International Journal of
Current Multidisciplinary Studies
Available Online at http://www.journalijcms.com
Vol. 5, Issue,11(A), pp. 1171-1176, November,2019

IJCMS

RESEARCH ARTICLE

PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT PROFILE OF PRODUCED PRODUCTS IN NORTHEAST BRAZILIAN SEMIARID

Josileide Gonçalves Borges*, Aline Bezerra Monteiro and Rafaela Clementino Carvalho

Department of Pharmacy, Federal University of San Francisco Valley, 56304917, Petrolina, Pernambuco, Brazil

ARTICLE INFO

Received 13th August, 2019
Received in revised form 11th September, 2019
Accepted 8th October, 2019
Published online 28th November, 2019

Keywords:

Honey, antioxidants, acidity, total phenolics, flavonoids.

ABSTRACT

Honey is nutritious food that has several enzymes, phenolic compounds that increase antioxidant capacity, this contributes to pharmacological use in treatment of various diseases. Aim of this work is to analyze physicochemical properties that contribute to conservation, antioxidant profile of honey samples produced, marketed in northeastern semiarid region of Brazil. Thirty samples of *Apis mellifera* honey were analyzed for pH, free acidity, lactonic acidity, total acidity. Phenolic, total flavonoid levels were quantified by spectrophotometry using calibration curves. Ferric reducing antioxidant power (FRAP), reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in assays were performed to determine antioxidant capacity. Samples showed very variable results in all tests, pH (3.4-4.2), free acidity (41.20-75.00 mEq Kg^{-1}), total acidity (48.00-109.64 mEq Kg^{-1}), total phenolics (0.12-0.98 EAG g^{-1}), flavonoids (0.03-0.32 mg g^{-1}), DPPH (0.32-87.39%), FRAP (25.76-645.44 $mMFe^{2+}100g^{-1}$. Results show that semiarid honeys have antioxidant properties that stimulate their consumption, although food preservation aspects must be obeyed to avoid spoilage and growth of microrganisms.

INTRODUCTION

Honey is viscous liquid produced by *Apis mellifera* bees from flower nectar, composed of several sugars, where fructose, glucose are major components. In addition to sugars it has naturally occurring compounds during its formation as enzymes (glucose oxidase, catalases, diastasis), vitamins, proteins, minerals, amino acids, phenolic compounds (carotenoids, flavonoids, phenolic acids), ascorbic acid favoring antioxidant capacity, high commercial value due to its therapeutic properties (Roshan *et al*., 2017; Pita-Calvo and Vázquez, 2017; Nascimento *et al*., 2018; Borges *et al*., 2019; Vasic *et al*., 2019).

Honey has high commercialization price due to its nutritional value, healing, functional, biological properties, which have been proven by several studies (Roshan *et al*., 2017; Borges *et al*., 2017; Al Farsi *et al*., 2018). Honey has been used medicinally because of its antioxidant compounds such as phenolics, flavonoids, well its ability as anti-inflammatory, antibacterial, antiviral, antiulcerative, anti-lipid, anticancer properties (Khalil *et al*., 2012; Al Farsi *et al*., 2018).

Phenolic compounds are biologically active secondary metabolites found in molecular-acting plants, collected as potent natural antioxidants (Martins, Petropoulos, and Ferreira, 2016; Nascimento *et al*., 2018), eliminating free radicals that damage DNA, are associated with many non-contagious diseases, different cancers. Honey's macro and micro-constituents associated with its phytochemicals play an important role in human health, reducing risk of heart disease, cataracts, obesity, diabetes, immune system decline, different inflammatory processes (Nayik and Nanda, 2016; Zarei, Ali Fazlara and Noushin Tulabifard, 2019). Poor conservation, improper storage of honey can influence its physicochemical properties, favoring growth of microorganisms that ferment honey, altering taste, nutritional composition. Aim of this work is to analyze physicochemical properties that contribute to conservation and antioxidant profile of honey samples produced, marketed in northeastern semiarid region of Brazil.

*✉ ***Corresponding author:*** **Josileide Gonçalves Borges**
Department of Pharmacy, Federal University of San Francisco Valley, 56304917, Petrolina, Pernambuco, Brazil

MATERIALS AND METHODS

Chemical reagents

Analytical standard reagents were used for experiments: sodium hydroxide, hydrochloric acid, gallic acid, methanol, sodium carbonate (Synth) sodium nitrite, quercetin, Folin-Ciocalteau, 2,2 diphenyl-1-pricril-hydrazine, 2,4,6-Tris (2-pyridyl) -s-triazine) TPTZ (Sigma Aldrich), aluminum chloride, ferrous sulfate, ethanol (Vetec).

Honey Samples

Thirty samples of honeys produced, marketed in northeastern semiarid, in free markets of cities of Petrolina-PE, Juazeiro-BA, were evaluated. Petrolina is municipality in state of Pernambuco, located in São Francisco Valley region, neighboring municipalities of Juazeiro, Sobradinho. Located at 380 meters altitude, latitude 9° 23 '39" South, longitude 40° 30' 35 " West. Juazeiro is city in state of Bahia. Neighboring municipalities of Petrolina, Sobradinho, Juazeiro is 5 km south-east of Petrolina. Located at 369 meters altitude, latitude: 9° 26 '18' 'South, longitude: 40 ° 30' 19 " West.

Study site

Honeys were analyzed at Analytical Chemistry laboratory, Department of Pharmacy, Federal University of Vale de São Francisco (UNIVASF) in Petrolina, Pernambuco, Brazil.

Physicochemical characterization

pH

To measure pH, method suggested by Moraes and Teixeira. (1998) was used, based on determination of concentration of hydrogen ions present in honey solution. For this purpose, 10 g of sample was diluted in 75 mL of distilled water in 100 mL Becker, followed by direct reading with a properly calibrated MS Tecnopon instruments, model mPA 210, pHmeter.

Free, lactonic and total acidity

Method used to determine free acidity was recommended by Associationof Official Analytical Chemists (AOAC, 2012) and Adolf Lutz Institute. (2008), is based on neutralization of acidic honey solution through use of sodium hydroxide solution. In procedure 5 g of honey was diluted in 40 mL of CO_2-free distilled water, and then titrated with 0.1 M sodium hydroxide. Titration was stopped when solution reached pH 8.5 (free acidity). Acidity value (milliequivalent acid per g honey) was determined by equation 1:

Free acidity (mEq kg^{-1}) = (NaOH 0.1M volume - Blank volume) X correction factor X 40 / sample mass (g) (Eq. 1)

In lactonic acid analysis procedure, 5 g of honey was diluted in 40 mL of CO_2-free distilled water, then 10 mL of 0.1 M sodium hydroxide was added, material was titrated with HCL 0.1M. Titration was stopped when solution reached pH 8.3 (lactonic acidity). Acidity value (milliequivalent of acid per kg of honey) was determined by equation 2:

Lactonic acidity (mEq kg^{-1}) = (10-V HCL) X 40 mL X f / mass (Eq. 2)

Total acidity was obtained by summing free acidity, lactonic acidity according to equation 3:

Total acidity = free acidity + lactonic acidity (Eq. 3)

Determination of total phenolic content (TPC)

Determination of total phenolics was performed according to methodology described by Singleton and Rossi. (1965) using Folin-Ciocalteau reagent. For this, 2 g of honey was diluted in 10 mL of methanol. From honey solution (0.2g mL^{-1}) a 1.0 mL aliquot was taken into a 10 mL volumetric flask, made up to volume with CO_2-free distilled water. 500 mL of this second solution was placed in test tubes, 0.5 mL of Folin-Ciocalteu reagent (1:10) was added, 5 minutes was waited, then 1.5 mL of $Na_2CO_3.10H_2O$ (7.5 mg) was added 100 mL^{-1}). Tubes were incubated in a 45 °C water bath (protected from light) for period of 15 minutes, and then placed in dark for 30 minutes (room temperature). Absorbance readings were taken at 765 nm with aid of spectrophotometer (Nova Instruments, model 1600UV), using distilled water in place of sample as blank. For total phenolic calculations, gallic acid calibration curve (5 to 80 mg mL^{-1}) was used, results were expressed in mg of gallic acid equivalent (honey EAG g^{-1}).

Total Flavonoid Content (TFC)

It was determined according to methodology described in literature (Woisky and Salatino, 1998), where honey solution of 0.2 g was first prepared. mL^{-1}. 0.5 mL of honey solution, 2 mL of distilled water (CO_2-free), 150 µL of $NaNO_2$ solution (2.5%) were added to test tubes (triplicate) for 5 minutes. To mixture was added 150 µL of $AlCl_3$ solution (5%), after 1 minute 1 mL of NaOH (1 Mol L^{-1}), 1.2 mL of distilled water. Mixture was first stirred, and then allowed to stand in a dark place for 15 minutes. Absorbance was read at 510 nm with aid of spectrophotometer (Nova Instruments, model 1600UV). Water instead of samples was used as white. Quercetin curve (5 to 400 mg mL^{-1}) was used as standard. Flavonoid content was expressed in mg quercetin (QE) g^{-1} honey.

Antioxidatin activity (DPPH)

Free radical scavenging activity of extracts was determined based on DPPH method (Braca *et al*., 2001). This method is based on reduction of 2,2 diphenyl-1-pricrylhydrazyl radical (DPPH), which, when fixing an H (removed from antioxidant under study), leads to decrease in absorbance, allowing to calculate, after equilibrium is established of reaction, amount of antioxidant spent to reduce 50% of DPPH radical. 1 mL of DPPH solution (50 µg mL^{-1}) was added to 2.5 mL of sample. DPPH solution (1.0 mL), ethanol (2.5 mL) was used as blank. After 30 min absorbance values were measured at 518 nm in spectrophotometer (Nova Instruments, model 1600UV), converted to antioxidant percentage (AA) using equation 4:

AA% = [(blank absorbance-sample absorbance) / blank absorbance] x 100 (Eq. 4).

Test for reducing power and antioxidating activity (FRAP)

Method was based on direct measurement of ability of sample's antioxidants (reducing agents) to reduce in acidic medium pH (3,6), Fe^{3+}/ (2,4,6-Tris (2-pyridyl) -s-triazine complex). TPTZ to form Fe^{2+}, blue in color, absorbing 595 nm. Honey (1 g) was dissolved in 40 mL of methanol, honey solution was filtered using filter paper. 90 µL aliquot of honey solution was mixed with 270 mL of distilled water, 2.7 mL of FRAP reagent,

incubated at 37 °C in water bath (SL 154, Solar) for 30 min. Absorbance readings were taken at 595 nm with aid of spectrophotometer (Nova Instruments, model 1600UV) using distilled water in place of sample as blank. For FRAP antioxidant activity calculations, standard ferrous sulfate heptahydrate curve (200 to 2500 mg mL^{-1}) was used, results were expressed mg ferrous sulfate equivalent per gram of product.

Statistical analysis

Data were submitted to analysis of variance (ANOVA) the results were expressed as average and standard deviation. Results were compared by Tukey test at 95% confidence to verify if there was a significant difference.

RESULTS AND DISCUSSION

Table 1 presents results of physicochemical profile of honey samples produced, marketed in northeastern semiarid. According to Brazilian law, ideal pH value of honey should be less than 4.00 but for floral honeys values are accepted in range of 3.20 to 4.40 because they are considered fit for consumption, pH values very low interferes significantly in sensory characteristics of product, as high values may cause a decrease in existing microbial control (BRAZIL, 2000).

pH (hydrogen potential) of honeys samples from city of Petrolina ranged from 3.40 to 4.20, from city of Juazeiro varied from 3.80 to 4.20. Most samples showed no significant difference by tukey test. Abadio Finco, Moura and Silva. (2018), when analyzing physical, chemical properties of *Apis mellifera* L. honey from Southern Tocantins, Brazil, found pH values ranging from 3.35 to 4.50. Moura *et al*. (2017) when analyzing different honeys produced marketed in different cities of Bahia, Brazil, found values ranging from 4.00 to 4.40. Vicinieski1, Cordeiro and Oliveira. (2018) in honey analyzes of small producers from interior of Rio Grande do Sul found pH values in range of 3.00. This study, others cited present values considered acceptable by current legislation.

According to Crane. (1983) pH value may be directly related to composition of flowers in collection areas, since honey pH may be influenced by nectar pH, differences in soil composition or association of plant species to final composition honey.

Table 1 Physicochemical properties of bee honeys produced in northeastern semiarid.

Samples	pH	Free Acidity* (mEq kg^{-1})	Acidity Lactônica* (mEq kg^{-1})	Total Acidity* (mEq kg^{-1})
Braz. Leg	Ideal <4,0 Floral 3.2-4.4	Máx 50	NE	NE
PNZ1	$4.10^{b} \pm 0.01$	$41.20^{fg} \pm 0.40$	$40.00^{a} \pm 0.10$	$81.20^{cd} \pm 0.01$
PNZ2	$4.40^{a} \pm 0.01$	$60.00^{c} \pm 0.01$	$5.40^{g} \pm 0.10$	$65.40^{h} \pm 0.10$
PNZ3	$4.10^{b} \pm 0.01$	$46.67^{f} \pm 0.10$	$8.00^{f} \pm 0.10$	$54.67^{k} \pm 0.01$
PNZ4	$4.20^{ab} \pm 0.01$	$56.00^{d} \pm 0.01$	$18.67^{de} \pm 0.20$	$74.67^{ef} \pm 0.10$
PNZ5	$4.10^{b} \pm 0.01$	$48,00^{f} \pm 0,10$	$16.00^{de} \pm 0.20$	$64.00^{h} \pm 0.10$
PNZ6	$4.20^{a} \pm 0.01$	$52.00^{e} \pm 0.01$	$5.40^{g} \pm 0.10$	$57.40^{j} \pm 0.01$
PNZ7	$4.10^{b} \pm 0,01$	$44.00^{fg} \pm 0.20$	$4.30^{h} \pm 0.10$	$47.30^{n} \pm 0.20$
PNZ8	$4.20^{ab} \pm 0.01$	$56.00^{de} \pm 0.01$	$26.67^{c} \pm 0.10$	$82.67^{c} \pm 0.01$
PNZ9	$3.40^{d} \pm 0.01$	$82.00^{a} \pm 0.01$	$4.67^{h} \pm 0.20$	$86.67^{b} \pm 0.01$
PNZ10	$3.40^{d} \pm 0.01$	$75.00^{b} \pm 0.01$	$34.64^{b} \pm 0.10$	$109.64^{a} \pm 0.01$
PNA11	$4.00^{b} \pm 0.01$	$44.00^{fg} \pm 0.20$	$21.00^{cd} \pm 0.10$	$65.00^{h} \pm 0.10$
PNZ12	$4.00^{b} \pm 0.01$	$43.00^{fg} \pm 0.25$	$6.00^{g} \pm 0.01$	$49.00^{n} \pm 0.20$
PNZ13	$4.00^{b} \pm 0.01$	$44.00^{fg} \pm 0.20$	$4.00^{h} \pm 0.10$	$48.00^{n} \pm 0.20$
PNZ14	$4.00^{b} \pm 0.01$	$44.00^{fg} \pm 0.20$	$4.00^{h} \pm 0.10$	$48.00^{n} \pm 0.20$
PNZ15	$4.00^{b} \pm 0.01$	$48.00^{f} \pm 0.10$	$25.00^{e} \pm 0.10$	$73.00^{f} \pm 0.10$
JUA16	$4.20^{ab} \pm 0.01$	$53.33^{e} \pm 0.01$	$26.67^{c} \pm 0.10$	$80.00^{d} \pm 0.01$
JUA17	$3.80^{c} \pm 0.01$	$56.00^{de} \pm 0.01$	$21.33^{cd} \pm 0.10$	$77.67^{e} \pm 0.10$
JUA18	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$8.00^{f} \pm 0.10$	$52.00^{m} \pm 0.20$
JUA19	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$18.67^{de} \pm 0.20$	$62.67^{i} \pm 0.10$
JUA20	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$21.33^{cd} \pm 0.10$	$65.33^{h} \pm 0.20$
JUA21	$4.10^{ab} \pm 0.01$	$47.00^{fg} \pm 0.10$	$25.00^{c} \pm 0.10$	$72.00^{f} \pm 0.10$
JUA22	$4.00^{ab} \pm 0.01$	$42.00^{fg} \pm 0.30$	$12.00^{e} \pm 0.20$	$54.00^{k} \pm 0.10$
JUA23	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$14.00^{e} \pm 0.20$	$58.00^{j} \pm 0.10$
JUA24	$4.00^{ab} \pm 0.01$	$46.00^{fg} \pm 0.10$	$18.00^{de} \pm 0.25$	$64.00^{i} \pm 0.10$
JUA25	$4.00^{ab} \pm 0.01$	$43.00^{fg} \pm 0.10$	$7.00^{f} \pm 0.10$	$50.00^{m} \pm 0.20$
JUA26	$4.00^{ab} \pm 0.01$	$46.00^{fg} \pm 0.10$	$22.00^{cd} \pm 0.10$	$68.00^{g} \pm 0.01$
JUA27	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$19.00^{de} \pm 0,20$	$63.00^{i} \pm 0.10$
JUA28	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.20$	$18.00^{de} \pm 0.25$	$62.00^{i} \pm 0.10$
JUA29	$4.00^{ab} \pm 0.01$	$43.00^{fg} \pm 0.20$	$16.00^{e} \pm 0.01$	$59.00^{j} \pm 0.10$
JUA30	$4.00^{ab} \pm 0.01$	$44.00^{fg} \pm 0.10$	$21.00^{cd} \pm 0.10$	$65.00^{h} \pm 0.10$

NE- not established by Brazilian law. PNZ and JUA produced in Petrolina and Juazeiro respectively. *Average ± standard deviation of triplicate analyses. Values that have same letter, in same column, do not show significant differences ($p < 0.05$) by Tuckey to 95% confidence.

In analysis of free acidity, it was found that five samples of Petrolina and two from Juazeiro were outside values established in Technical Regulation of Honey Identity, Quality (BRAZIL, 2000), which states that free acidity values should not be exceed 50 milliequivalents of acidity kg^{-1} of honey. Exception of these samples that had values that were very different from established by law, other samples did not present significant differences. Lactonic acidity presented values ranging between 4,00 and 40,00 mEq Kg^{-1} for samples produced, marketed in Petrolina, values between 7.00 and 26.67 mEq Kg^{-1} for samples of Juazeiro. Total acidity values ranged from 48.00 to 109.64 mEq Kg^{-1} when analyzing samples from both cities.

Garcia *et al*. (2018) when analyzing honeys from different flowering found acidity values ranging between 13.07 and 6.31 mEq Kg^{-1}. In studies by Garcia *et al*. (2018) did not evaluate lactonic, total acidity. Vicinieski, Cordeiro and Oliveira. (2018) when analyzing honeys from small gaucho producers found for free acidity values between 27.54 to 42.14 mEq Kg^{-1}, lactonic acidity ranging from 8.70 to 19.18 mEq Kg^{-1}, total acidity values in range between 36.09 and 57.85 mEq Kg^{-1}. Borges *et al*. (2017) when analyzing honeys from Salvador, Petrolina found values for city of Petrolina between 14.1 and 45.5 meq kg^{-1}, for city of Salvador values between 65.6 and 69.9 meq kg^{-1}, values higher than those allowed by current legislation.

According to Paiva *et al*. (2012), free acidity, can be used as indication of degree of honey deterioration. Increase in acidity is probably due to fermentative or enzymatic process of honey at room temperature conditions. In addition, acidity in honey results from variation of organic acids from nectar by action of enzyme glucose oxidase, which in turn originates gluconic acid through bacteria during honey maturation process (Abadio Finco, Moura and Silva, 2009; Finola *et al*., 2007; Vilhena, Almeida-Muradian, 1999; Vicinieski, Cordeiro and 2018). In addition, environmental factors, plants available for use in honey composition vary greatly between states, municipalities, countries.

Table 2 presents results found in analysis of total phenolics, flavonoids, antioxidant activity by DPPH and FRAP. Total

phenolics tests are widely used as indirect measure to know antioxidant power of samples, since largest contribution to antioxidant activity comes from total phenolics present in sample, so higher total phenolic content, and better antioxidant activity.

Total phenolic values were calculated using gallic acid calibration curve y = 16.155x-0.0041, R^2 = 0.9969, where values ranging from 0.06 to 0.98 mg EAG g^{-1} (Table 2). Zarei *et al*. (2019) when studying multifloral honeys found values between 0.46 and 0.61mg EAG g^{-1}.

Gul and Pehlivan. (2018) when analyzing antioxidant activity floral honeys from Peru found contents ranging from 0.34 to 4.7 mg g^{-1}. Nascimento *et al*. (2018), when analyzing antioxidant capacity of Brazilian monofloral honeys, found total phenolic contents ranging between 0.26 to 1.0 mg g^{-1}.

Table 2 Antioxidant activity of honeys produced, marketed in northeastern semiarid.

Samples	Phenolic Totals (EAG g^{-1})	Flavonoids Totais (mg g^{-1})	DPPH (%)	FRAP (mMFe^{2+} 100g^{-1})
PNZ1	$0.72^{b} \pm 0.03$	0.16 ± 0.08	$64.91^{i} \pm 0.61$	$565.20^{f} \pm 0.20$
PNZ2	$0.43^{g} \pm 0.05$	0.05 ± 0.08	$38.66^{g} \pm 0.53$	$425.06^{i} \pm 0.05$
PNZ3	$0.26^{i} \pm 0.02$	0.24b± 0.06	$12.94^{p} \pm$ 0.77	$516.82^{g} \pm 0.05$
PNZ4	$0.49^{f} \pm 0.02$	0.22 ± 0.28	$43.57^{f} \pm 0.59$	$603.21^{d} \pm 0.15$
PNZ5	$0.71^{b} \pm 0.05$	0.17 ± 0.14	$64.89^{b} \pm 0.15$	$286.07^{o} \pm 0.10$
PNZ6	$0.40^{g} \pm 0.14$	0.09 ± 0.05	$35.87^{h} \pm 0.59$	$339.82^{l} \pm 0.05$
PNZ7	$0.60^{d} \pm 0.08$	$0.32^{a} \pm 0.18$	$53.97^{b} \pm 0.14$	$59.16^{r} \pm 0.01$
PNZ8	$0.63^{c} \pm 0.03$	$0.24^{b} \pm 0,08$	$56.54^{c} \pm 0.40$	$611.65^{c} \pm 1.00$
PNZ9	$0.64^{c} \pm 0.04$	$0.17^{e} \pm 0.15$	$57.37^{o} \pm 0.01$	$645.44^{b} \pm 0.10$
PNZ10	$0.98^{a} \pm 0.01$	0.27 ± 0.08	$87.39^{a} \pm 0.14$	$450.01^{h} \pm 0.15$
PNA11	$0.30^{h} \pm 0.04$	0.12 ± 0.10	$26.99^{l} \pm 0.29$	$400.49^{j} \pm 0.15$
PNZ12	$0.42^{g} \pm 0.01$	0.26 b ± 0.03	$37.65^{g} \pm 0.58$	$464.22^{h} \pm 0.26$
PNZ13	$0.22^{j} \pm 0.32$	$0.12^{hi} \pm 0.10$	$17.26^{o} \pm 0.01$	$334.07^{m} \pm 0.01$
PNZ14	$0.19^{j} \pm 0.02$	$0.06^{ab} \pm 0.10$	$5.27^{rs} \pm 0.14$	$610.50^{c} \pm 1.00$
PNZ15	$0.17^{j} \pm 0.21$	$0.13^{mn} \pm 0.06$	$4.45^{rs} \pm 0.01$	$704.18^{a} \pm 0.10$
JUA16	$0.64^{c} \pm 0.03$	$0.20^{d} \pm 0.06$	$56.94^{c} \pm 0.15$	$550.22^{f} \pm 0.15$
JUA17	$0.53^{e} \pm 0.02$	$0.24^{bc} \pm 0.10$	$47.04^{e} \pm 0.29$	$565.58^{f} \pm 0.19$
JUA18	$0.54^{e} \pm 0.02$	$0.04^{op} \pm 0.03$	$48.14^{d} \pm 0.13$	$461.53^{h} \pm 0.29$
JUA19	$0.39^{g} \pm 0.15$	$0.04^{nop} \pm 0.00$	$35.24^{h} \pm 0.01$	$256.51^{p} \pm 0.10$
JUA20	$0.49^{f} \pm 0.03$	$0.06^{mn} \pm 0.08$	$43.91^{f} \pm 0.44$	$25.76^{s} \pm 0.06$
JUA21	$0.25^{i} \pm 0.04$	$0.10^{jl} \pm 0.03$	$22.67^{n} \pm 0.29$	$154.00^{q} \pm 0.04$
JUA22	$0.33^{h} \pm 0.13$	$0.24^{bc} \pm 0.13$	$29.65^{j} \pm 0.08$	$461.92^{h} \pm 0.09$
JUA23	$0.18^{j} \pm 0.02$	$0.15^{fgh} \pm 0.09$	$7.53^{q} \pm 0.32$	$295.67^{o} \pm 0.01$
JUA24	$0.16^{j} \pm 0.01$	$0.13^{ghi} \pm 0.10$	$5.64^{r} \pm 0.02$	$244.99^{p} \pm 0.22$
JUA25	$0.12^{l} \pm 0.01$	$0.08^{lm} \pm 0.09$	$2.54^{r} \pm 0.15$	$257.28^{p} \pm 0.10$
JUA26	$0.13^{l} \pm 0.02$	$0.06^{mn} \pm 0.05$	$3.16^{b} \pm 0.02$	$388.97^{k} \pm 0.07$
JUA27	$0.09^{m} \pm 0.01$	$0.07^{lm} \pm 0.10$	$0.32^{m} \pm 0.58$	$496.47^{g} \pm 0.08$
JUA28	$0.27^{i} \pm 0.22$	$0.03^{p} \pm 0.05$	$6.35^{rs} \pm 0.04$	$589.00^{e} \pm 0.10$
JUA29	$0.13^{l} \pm 0.01$	$0.06^{mn} \pm 0.13$	$3.17^{b} \pm 0.06$	$412.39^{j} \pm 0.02$
JUA30	$0.25^{i} \pm 0.11$	$0.17^{e} \pm 0.13$	$15.87^{o} \pm 0.02$	$308.73^{n} \pm 0.05$

*Average ± standard deviation of triplicate analyses. Values that have same letter, in same column, do not show significant differences ($p < 0.05$) by Tukey to 95% confidence

Vasic *et al*. (2019) when analyzing honey of honey of botanical origin found 41 different compounds found in all studied samples with average values of total phenolics ranging from 0.57 to 1.60 mg of GAE g^{-1} honey. Results reported by him indicate high variability of TPC values for honeydew honey, since they contain more phenolic compounds compared to floral honeys. Other authors also confirm this hypothesis (Pita-Calvo and Vázquez, 2017; Alves *et al*., 2013).

Antioxidants in honey include both enzymatic substances such as glucose oxidase, catalase, peroxidase and non-enzymatic substances such as tocopherol ascorbic acid, carotenoids, over 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins, cinnamic acid derivatives (Al Mamary *et al*., 2002; Khalil *et al*., 2012; Al Farsi *et al*., 2018).

Flavonoid contents were calculated using standard quercetin curve y = 1.2136x + 0.0211, R^2= 0.9992, values ranging from 0.03 to 0.32 mg g^{-1} were found. Nascimento *et al*. (2018) found in honey flavonoid values ranging from 0 to 0.026 mg g^{-1}. In most samples there was significant difference between them. Bueno-Costa *et al*. (2016) in studies on flavonoid content of honeys from southern Brazil found values in range of 0.03 to 0.10 mg g^{-1}. Al-Farsi *et al*. (2018) when analyzing flavonoid contents in honey producing regions of Sultanate of Oman in Asia found values ranging between 1.61 and 2.89 mg g^{-1}. Flavonoids, simple phenolic derivatives such as phenolic acids represent majority of plant polyphenols, are considered to be main antioxidants in honey (Bravo, 1998; Al – Farsi *et al*., 2018).

In recent years, there has been increasing interest in determining antioxidant activity of honey. Amount of components responsible for antioxidant activity of honey varies widely according to floral, geographical origin of honey (Nayk and Nanda, 2016; Nascimento *et al*., 2018; Zarei *et al*., 2019). Many methods have been used to determine honey's antioxidant activity, such as DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducer/ Antioxidant), ORAC (Oxygen Radical Absorption Capacity) Test, TEAC (antioxidant activity equivalent to Trolox).

In DPPH radical reduction tests, values ranged from 0.32 to 87.39%, showing significant strength among most samples. Figure 1 shows some results of antioxidant activity by DPPH in honey samples, in this test greater discoloration of DPHH radical (purple color), higher phenolic content, and better antioxidant activity.

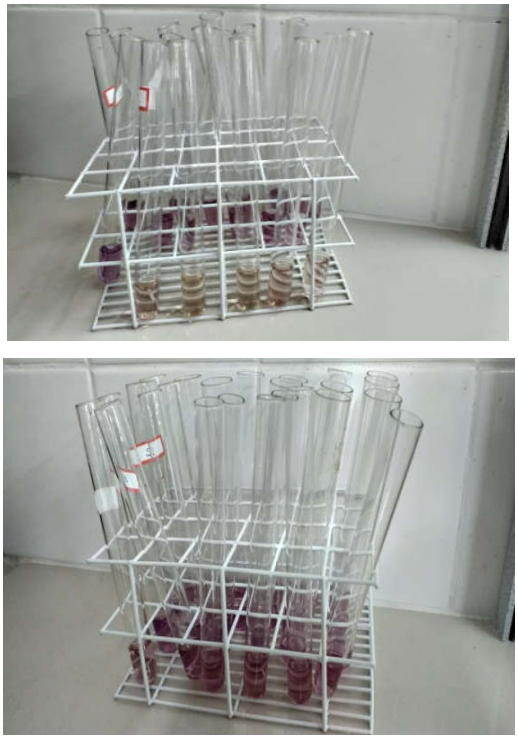

Figure 1 Honey samples submitted to DPPH test

In studies by Vasic *et al*. (2020) DPPH radical scavenging activity was found between 12.20 and 48.89%. Zarei *et al*. (2019) found DPPH values between 43.1 and 6.8% for multifloral honey samples, 61.3 and 7.6% for thyme honey. Maximum values found in this study were higher than those found by researchers cited. Since DPPH assay procedure reflects only antioxidant activity of water soluble substances (Aljadi and Kamaruddin, 2004; Zarei *et al*., 2019), FRAP test was used to assess total antioxidant activity.

Ferric Reducing Antioxidant Power Test (FRAP) is simple, fast, accurate, able to measure antioxidants' ability to convert ferric ions (Fe^{3+}) to ferrous (Fe^{2+}), resulting in formation of colored ferrous-tripyridyltriazine complex, pH reduction low causes a color change from colorless to blue, which can be measured by absorbance from 593 to 600 nm. Antioxidant activity levels by FRAP were found using ferrous sufate calibration curve $y = 0.3123x + 0.1543$, $R^2 = 0.9595$, with values in range between 25.67 and 704.18 mM 100 g^{-1}.

Tuksitha *et al*. (2017) in their analyzes of Sarawak honey found FRAP values between 50.66 ± 5.77 mM Fe^{2+} 100 g^{-1}. According to Nayik and Nanda. (2016), during heat treatment, honey loses most of its natural antioxidants, which can be offset by formation of non-nutritional antioxidants, products of Maillard reaction. Although heat treatment is essential to eliminate possible *Clostridium botulinum* spores, they may influence antioxidant capacity of honeys.

CONCLUSION

Honeys showed varied profile in all tests performed with significant differences. In pH analyzes all samples were within established standards for floral honeys. In free acidity tests 7 samples exceeded values established by current legislation. Antioxidant profile was quite varied in samples, but they prove that honeys can be used as natural antioxidants, are used to reduce free radical damage to human body.

Acknowledgment

Authors acknowledgment Federal University of Valley of San Francisco for use of laboratories for experiments.

References

1. Roshan, A., Gad, H. A., El-Ahmady, S.H., Abou-Shoer, M.I., Khanbash, M.S., Al-Azizi, M.M. 2017. Characterization and discrimination of the floral origin of sidr honey by physicochemical data combined with multivariate analysis. Food Anal. Methods., 10:137-146.
2. Pita-Calvo, C., Vázquez, M. 2017. Differences between honeydew and blossom honeys: A review. Trends Food Sci Tech.,59:79-87.
3. Nascimento,K. S., Sattlera, J. A. G., Macedo, L. F. L., González, C. V. S., Melo, I. L. P., Araújo, E. S., Granato, D., Sattlerd, A., Almeida-Muradian, L. B. 2018. Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian *Apis mellifera* honeys. LWT - Food Sci Technol., 91:85–94.
4. Borges, J. G., Carvalho, A, C., Santana, L. L. D. S and Mota, C. M. C. 2019. *Appis melifera* honey marketed in cities of Petrolina-PE and Juazeiro-BA: Physicochemical properties and adulterating. Int. J. Acad. Res., 01(07):328-332.
5. Borges, J. G., Pinheiro, J. V. R., Telles, R. B. A and de Quadros, C. P. 2017. Determination of honey quality sold in salvador and petrolina free market. Braz. J. agri-industrial prod., 19(3): 231-240.
6. Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A and Al-Belushi, S. 2018. Color, flavonoids, phenolics and antioxidants of Omani honey. Heliyon., 4:00874.
7. Khalil *et al*., 2012. Khalil, I., Moniruzzaman, M., Boukraâ, L., Benhanifia, M., Islam A., Islam, N., Sulaiman, S. A., Gan, S. H. 2012. Physicochemical and antioxidant properties of Algerian honey. Molecules., 17(9):11199-11215.
8. Martins, N., Petropoulos, S and Ferreira, I. C. F. R. 2016. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem., 211:41-50.
9. Nayik, G. A and Nanda, V. 2016. Effect of thermal treatment and pH on antioxidant activity of saffron honey by using response surface methodology. Food Mea., 10 (1): 64-70.
10. Zarei, M., Ali Fazlara, and Tulabifard, N. 2019. Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon., 5(6): 01894.
11. Moraes, R. M and Teixeira, E. W. 1998. Honey Analysis. Pindamonhangaba, 42p. (Technical manual).
12. AOAC- International Official methods of analysis. 2012. 19th ed. Gaithersburg, MD: Association of Official Analytical Chemists, 300p.
13. Adolfo Lutz Institute. 2008. Physicochemical methods for food analysis**.** São Paulo, 1020 p.
14. Singleton, V. L., Rossi, J. A. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol Vitic., 16: 144-158.
15. Woisky, R.G. and Salatino, A. 1998. Analysis os propolis: some parameters ond prodecore for chemical fuality control. J. Apic. Res., 37(2):99-105.
16. Braca, A., Tommasi, N. D., Bari, L. D, Pizza, C., Politi, M and Morelli, I. 2001. Antioxidant principles from Bauhinia terapotensis. J. Nat. Prod., 64: 892–895.
17. BRAZIL, 2000. BRASIL. Ministry of Agriculture, Livestock and Supply. Normative Instruction 11, October 20, 2000, Technical Regulation of Honey Identity and Quality**.** Available in<http://www.agricultura.gov.br/sda/dipoa/anexo_intrnorm11.htm> Accessed on: November 26, 2018.
18. Abadio Finco, F. D. B., Moura, L. L and Silva, I. G. 2010. Propriedades físicas e químicas do mel de *Apis mellifera* L. Ciênc. Tecnol. Aliment., 30(3): 706-712.
19. Moura, L. R., Sanches, I. S., Tesch, M. F. T., Armesto, C and Souza, L. A. 2017. Physicochemical analysis of different honeys produced and marketed in Brazil. Focus on Manag. Magazine., 9.
20. Viciniescki, R. P., Cordeiro, S. G and Oliveira, E. C. 2018. Adulteration detection and physicochemical characterization of bee honey from small producers in the interior of Rio Grande do Sul. Acad. Hig. Magazine., 10 (4).

21. Crane, E. 1985. The book of honey. 2ª Edição. São Paulo-SP. Editora Nobel, S.A.
22. Garcia, L. N. H., Castro, B. G., Oliveira, J. F.,Velame, M. S., Raghiante, F., Pinto, J. P. A. N., Possebon, F. S and Martins, O. A. 2018. Physicochemical quality of bee honey *Apis mellifera* of different flowering. Rev. Braz. Hig. San. Anim.,12 (1): 11-20.
23. Paiva, C. A., Queiroz Tomaz, H. V., Aroucha, E. M. M., Nunes, G. H. S and Oliveira, A. J. F. 2012.Vida de prateleira do mel produzido por abelhas africanizadas. Acta Vet. Bras., 6 (2):151-159.
24. Finola, M. S., Lasagno, M. C and Mario, J. M. 2007. Microbiological and chemical characterization of honeys from central Argentina. Food Chem., 100:1649-1653.
25. Vilhena, F and Almeida-Muradian, L. B. 1999. Manual of Physicochemical analyzes of honeys from São Paulo. Mensagem Doce., 53:17-19.
26. Gül, A and Pehlivan, T. 2018. Antioxidant activities of some monofloral honey types produced across Turkey. Saudi. J. Biol. Sci., 25, 1056-1065.
27. Vasić, V., Gašić, U., Stanković, D., Lušić, D., Vukić-Lušić, D., Milojković-Opsenica, D., Tešića, Z andTrifković, J. 2019. Towards better quality criteria of European honeydew honey: Phenolic profile and antioxidant capacity. Food Chem., 274:629–641.
28. Alves, A., Ramos, A., Gonçalves, M. M., Bernardo, M., Mendes, B. 2013. Antioxidante activity, quality parameters and mineral contente of Portuguese monofloral honeys. J. Food Comp. Anal., 30(2):130-138.
29. Al-Mamary, M., Al-Meeri, A., Al-Habori, M. 2002. Antioxidant activities and total phenolics of different types of honey. Nutr. Res., 22:1041-1047.
30. Khalil, I., Sulaiman, S., Alam, N., Ramli, N., Mohamed, M., Bai'e, S., Hua, G. 2012. Content and antioxidant properties of processed Tualang honey (agromas) collected from different regions in Malaysia. Int. J. Pharm. Pharm. Sci., 4 (3): 214-219.
31. Bueno-Costa, F. M., Zambiazi, R. C., Bohmer, B. W., Chaves, F. C., Silva, W. P., Zanusso, J. T and Dutra, I. 2016. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. Food Sci.Toxicol., 65:333–340.
32. Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolis, and nutritional significance. Nutr. Rev., 56 (11): 317-333.
33. Nayik, G. A and Nanda, V. 2016. A chemometric approach to evaluate the phenolic compounds, antioxidant activity and mineral content of different unifloral honey types from Kashmir, India. LWT - Food Sci Tech., 74: 504–513.
34. Aljadi, A. M and Kamaruddin, M. Y. 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem., 85:513–518.
35. Tuksitha,L., Chen, S. Y. L., Chen, Y.L ., Wong, K.Y and Peng, C.C. 2018. Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). J. Asia-Pacific Ent., 21 (2):563-570.

****