Bee's Honeys With High Concentration of Dicarbonyl Molecules Are Rich in Vitamin C and Deficient in Hydrogen Peroxide

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Abstract

The altitude is well known to affect the temperature, parometric and oxygen pressure and the amount of UV radiation which affect the bees and the physicochemical properties of bee's honey. This study investigated the relations between the concentration of hydrogen peroxide, vitamin C and dicarbonyl molecules in honey samples from different floral origins and altitudes. Ten Ziziphus and twenty Acacia honey samples were collected directly from their bee farms. The hydrogen peroxide and vitamin C were measured using redox titartions while the dicarbonyl molecules concnetration was determined spectrophotometerically. The results were statisticaly analyzed by the ANOVA and t-test of the SPSS. The mean concentration of vitamin C in the *Acacia* and *Ziziphus* honey samples were 275.14± 82.3 and 239.16± 91.5 mg/100g, respectively. The mean hydrogen peroxide percentages in the *Acacia* and *Ziziphus* honey samples were 2.66± 0.81 and 4.94± 1.85%, respectively. The mean concentrations of the dicarbonyl molecules in the *Acacia* and *Ziziphus* were 324.62± 291.03 and 115.75± 94.9 mg/Kg, respectively. Significant variations were reported in the case of the hydrogen peroxide and the dicarbonyl molecules concentrations in the *Acacia* and *Ziziphus* honeys (p- values = 0.004 and 0.007, respectively). The altitude significantly afected the studied parameters. Honeys rich in dicarbonyl molecules have a high concentration of vitamin C and low content of hydrogen peroxide.

1. Introduction

Honey is composed of sugars, water, proteins, enzymes, lipids, minerals, free amino acids, organic acids, polyphenols, minerals and some water and fat soluble vitamins. The sources of the honey molecules are the plant nectar, pollens and the honeybees. (Brudzynski et al., 2017).

Vitamin C in the honey is obtained from the plants nectar and can be syntheized by the honeybees (Smirnoff, 2018; Herbert, Vanderslice and Higgs, 1985). Honey samples rich in vitamin C are well known for their usage as antioxidnats because of their fee radical removal activity (Chua et al., 2013). Vitamin C is used as a parameter to discriminate between honey samples of different botanical origins (León-Ruiz et al., 2011).

Hydrogen peroxide is found in honey samples as a result of the glucose oxidase and catalase acitivites. The Glucose oxidase is the source of the hydrogen peroxide in bee's honey which is responsible for the conversion of glucose to gluconic acid and hydrogen peroxide (Bucekova et al., 2018). The catalase enzyme origin is the nectar pollens, it removes the hydrogen peroxide by converting it to oxygen and water (Mandal and Mandal, 2011). The concnetration of the hydrogen peroxide in honey samples increases with dalution because the dalution increases the activity of the glucose oxidase enzyme. The antibacterial activity of honey samples depends majorly on the concnetration of the hydrogen peroxide (Mama et al., 2019).

The honey contains nine dicarbonyl compounds include glyoxal, methylglyoxal , 3-deoxyglucosone, and 2,3- butanedione (Alvarez-Suarez et al., 2014; Marceau and Yaylayan, 2009). The dicarbonyl compounds

are synthesized either non enzymatically or by enzymes from intermediates of glycolysis (Alvarez-Suarez et al., 2014; Kunieda et al., 2006). Honey samples rich in dicarbonyl compounds are used as an antibiotic. The famous Manuka honey is usd as an antibacterial because of its methylglyoxal high concentration (Johnston et al., 2018).

The well-known factors that affect the biochemical composition of bee's honey are the botanical origin, geographical area, climate and storage conditions, handling, effect of biochemical constituents on each other and bee species, health and nutrition (El Sohaimy et al., 2015; Burns et al., 2018; Majtan et al., 2014).

The aim of this article was to Investigate the relations between the concentration of hydrogen peroxide, vitamin C and dicarbonyl molecules in honey samples from different floral origins and altitudes.

2. Materials And Methods

2.1. Honey samples description and treatment

Ten Ziziphus spina christi and twenty Acacia spp honey samples were collected from their bee farms and in their sealed hives. The honey samples were filtered through a nylon filter and kept at room temperature and at darkness. As as second authentication step, we measured the pH and conductivity of the honey samples and compared to the standards. The botanical source was confirmed by pollen analysis. The Ziziphus honey samples were collected from two altitudes; 113 and 511 meters above sea level and the Acacia honey samples were harvested from four different altitudes; 14, 113, 576 and 2247 meters above sea level.

2.2. Work flow

The parctical work was carried out according to the following steps:

1- Microscopic confiramation of the botanical origin of the honey samples.

2- Authentication of the honey samples by measuring the pH and conductivity and comparing their values to honey standards.

3- Measurement of vitamin C, hydrogen peroxide and dicarbonyl molecules

4- Statistical analysis and interpretations

2.3. Confirmation of the botanical source

The botanical source of each honey sample was confirmed microscopically (Louveaux et al., 1978). Any honey sample was considered monofloral, if 50% of the pollens were from one source (Song et al., 2012). However, all the honey samples were monofloral.

2.4. Determination of the honey pH

Ten grams of honey were dissolved in 75 ml of deionized water (13.3%) . Then the pH was measured using a pH meter after it was calibrated with two buffers; pH 9 (Tris-EDTA buffer solution) and pH 4 (Acetic acid- sodium acetate buffer solution) (Bogdanov et al., 2009).

2.5. Measurement of the conductivity

Twenty grams of each honey sample were dissolved in 100 ml deionized water (20%) and the conductivity was measured using a calibrated conductometer (Bogdanov, Martin & Lüllmann, 2009). The conductivity meter was calibrated by Standard solution of potassium chloride (0.1 M) freshly prepared by dissolving 0.7456 g in 100 ml deionized water.

2.6. Measurement of vitamin C

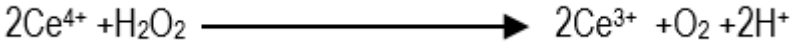
60 mls of distilled water were used to dissolve 1.5 gram of each honey and divided to three beakers (20 ml each; containing 0.5 g of honey). One ml of starch indicator (0.5% w\v) was added to each beaker. The honey and indicator solution was titrated (triplicate) against 0.005 M iodine solution. The added volume of iodine solution was taken at the point of a permanent pale dark blue color appearance (Settaluri et al., 2015; Bailey, 1974).

Vitamin C concentration was calculated following the bellow equation:

Conc of vitamin C (mg\100 g) =0.005(iodine molarity)×MWof vitamin C×volume of iodine (ml)×200

2.7. Determination of the hydrogen peroxide concentration

The concentration of the hydrogen peroxide was measured by titration with ceric sulfate following the equation:



2.7.1. The titration step

Two hundred and fifty mililiters of diluted sulfuric acid (1: 19 V/V) were added to 0.4 g of honey. The indicator used was the Ferroin indicator and it was prepared by dissolving 0.174 gram of ferrous sulfate hepta hydrate in 25 ml distilled water and addition of 0.37 gram of O- phenanthroline monohydrate. The titration of the honey samples was done using 0.1 N ceric sulfate solution. Appearance of a pale blue color was taken as the end point of the titration and the calculations were done according to the procedure of (Solvay Chemicals, 2014).

2.8. Estimation of the dicarbonyl molecules concentration

In order to determine the concentration of the dicarbonyl molecules, they were reacted with alakaline dinitrophenyl hydrazine (DNPH) and the product color intensity was measured using spectrophotometer (Kwok et al., 2016).

2.8.1.Standard curve

Glyoxal was used as a standard dicarbonyl molecule and six standard solutions were prepared (0, 0.16, 0.32, 0.63, 1.25, 2.5 and 5 mM). From each standard concentration, 25 µl was added to 975 µl of distilled water in a test tube. To the diluted standards, Thousand microliters of acidified Dintrophenylhydrazine (DNPH) (0.9 mM in 1 N HCl) was added to each of the standard tubes and they were stored for 10 minutes at 37°C. Finnally, thousand microliters of 1.5 N sodium hydroxide solution was added and the absorbance was determined at 525 nm (JASCO UV\VIS Spectrophotometer, SN B184160512- Japan).

2.8.2 Sample treatment

One gram of each honey sample was dissolved in 5 ml distilled water 20% (w/v) and they were reacted with the acidifies DNPH and hydrogen peroxide as the standards were treated.

2.8.3. Calculation of the results

The dicarbonyl compounds concnetration was determined following the equation bellow.

Concentration (mg/Kg) = (Concentration (mM)×58×1000)/200

58= molecular weight of glyoxal

200= conversion of one liter to five mls (one gram of honey)

1000= concentration of the dicarbonyl molecules in Kg of honey..

2.9. Statistical analysis

The SPSS program was used for the analysis of the obtained results. The means of the studied parameters were compared using the independant sample t-test and the LSD post hoc test of ANOVA. The significance level of difference between the means was set as the level of ≤ 0.05.

3. Results

3.1. results of the pH and conductivity

The pH was within the honey standards of the USA national honey board reference guide (National Honey Board, 2005). The conductivity of some of the Ziziphus and Acacia honey samples exceeded the reference value of the Codex standards (Codex Alimentarius, 2001). The high conductivity of the honey samples may be due to the altitude since the samples with high conductivity values were recruited from farms in high altitude areas. However, the pH of the *Ziziphus* and *Acacia* honey were almost the same

while the *Acacia* honey had slightly and insignificantly increased conductivity (p- value= 0.074) compared to the *Ziziphus* honey (Table.1).

3.2. Effect of floral origin on the studied parameters

The floral origin significantly affected the hydrogen peroxide and the dicarbonyl compounds. The Acacia honey was characterized by high concentration of vitamin C and dicarbonyls and low concentration of hydrogen peroxide (Table.1).

Moreover, the Ziziphus honey was characterized with low vitamin C and dicarbonyls and high hydrogen peroxide concentration i.e. there was inverse relationship between the hydrogen peroxide and the concentration of vitamin C and dicarbonyl molecules (Table.1).

3.3. Effect of altitude on the studied parameters

The altitude insignificantly affected the concentration of vitamin C in the Ziziphus honey samples (p-value= 0.16), however, the concentrations of vitamin C in the 113 and 511 meters were 194.48± 16.36 and 283.84± 116.54 mg/100 g, respectively [Fig.1]. The high altitude (2247 meters) significantly affected the concentration of vitamin C in the Acacia honey compared to the three other altitudes; 14, 113 and 576 meters above sea level [Fig.1]. The concentration of vitamin C in the 14, 113, 576 and 2247 meters above sea level were 254.8± 33.48, 193.96± 5.01, 272.8± 82.03 and 378.98± 45.78 mg/ 100 g, respectively.

The altitude significantly affected the concentration of the hydrogen peroxide in the Ziziphus and Acacia honey samples (p- value≤ 0.000) [Fig.2]. The concentration of the hydrogen peroxide in the Ziziphus honey from the 113 and 511meters were 6.68± 0.23 and 3.19± 0.18 g/Kg, respectively. The concentration of the hydrogen peroxide in the Acacia honey from the altitude of 2247 meters was significantly different than its concentration in the Acacia honey from the altitudes 14 and 113 meters (p- value≤ 0.000) [Fig.2]. The concentration of the hydrogen peroxide in the Acacia honey from the altitudes 14, 113, 576 and 2247 meters above sea level were 3.84± 0.28, 1.76± 0.28, 2.47± 0.22 and 2.59± 0.27 g/Kg, respectively.

The mean concentration of the dicarbonyl compounds in the Ziziphus honey from the altitudes of 113 and 511 meters above sea level were 55.1± 38.09 and 176.4± 98.06 mg/Kg, respectively (p- value= 0.044). The mean concentration of the dicarbonyls in the Acacia honey from the altitude 2247 was 755.2± 16.56 mg/Kg. The mean concentration of the dicarbonyl compounds in the Acacia honey from the 2247 meter was significantly different when compared to their concentration in the Acacia honey from the altitudes 14 (352.2± 23.15 mg/Kg), 113 (114.4± 97.47 mg/Kg) and 576 meters (76.66± 41.38 mg/Kg) (p- value≤ 0.000) [Fig.3].

4. Discussion

Regarding the effect of the floral origin on the physicochemical properties of honey, it is well known that the botanical origin significantly affects the physicochemical properties of honey including its color, flavor, conductivity, acidity and its content of sugars, flavonoids, total phenols, amino acids. Also, the

floral origin determines the bilogical activities of honey (Pasias et al., 2018; Abdulkhliq and Swaileh, 2017; Kaškonienė and Venskutonis, 2010).

This study mentioned that the floral origin of the honey affected the concentration of vitamin C, but insignificantly (p- value= 0.3), however, we have previously reached to the same conclusion (Al-Mosa et al., 2019). Perna et al. (2013) reported that the multifloral honey had the highest vitamin C concnetration. However, the concentration of vitamin C in previous studies showed that it was ranging from less than 0.01 to 378.3 mg/100g (Bonta et al., 2013; Ciulu et al., 2013; Perna et al., 2013; Dorbinas et al., 2006; Ahmed et al., 2016; Chua et al., 2013; Kesić et al., 2008).

Regarding the effect of floral origin on the hydrogen peroxide concnetration, we have reported significant effcet (p- value= 0.004). Al- Shehri (2017) found that the hydrogen peroxide concentration in the Ziziphus honey was significantly high than its concentration in the Acacia honey. However, the hydrogen peroxide percentages of this study were different from the findings of some of the previous studies due to the fact that we used dilution percentage in weight per volume compared to volume per volume in most of the previous studies.. The results of this study were ten times high than those of Pasias et al. (2018) who reported mean concentration 184, 280, 301 and 412 mg/Kg. However, Pasias and his colleagues studied three different honey samples from Greece. An Egyptian study reported a 3.98 ppm as the highest concentration of hydrogen peroxide in cotton honey sample with 20% dilution (Awad and Ayman, 2006). Halawani and Shohayeb (2011) determined the hydrogen peroxide concentration in different Ziziphus honey samples from four countries and found that their hydrogen peroxide percentages were between 6.8% and 8.6%. The results of Halawani and Shohayeb (2011) study were very high than the results of this study.

With regard to the effect of floral origin, this study reported a significant effect of the floral origin on the concentration of the dicarbonyl molecules (p- value= 0.007) and this conclusion was similar to the conclusion of our previous study in a different harvesting time (Mohammed et al., 2019). However, Arena et al. (2011) classified different origin honey depeding on the diacrbonyl compounds and the phenols. Concerning the effect of altitude we have reported in this study and in our previous study (Mohammed et al., 2019) that the altitude significantly affected the concentration of the dicarboyl molecules in bee's honey. Regarding the concentration of the dicarbonyl compounds in the previous studies , Arena and her research group measured the concentration of three dicarbony compounds including the glyoxal in different honey samples. The concentration ranges of Arena study were: 3-deoxyglucosone (75.9 - 808.6 mg/kg), glyoxal (0.1-10.9 mg/kg) and methylglyoxal (0.2-2.9 mg/kg) (Arena et al., 2011).

Concerning the effect of altitude on the physicochemical properties of honey, some previous studies reported the effect of altitude on some physicochemical properties honey such as the pH, conductivity, moisture, specific gravity and acidity (Popov-Raljića et al., 2015; Mohammed et al., 2017). In a previous study, We have reported that the altitude significantly affected the concnetration of vitamin C in Acacia and Ziziphus honey samples in different harvesting season (Al-Mosa et al., 2019). Siimilar to the conclusion of this study, We have concluded in another study that the altitude significantly affected the

hydrogen peroxide percentage (Mohammed et al., 2019). Similar to our previous study finding (Mohammed et al., 2019), the altitude of this study significantly affected the concentration of dicarbonyl compounds in the Ziziphus and Acacia honey samples. However, the two studies were conducted in different seasons.

This study proved that honey samples with high diacrbonyl compounds are with low hydrogen peroxide percentage similar to the finding of Majtan who added methylglyoxal to honey samples and found that the addition of methylglyoxal (MGO) decreased the hydrogen peroxide percentage because of the inhibition effect of MGO on glucose oxidase (Majtan et al., 2014). Also we reported that honey samples with high vitamin C are associated with low hydrogen peroxide concentration which may be due to the antioxidant activity of vitamin C (Kerkvliet,1996). Moreover, honeys with high hydrogen peroxide percentage are with low concentration of dicarbonyl molecules (removal of glucose oxidase inhibition) and low vitamin C concnetraion due to the ability of hydrogen peroxide to oxidize vitamin C (Deutsch, 1998).

5. Conclusion

This study concludes that honey samples with high dicarbonyl compounds concentration have low hydrogen peroxide production and high concentration of vitamin C.

Declarations

Ethics approval and consent to participate

Not Appilcable

Consent for publication

The authors agree that the BMC chemistry Journal has the right to publish this article

Availability of data and material

The data of this research is avaible for the journal

Competing interests

The author declare no conflict of interest

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Authors' contributions

Alaergani W and Al-Musa A did the practical work, wrote the manuscript draft and apprved the final copy of the manuscript. Mohammed MEA designed the research, statistically analyzed the results and revised and approved the final copy of the manuscript

Authors' information (optional)

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Tables

Table.1: Effect of floral origin on the concentration of the studied parameters in the honey samples

p- value	Mean ± SD	Honey type	Parameter
0.5	5.19± 0.19	Ziziphus	pH
	5.12± 0.29	Acacia	
0.07	661.4± 172.37	Ziziphus	Conductivity
	865.31± 426.25	Acacia	µS/cm
0.3	239.16± 91.5	Ziziphus	Vitamin C
	275.14± 82.3	Acacia	mg/ 100 g
0.004	4.94± 1.85	Ziziphus	Hydrogen peroxide
	2.66± 0.81	Acacia	g/Kg
0.007	115.75± 94.9	Ziziphus	Dicarbonyls
	324.62± 291.03	Acacia	mg/ Kg

The Acacia honey was rich in vitamin C and the dicarbonyl compounds while the Ziziphus honey was rich in hydrogen peroxide. The results suggested a negative effect of the dicarbonyl compounds and vitamin C on the concentration of the hydrogen peroxide. The pH of the two honeys was within the range of the USA national honey board reference guide (3.9- 6.1) (National Honey Board 2005). The conductivity of some of the Ziziphus and Acacia honey exceeded the conductivity value of the Codex standards ≤ 800µS/cm (Codex Alimentarius 2001).

Figures

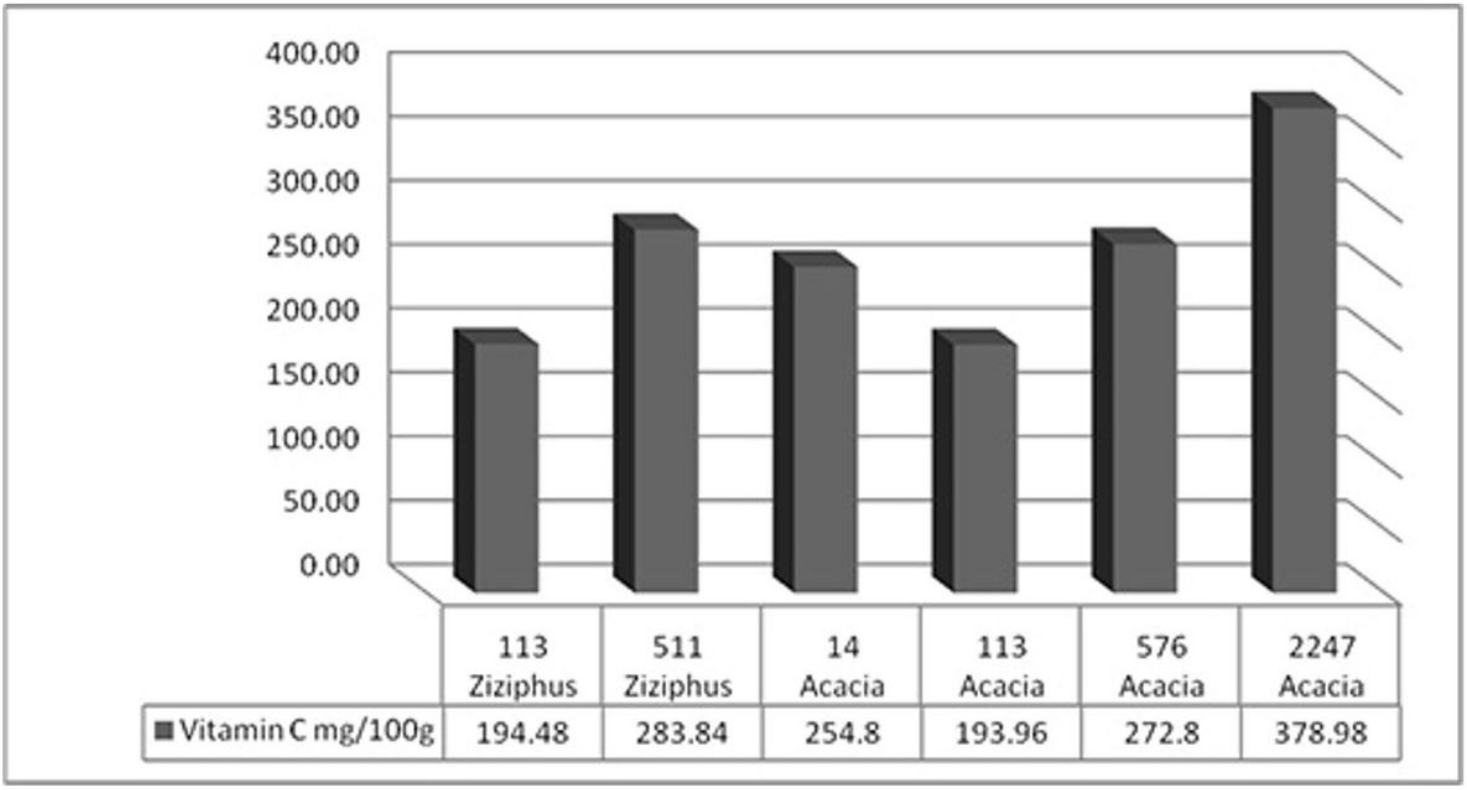

Figure 1

Effect of altitude on the concentration of vitamin C in the Ziziphus and Acacia honey samples. The altitude insignificantly affected the concentration of vitamin C in the Ziziphus honey samples (p- value= 0.16). The comparison of the vitamin C mean value in the Acacia honey from the altitude 2247 meters was significantly different when compared to the honey samples from the altitudes 14, 113 and 576 meters (p- value= 0.005, p- value≤ 0.000 and p- value= 0.014, respectively).

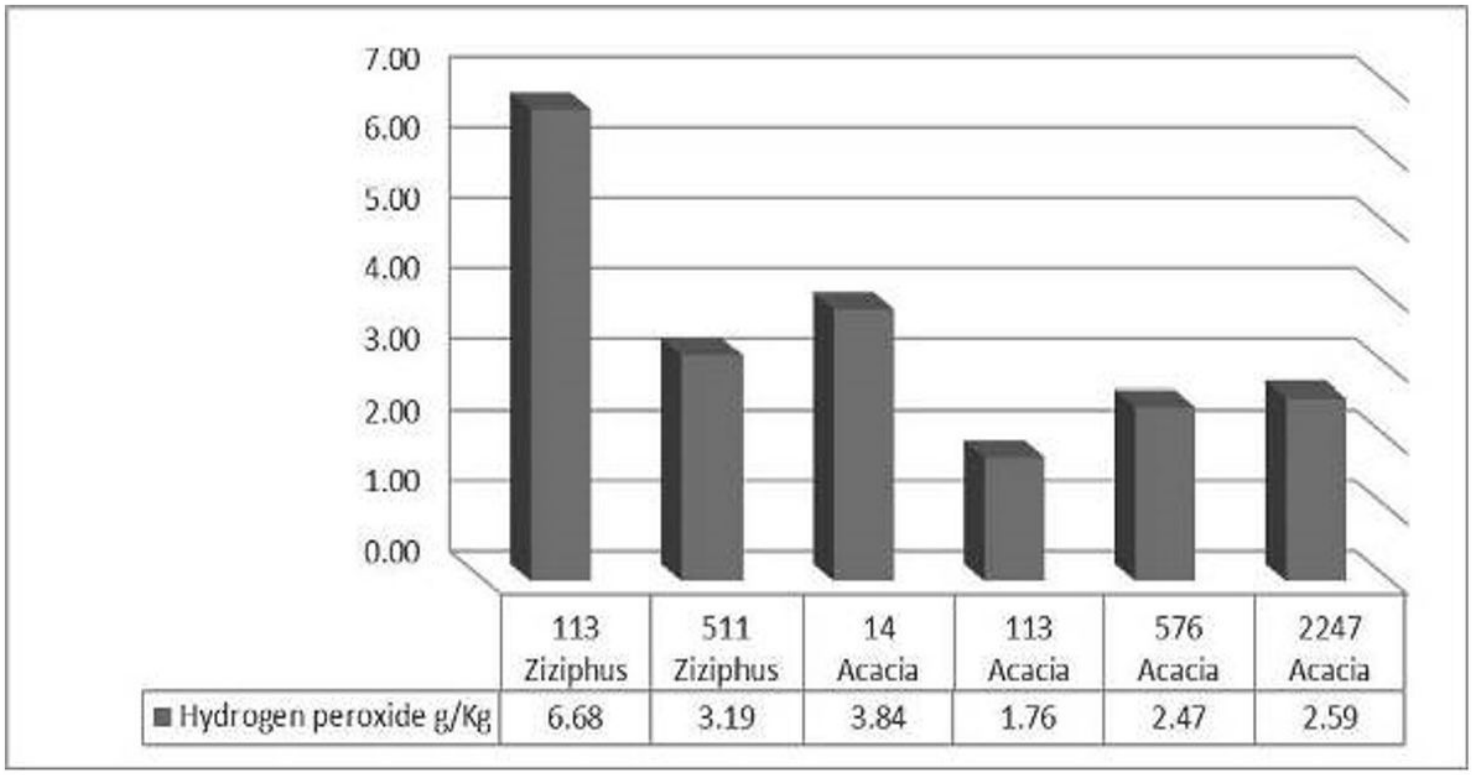

Figure 2

Effect of altitude on the hydrogen peroxide concentration in the Ziziphus and Acacia honey samples. The altitude significantly affected the concentration of the hydrogen peroxide in the Ziziphus and Acacia honey samples (p- value≤ 0.000). However, the hydrogen peroxide in the Acacia honey from the highest was insignificantly different when compared to its concentration in the Acacia honey from the altitude 576 meters (p- value= 0.45).

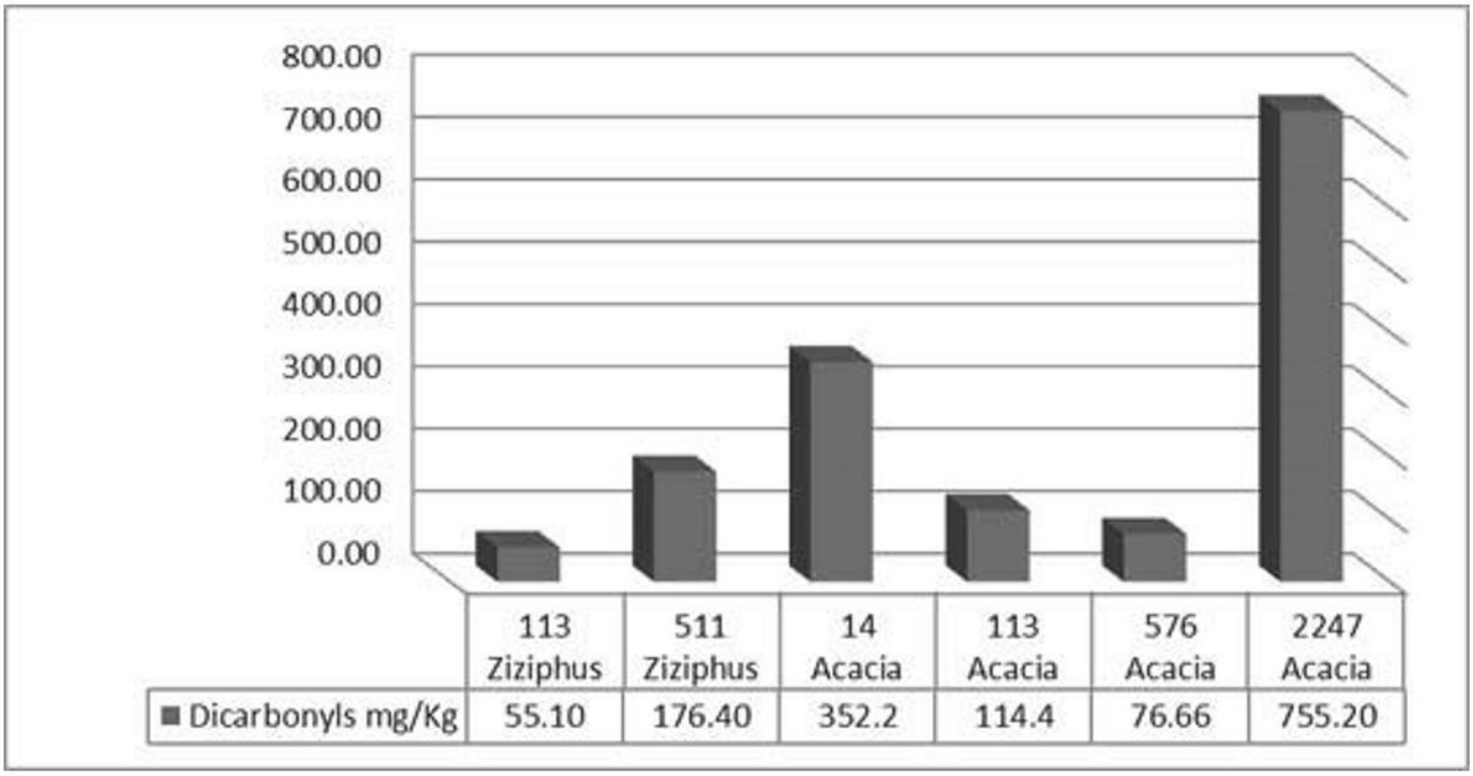

Figure 3

Effect of altitude on the dicarbonyl compounds concentration in the Ziziphus and Acacia honey samples. The altitude significantly affected the concentration of the dicarbonyl compounds in the Ziziphus and Acacia honey samples (p- value= 0.044 and p- value≤ 0.000, respectively).