



Hydrogen Peroxide and Dicarbonyl Compounds Concentration in Honey Samples from Different Botanical Origins and Altitudes in the South of Saudi Arabia

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Abstract

Different factors are known to affect the chemical constituents of the bees honey including the climate and the floral origin. The objective of this study was to investigate the effect of floral origin and altitude on some physiochemical properties of honey samples from the south western part of Saudi Arabia. The investigated physiochemical properties were the pH, conductivity and the concentration of hydrogen peroxide and dicarbonyl molecules. Fifteen *Ziziphus spina christi* honey samples were collected from three different altitudes in Asir Region (113, 317 and 511 meters above sea level) and twenty five *Acacia* spp honey samples (14, 113, 567, 576 and 2247 meters above sea level). Honey pollens were analyzed to confirm the botanical origin of the honey samples. The pH and conductivity of the honey were determined and compared to three honey standards (Gulf countries standards, CODEX and USA national honey board). The hydrogen peroxide percentage (ww) was determined by titration with ceric sulfate and ferroin as indicator while the dicarbonyls were determined as glyoxal equivalent using spectrophotometer. The pH and conductivity were measured using pH meter and conductometer.



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The SPSS version 20 program was used for the analysis of the obtained results. The honey samples contained high amounts of the plants pollens and the pH and conductivity were within the standards ranges. The *Ziziphus* honey was characterized by significantly high mean percentage of hydrogen peroxide ($3.9\% \pm 2.2$) compared to the *Acacia* honey ($2.5\% \pm 0.83$) and the *Acacia* honey contained the highest mean concentration of the dicarbonyl molecules ($278.7\text{mg}/100\text{g} \pm 278.4$) compared to ($147.6\text{mg}/100\text{g} \pm 93.2$) in the *Ziziphus* honey. Regarding the effect of altitude, the high altitudes were characterized by low concentrations of hydrogen peroxides and high concentration of dicarbonyl molecules. The altitude significantly increased the dicarbonyl molecules concentration and significantly decreased the hydrogen peroxide percentage. The *Ziziphus* honey was characterized by significant high percentage of hydrogen peroxide while the *Acacia* honey was characterized by significant high concentration of dicarbonyl molecules.

Introduction

Honey is a natural nutrient produced by honey bees from different sources including the nectar of the plants, secretions from different parts of the plants and from the secretions of plant insects. There are two types of honey depending on its source; nectar or blossom honey and honeydew honey. The nectar honey is produced by the honey bees depending on the plants nectar as a nutrient source while the nutrient source of the honeydew honey is the plant or plant insects secretions.¹

The two types of honey are rich in carbohydrates including monosaccharide sugars like fructose and glucose, disaccharides like sucrose, trehalose and maltose and oligosaccharides such as raffinose. However, the honeydew is rich in oligosaccharide compared to the blossom honey.²

The honey contains small amount of proteins (0.5%) and it contain three protein enzymes; diastase (amylase), sucrase (invertase or glucosidase) and glucose oxidase. The diastase and invertase break down the glycosidic bonds of polysaccharide (starch and glycogen) and disaccharides (sucrose), respectively. The glucose oxidase converts glucose to hydrogen peroxide and gluconic acid. It is well known that the honey is used as topical antibiotic because of its high hydrogen peroxide content.²

The honey is also rich in acids like the gluconic acid and poly phenols like flavonoids, phenolic acid and its derivatives. The dominant flavonoids in honey are quercetin, chrysin, galangin, luteolin, kaempferol and apigenin. However, the aroma and color of the honey are mostly due to their content of acids and polyphenols.^{3,4}

Different factors are well known to be involved in the determination of the physiochemical properties of honey including the botanical and geographical origin and the conditions of processing and storage.⁵ The aim of this manuscript was to investigate the effect of floral origin and altitude of honey samples on some biochemical parameters including the pH, conductivity and the concentration of hydrogen peroxide and dicarbonyl molecules.

Material and Methods

Samples collection

Forty honey samples were collected directly from bee farms in Asir region at the south western part of Saudi Arabia. The honey samples were of two floral origins and six different altitudes. The floral origins of the honey samples were *Ziziphus spina christi* and different *Acacia spp.* The altitude of the bee farms was determined using the google earth program.⁶ Fifteen *Ziziphus spina christi* honey samples were collected from three altitudes; 113, 317 and 511 meters above sea level and twenty five different *Acacia spp* honey samples were from five altitudes; 14, 113, 567, 576 and 2247 meters.

Sample Analysis

The parameters were measured in triplicates and the mean value was considered as the final result for each parameter. The mean value of each parameter was approved when the coefficient of variation (CV) between the three results was $\leq 10\%$.

Determination of the Floral Origin

The floral origin of the honey samples was confirmed by microscopic pollen analysis following the method of Louveaux and his colleagues published in

1978.⁷ The floral origin was judged by 50% pollen dominance.

Determination of the pH

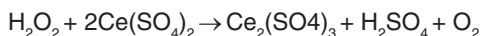
The pH of each honey sample was determined in a 13.3% solution (10g\75ml deionized water). However, the pH meter was calibrated by two buffers before the analysis; pH 4 and pH 9.⁸

Determination of the Conductivity (mS\cm)

The conductivity of the honey samples was determined in a 20% (w/v) honey solution according to the method of the International honey commission.⁹

Determination of the Hydrogen Peroxide Percentage (w/w)

The hydrogen peroxide concentration was determined by titration with Ceric sulfate according to the equation:



The Titration Procedure

0.5 g of honey was weighed in a 500 ml beaker and 250 ml of diluted sulfuric acid (1: 19 V/V) were added to the honey. The honey samples were titrated with 0.1 N ceric sulfate solution using the Ferriin indicator. The ferriin indicator was prepared by dissolving 0.174 gram of ferrous sulfate hepta hydrate in 25 ml distilled water and 0.37 gram of O- phenanthroline monohydrate was added and dissolved. The end point of the titration was the appearance of pale blue color.

The concentration of the hydrogen peroxide was determined according to the equation:

H_2O_2 weight percentage = $A \times N \times 1.701 \div W$
Where A is the volume of the ceric sulfate (ml)
N is the normality of the ceric sulfate
W is the weight of the honey sample (0.5g)¹⁰.

Determination of the Dicarbonyl Molecules

The dicarbonyl molecules (alpha keto acids) were determined spectrophotometrically after reaction with dinitrophenyl hydrazine (DNPH) in basic media.

Standard Curve

Standard solutions of glyoxal were prepared with concentration between 0 to 0.6 mM. 25 μ l of each

standard was pipette in a test tube and 975 μ l of distilled water was added to each tube. To the diluted standards, 1000 μ l of DNPH (0.9 Mm in 1N HCl) was added and the mixture was kept at 37°C for 10 min. After that 1000 μ l of soium hydroxide (1.5 N) was added and the absorbance was read spectrophotometrically at the wave length of 525 nm ((JASCO UV\VIS Spectrophotometer, SN B184160512- Japan).

Sample Treatment

10% (w/v) solution of honey samples was prepared and the diluted samples were treated as the standards for the purpose of measuring the concentration of the dicarbonyls. The diacrobonyls were measured according to the method of Kwok *et al.*¹¹

Statistical Analysis

The mean values of studied parameters were analyzed by the SPSS program version 20. The effect of the floral origin was determined using the t- test while the effect of the altitude was investigated using the one way ANOVA test.

Results

Pollen Results Analysis

The *Ziziphus* and *Acacia* honey samples contained 50% of the pollens. However, each honey sample contained four to five different types of pollens.

Results of the pH

pH results are presented as mean pH \pm SD (range). The pH results of the *Acacia* honey were 5.2 \pm 0.34 (4.35- 5.74) while the *Ziziphus* honey pH results were 5.6 \pm 0.2 (5.27- 5.75). The t-test showed that the variation of the pH between the *Ziziphus* and the *Acaica* honey was significant (p- value= 0.005). The altitude mostly had significant positive and negative effects on the pH value of the *Acacia* and *Ziziphus* honey samples (p- value \leq 0.05). However, some insignificant variations were obtained [Fig.1, Fig.3, Fig.5].

Results of the Conductivity (μ S\cm)

The mean \pm SD and the ranges of the conductivity in the *Acacia* and the *Ziziphus* honey were 751 \pm 258.3 μ S\cm (166- 1200 μ S\cm) and 662.1 \pm 138.2 μ S\cm (452- 842 μ S\cm), respectively. The variation between the two conductivity means was

insignificant (p - value= 0.16). Variable effects of the altitude on the conductivity of the two honey types were achieved [Fig.2, Fig.4, Fig.6]

samples while variable effects (significant and insignificant) were seen in the case of the *Acacia* honey [Fig.1, Fig.3, Fig.5].

Results of the Hydrogen peroxide percentage (weight\weight)

The mean value \pm SD and the range of the hydrogen peroxide in the *Acacia* and *Ziziphus* honey were $2.5 \pm 0.83\%$ (1.36- 4.18%) and $3.9 \pm 2.2\%$ (1.22- 6.91%) respectively. The mean value of the hydrogen peroxide percentage in the *Ziziphus* honey was significantly more than its percentage in the *Acacia* honey (p - value= 0.007).

Dicarbonyl Molecules Results (mg\ Kg)

The mean concentration and the range of the dicarbonyl molecules in the *Acacia* honey were 278.7 ± 278.4 mg\ Kg and (49.3- 928 mg\ Kg), respectively. The *Ziziphus* honey was characterized by mean value of 147.6 ± 93.2 mg\ Kg and a range of (5.8- 263.9 mg\ Kg). The dicarbonyl molecules were significantly high in the *Acacia* honey compared to the *Ziziphus* honey (p - value= 0.04). Most of the altitudes significantly increased the concentration of the dicarbonyl molecules in the *Acacia* and the *Ziziphus* honey samples [Fig.2, Fig.4, Fig.6].

The altitude significantly affected the percentage of the hydrogen peroxide in the *Ziziphus* honey

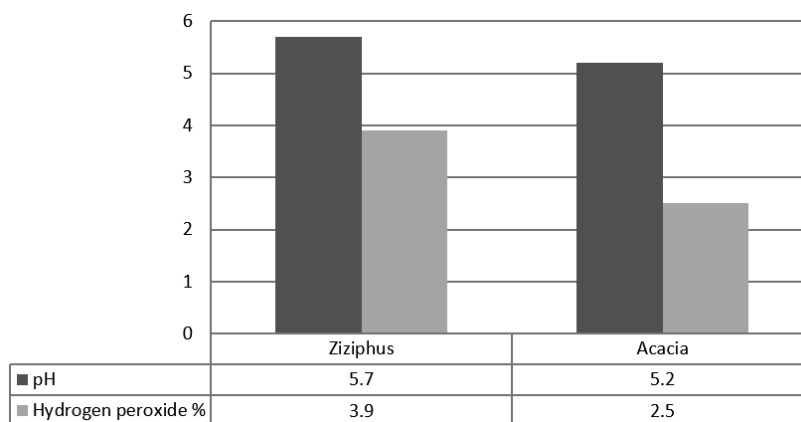


Fig.1: Effect of Floral Origin on the pH and Hydrogen Peroxide in the Acacia and Ziziphus honey samples

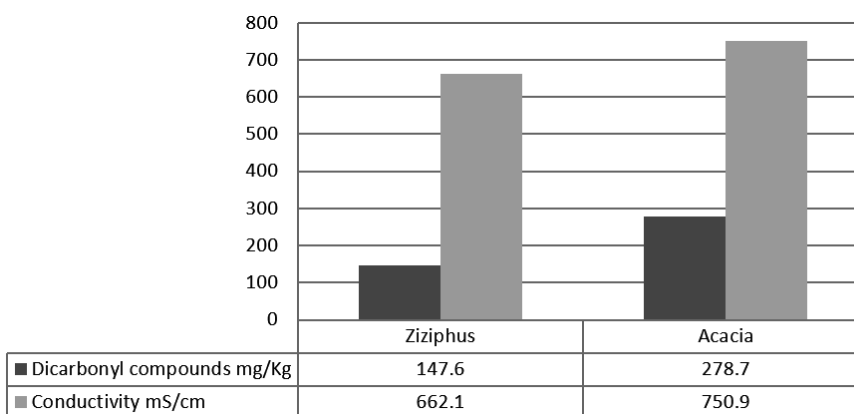


Fig. 2: Effect of Floral Origin On the Conductivity and the Dicarbonyl Compounds in the Acacia and Ziziphus honey samples.

Results of the Altitudes 113, 317, 567 and 576 Meters

The results of the mid altitudes; 113, 317, 567 and 576 meters above sea level were with different

pattern of results of all the studied parameters [Fig.3- Fig.6]. The different pattern of the results in these altitudes may be due to their mid way altitude. High altitudes are characterized by low barometric

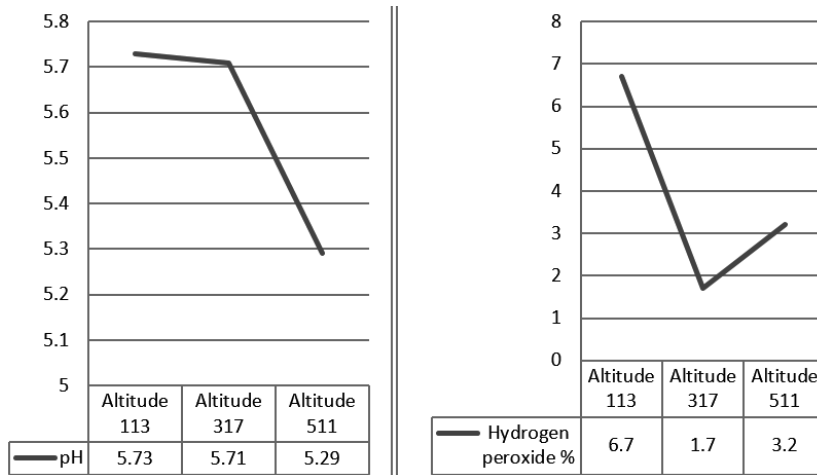


Fig.3: Effect of Altitude on the pH and hydrogen peroxide percentage of the *Ziziphus* honey samples.

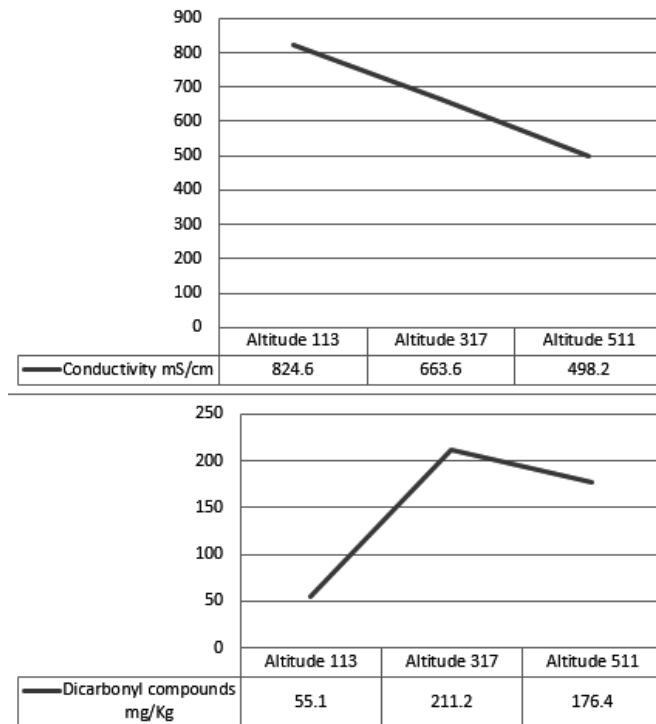


Fig. 4: Effect of altitude on the concentration of the dicarbonyl compounds and the conductivity of the *Ziziphus* honey samples.

pressure and temperature while low altitudes near the sea are characterized by high barometric pressure, temperature and humidity. The characteristics of the high and low altitude areas are missed in the mid altitude areas, this may be the reason of why the midway altitudes results were different from the results of low and high altitude area.

The pH of the *Ziziphus* honey was significantly more than the pH of the *Acacia* honey (p- value= 0.005). The hydrogen peroxide percentage in the *Ziziphus* honey was significantly more than that of the *Acacia* honey (p- value= 0.007).

The *Acacia* honey conductivity was insignificantly increased compared to its value in the *Ziziphus* honey (p- value= 0.16). The dicarbonyl compounds concentration in the *Acacia* honey was significantly more than its concentration in the *Ziziphus* honey (p- value= 0.04).

Significant pH decrease was reported when the altitude decreased from 113 to 511 meters (p- value= 0.007). The conductivity significantly decreased by the increase of the altitude (p- value≤ 0.000).

There was significant increase in the dicarbonyl compounds concentration in the *Ziziphus* honey

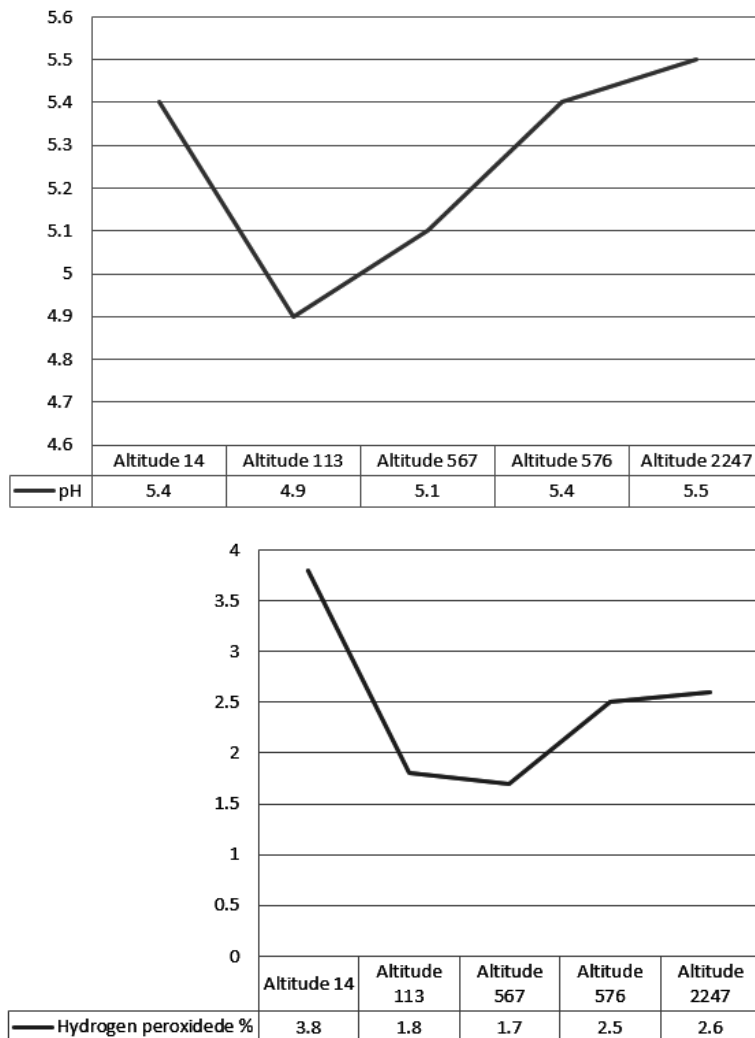


Fig. 5: Effect of altitude on the pH and hydrogen peroxide percentage of the *Acacia* honey samples

from the altitude 113 compared to the altitudes of 317 meters (p- value= 0.008) and 511 (p- value= 0.036). the conductivity was decreasing with the increase of the altitude significantly (113 vs 511) and insignificantly (113 vs 317).

Significant variations were reported when the conductivity of the 14 meters honey was compared to the honey from the altitudes of 113 and 567 meters. The hydrogen peroxide percentage of the *Acacia* honey from the altitude 14 was significantly decreased when comparing it to its concentration in the *Acacia* honey from all the other altitudes.

The concentration of the dicarbonyl compounds in the *Acacia* honey from the altitude 14 meters was significantly different (decreased and increased) from their concentration in the honey from the other altitudes. The conductivity of the *Acacia* honey from the 14 meters altitude was significantly different when compared to the honey from the altitude 113 (p- value= 0.01) and 567 (p-value≤ 0.000).

Discussion

The values of the pH of the *Acacia* and *Ziziphus* honey samples were within the range of the pH of the reference guide of the USA national honey board

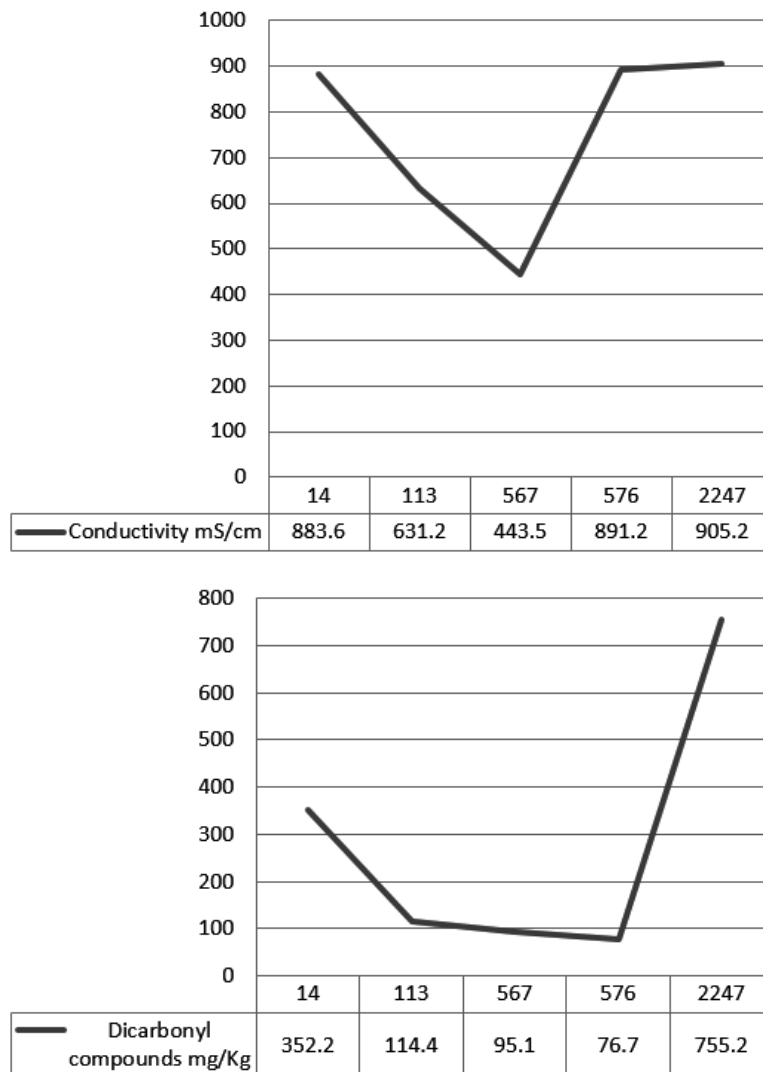


Fig. 6: Effect of altitude on the concentration of dicarbonyl compounds and conductivity of the *Acacia* honey samples

(3.4- 6.1).¹² This study revealed significant negative and positive effects of altitude on the pH value of the honey samples. However, it is mentioned, in two previous studies, that the altitude positively increased the pH and affected the physiochemical properties of honey samples from Saudi Arabia and Serbia.^{13,14}

This study indicated that the *Ziziphus* honey was slightly alkaline (5.6) compared to the *Acacia* honey (5.2). However, our results regarding the *Ziziphus* honey was comparable to the previous results from Algeria and most of the previous results.^{15,16} The mean value of the pH of our *Acacia* honey was 5.2, this value was higher than most of the previously measured values and it was comparable to honey samples from Lithuania (3.55- 4.88) and Germany (5.4).¹⁷⁻¹⁸

The conductivity of the *Acacia* honey in our study was $751 \pm 258.3 \mu\text{S}/\text{cm}$ (166- 1200 $\mu\text{S}/\text{cm}$), this value was more than most of the previous studies in different countries like Ethiopia ($170 \pm 2 \mu\text{S}/\text{cm}$)¹⁹, Romania $156.5(109.9-244.7\mu\text{S}/\text{cm})$ ²⁰ and Hungary ($101- 185 \mu\text{S}/\text{cm}$)²¹. However, the conductivity of our *Acacia* honey was comparable to the conductivity of *Acacia* honey from Malaysia ($760 \mu\text{S}/\text{cm}$).²²

The *Ziziphus spina christi* honey of this study was with a mean value of conductivity of $662.1 \pm 138.2 \mu\text{S}/\text{cm}$. The conductivity of the *Ziziphus spina christi* honey was similar to the conductivity of the *Ziziphus* lotus honey produced in Algeria ($654 \mu\text{S}/\text{cm}$)¹⁵ and *Ziziphus* honey from Morocco ($673.42 \pm 150.04 \mu\text{S}/\text{cm}$).²³

Similar to the range of the conductivity of our *Ziziphus spina christi* ($452- 842 \mu\text{S}/\text{cm}$), Abdul-Rahman and his colleagues measured the conductivity of *Ziziphus spina christi* honey samples from Yemen and found that the range of the conductivity was ($439.9- 751.9 \mu\text{S}/\text{cm}$).²⁴ However, 23 *Ziziphus* Jujube honey samples from China registered low mean conductivity value compared to our study or to the other previous studies. The mean value of the China *Ziziphus* Jujube honey was $474 \mu\text{S}/\text{cm}$.²⁵

Regarding the hydrogen peroxide percentage in the diluted *Ziziphus* honey (50% w/v) of Asir region, we

have achieved a mean value and a range of $3.9 \pm 2.2\%$ (1.22- 6.91%). However, our results were not comparable to most of the previous studies because we used dilution percentage in weight per volume while they used dilution in volume per volume. Generally our conclusion is similar to the conclusion of Al- Shehri²⁶ who found that the hydrogen peroxide concentration in the *Ziziphus* honey was significantly high than its concentration in the *Acacia* honey. We compared our results to the results of Chen *et al.*²⁷ which they investigated the effect of processing on the hydrogen peroxide concentration in Australian honey and we found that our results were comparable since they obtained a range of hydrogen peroxide between 0- 1017 μM (0- 6.92%) in the unprocessed honey. However, the honey samples of Chen *et al.*²⁷ were of different botanical origins rather than the *Ziziphus* and their dilution was weight per volume as our study. Another comparable previous study findings is that of Juraj *et al.*²⁸ who studied the effect of methylglyoxal (MGO) addition on the production of hydrogen peroxide in Manuka honey and honeydew samples and found that the highest production of hydrogen peroxide was obtained in the 50% (w/v) dilution of the honeydew samples. The highest hydrogen peroxide concentration range of the Juraj study was (306.9- 495.8 μM) (2.09- 3.37% w/w).²⁸ However, Eman Halawani and Mohamed Shohayeb analyzed different *Ziziphus* honey samples from Saudi Arabia, Yemen, Pakistan and Turkey and their hydrogen peroxide percentages were 8.3%, 6.8%, 8.6% and 7.5% respectively.²⁹

Similar to Al- Shehri,²⁶ we found that the hydrogen peroxide percentage in the *Acacia* honey was less than its percentage in the *Ziziphus* honey. The mean hydrogen peroxide percentage in the *Acacia* honey was $2.5\% \pm 0.83$. Our result was less than the results of another Saudi honey study which registered 4.8% for German honey.²⁹

Concerning the concentration of the dicarbonyl molecules in the *Acacia* and *Ziziphus* honey samples from Asir region, we have reported significantly high concentration in the *Acacia* honey (49.3- 928 mg/Kg) compared to the *Ziziphus* honey (5.8- 263.9 mg/Kg). Our results were comparable to the majority of the previous studies like the study of Marshal³⁰ who

reported a total carbonyl compounds concentration in monofloral honey samples range of (212- 3679 µg/g). Another study measured the concentration of 3 deoxyglucosone, glyoxal and methylglyoxal in different honey samples and registered their concentration ranges as follows: (75.9 - 808.6 mg/kg), (0.1-10.9 mg/kg) and (0.2-2.9 mg/kg), respectively.³¹

Our results showed that there was an inverse relationship between the hydrogen peroxide percentage and the concentration of the dicarbonyl compounds in the honey samples, this results was similar to the finding of Juraj²⁸ who stated that addition of methylglyoxal to honey samples led to the reduction of the hydrogen peroxide percentage.²⁸

With regard to effect of altitude on the percentage of hydrogen peroxide and dicarbonyl molecules in the honey, no previous study was found. There are two published papers associated with the effect of altitude on the physiochemical properties of honey and they reported a significant positive and

negative effects on the studied physiochemical characteristics.^{13,14}

Conclusions

The *Ziziphus* honey of Asir region was characterized by significantly high percentage of hydrogen peroxide compared to the *Acacia* honey while the *Acacia* honey was associated with significantly increased concentration of the dicarbonyl compounds compared to the *Ziziphus* honey.

The altitude significantly increased the dicarbonyl molecules concentration and significantly decreased the hydrogen peroxide percentage irrespective of their floral origin.

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Conflict of Interest

All the authors have declared no conflict of interest.

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