DOI: https://doi.org/10.17921/1415-6938.2022v26n3p251-260

Microbiological and Physicochemical Quality and Antioxidant and Antimicrobial Potential of Commercial Honeys from the Sertão of North-eastern Brazil

Qualidade Microbiológica e Físico-química e Potencial Antioxidante e Antimicrobiano de Méis Comerciais do Sertão Nordestino do Brasil

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Abstract

The study aimed to evaluate the microbiological and physicochemical quality and the antioxidant and antimicrobial potential of commercial honeys from the northeastern Sertão of Brazil. The quality of thirty commercial honey samples was investigated through microbiological and physicochemical analyzes (color, moisture, total soluble solids, acidity, formaldehyde index, ash, hydroxymethylfurfural, diastasis, Lund test and electrical conductivity). Samples that complied with national and international legislation were analyzed for reducing sugars, proline, total proteins, antioxidant and antibacterial activity. In all honey samples studied, no total coliforms, E. coli, S. aureus, yeasts or Clostridium spores were detected. However, only 26.67% of the samples were within the parameters determined by the normative committees regarding the physical-chemical quality. Both antioxidant and antimicrobial activity were determined and showed the highest levels for samples P9 and F9. From the results it is possible to suggest that the honey samples collected in the north-eastern Sertão have microbiological quality, antimicrobial and functional potential. However, it is necessary that the product meets the physicochemical standards so that it can be used safely in the promotion of human health. These data direct public policies to maximize technical assistance and technology transfer to producers in the northeastern Sertão of Brazil, in order to improve the quality of honey so that they meet the standards established by the normative committees.

Keywords: *Apis mellifera*. Bee Products. DPPH. Multifloral Honey. Polyphenols. Proline. *Salmonella typhi*.

Resumo

O estudo teve como objetivo avaliar a qualidade microbiológica e físico-química e o potencial antioxidante e antimicrobiano de méis comerciais do Sertão nordestino do Brasil. A qualidade de trinta amostras de méis comerciais foi investigada por meio de análises microbiológicas e físico-químicas (cor, umidade, sólidos solúveis totais, acidez, índice de formaldeído, cinza, hidroximetilfurfural, diástase, Teste de Lund e condutividade elétrica). As amostras que atenderam à legislação nacional e internacional foram analisadas quanto a açúcares redutores, prolina, proteínas totais, atividade antioxidante e antibacteriana. Em todas as amostras de mel estudadas não foram detectados coliformes totais, E. coli, S. aureus, leveduras ou esporos de Clostridium. Contudo, apenas 26,67% das amostras estavam dentro dos parâmetros determinados pelos comitês normativos quanto à qualidade físico-química. Tanto a atividade antioxidante quanto a antimicrobiana foram determinadas e apresentaram os maiores teores para as amostras P9 e F9. A partir dos resultados é possível sugerir que as amostras de mel coletadas no Sertão nordestino apresentam qualidade microbiológica, potencial antimicrobiano e funcional. No entanto, é necessário que o produto atenda aos padrões físico-químicos para que seja utilizado com segurança na promoção da saúde humana. Estes dados direcionam políticas públicas à maximização da assistência técnica e transferência de tecnologia aos produtores do Sertão nordestino do Brasil, no intuito de melhorar a qualidade do mel para que atendam os padrões estabelecidos pelos comitês normativos.

Palavras-chave: *Apis mellifera. Produtos Apícolas. DPPH. Mel Multifloral. Polifenóis. Prolina. Salmonella typhi.*

1 Introduction

Honey production in the Sertão of the San Francisco, dry interior region of north-eastern Brazil, is based on the flowering of an exclusively Brazilian biome, the Caatinga. This production also contributes to agriculture in the region, which is an important polo of production and export of fruit (PEREIRA *et al*., 2019). Bees as pollinators of native and cultivated plants are important for the economy, in addition to providing bee products they also contribute to agricultural quality and productivity, as well as generating a secondary income for farmers by producing honey (CARNEIRO-NETO *et al*., 2017).

Honey is a natural food of high nutritional value that is composed of substances beneficial to human health (MITITELU *et al*., 2022). Some of the benefits are related to antimutagenic, anti-inflammatory, antioxidant and antimicrobial properties (ALVAREZ-SUAREZ *et al*., 2013; LEME *et al*., 2018; ZAREI *et al*., 2019). In addition, honey has therapeutic potential for diseases including cancer, diabetes, and cardiovascular, liver, neurodegenerative, and pulmonary diseases (ALVAREZ-SUAREZ *et al*., 2013). However, the health protection characteristics depend on the composition and quality of the honey, which is strongly influenced by its botanical and geographical origin, as well as climatic and environmental conditions (EL-HASKOURY *et al*., 2018).

Factors related to the production, extraction, handling, and

processing of honey can also affect its quality, which justifies the need for constant supervision in establishments responsible for the processing of bee products and its derivatives. Honey quality is assessed according to its physicochemical and microbiological characteristics, which are regulated in the Brazil by identity and quality document (BRASIL, 2000), based on the codex standard for honey published by the Codex Alimentarius Commission (CODEX ALIMENTARIUS, 2001).

Studies that simultaneously investigate microbiological and physicochemical quality and beneficial aspects of honey samples do not exist in North-eastern Brazil. According to Gomes *et al*., (2010) these studies would be important for the regional honey chain, as they could signal opportunities for improvement and direct the supply of quality products, which result in benefits to the health of the consumer. Therefore, this is a pioneer study and aimed to evaluate the microbiological and physicochemical quality and the antioxidant and antimicrobial potential of commercial honeys from the Sertão of North-eastern Brazil.

2 Material and Methods

2.1 Honey samples

Thirty samples of commercial honeys of *Apis mellifera* and multifloral origin from the city of Petrolina, Sertão of North-eastern Brazil, were analysed in this study. Among the samples, 10 were collected from Association of Petrolina Bee Breeders (ASCAMP), 10 from local supermarkets, and 10 from fairs (street markets). After their collection, the samples were immediately transported to the laboratory for analysis.

2.2 Microbiological analysis

The enumeration of total and thermotolerant coliforms was performed in triplicate according to Heizmann *et al.* (1988), with some modifications, results were expressed as Most Probable Number (MPN) per gram of sample. For the counts of *Staphylococcus aureus* and moulds and yeasts was performed according to (BRASIL, 2003), results were expressed as CFU/g of honey. The detection of *Clostridium* sp. spores was performed as reported by Ragazani *et al.* (2008), for tubes with turbidity, Wirtz-Conklin spore staining was performed.

2.3 Physicochemical analysis

The coloring, moisture content, soluble solids, acidity, formaldehyde index, ash, Lund's test and diastatic activity of the samples were required according to the methodology reported by Brasil (1981), in triplicate. As for the hydroxymethylfurfural index (HMF) and electrical conductivity of honey, it was measured according to Bogdanov (2009). The honey samples that fully met the legal parameters valid in the corresponding legislation (BRASIL, 2000; CODEX ALIMENTARIUS, 2001) were selected for the other necessary tests in the following ones.

2.4 Determination of the reducing sugar and total protein content and proline concentration of selected honey samples

The reducing sugar content of the selected honeys was determined by oxide-reduction titration using the Fehling method (BRASIL, 1981), in triplicate. According to the method of Bradford (1976), the total protein content of the samples was measured using a Pierce™ Coomassie Plus kit (Bradford) (Thermo Fisher Scientific, Carlsbad, CA, USA), according to the manufacturer's instructions.

To determine the proline concentration, aqueous honey solution (50 mg/mL) and proline standard solution (12.5, 25, 50, and 100 mg/mL) were prepared was performed according to (BOGDANOV, 2009). The result was expressed in mg proline per kg of honey, where the proline standard curve presented a linearity of 0.999 (R^2).

2.5 Antioxidant activity of selected honey samples

2.5.1 Total phenol content (TPC)

Deionized water (10 mL) was used to dilute the honey samples (1 g) for subsequent filtration through Whatman filter paper (no. 1). Under stirring, an aliquot (0.2 mL) of the solution was mixed with 0.2 N Folin-Ciocalteu reagent (1 mL) by 5 minutes After adding 0.8 mL of 7.5% sodium carbonate (Na_2CO_3) and resting for 2 h at room temperature away from light, the absorbance was read at 760 nm. Methanol was used as blank. For the calibration curve, aqueous gallic acid solutions (0–100 μg/mL) were prepared and the linearity was 0.999. The result was expressed as mg of gallic acid equivalents (GAE)/100 g of honey (AL *et al*., 2009).

2.5.2 Total flavonoid content (TFC)

To determine the TFC, 0.3 mL of sodium nitrite ($NaNO_2$) 5% was added to 1 mL of methanolic honey solution (1 mg/mL). After 5 minutes, 0.3 mL of aluminium chloride ($AlCl_3$) 10% was also added. The mixture was stirred and after 6 minutes, there was the addition of 2 mL sodium hydroxide (NaOH) solution (1 M). The absorbance was measured at 510 nm. Quercetin methanolic solution at concentrations ranging from 0 to 120 μg/mL were used in the calibration curve with a linearity of 0.9974. The results were expressed in mg quercetin equivalents (QE)/100 g of honey (AL *et al*., 2009).

2.5.3 DPPH free radical-scavenging activity

As reported by Al *et al.* (2009) with some modifications, 1.5 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) methanolic solution was added to 0.75 mL of each methanolic honey solution. The samples were incubated away from light at room temperature for 15 minutes before measuring the resulting absorbance at 517 nm. For this analysis, the methanolic DPPH solution and methanol were

used as the controls. The blanks were composed of each methanolic honey solution and methanol. Standard solutions (DPPH solution, 0–120 µM, linearity of 0.9983) were used as the positive controls. The following formula was used to evaluate the scavenging activity: Scavenging ability (%) = $[1 - (A_{sample} - A_{blank}) / A_{control}] \times 100$.

2.5.4 Ferric reducing antioxidant power (FRAP)

One gram of each of the selected honeys was added to 10 mL of methanol. Then, 270 µL of water and 2.7 mL of FRAP reagent were added to 90 µL of honey solution. The FRAP solution consisted of 25 mL acetate buffer (0.3 M), 2.5 mL 2,4,6-tris (2-pyridyl)-s-triazine solution (TPTZ) (10 mM) (Sigma-Adrich) in HCl (40 mM) and 2.5 mL of aqueous ferric chloride hexahydrate ($FeCl_3 .6H_2O$) solution (20 mM). The mixture was stored in a water bath at 37 °C for 30 minutes, followed by the measurement of absorbance at 595 nm. For the calibration curve, aqueous solutions of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$, 500–2000 µM/mL) were prepared and the linearity obtained was 0.9967. The results were expressed as µM ferrous sulphate per 100 g honey sample (RUFINO *et al*., 2006).

2.6 Antibacterial activity of selected honey samples

The antibacterial activity of the honey samples was tested by disk agar diffusion, according to Almeida Júnior et al. (2015). Pure honey (100%) and 80% aqueous solution were used. For the test, the cultures of the pathogens *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Salmonella typhi* (ATCC 6539) were standardized to 10^8 CFU/mL.

2.7 Statistical analysis

The tests were performed in triplicate, except the colouration, moisture content, and TSS analyses. The results were evaluated using analysis of variance (ANOVA) and the means compared with the Scott-Knott test ($p < 0.05$) in Sisvar® software (version 5.6, Lavras, MG, Brazil). Principal component analysis (PCA) was used to evaluate the relationship between the biochemical variables, as well as to visualize the differences between the honey samples. PCA with confidence ellipses (confidence level 95%) for the set of honey samples obtained at each point of sale (producer associations, fairs, and supermarkets), as well as PCA with Varimax rotation for honey samples selected after physicochemical analysis, were used in Origin® software (student version 2020, Northampton, MA, USA).

3 Results and Discussion

3.1 Microbiological and physicochemical quality of honey

Among all honey samples studied, total coliforms, *E. coli, S. aureus*, yeasts or *Clostridium* spores were not detected (Table 1). The absence of these microorganisms may be related to the low moisture content of the honey parts.

Table 1 - Microbiological analyses, moisture content, colouration, and total soluble solids (TSS) of commercial honey samples from Petrolina in Sertão of North-eastern Brazil

Origin	Samples	Microbiological analyzes				First physicochemical analyzes		
		E. coli	*S. aureus*	Yeasts	*C.* spores	Moisture (%)	Colouration	TSS (°Brix)
Producer association	P1	absent	absent	absent	absent	15.2	Dark amber	84.8
	P2	absent	absent	absent	absent	16.6	Amber	83.4
	P3	absent	absent	absent	absent	17.2	Amber	82.8
	P4	absent	absent	absent	absent	16.0	Amber	82.4
	P5	absent	absent	absent	absent	15.0	Dark Amber	83.6
	P6	absent	absent	absent	absent	14.8	Amber	83.8
	P7	absent	absent	absent	absent	18.8	Light amber	79.8
	P8	absent	absent	absent	absent	16.0	Amber	82.6
	P9	absent	absent	absent	absent	16.0	Dark amber	82.6
	P10	absent	absent	absent	absent	16.0	Amber	82.6
Fairs	F1	absent	absent	absent	absent	15.8	Amber	82.8
	F2	absent	absent	absent	absent	19.2	Amber	79.2
	F3	absent	absent	absent	absent	17.8	Extra light amber	80.6
	F4	absent	absent	absent	absent	16.2	Amber	82.2
	F5	absent	absent	absent	absent	18.2	Amber	80.8
	F6	absent	absent	absent	absent	15.6	Dark amber	83.0
	F7	absent	absent	absent	absent	18.8	Dark amber	79.8
	F8	absent	absent	absent	absent	17.0	Amber	81.0
	F9	absent	absent	absent	absent	15.6	Dark amber	83.0
	F10	absent	absent	absent	absent	16.8	Dark amber	81.8

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Origin	Samples	Microbiological analyzes				First physicochemical analyzes		
		E. coli	*S. aureus*	Yeasts	*C.* spores	Moisture (%)	Colouration	TSS (°Brix)
Supermarkets	S1	absent	absent	absent	absent	17.6	White	80.8
	S2	absent	absent	absent	absent	17.6	Light amber	82.0
	S3	absent	absent	absent	absent	17.8	Light amber	80.8
	S4	absent	absent	absent	absent	16.0	Extra light amber	82.6
	S5	absent	absent	absent	absent	17.2	Light amber	81.0
	S6	absent	absent	absent	absent	16.2	Amber	82.2
	S7	absent	absent	absent	absent	17.0	Light amber	81.2
	S8	absent	absent	absent	absent	15.8	Amber	82.8
	S9	absent	absent	absent	absent	19.0	Light amber	91.2
	S10	absent	absent	absent	absent	16.0	Light amber	82.2
Reference values	*	-	-	-	-	≤20%	Almost colorless to dark brown	-
	**	-	-	-	-	≤20%	Almost colorless to dark brown	-

*Brasil (2000). **Codex Alimentarius (2001). *E. coli*= *Escherichia coli*. *S. aureus*= *Staphylococcus aureus*. *C.* spores= *Clostridium* spores.
Source: Research data.

The microbiological quality of honey is of fundamental importance for the consumer's health (ADADI; OBENG, 2017; MITITELU *et al*., 2022). Although the microbial characteristics of honey depend on the intrinsic biota of honey, several factors can influence its contamination (MITITELU *et al*., 2022). The main sources of contamination in honey are related to its production within the bee hive, which are thus difficult to control. However, secondary sources of contamination, related to the extraction, handling, and processing of honey, can be controlled by maintaining good manufacturing practices (ADADI; OBENG, 2017). Adadi e Obeng (2017) also reported on the absence of microorganisms in honey samples taken from two production sites in Tamale metropolis (Ghana).

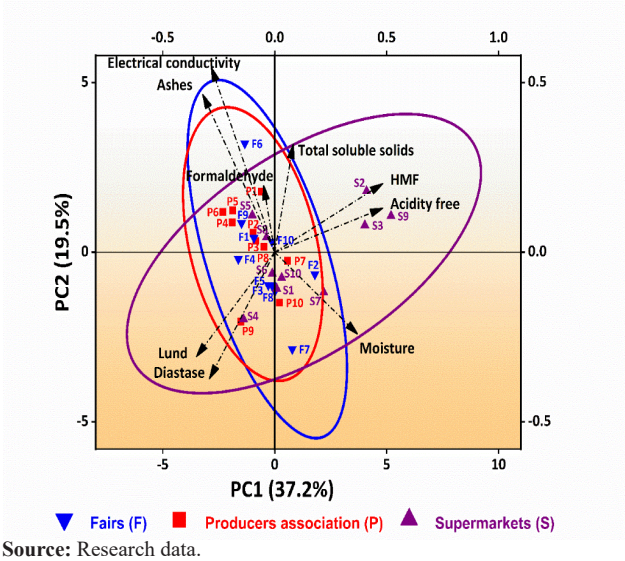
All samples were in concordance with the legal limits in force for moisture (≤20%). The results of the physicochemical analyses for the classification of colouration, moisture content, and TSS, as well as the recommendations according to brazilian (BRASIL, 2000) and international (CODEX ALIMENTARIUS, 2001) standards, are shown in Table 1.

The moisture content of the samples analysed varied from 14.8 to 19.2%, that is, within the limits established by the legislation (≤ 20%). Similarly, Tôrres et al. (2020) found moisture ranging from 16.8 to 18.0% in certified and uncertified honeys from the Brazilian Semi-Arid Region. The low moisture provides a supersaturated environment that deters the growth of many microorganisms. The moisture content is affected by the source of nectar collected by the honey bee, product maturity, environment and time of harvest, and handling during processing (EL-HASKOURY *et al*., 2018; GOMES *et al.,* 2010). A high moisture content (> 20%) predisposes the honey to microbial development, including osmotolerant fermentative yeasts, which are active in the deterioration of honey (GOMES *et al.,* 2010). A low moisture content, on the other hand, reduces or impedes the growth of most microorganisms, as observed in the honey samples in the present study, and contributes to increasing the product's lifetime (KARABAGIAS *et al*., 2014).

Colour is the first parameter to be evaluated by consumers. In the present study, the colour of the honey samples varied from extra light amber to dark amber (Table 1). Color it is a determining factor of consumer acceptance and, consequently, of market value (LEME *et al*., 2018; ZAREI *et al*., 2019). The colour of honey is dependent on ingredients, such as minerals, and can be influenced by a variety of factors, including floral source, storage time, and humidity (SILVA *et al*., 2016). According to Zarei *et al*. (2019), honeys become darker after heat treatment (63 °C for 30 minutes) due to a reduction in the moisture content and an increase in the concentration of the components responsible for the colour of the honey. Colour is the first parameter to be evaluated by consumers. In the present study, the colour of the honey samples varied from extra light amber to dark amber.

In the present study, there was a variation from 79.2 to 91.2 ºBrix (Table 1) in the samples analysed, and TSS correlated positively with PC2, whiles moisture value correlated with PC1 in positive direction (Figure 1) with negative correlation (r = 0.7263). National and international laws have not established a standard or recommendation for the TSS (ºBrix) content of honey. However, this parameter can be applied as a general indicator of sugar content. Furthermore, the TSS content is inversely proportional to the moisture content (GUO *et al*., 2010), as reported by El-Haskoury *et al.* (2018), who found a strong negative correlation between the two variables (r = 0.927). Similar TSS values, ranging from 76.55 to 84.15 °Brix, have been previously reported in honeys from Morocco (EL-HASKOURY *et al*., 2018).

Figure 1 - Biplot of 30 commercial honey samples with confidence ellipses (confidence level 95%) for each set of honey samples (10) obtained at each point of sale (fairs, producers association, and supermarkets). The first two principal components based on 9 physicochemical characteristics (39.8%, PC1 and 18.2%, PC2) were extracted in the principal component analysis (PCA)



Source: Research data.

In PCA biplot (Figure 1), confidence ellipses with 95% confidence intervals are included for each set of honey samples. PC1 was found to have a strong influence on the moisture, free acidity, and HMF of honey with positive eigenvectors, and on the Lund value with negative eigenvector. On the other hand, PC2 highlighted the differences in the TSS, electrical conductivity, formaldehyde content, and ash content with positive eigenvectors, and on diastase with negative eigenvector. The ellipse of the supermarket samples (Figure 1) was found more to the right than the other ellipses due to their high values of HMF, free acidity, and moisture (positive part of PC1). Higher moisture and acidity favour the formation of HMF. On the left, the ellipses were found to overlap, with samples collected from producer associations found more within the negative parts of the PC1 and PC2 than the samples collected from fairs. Furthermore, the samples collected from fairs were found to be prominently grouped on the positive side of PC2, compared to the samples collected from producer associations. These results indicate that the producer association samples were more related to diastase and Lund, while samples obtained from fairs were more related to TSS, electrical conductivity, ash content, and formaldehyde content. These last three parameters are indicative of the botanical and geographical origin of the honey, as well as its authenticity. In addition, as the electrical conductivity indicates the ionizable organic and inorganic substances found in the honey, there is therefore a strong correlation between this parameter and ash content (ALVAREZ-SUAREZ *et al*., 2010).

In these honey samples analysed, the acidity ranged from 31.33 to 167.33 mEq/kg. A total of 21 samples exceeded the maximum value established by national and international regulations (Table 2). An increased free acidity in honey is often related to an increase in the organic acid content, resulting from the fermentation of sugars. Free acidity is an important parameter of quality in honey and is determinutesed by the content of organic acids, inorganic ions, such as phosphate, esters, lactones, and phenolic acids, vitamin C, and proteins (GOMES *et al.,* 2010).

Table 2 - Physicochemical characterization of the commercial honey samples from Petrolina in Sertão of north-eastern Brazil

Origin	Samples	Acidity[1] (mEq/kg)	Formaldehyde[2] (mL/kg)	Ashes[3] (%)	HMF[4] (mg/Kg)	Diastase[5] (°Gothe)	Lund[6] (ml)	Elect. Cond.[7] (mS/cm)
Producer association	P1	56.53^{f}	9.33^{e}	0.29^{e}	13.22^{i}	1.00^{l}	1.20^{e}	0.76^{g}
	P2	36.33^{g}	9.66^{b}	0.29^{e}	13.52^{i}	16.35^{c}	1.00^{e}	0.66^{j}
	P3	81.66^{d}	13.66^{a}	0.27^{e}	2.09^{j}	11.38^{g}	2.00^{a}	0.95^{b}
	P4	33.66^{g}	9.00^{c}	0.36^{d}	7.18^{j}	14.78^{d}	1.33^{c}	0.77^{f}
	P5	33.33^{g}	10.00^{b}	0.42^{c}	8.13^{j}	13.04^{f}	1.23^{c}	0.74^{h}
	P6	38.66^{g}	10.33^{b}	0.40^{c}	9.08^{j}	17.47^{c}	1.63^{b}	0.74^{h}
	P7	83.00^{d}	10.33^{b}	0.13^{g}	18.93^{h}	11.33^{g}	1.00^{e}	0.92^{c}
	P8	68.00^{e}	9.00^{c}	0.13^{g}	2.02^{j}	9.56^{h}	1.33^{c}	0.75^{h}
	P9	38.33^{g}	5.00^{d}	0.12^{g}	6.93^{j}	22.04^{b}	2.23^{a}	0.32^{p}
	P10	121.00^{c}	1.00^{e}	0.06^{h}	14.61^{i}	13.40^{e}	1.53^{b}	0.25^{s}
Fairs	F1	73.33^{e}	11.66^{b}	0.26^{e}	52.44^{g}	11.19^{g}	2.23^{a}	0.73^{i}
	F2	78.66^{e}	14.66^{a}	0.15^{g}	82.03^{e}	0.00^{l}	1.63^{b}	0.27^{q}
	F3	73.66^{e}	7.66^{c}	0.18^{f}	16.27^{i}	10.59^{h}	1.83^{b}	0.43^{k}
	F4	31.33^{g}	8.00^{c}	0.26^{e}	5.78^{j}	21.54^{b}	1.13^{c}	0.78^{f}
	F5	88.16^{d}	16.00^{a}	0.33^{d}	16.32^{i}	14.39^{d}	2.13^{a}	0.20^{u}
	F6	93.66^{d}	2.66^{e}	0.60^{a}	64.27^{f}	5.24^{k}	1.70^{b}	1.52^{a}
	F7	96.33^{d}	2.00^{e}	0.02^{h}	26.69^{h}	11.96^{g}	2.50^{a}	0.26^{r}
	F8	52.33^{f}	5.33^{d}	0. 16^{g}	13.17^{i}	10.39^{h}	1.33^{c}	0.39^{m}
	F9	39.33^{g}	14.00^{a}	0.16^{g}	27.49^{h}	10.52^{h}	1.83^{b}	0.79^{e}
	F10	51.33^{f}	8.66^{c}	0. 23^{f}	45.36^{g}	7.37^{j}	1.31^{c}	0.66^{j}

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Origin	Samples	Acidity[1] (mEq/kg)	Formaldehyde[2] (mL/kg)	Ashes[3] (%)	HMF[4] (mg/Kg)	Diastase[5] (ºGothe)	Lund[6] (ml)	Elect. Cond.[7] (mS/cm)
Supermarkets	S1	67.66^{e}	6.33^{d}	0.19^{f}	27.99^{h}	12.58^{f}	1.53^{b}	0.41^{l}
	S2	167.33^{a}	10.33^{b}	0. 14^{g}	197.10^{a}	0.00^{l}	0.60^{d}	0.66^{j}
	S3	140.66^{b}	13.33^{a}	0.12^{g}	139.72^{c}	0.00^{l}	0.00^{e}	0.11^{w}
	S4	35.33^{g}	8.00^{e}	0.15^{g}	5.49^{j}	26.07^{a}	1.66^{b}	0.36^{n}
	S5	75.66^{e}	10.00^{b}	0.49^{b}	6.83^{j}	8.49^{i}	1.50^{b}	0.83^{d}
	S6	64.00^{e}	11.00^{b}	0.11^{g}	62.52^{f}	16.72^{c}	1.13^{c}	0.27^{q}
	S7	129.33^{c}	4.33^{d}	0.04^{h}	114.87^{d}	16.64^{c}	1.08^{c}	0.24^{t}
	S8	69.66^{e}	9.33^{c}	0.15^{g}	23.10^{h}	10.59^{h}	1.23^{c}	0.76^{g}
	S9	156.00^{a}	3.00^{e}	0.01^{h}	157.08^{b}	6.85^{j}	0.00^{e}	0.19^{v}
	S10	40.00^{g}	2.66^{e}	0.14^{g}	32.37^{h}	4.04^{k}	1.60^{b}	0.35^{o}
Reference values	*	≤50	-	≤0.6	≤60	≥8 ou 3****	-	-
	**	≤50	-	-	≤40 or ≤ 80***	≥8 ou 3*****	-	≤0.8

In each column, mean values with different letters denote significant results ($p < 0.05$), according to the Scott–Knott test. Cells highlighted in dark gray denote values in discordance with legal limits in force (Brasil, 2000; Codex Alimentarius, 2001). *Brasil (2000). **Codex Alimentarius (2001). ***Regions with tropical ambient temperatures, where the HMF content is not to be more than 80 mg/kg.****The minimum value is 8, with a minimum tolerance of 3 for honey with a low enzyme content, since HMF does not exceed 15 mg/kg. *****The minimum value is 8, with a minimum tolerance of 3 for honey with a low enzyme content. [1] Standard Error (SE) = 5.0338; [2]SE = 0.7935; [3]SE = 0.0193; [4]SE = 3.7297; [5]SE = 0.4405; [6]SE = 0.1327; [7]SE = 0.0011. Elect. Cond= Electrical Conductivity.

Source: Research data.

Already the formaldehyde index in the honey samples ranged from 1 to 16 mL/kg (Table 2). Similarly, Leme *et al*. (2018) reported on honeys from northern Brazil with values ranging from 0.99 to 14.05 mL/kg. The formaldehyde index of honey indicates its content of amino compounds, including peptides, proteins, and amino acids (FINCO *et al.,* 2010). The addition of artificial products generally reduces the nitrogen content in honey, which results in a low formaldehyde index. Therefore, this index serves as a parameter of the authenticity of honey. However, an excessively high formaldehyde index may be indicative of the introduction of hydrolysed proteins in the honey bees' diet (LEME *et al*., 2018).

Six and one samples showed results that were lower and higher than the recommended value, respectively. Some samples, S7 and S9, with lower ash contents (Table 2) showed lighter colours (Table 1), and the opposite was also observed (samples P5 and F6). The ash content is used to determinutese the minuteseral content of honey. This parameter indicates the minerals that were present in the soil of the plants from which the honey bees collected their nectar (KARABAGIAS *et al*., 2014; SILVA *et al.,* 2016). Thus, the ash content is associated with the botanical and geographical origin of honeys (ALMEIDA-MURADIAN *et al*., 2013). The ash content influences the colour of the honey, usually with a positive correlation between the two characteristics (KARABAGIAS *et al*., 2014). Among the samples analysed, only the value relative to sample F6 exceeded the maximum limit established by national legislation (BRASIL, 2000), which suggests a deficiency in the processing of this honey sample, particularly in the stages of filtration or decantation.

The HMF index is an important parameter in the evaluation of the purity and freshness of honey (CODEX ALIMENTARIUS, 2001). In this study (Table 2), the HMF values of 12 samples were found above the maximum limit (40 mg/kg) recommended by international legislation (CODEX ALIMENTARIUS, 2001), while seven samples were above of the legal limit in force (60 mg/kg) in the Brazil (BRASIL, 2000). In the present study, eight of the samples analysed were found to be below this value. Of these, four samples showed values above 3° Gothe with an HMF exceeding 15 mg/kg (Table 2). Freshly harvested honey has a small amount of HMF (GOMES *et al.*, 2010), which justifies the low value of samples collected directly from producer associations. A high HMF content is indicative of aging, storage under precarious conditions, and/or overheating (ZAREI *et al*., 2019). Of the samples with values of HMF ≥60 mg/kg (BRASIL, 2000), two were collected at fairs and five at supermarkets (Table 2). However, the highest rates of HMF were identified in honeys collected in supermarkets (samples S2, S3, S7, and S9), which suggests that the honey was stored at inadequate temperatures and consequent overheating. Due to the warm climate in the Sertão of north-eastern Brazil, attention should be paid to the conditions under which honey is being stored.

Similar to the HMF index, the diastase value is used to assess the maturity and heating of honey (GOMES *et al*., 2010). Diastase is a thermolabile enzyme naturally present in honey that originates from the saliva of honey bees and which is denatured at temperatures above 60 ºC (SILVA *et al.,* 2016). The minimum level is 8 units on the Gothe scale, with a minimum of 3 units allowed if the HMF does not exceed 15 mg/kg (BRASIL, 2000). According to Almeida-Muradian *et al.* (2013), substandard diastase values are indicative of inadequate conditions in the processing or storage of honey, which influences its storage life. In addition, 3 samples (F2, S2, and S3) did not show any diastatic activity.

Samples S3 and S9 from the supermarkets did not show

any precipitate (Table 2). The fact that these samples did not have any albuminoid in their composition suggested that they are artificial honey (BRASIL, 1981). In particular, the results of the Lund test for sample S3 indicated that it had a high HMF content and no precipitate, and consequently, no albinoid content (Table 2). This suggests that the sample was not honey, or was honey that had been adulterated with commercial sugar and that had undergone heating. The Lund test is used to determine the presence of albuminoid substances in honey, which precipitate in the presence of tannic acid (ALMEIDA-MURADIAN *et al*., 2013; FINCO *et al*., 2010).

In the honey samples analysed, the electrical conductivity ranged from 0.116 to 1.526 mS/cm (Table 2). A total of 27 samples (90%) were in accordance with international recommendations (CODEX ALIMENTARIUS, 2001), with an electrical conductivity between 0.2 to 0.8 mS/cm and beneath 0.8 mS/cm, respectively. Sample F6 was found to have the highest electrical conductivity (1.526 mS/cm) and ash content (0.609%), and was dark amber in colour. In current Brazilian legislation (BRASIL, 2000), there are no specific standards for electrical conductivity. However, Bogdanov et al. (1999) state that this analysis is important for the international characterization of honeys, allowing a maximum value of 800 µS/cm. Electrical conductivity is a property of electron mobility. Traditionally, electrical conductivity is used as proof of botanical origin (CAN *et al.,* 2015). The parameters of conductivity and colour of honey are influenced by its mineral content.

Although the microbiological quality was verified in all the samples, only eight samples, namely P2, P4, P5, P6, P9, F4, F9, and S4 (26.67%), met the physicochemical standards fixed by the national and international legislations, five of which were obtained from producer associations, two from fairs, and one from a supermarket. These samples proceeded to the other tests of this study.

3.2 Determination of apparent reducing sugars total, total proteins, proline, and antioxidant properties

The percentage of reducing sugars in the honey samples ranged from 67.257 to 72.140% (Table 3). All the samples had a percentage above 65%, thereby meeting the recommendation of the Brazilian legislation (BRASIL, 2000). Honey is a naturally sweet substance because it is mainly composed of reducing sugars, such as fructose and glucose. The sugar content of honey can be influenced by the time of collection and storage of honey, in addition to the source of nectar used by the honey bees (BRASIL, 2000; CHEN *et al*., 2019). Reducing sugars values below 65% are indicative of the collection of honey not yet mature, so the product can have high moisture and be more susceptible to undergo fermentation by microorganisms. The evaluation of the reducing sugar content of honey is one strategy used for quality control of honey and preservation of consumer health (CHEN *et al*., 2019).

Table 3 - Reducing sugars, total protein, proline content, and antioxidant activity of eight selected commercial honey samples from Petrolina in Sertão of north-eastern Brazil.

Samples	Reducing sugars[1] (%)	Protein[2] (mg BSA/100 g)	Proline[3] (mg/kg)	TPC[4] (mgGAE/100 g)	TFC[5] (mg QE/100 g)	DPHH[6] (% RSA)	FRAP[7] (µM ferrous sulphate/100 g)
P2	67.55^{d}	54.59^{g}	784.09^{d}	61.59^{c}	1.70^{d}	43.56^{c}	133.24^{d}
P4	67.25^{d}	55.43^{e}	603.72^{f}	66.02^{c}	1.45^{f}	38.77^{d}	132.98^{d}
P5	67.32^{d}	55.65^{d}	683.88^{e}	51.85^{d}	1.56^{e}	36.91^{e}	121.07^{f}
P6	67.90^{d}	56.12^{c}	811.64^{c}	53.26^{d}	2.74^{c}	54.23^{b}	163.07^{c}
P9	70.40^{b}	56.29^{b}	1082.19^{b}	76.55^{b}	3.49^{b}	54.64^{b}	176.04^{b}
F4	72.14^{a}	54.97^{f}	537.34^{g}	60.12^{c}	1.26^{g}	34.78^{f}	130.66^{e}
F9	70.43^{b}	56.64^{a}	1350.24^{a}	85.28^{a}	6.36^{a}	56.34^{a}	180.84^{a}
S4	68.62^{c}	55.62^{d}	300.61^{h}	48.75^{d}	1.09^{h}	35.73^{f}	131.58^{e}
Average	68.95	55.66	769.21	62.93	2.46	44.37	146.18

In each column, mean values with different letters denote significant results ($p < 0.05$), according to the Scott–Knott test. TPC= Total phenolic content; GAE = Gallic acid equivalent; TFC = Total flavonoids content; QE = Quercetin equivalent; DPPH (% RSA) = 2,2-diphenyl-1-picrylhydrazyl (% radical scavenging activity); FRAP = Ferric reducing antioxidant power. [1]SE = 0.2135; [2]SE = 0.0173; [3]SE = 4.7282; [4]SE = 2.7962; [5]SE = 0.0377; [6]SE = 0.4079; [7]SE = 0.3237.

Source: Research data.

The total protein content of the eight samples analysed ranged from 54.591 to 56.649 mg BSA/100 g, with an average of 55.667 mg BSA/100 g (Table 3). The protein content of honey is comprised of the enzymes, glycoproteins, and

antimicrobial peptides, and varies according to the floral source and the storage time of the product (ANAND *et al*., 2018). In addition to evaluating the degree of maturity of honey, the evaluation of the protein content can be used to determine the degree of adulteration (LEME *et al*., 2018). In previous studies, Chen *et al.* (2019) and Alvarez-Suarez *et al*. (2010) reported superior and inferior results, respectively, compared to those found in this study. The differences in protein content of the honey samples are mainly attributed to their floral origins and geographical locations. According to Alvarez-Suarez *et al*. (2010), the samples in this study have a medium protein content (40–100 mg BSA/100 g).

The values found from Proline of the eight honey samples tested ranged from 300.61 to 1350.24 mg/kg (average: 769.219 mg/kg) (Table 3). Proline is the most abundant amino acid in honey, which originates from the saliva of honey bees. The proline index is used as an indicator of honey purity. A low index is often associated with adulteration (CAN *et al.,* 2015), while a high index suggests high antioxidant activity, where the correlation between these two factors has been previously reported by Moniruzzaman *et al*. (2014). Similar values were observed by Moniruzzaman *et al*. (2014) in their study on samples of monofloral honey from Bangladesh, with proline values ranging from 237.51 to 1419.33 mg/kg.

The antioxidant capacity of honey is of great medicinal interest and is one of the main benefits of its consumption. The antioxidant substances present in honey provide cells with protection against damage caused by free radicals, reducing the occurrence of oxidative stress as a result (ALVAREZ-SUAREZ *et al*., 2013; ANAND *et al*., 2018). In this study, sample F9, which was dark amber in colour (Table 1), was found to have the highest antioxidant activity (Table 3, Figure 2). In contrast, sample S4, with an extra light amber colour (Table 1), showed the lowest antioxidant activity ($p < 0.05$) according to the result of test for TFC (Table 3). According to Alvarez-Suarez *et al*. (2010), the antioxidant capacity of honey is correlated to its colour. Amber and more crystallized honeys have greater antioxidant activity compared to lighter and more transparent honeys. Moniruzzaman *et al*. (2014) reported that the antioxidant properties of honey are attributed to it composition, including colour pigments, TPC, TFC, proline, and ascorbic acid. Among these, TPC and TFC are the most significant determinants for the ability of honey to reduce oxidative stress and eliminate free radicals. Therefore, the quantification of these compounds can be used as an indicator of the benefits of honey for human health (ALVAREZ-SUAREZ *et al*., 2010).

The in the honey samples analysed was 62.930 mg GAE/100 g, varying between 48.750 to 85.280 mg GAE/100 g. While Galhardo *et al.* (2021) found a mean value of TPC of 34.20 mg GAE/100 and a variation from 11.39 to 61.27 mg GAE/100 in honey samples from southern Brazil. The TPC is comprised a group of secondary metabolites that are abundantly distributed in plants, and which have antioxidant properties. By using the nectar of plants, honey bees transfer these phenolic compounds into the honey they produce (ALVAREZ-SUAREZ *et al*., 2013).

The TFC of the evaluated honeys ranged from 1.092 mg GAE/100 g in sample F4, with an extra light amber colour, to 6.364 mg GAE/100 g in sample F9 with a dark amber colour (Tables 1 and 3). The darker the colour of the honey, the greater the TFC value and the antioxidant activity, as described previously. A lower variation in the TFC of honeys was observed by El-Haskoury *et al*. (2018), with values ranging from 2.26 to 4.79 mg QE/100 g.

The samples analysed were found to range from 34.789 to 56.344% in terms of their radical scavenging activity, with an average of 44.376% (Table 3). The DPPH test is commonly used to determine the percentage of radical scavenging activity (% RSA), which is directly proportional to the antioxidant potential (ZAREI *et al*., 2019). A similar result was observed by Moniruzzaman *et al.* (2014), who reported an average % RSA of 36.95%. Gül e Pehlivan (2018) found that the floral origin is an important determinant in the honey's ability to eliminate DPPH radicals, with values of 12, 48.95, and 65.52% for Citrus, Rhododendron, and Pine, respectively. In the present study, sample F9 was found to have the highest % RSA ($p < 0.05$), which could be due to its higher flavonoid and total phenol content, as suggested by Moniruzzaman *et al*. (2014).

The average FRAP result was 146.189 μM ferrous sulphate/100 g, ranging from 121.077 to 180.845 among the honey samples. FRAP allows to estimate the amount of antioxidants or reducers from the capacity of the Fe^{3+} to Fe^{2+} reduction samples. Therefore, in the presence of antioxidants, the ferric complex of 2,4,6-tripyridyl-striazine is reduced in its ferrous and coloured form (Fe^{2+} - TPTZ) (GÜL; PEHLIVAN, 2018). Can *et al.* (2015) reported on a much wider variation, from 59 to 430 μM $FeSO_4.7H_2O$/100 g in the Turkish honeys

3.3 Antibacterial activity

In addition to the antioxidant profile, the antimicrobial activity of honey is an interesting property for its medicinal use (GOMES *et al*., 2010). In the present study, the inhibition of microbial growth was verified only at concentrations of 80% and 100% (Table 4). At lower concentrations (60%, 40%, and 20%), the growth of microorganisms was not inhibited (data not shown). At 80% and 100%, a greater susceptibility to *S. typhi* was observed, followed by *E. coli*, and *S. aureus*. At a concentration of 100%, a larger halo of inhibition was observed for the three bacteria, compared to at a concentration of 80%. At both concentrations, samples P9 and F9 showed the greatest antibacterial activity for the three microorganisms tested ($p < 0.05$). At 80%, samples P6 and P5 also showed outstanding bacterial activity against for *E. coli* and *S aureus* ($p < 0.05$), respectively.

Table 4 - Inhibitory action of honey against pathogens at a concentration of 80% (aqueous solution) and 100%

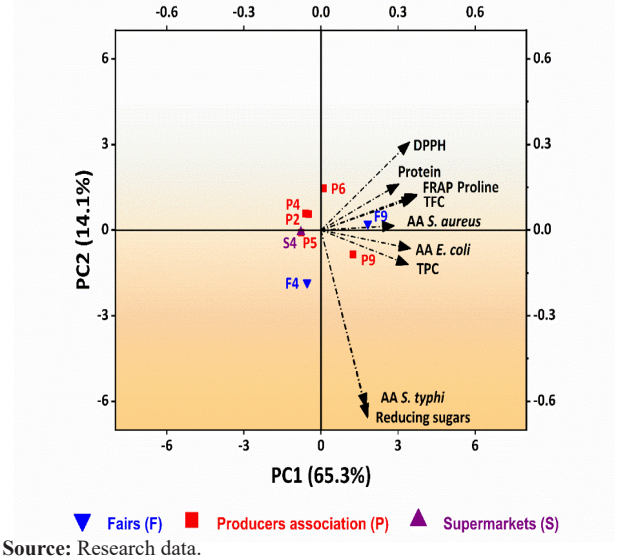
Pathogen	Honey (%)	Diameter of inhibition halo (mm)										
		Selected honey samples								Average	Positive control	
		P2	P4	P5	P6	P9	F4	F9	S4		Norfloxacin (10 µg)	Ampicillin (30 µg)
E. coli	80[1]	0.00^{b}	0.00^{b}	0.00^{b}	8.67^{a}	9.00^{a}	0.00^{b}	8.33^{a}	0.00^{b}	3.25	6	13
	100[2]	9.00^{b}	8.67^{b}	9.00^{b}	9.67^{b}	10.66^{b}	9.67^{b}	13.67^{a}	9.67^{b}	10.00		
S. aureus	80[3]	0.00^{b}	2.33^{b}	0.00^{b}	3.33^{a}	7.66^{a}	0.00^{b}	5.00^{a}	0.00^{b}	2.29	15	10
	100[4]	11.00^{b}	8.67^{c}	5.33^{c}	9.00^{c}	14.33^{a}	7.33^{c}	11.33^{b}	9.00^{c}	9.5		
S. typhi	80[5]	0.00^{d}	0.00^{d}	7.67^{a}	0.00^{d}	8.33^{a}	7.00^{b}	8.00^{a}	6.00^{c}	4.62	10	6
	100[6]	9.33^{c}	9.33^{c}	10.67^{b}	9.00^{c}	12.00^{a}	10.67^{b}	12.33^{a}	9.67^{c}	10.37		

In each line, mean values with different letters denote significant results ($p < 0.05$) according to the Scott–Knott test.
[1]SE = 0.16; [2]SE = 0.54; [3]SE = 1.02; [4]SE = 1.03; [5]SE = 0.26; [6]SE = 0.41. *E. coli*= *Escherichia coli* (ATCC 25922). *S. aureus*= *Staphylococcus aureus* (ATCC 25923). *S. typhi*= *Salmonella typhi* (ATCC 6539)
Source: Research data.

The inhibitory success of samples P9 and F9 against pathogenic bacteria can be explained by their high TPC ($p < 0.05$). This antimicrobial activity is the results of several factors, including composition and physical nature. In terms of the composition, the existence and abundance of phenolic compounds, as well as hydrogen peroxide, confers honey with a greater capacity to inhibit the growth of microorganisms. Goslinski *et al*. (2020) reported that manuka honey with high antioxidant properties also presented strong antimicrobial activity against *S. aureus*. Regarding the physical nature of honey, the high osmolarity, acidity, and natural viscosity of honey proves to be disadvantageous to microbial growth and plays an important role in its biological activity (ALVAREZ-SUAREZ *et al*., 2013; GOSLINSKI *et al*., 2020).

As result, samples F9 and P9 showed high antioxidant and antibacterial activities (Figure 2). However, it should be noted that the other samples also demonstrated antioxidant properties, indicating that the consumption of honey from Sertão of north-eastern Brazil, has potential benefits to human health.

Figure 2 - Representation of original nine biochemical variables loaded heavily on two dimensions (65.3%, PC1 and 14.1%, PC2) with the observation of the eight honey samples using varimax rotation



Source: Research data.

4 Conclusions

All honey samples from the Sertão of north-eastern Brazil analyzed in this study presented microbiological quality, while the smallest part presented physicochemical quality. The honeys also showed important antioxidant and antimicrobial activities, especially in two samples. Since the honey samples from the region that meet the normative quality standards, have biofunctionality, they are a potential alternative in the promotion of human health, particularly in the fight against oxidative damage and in the prevention of diseases.

Acknowledgements

The authors acknowledge the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for scholarships.

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