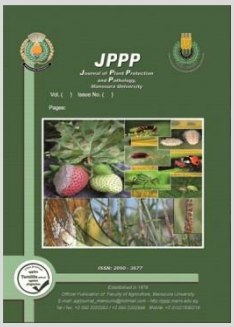
Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www. jppp.journals.ekb.eg

Physicochemical Characterization and Antimicrobial Activity of Sidr Honey Produced by Dwarf Honey Bees (*Apis florea* F.)

Taha, A. A.[1, 2*]; Naglaa M. Balabel[3] and H. M. Elshishtawy[4]

[1]Research and Training Station, King Faisal University, Al-Ahsa, Saudi Arabia.
[2]Beekeeping Research Dept., Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.
[3]Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.
[4]Microbial Molecular Biology Department, Agriculture Genetic Engineering Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.



ABSTRACT

The objective of the study was to evaluate the physicochemical properties and the antimicrobial activity of sidr honey produced by dwarf honey bees, *Apis florea* F. Three sidr honey combs presents three samples from Ziziphus trees (*Ziziphus spinchristis* L.) were collected during the blooming period (September and October, 2017) from Al-Ahsa, Saudi Arabia. *Apis florea* (*A. f.*) sidr honey had a high water content value (23.9%). The average results of pH no (4.52), free acidity (49.5 meq/kg), Hydroxymethylfurfural (HMF) (1.7 mg/kg), Ash content (0.95 %) and electrical conductivity (EC) (1.79 mS/cm) for *Apis florea* sidr honey, respectively. The average sugar composition was (25.39, 39.47 and 3.29%) for Glucose, Fructose and Sucrose, respectively. Vitamins B3, B6, B12 and Ascorbic acids were detected in sidr honey. Vitamin B6 was the superior (7.87 mg/kg) of all tested vitamins followed by Ascorbic acid giving 4.04 mg/kg. The results suggested that *Apis florea* sidr honey is a good source of antioxidants and Vitamins. The antimicrobial activity of *Apis florea* sidr honey was tested for fungus (*Rhizoctonia solani & Fusarium solani*) and bacteria (*Ralstonia solanacearum & Agrobacterium rhizogenes*). There were positive results with the bacteria mentioned in each of the concentrations (15 - 50 - 100% with *Ralstonia solanacearum*) while the concentration 100% gave a positive result with the bacteria (*Agrobacterium rhizogenes*). Honey did not show antifungal activity. In addition, further studies are needed in order to investigate the therapeutic effect of sidr honey produced by *Apis florea* honey bees.

Keywords: *Apis florea* bees, Sidr honey, physicochemical, antimicrobial, inhibitory, plant Pathogens.

INTRODUCTION

From all the global natural products honey becomes one of the most orders as it has a lot of benefits in medicine and food. It is high valuable to determine the food products standard criteria because food validity, quality and consumption depend on it. In addition, consumer health is affected by the healthy and purity of food products (Serrano *et al*., 2007). Variation in honey characteristics differs greatly in composition according to its geographical and botanical origin (Joseph *et al*., 2007). Several researchers have been studied the chemical composition and physical properties of honey (Iglesias *et al*., 2004; Qamer *et al*., 2005). Honey has many important constituents which influence on the medical and nutritional quality, flavor, crystallization, texture and the storage quality (Joshi *et al*., 2000). The honey components mainly depend on nectar sources as well as the regional and climatic conditions (Abu-Tarboush *et al*., 1992; Ferreres *et al*., 1994; Russo-Almeida, 1997). Certain constituents have been proposed as quality criteria for honey by the international honey commission (IHC). These include water content, electrical conductivity, reducing sugars (fructose and glucose), sucrose content, free acidity, ash content, diastase and invertase activity, HMF content, proline content and specific rotation (Bogdanov *et al*., 1997; Joshi *et al*., 2000). There are approximately 20,000 species of bees exhibiting terrestrial life, only 6–11 species of them are known to produce honey (Ball, 2007).

There are two honeybee species in kingdom of Saudi Arabia among them is *Apis florea*, are indigenous species, constructive their nest in open places and living wildly in nature. Sidr honey produced by *A. florea* bees is commonly used in folk and traditional medicine.

Thus the objective of the study was (I) to evaluate the physicochemical properties of *Apis florea* sidr honey (II) to detect the antioxidant contents (III) to increase the scientific knowledge about the antimicrobial activities of *Apis florea* sidr honey collected from Al-Ahsa region, KSA (IV) to increase knowledge on sensitivity of certain plant pathogens to the preparations of honey with special of bacteria and fungi pathogens to enrich literature with records on the inhibitory effect of honey on some plant pathogens.

MATERIALS AND METHODS

The present investigation was conducted at Food Safety& Quality Control Lab, Faculty of Agriculture, Cairo University, Egypt during 2017, to study physicochemical

* **Corresponding author.**
E-mail address: aaismail@kfu.edu.sa
DOI: 10.21608/jppp.2019.78154

properties of the sidr honey collected from the dwarf honey bee, *Apis florea* colonies. Three sidr honey combs represented three different *Apis florea* honey bee colonies were collected. For each parameter, the tests were replicated three times and the mean values were taken.

Collecting honey samples:

During the sidr trees (*Ziziphus* spp.) blooming season (September and October, 2017), three sidr honey combs represented three different *Apis florea* colonies were collected from Al-Ahsa province, Eastern Region, Kingdom of Saudi Arabia. Al-Ahsa lies at latitude 25° 25' 46" N, longitude 49° 37'19'' E and an altitude of 121 m above sea level. The Unprocessed (raw) sidr honey from sidr trees (*Ziziphus* spp.) in Al-Ahsa province (Fig, 1) about 400 km. east Riyadh city, Saudi Arabia, collected by cutting a honey comb from three different colonies of the species *Apis florea* and put in light plastic pages kept in freezing conditions until analyses. Each sidr honey comb was squeezed with mesh to collect three honey samples. Each sample was kept in tied glass bottles (200 gm/colony) and put directly in the refrigerator until the experimental analysis was done.

Fig. 1. *Apis florea* honey bee comb standing on sidr tree in Al-Ahsa region, KSA

Analytical procedures:

a. Determination of Water content

Determination of moisture content of honey was conducted by measurement its refractive index value (RI) using Abbe's refractometer at 20 ºC (A.O.A.C, 1995).

b. Determination of pH, free acidity

Based on the method of White *et al*. (1962), the pH, free acidity and total acidity were determined. The amount of NaOH added from the burette, minus the 'blank' correction is considered the measure of the free acid present, and the amount of HCl used substrated from 10 mL is measured of the lactone content. The sum of free acid and lactone is the total acidity.

c. Determination of Ash and Electrical conductivity (EC) content

Ash content was detected according to the standard methods of the Association of the Official Analytical Chemists (AOAC, 1995).The Electrical conductivity (EC) of a honey solution, at 20% (w/v) (dry matter basis) in ultrapure water, was measured at 20 ºC using a Conductivity meter (Mettler, Switzerland). The results were presented as milli Siemens per centimeter (mS/cm).

d. Determination of Hydroxymethylfurfural (HMF)

The HMF content was determined colorimetrically after dilution with distilled water and addition of P-toluidine solution. Absorbance of the solution was determined at 550 nm by UV Spectrophotometer (Winkler (1955). The equation by which results may be roughly worked out is: HMF (mg /1000 g) = absorbance /thickness of layer *192 Results are expressed as mg HMF/Kg honey.

e. Free Radical Scavenging Activity (DPPH %)

According to Da Silva *et al*. (2013) the scavenging activity of the free radical of honey samples was determined by screening the DPPH (1,1-diphenyl-2-picrylhydrazyl). After 15 min of incubation at 25°C, the absorbance was detected at 517 nm. Ascorbic acid was used as positive control. The following regression equation was used to calculate the scavenging activity of DPPH , where A control and A sample are the absorbance's of control and sample, respectively. The concentration of honey sample required to scavenge 50% of (DPPH) EC 50% was determined based on the ascorbic acid calibration curve (0–10 mg/L).

$$\text{DPPH Scavenging Activity (\%)} = 1 - [(A_{control} - A_{sample}) / A_{control}] \times 100$$

f. Determination of Invertase activity:

According to Harmonized methods of international honey commission, 2009, the instrument UV/Vis Spectrophotometer, Jenway, England was used to determine invertase activity. The invertase number (IN) indicates the amount of sucrose per gram hydrolyzed in 1 h by the enzymes contained in l00g of honey under test conditions (Bogdanov *et a1*., 1997).

g. Determination of Diastase activity:

Determination of diastase activity was evaluated spectrophotometrically based on the method of Schade *et al*. (1958) using the Shade method (UVA/IS Spectometer Lambda ll, Perkin Elmer, USA). The diastase activity was presented as diastase number (DN) (Bogdanov *et al*., 1997).

h. Determination of water soluble vitamins (WSV) in honey:

For determine the WSV in honey the Agilent 1260 infinity HPLC Series (Agilent, USA) equipped with Quaternary pump, a Kinetex XB-C18 column 100 x 4.6 mm (Phenomenex, USA), operated at 35 C°. Detection VWD detector set at 254 nm for ascorbic acids and 220 nm for vitamins B3, B6 and B12. (Ciulu *et al*., 2011)**.**

i. Determination of Sugars in honey:

The total reducing sugar, reducing and non-reducing sugars were determined by HPLC Knauer Instrument, Germany according to Codex Alimentarius, 1993.

j. Antimicrobial assay:

Honey was diluted to the required concentrations (5, 10, 15, 25, 50 and 100 %, v/v) to prepare the solutions. Then, incubation was done for all the samples for (30 minutes / 37°C) in a shaking water bath for the solutions aeration. As both glucose oxidase and hydrogen peroxide are sensitive to light, so incubation was conducted in the dark (White and Subers, 1964).

Bacterial and fungal strains and growing conditions

Bacterial and fungal strains were provided by Potato Brown Rot Project (PBRP), Dokki, Egypt. Ralstonia solanacearum and Agrobacterium rhizogenes were routinely

grown for 2-3 days at 28oC on King's B (KB) medium. Otherwise, Rhizoctonia solani and Fusarium solani were grown for 5 days at 25oC on Potato Dextrose Agar (PDA) medium. For long term storage, the bacteria were kept in Luria Bertani (LB) broth medium, supplemented with 20% glycerol and was kept at -80oC.

1. Evaluation of inhibition ability of *Apis florea* sidr honey on pathogenic bacteria *Ralstonia solanacearum* and *Agrobacterium rhizogenes* (in vitro)

After 24-h growth of Ralstonia solanacearum and Agrobacterium rhizogenes on Nutrient Glucose Agar (NGA) medium at 30°C (Dowson, 1957), the bacterium was re-inoculated separately into Nutrient Broth (NB) medium for 24 h (Ramaley and Burden, 1970). 1mL of each bacterial suspension was inoculated in to 9 cm plate then 10 mL of (NGA) was mixed gently with the bacterial inoculum. Once the agar has solidified, 8 mm well was made by using cork borer. The effectiveness of different concentrations of sidr honey (5, 10, 15, 50, 100 %, w/v) on the inhibition of pathogenic bacterial growth was evaluated by using agar diffusion technique. 100 µL of each honey concentration was poured into the well. Plates were incubated for 24 hr at 30°C. Control plates were prepared and incubated similarly, substituting the sidr honey with sterile distilled water. The diameter of inhibition zone was determined after 24 hr.

2. Evaluation of inhibition ability of *Apis florea* sidr honey on plant pathogenic fungi, *Rhizoctonia solani* and *Fusarium solani* (in vitro).

Mycelia plugs of actively growing ***R. solani*** and ***F. solani*** were placed on the center of (NGA) agar plates and surrounded with four wells filled with specific concentration of honey solution (15, 50, 100 %), 3 plates for each concentration and plates with wells filled with dist. Sterilized water served as a control. After 3 days of incubation at 28°C the diameter of fungal mycelia growth was determined.

k. Statistical analysis

The obtained data were tabulated to analysis of variance program (ANOVA) (Gomez and Gomez, 1984) followed by Multiple Range Test to compare means (Duncan, 1955).

RESULTS AND DISSCUSSION

The composition of sidr honey samples

Table (1) presents the analysis of *Apis florea* sidr honey collected from Research & Training Station, King Faisal University, Kingdom of Saudi Arabia. The physico-chemical parameters were within the standards of the regulations. Relationship between geographical origin of production honey and physical and chemical activity was illustrated in Tab. (1-5) and Fig. (2-5). Table (1) shows means and standard deviations of the various chemical parameters analyzed: moisture, ash, free acidity, pH, EC, HMF and DPPH % Radical-Scavenging activity.

Water content

The initial moisture content of *Apis florea* sidr honey was measured. Data in table (1) showed that *A. f.* sidr honey exceed the 20% allowed by (Codex Alimentarius, 1993). Average moisture content was 23.9 %. It is due to the exposure of the honey bee combs to the direct environmental condition of low temperature and moderate relative humidity which reaches 20 °C and 48% respectively, during November month (month of collecting sidr honey) in Al-Ahsa region. Harvesting season, beekeeping practices and nectar composition are responsible for the moisture content of honey. The result is in contrary with Al-qarni *et al*. (2012) and Al-Ghamdi, *et al*. (2017) they found that all the tested Saudi honeys produced by *Apis mellifera* L. had relatively low water content (12.12%- 18.5%) in Riyadh region. Abu- Tarboush *et al*. (1992) and Kaakeh and Gade-Elhak (2005) stated that water content in honey is responsible for its stability against fermentation and granulation.

Determination pH and free acidity

The pH value of *Apis florea* sidr honey representing 4.52 (Table, 1). These findings agreed with Bogdanov (1999) and Codex Alimentarius (1998) they specified a pH range of (3.42 to 6.10). This parameter is of great importance during extraction and storage of honey as it influences the texture, stability and shelf life of honey (Terrab *et al*., 2004). According to Kamal *et al*. (2002) they reveal the difference in pH to present differ acids and minerals in honey. Higher pH values obtained from honey harvested using traditional method could be as a result of fermentation due to inappropriate method of harvesting (Babarinde *et al*., 2011). All of the investigated honey samples were acidic and were within the limit that indicates freshness.

The value of free acidity obtained is 49.5 meq/kg for *A.f.* sidr honey (Table,1). It's obvious that free acidity value exceed the maximum standard specified by Codex Alimentarius, 1998, (40 meq/kg) (Babarinde *et al*., 2011). The acidity indicates the history of honey and possible alcohol and acid production by bacterial fermentation. According to Costa *et al*. (1999) high total acidity *may due to Xerotolerant* yeast activity. Furthermore, Al-Doghairi *et al*. (2007) stated that the total acidity of Saudi honeys ranged between (9.12 to 93.02 meq/kg). Moreover, high acidity value in honey could be due to floral sources and inappropriate method of harvesting which involved unripening honey combs and brood that accelerate fermentation rate.

Table 1. Chemical composition of *Apis florea* sidr honey

Parameter	Water content g/100g (%)	pH No	Free Acidity meq/Kg	HMF mg/kg	Ash Content %	Electrical conductivity (mS/cm) EC No.	DPPH % Radical-Scavenging activity
Sidr honey	23.9c± 0.1527	4.52d±0.347	49.5b±0.2993	1.7e±0.1	0.95e±0.0378	1.79e±0.0529	91.64a±1.245

Data presented are means of three samples ± SD of triplicate readings.

Hydroxymethylfurfural (HMF)

The HMF content of *A.f.* sidr honey was 1.7 mg/kg (table, 1). It's obvious that HMF content did not exceed the maximum standard of 80 mg/kg specified by Codex Alimentarius (1993) for Arab Gulf countries. In addition, the HMF value in honey is affected by the harvesting ways of honey even modern or traditional, the pH and acidity values and the overheating and long period's storage of honey (Saxena *et al*., 2010 and Babarinde *et al*., 2011).

Total minerals (Ash) content

Ash content of *A. f.* sidr honey was analyzed as it presents the minerals content of the honey. The ash content value of the honey samples was 0.95 g/100g, table (1). Moreover, Saxena *et al*. (2010) stated that the range of ash content in some Indian honeys were 0.03%-0.43%. Meanwhile, Taha and Asmaa (2011) reported the range of ash content from 0.03% to 0.26% for Egyptian and Libyan honeys. Dark honey contains significant qualities and quantities of minerals higher than lighter honey. In general, the presence of these minerals is indication of contamination during processing, shipping or storage due to the use of steel galvanized containers (Corbella and Cozzolino, 2006).

The Electrical Conductivity (E.C.):

The electrical conductivity (E.C.) of *Apis florea* sidr honey showed high value (1.75 mS/cm), table (1). Iftikhar *et al*., 2011 stated that the E.C of *Apis florea* honey from Pakistan is highest (0.76 mS/cm), while the E.C. of *Apis mellifera* honey is lowest (0.23 mS/cm). Furthermore, *Apis florea* honey was superior of *Apis cerana, Apis dorsata, and Apis mellifera* honey in EC value. The botanical origin of honey effects on the minerals contents and sequence the EC of honey. Our results stated that the E.C. of *Apis florea* honey was high which indicate it had a high minerals amount (0.95 %). As *Apis florea* bees have the smallest size, it suggests the ability to visit the smallest flowers to get nectar and pollen.

Antioxidant activity, (1,1-diphenyl-2-picrylhydrazyl, DPPH):

The DPPH free radical is a stable free radical, which has been used widely as a tool to determine the free radical-scavenging activity of antioxidants. Antioxidants, when interacting with DPPH, transfer either electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Devasagayam *et al*., 1995). The value of the DPPH in honey samples was illustraited in Table (1). The mean values of the DPPH were 91.64 ± 1.24 mg/mL for *Apis florea* sidr honey. The antioxidant capacity of honey and of its components poses as useful parameters to correlate phytochemical determinations (Alvarez-Suarez *et al*., 2010).

Sugar composition

The range and mean levels of Glucose, Fructose and Sucrose in *Apis florea* sidr honeys were analyzed (Table, 2).

Table 2. Analysis of glucose, fructose and sucrose in *Apis florea* sidr honeys.

Sugar	Fructose (%)	Glucose (%)	Sucrose (%)	LSD 5%
Sidr honey	39.47a±0.0066	25.39b±0.0057	3.29c±0.0057	0.0210

Data presented are means of three samples ± SD of triplicate readings.

The sucrose content of honey samples giving 3.29% for sidr honey. The presence of sucrose below 5% as specified by Codex Alimentarius (1998) indicates that the bees were not feeding artificially. In table (2) the mean percentages of glucose and fructose were (25.39 and 39.47%), consecutively. Glucose and fructose (reducing sugars) are the major constituent of honey (64.86%) and the main factor in determining the tendency of honey to crystallize. (Kucuk *et al*., 2007).

Fructose was the predominant sugar followed by glucose

The superior sugar of the tested honey was fructose followed by glucose, while sucrose was present in low amounts in all tested samples. Obviously, invertase analyses sucrose to glucose and fructose (O¨zcan *et al*., 2006). In addition, the fructose/glucose ratio tells about the tendency of honey to crystallize, i.e. when fructose is higher than glucose the honey is fluid (Venir *et al*., 2010). Furthermore, the fructose/glucose ratio may also have an impact on the honey flavor since fructose is much sweeter than glucose (Alvarez-Suarez *et al*., 2010). It is clear that *Apis florea* sidr honey samples were fluid (ratio great than 1). Generally, the higher the glucose, the faster honey crystallizes, and the higher the fructose, the slower it crystallizes.

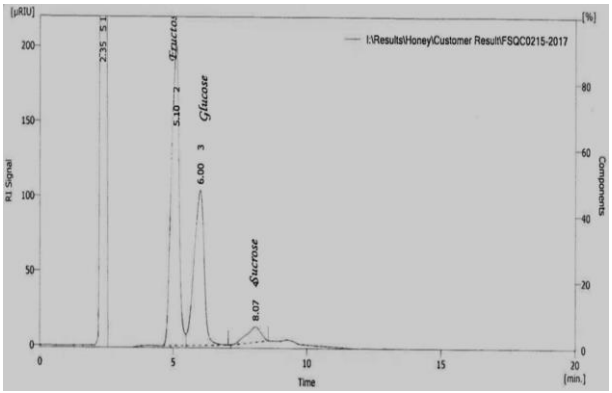


Fig. 2. Estimation of *Apis florea* sidr honey sugars

Enzymes in honey samples

Table (3) showed the invertase and diastase activity values for *Apis florea* sidr honey samples. The invertase activity in sidr honey gave 18.12µ/kg. Invertase secrete by the salivary glands and the gut of plant-sucking insects. In addition, the reduction in invertase activity may be due to heating processes during honey bottling or transport as invertase is more heat-sensitive than diastase (Beckmann *et al*., 2011). Results indicated that Diastase number (DN) in sidr honey was in an acceptable range not less than 8 on Goth standard presented 29.7µ/g. The relation between the two enzymes had expressed by the invertase/diastase ratio. The invertase/diastase ratio for *A.f.* sidr honey was 0.61.

Table 3. Values of some enzymes characteristics of *Apis florea* sidr honey.

Enzymes	Invertase µ/kg	Diastase µ/g	LSD 5%
A.f. Sidr honey	18.12b±0.0057	29.70a±0.1560 D.N	0.4334

Data presented are means of three samples ± SD of triplicate readings.

The diastase activity (α-, β-, γ-amylase) is the important quality parameter of honey as it mustn't be less than or equal to 8. Even the honey is fresh or overheating can be evaluated by Diastase number. The reduction of diastase number can take a marker of honey adulteration by addition of inverted sucrose or hydrolysed starch namely high fructose corn syrup (HFCS). Enzymes are the most important and also the most interesting honey components. They are accountable for the conversion of nectar and honeydew to honey, and serve as a sensitive indicator of the honey treatment (Codex Alimentarius, 1993). The results suggest that the proteolytic enzymes of honey can significantly change honey protein profile and thereby strongly influence quality and nutritional value of honey (Rossano *et al*., 2012).

Water-soluble vitamins (WSV)

According to Table (4) average concentration of water-soluble vitamins (mg/kg) of sidr honey produced by *Apis florea* honey bees were detected. Each of vitamin B3 (Nicotinic acid), B6 (Pyridoxine), B12 (Cobalamin) and Ascorbic acid was detected.

Table 4. Values of some vitamins characteristics of *Apis florea* sidr honey (mg/kg).

Vitamins	Ascorbic acid	Vit. B3	Vit. B6	Vit. B12	LSD 5%
Sidr honey	4.04 b ±0.0057	0.37 d ±0.0057	7.87 a ±0.1527	0.96 c ±0.0057	0.0297

Data presented are means of three samples ± SD of triplicate readings.

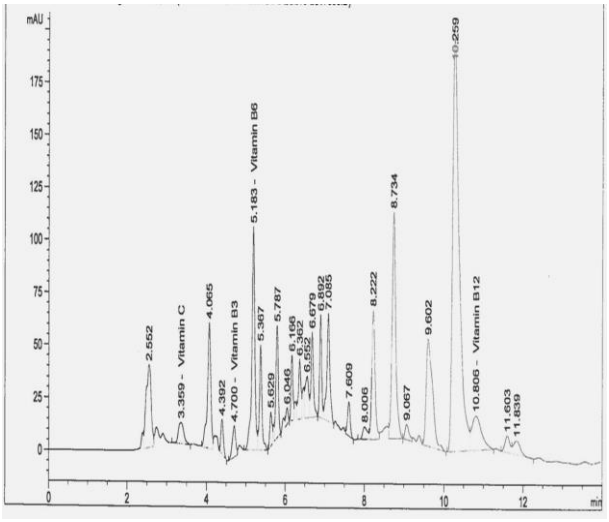


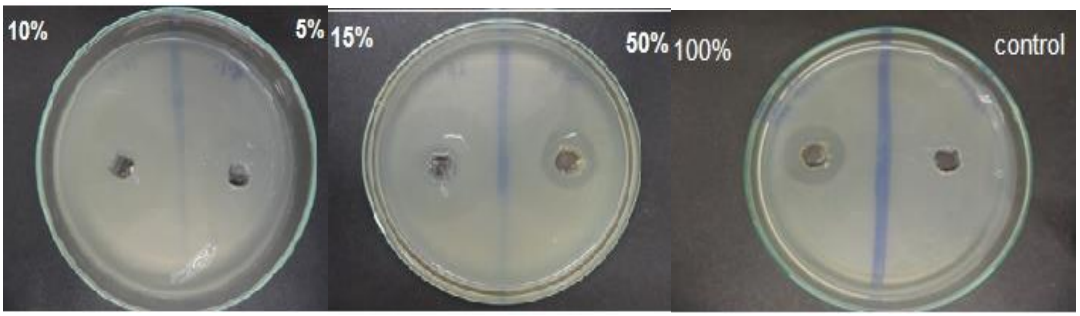
Fig.3. Estimation of *Apis florea* sidr honey vitamins

Table (4) reports the amount of the water soluble vitamins (WSV) for *A. f.* sidr honey. It's cleared that Vitamins B6 and Ascorbic acids were in high values presented (7.87 and 4.04 mg/100g), respectively. Also, B12 and B3 vitamins were detected in sidr honey. These results are in agreement with Taha and Asmaa, 2011 they found that Cotton honey was only superior of vitamin B6 giving 0.031 mg/100g. Interestingly, the concentration of vitamin B6 was observed to be as high as 7.87 mg/100g and it may be strongly dependent on the botanical origin of the honey samples (Ciulu *et al*., 2011). The Recommended Daily Allowance (RDA) for ascorbic acid is 75 mg/daily for adult. The RDA for vitamin B3 (Niacin) vary with age, 14 and 16 mg/daily for women and men, consecutively.

h. In vitro evaluation of inhibition ability of honey on pathogenic bacteria *Ralstonia solanacearum* and *Agrobacterium rhizogenes*

Treatment concentration	Diameter of inhibition zone of *Agrobacterium rhizogenes* R1	R2	R3
100%	15	18	15
50%	0	0	0
15%	0	0	0
10%	0	0	0
5%	0	0	0

Treatment concentration	Diameter of inhibition zone of *Ralstonia solanacearum* R1	R2	R3
100%	22	20	22
50%	19	20	20
15%	0	0	0
10%	0	0	0
5%	0	0	0



Inhibition ability of honey on *Ralstonia solanacearum*

Inhibition ability of honey on *Agrobacterium* rhizogenes

Fig .6. Inhibition ability of honey on plant pathogenic bacteria

i. In vitro evaluation of inhibition ability of honey on plant pathogenic fungi, *Rhizoctonia solani and Fusarium solani.*

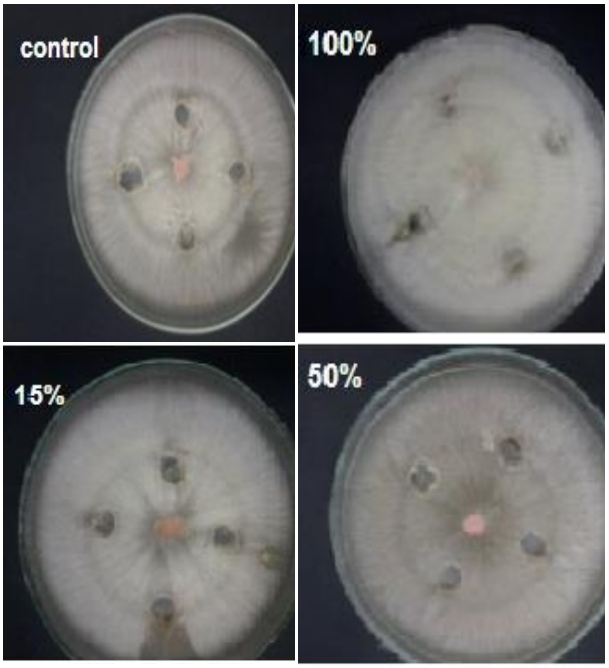


Fig. 7. Inhibition ability of honey on plant pathogenic fungi

Furthermore, Niacin can boost levels of good HDL cholesterol and lower triglycerides. In addition, vitamin B6 (Pyridoxine) developed by the US Food and Drug Administration (FDA) 2 mg for adult as its effective to anemia and high homocysteine blood levels. As our body does not make vitamin B12, we have to get it from animal based foods or from supplements. The RDA for adult (2.4 microgram/daily) for B12 and it helps make our DNA and the red blood cells (FAO, 2001).

Effect on *Rhizoctonia solani*

There is a positively correlation between the sidr honey concentration and the growth of the fungus. On the other hand, the tested honey stimulated the growth of pathogenic *Rhizoctonia solani*, thus we can't considered the tested honey as antifungal agent. The present results disagree with AL-Mughrabi, (2003) who reported the antifungal properties of wild honey when used at low concentration (1000 ppm). High osmolarity, acidity, hydrogen peroxide, bee-origin and floral source are the factors responsible for the antimicrobial activity of honeys (Alvarez-Suarez *et al*., 2010; Al-Habsi and Niranjan, 2012 and Eleazu *et al*, 2013). It is possible that the honeys with high antimicrobial activities could contain high quantities of glucose oxidases or polyphenols or both as these have also been reported to possess antibacterial properties (Khalil *et al*., 2010). Thus for optimum antibacterial activity, honey should be stored in a cool, dark place and be consumed when fresh (Ndife *et al*., 2014).

CONCLUSION

It can be concluded that *Apis florea* sidr honey contains more moisture content because they building their nest in open places and living widely in nature. Possibly they process honey differently during the mechanical stage of honey ripening and it may be related to the maturity level in the hive and the harvest season. For pH value none of the investigated samples exceeded the allowed limit of international standards (pH of 3.42–6.10). Also, *A. florea* honey contains higher acidity, ash, DPPH, Vitamin B6 and Ascorbic Acid due to the different foraging preference of *Apis florea* species. Many of the components of honey are unstable over time and are thermolabile. Moreover, *A. florea* sidr honey tolerated temperature regimes applied regarding HMF production. HMF value was within the recommended standard (maximum of 40 mg/kg) and the low HMF concentrations confirmed that this honey is of good quality. The free acidity values of the analyzed honey samples are within the international standard (not more than 50 meq/kg). The EC values of the investigated honey samples are within the allowed limit of international standards (not more than 0.8 mS/cm). The sugars contents values are within the limits (Reducing sugar—not less than 60% (g/100 g) and sucrose-not more than 5% (g/100 g) set by the Codex Alimentarius (2015) and European Commission (2002). This is the first report on the antimicrobial activity of sidr honey produced by *Apis florea* honey bee in the Arab Gulf Countries. Our results suggested that *Apis florea* sidr honey is a good source of Antioxidants, Ascorbic acid and vitamin B6.

REFERANCES

A. O. A. C., 1995. Official methods of analysis. (16th ed).Washington, DC: USA: Ass. Off. Ana. Chem.

Abu-Tarboush, H.M., Al-Kahtani, H.A., El-Sarrag, M.S.A., 1992. Floral-type identification and quality evaluation of some honey types. Food Chem., 46, 13-17.

Al-Doghairi, M.A., Al-Rehiayani, S., Ibrahim, G.H., Osman, K.A., 2007. Physicochemical and antimicrobial properties of natural honeys produced in Al-Qassim region, Saudi Arabia. Met. Environ. Arid Land Agric. Sci., 18 (2), 3-18.

Al-Ghamdi, A., Seif Eldin, A.M., Ansari, M.J., Adgaba, N., 2017. Comparison of physicochemical properties and effects of heating regimes on stored *Apis mellifera* and *Apis florea* honey. Saudi Journal of Biological Sciences, http: // dx. doi. Org/ 10.1016/j . sjb .06.002

Al-Habsi, N., Niranjan, K., 2012. Effect of high hydrostatic pressure on antimicrobial activity and quality of manuka honey. Food Chemistry, 135, 1448-1454.

AL-Mughrabi, k. I., 2003. Wild honey inhibits growth of some phytopathogenic fungi in vitro. Phytopathol. Mediterr. 42, 280-283.

Alqarni, A.S., Owayss, A.A., Mahmoud, A.A., 2012. Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. Arabian Journal of Chemistry, http://dx.doi.org/10.1016/j.arabjc.2012.11.013.

Alvarez-Suarez, J., Tulipani Romandini, S., Bertoli, E., Battino, M., 2010. Contribution of honey in nutrition and human health: A review. Mediterr J. Nutr. Metab. 3, 15-23.

Babarinde, G.O., Babarinde, S.A., Adegbola, D.O., Ajayeoba, S.I., 2011. Effects of harvesting methods on physicochemical and microbial qualities of honey. J. Food Sci. Technol., 48 (5), 628-634.

Ball, D.W., 2007. The chemical composition of honey. J. Chem. Educ., 84, 1643-1646.

Beckmann, K., Beckh, G., Luellmann, C., Speer, K., 2011. Characterization of filtered honey by electrophoresis of enzyme fractions. Apidologie, 42, 59-66. Doi:10.1051/apido/2010036

Bogdanov, S., 1999. Honey quality and international regulatory standards: Review by the International Honey Commission. Bee World, 80, 61-69.

Bogdanov, S., Martin, P., Lullman, C., 1997. Harmonised method of the European Honey Commission. Apidologie (extra issue), pp. 59.

Ciulu, M., Solinas, S., Floris, I., Panzanelli, A., Pilo, MI., Piu, P.C., Spano, N., Sanna, 2011. RP-HPLC determination of water-soluble vitamins in honey. Talanta, 83 (3), 924-929.

Codex Alimentarius Commission, 2015. Codex standard 12, Revised codex standard for honey, standards and standard methods, vol. 11; 2002. http://www.codexalimentarius.net.

Codex Alimentarius Commission, CAC, 1998. Codex Alimentarious Draft revised for honey. CAD CX P 5/102, CI, 1998/12-S, FAO, Rome, Italy.

Codex Alimentarius Standard for honey, 1993. Standard for Honey, Ref. no. CL 1993/14, SH, CAC, FAO/WHO, Rome, Italy.

Corbella, E., Cozzolino, D., 2006. Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics. LWT- Food Science and Technology, 39, 534-539.

Costa, LSM, Albuquerque, MLS., Trugo, LC., Quinteiro, LMC., Barth, OM., Ribeiro, M, De Maria, CAB., 1999. Determination of non-volatile compounds of different botanical origin Brazilian honeys. Food Chem., 65 (3), 347-352.

Da Silva, E.C., Muniz, M.P., Nunomora, R.D.S., Nunomora, S.M., Zilse, G.A.C, 2013. Phenolic constituents and antioxidants activity of geopropolis from two species of Amazonian stingless bees. Quimica Nova, 36(5), 628-633.

Devasagayam, T.P.A., Subramanian, M., Singh, B.B., Ramanathan, R., Das, N.P., 1995. Protection of plasmid pBR322 DNA by flavonoids against single strand breaks induced by singlet molecular oxygen. J. Photochem. Photobiol., B 30, 97-103.

Dowson, W.J., 1957. Plant Disease due Bacteria,(second ed.), The University Press, Cambridge, London, pp. 231.

Duncan, D.B., 1955. Multiple range and multiple F-Test. Biometries, 11, 1-42.

Eleazu, C.O., Iroaganachi, M., Okoronkwo, J., 2013. Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: The effect of varying colours. J. Nutr. Food Sci., 3(2), 189.

EU Council, 2002. European Union Council directive 2001/110/EC of 20 December, 2001, relating to honey. Off J. Eur Communities. L10, 47-52.

Ferreres, F., Andrade, P., Tomás-Barberán, F.A., 1994. Flavonoids from Portuguese heather honey, Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 199(1), 32-37.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agriculture research. 2nd Ed., June Wiley& Sons. Inc. New York. pp. 680.

Harmonized methods of the international honey commission, 2009. Determination of sugars by HPLC. Pp, 46.

Human vitamin and mineral requirements, 2001. Report of a joint FAO/WHO Expert Consultation, Bankok, Thailand, 2001, pp, 303.

Iftikhar, F., Masood, A.M., Waghchoure, E.S., 2011. Comparison of *Apis cerana, Apis dorsata, Apis florea and Apis mellifera* Honey from Different Areas of Pakistan. Asian J. Exp. Biol. Sci., 2(3), 399-403.

Iglesias, MT., De Lorezo, C., Polo, MDC., Martin-Alvarez, PJ., Pueyo, E., 2004. Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. J. of Agricultural Food Chemistry, 52, 84-86.

Joseph, T. Julius, AN., Florence, F., Delphine, DN., Jonnas, P., Antoine, MZ., 2007. Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon. African Journal of Biotechnology, 6 (7), 908-913.

Joshi, S.R., Hermann, P., William, A., Werner, O., 2000. Physico-chemical characteristics of Apis dorsata, Apis cerana and Apis mellifera honey from Chitwan district, central Nepal. Apidologie, 31, 367-375.

Kaakeh, W., Gadelhak, GG., 2005. Sensory evaluation and chemical analysis of *Apis mellifera* honey from the Arab Gulf Region. J. Food Drug Anal., 13(4), 331-337.

Kamal, A., Raza, S., Rashid, N., Hameed, T., Gilani, M., 2002. Comparative study of honey collected from different flora of Pakistan. Online J. of Biol. Sciences, 2, 626-627.

Khalil, M., Sulaiman, S., Boukraa, L., 2010. Antioxidant properties of honey and its role in preventing health disorder. The Open Nutraceuticals Journal, 3, 6-16.

Kucuk, MK., Kolayl, S., Karaoglu, S., Ulusoy, E., Baltac, C., Candan, F., 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem., 100, 526-534.

Ndife, J., Abioye, L., Dandago, M., 2014. Quality Assessment of Nigerian Honey Sourced from Different Floral Locations. Nigerian Food Journal NIFOJ, 32(2), 48-55.

O¨ zcan, M., Arsla, D., Ali, Ceylan, D., 2006. Effect of inverted saccharose on some properties of honey. Food Chem., 99, 24-29.

Qamer, S., Muzaffar, N., Ali, SS., Shakoori, AR., 2005. Physicochemical analysis of Pakistani unifloral honey from different nectar sources. Proceeding of Pakistan Congress of Zoology. 25, 123-130.

Ramaley, R., Burden, L., 1970. Replacement sporulation of bacillus subtilis 168 in a chemically defined medium. J. Bacteriology, 101(1), 1-8.

Rossano, R., Larocca, M., Polito, T., Perna, AM., Padula, MC., Martelli, G., Riccio, P., 2012. 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, 7(11), e49164. doi: 10. 1371 /journal. pone.0049164.

Russo-Almeida, PA., 1997. Some chemical parameters of honey from transmontane Terra Quente. Apicultor., 5, 39-35.

Saxena, S., Gautam, S., Sharma, A., 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem., 118 (2), 391-397. doi:10.1016/ j. food chem. 2009. 05.001.

Schade, JE., Marsh, GL., Eckert, JE., 1958. Diastase activity and Hydroxymethylfurfural in honey and their usefulness in detecting heat alteration. J. Food Res., 23, 446-464.

Serrano, S., Espejo, R., Villarejo, M., Jodral, ML., 2007. Diastase and invertase activities in Andalusian honeys. Int. J. Food Sci. Technol., 42, 76-79.

Taha, A.A., Asmaa, A.E., 2011. Chemical and physical properties of some Egyptian and Libyan honeys. J. Plant Prot. and Path., Mansoura Univ., 2(8), 731-740.

Terrab, A., Recamales, AF., Hernanz, D., Heredia, FJ., 2004. Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chemistry, 88, 537-542.

Venir, E., Spaziani, M., Maltini, E., 2010. Crystallization in ''Tarassaco'' Italian honey studied by DSC. Food Chem., 122, 410- 415.

White, JW., Subers, MH., 1964. Studies on honey inhibine. 3. Effect of heat. J. Apic. Res., 3,45-50.

White, JW., Riethof, ML., Sobers, MH., Kushnir, I., 1962. Composition of American honeys. U.S., Dept. Agric., Tech. Bull., pp, 1-124.

Winkler, O., 1955. Beitrag zum Nachweis und zur Bestinunung von Oxymethylufural in Honig und Kunsthonig z. Lebensmittelunters. u. Porsch, 102, 161-167.

www.accessdata.fda.gov.scripts/interactivenutritionfactslabe l / factsheets/vitamin and mineral.pdf

www.fao.org/3/a-y 2809e.pdf.

الخصائص الفيزيقية-كيماوية والنشاط المضاد للميكروبات لعسل السدر المجموع بواسطة نحل العسل القزم (*Apis florea* F.)

عمـــرو أحمد طـه [1،2]*، نجلاء موسى بلابل[3،4] و هشام محمد الششتاوى[5]

[1]محطة الأبحاث والتدريب- جامعة الملك فيصل- الاحساء- المملكة العربية السعودية

[2]قسم بحوث النحل- معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقى- الجيزة- مصر

[3]قسم بحوث الأمراض البكتيرية- معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الدقى-الجيزة- مصر.

[4]مشروع العفن البنى فى البطاطس- وزارة الزراعة- الدقى- الجيزة- مصر

[5]قسم بحوث المكروبيلوجيا الجزئيه - معهد بحوث الهندسة الوراثية الزراعية-مركز البحوث الزراعية-وزارة الزراعة- مصر

أجريت هذة الدراسة بهدف تقييم بعض الخصائص الفيزيقية-كيميائية والتأثير المضاد للميكروبات لعسل السدر الناتج من النحل القزم (النحل البرى الصغير *Apis florea* F.). تم جمع ثلاثة أقراص شمع عسلية تمثل ثلاثة عينات لأشجار السدر و ذلك خلال موسم تزهير السدر (سبتمبر- اكتوبر 2017 فى الاحساء- المنطقة الشرقية- المملكة العربية السعودية . وجد أن عسل سدر النحل البرى الصغير يحتوى على نسبة عالية من المحتوى المائى (الرطوبة) 23,9% . كان متوسط رقم الحموضة (4,52), الحموضة الحرة (49,5ملليمكافئ / كجم), وهيدروكسى ميثايل فورفورال (1,7 ملليجرام/كجم), محتوى المعادن الكلية (0,95%) والتوصيل الكهربى (1,79ملليسيمنز/ سم). كان متوسط نتائج محتوى السكريات 25,39 ,39,47 و 3,29% لسكر الجلوكوز, الفركتوز والسكروز , على التوالى. تم تقدير فيتامينات B3 B6, B12 وحمض الاسكوربيك فى عسل السدر. تفوق فيتامين B6 معطيا (7,87 ملليجرام/كجم) على كل الفيتامينات , يليه حمض الإسكوربيك معطيا (4,04 ملليجرام/كجم). تشير النتائج إلى أن عسل سدر النحل البرى الصغير هو مصدر جيد لمضادات الاكسدة والفيتامينات. تم إختبار النشاط المضاد للميكروبات لعسل سدر النحل البرى الصغير على فطريات *Rhizoctonia solani & Fusarium solani* وعلى بكتيريا *Ralstonia solanacearum* ، *Agrobacterium rhizogenes*. كانت هناك نتائج ايجابية مع البكتيريا المذكورة فى كل من التركيزات (15 – 50 -100% مع بكتيريا *Ralstonia solanacearum*، بينما التركيز 100% أعطى نتيجة ايجابية مع بكتيريا *Agrobacterium rhizogenes* . لم يظهر النشاط المضاد للعسل ضد الفطريات. بالاضافة الى ذلك هناك حاجة ماسة للمزيد من الدراسات لبيان التاثير العلاجى لعسل السدر الناتج من النحل البرى الصغير.