and polyphenols. *Food Chem.* **2019**, *272*, 580–585. [CrossRef] [PubMed]
44. Drivelos, S.A.; Danezis, G.P.; Halagarda, M.; Popek, S.; Georgiou, C.A. Geographical origin and botanical type honey authentication through elemental metabolomics via chemometrics. *Food Chem.* **2012**, *338*, 127936. [CrossRef]