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Physicochemical attributes of honey as a measure of enhanced beekeeping in the Bois River Valley, Brazil

Atributos físico-químicos do mel como medida de melhoria da apicultura no Vale do Rio Bois, Brasil

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ABSTRACT

The Local Production Arrangement (LPA) of beekeeping in the Bois River Valley in Goiás is at an initial stage of development and requires initiatives that would contribute to its development. The Goiás state has favorable conditions for beekeeping, and, therefore, the present study aimed to evaluate the quality of honey produced from different floral sources in the Bois River Valley Beekeeping LPA. Physicochemical analyses were, therefore, conducted to determine the moisture, water-insoluble solids, reducing sugars, apparent sucrose, total soluble solids, acidity, pH, diastatic activity, hydroxymethylfurfural, and color attributes of this honey. The determined values of these attributes were compared to the values recommended by the Technical Regulation of Identity and Quality of Honey (TRIQH). The results revealed significant correlations, especially between moisture and total soluble solids (r = –1.00), acidity and absorbance (r = 0.91), minerals and pH (r = 0.79), and reducing sugars and absorbance (r = –0.72). In general, the honey samples exhibited satisfactory quality, with the values of just a few samples being different from the limits established by the legislation. These results highlighted the importance of using Good Beekeeping Practices by those involved in the beekeeping production chain and honey commercialization. This initiative would contribute to strengthening the beekeeping LPA in the Bois River Valley by improving the quality of honey, ensuring higher income for beekeepers, and strengthening the regional economy.

Index terms: Local production arrangements; legislation; honey analysis.

RESUMO

O Arranjo Produtivo Local (APL) de apicultura do Vale do Rio Bois em Goiás está em estágio inicial de desenvolvimento e necessita de iniciativas que contribuam para seu desenvolvimento. O estado de Goiás possui condições favoráveis para a apicultura e, portanto, o presente estudo teve como objetivo avaliar a qualidade do mel produzido a partir de diferentes floradas no APL Apícola do Vale do Rio Bois. Portanto, foram realizadas análises físico-químicas para determinação dos atributos umidade, sólidos insolúveis em água, açúcares redutores, sacarose aparente, sólidos solúveis totais, acidez, pH, atividade diastática, hidroximetilfurfural e cor do mel. Os valores desses atributos foram comparados aos valores recomendados pelo Regulamento Técnico de Identidade e Qualidade do Mel (RTIQM). Os resultados revelaram correlações significativas, principalmente entre umidade e sólidos solúveis totais (r = –1,00), acidez e absorbância (r = 0,91), minerais e pH (r = 0,79) e açúcares redutores e absorbância (r = –0,72). De modo geral, as amostras de mel apresentaram qualidade satisfatória, com valores de apenas algumas amostras estando fora dos limites estabelecidos pela legislação. Esses resultados evidenciaram a importância da utilização das Boas Práticas Apícolas pelos envolvidos na cadeia produtiva da apicultura e na comercialização do mel. Essa iniciativa contribuiria para o fortalecimento do APL apícola do Vale do Rio Bois, melhorando a qualidade do mel, garantindo maior renda aos apicultores e fortalecendo a economia regional.

Termos para indexação: Arranjos produtivos locais; legislação; análise do mel.

Introduction

Beekeeping is being recognized in Brazil as a profitable activity with rapid returns on investment (Mendes et al., 2009). Honey is a well-known beekeeping product recognized for its aroma, flavor, color, consistency, and health benefits (Albuquerque, Sobrinho, & Lins 2021). Honey consumption in Brazil is, however, relatively low compared to the European nations. A record of honey production was achieved in Brazil in the year 2021. Brazilian honey has gained a good acceptance level in the international market, suggesting a great growth potential in this sector (Almeida, 2022).

Honey has great nutritional value as it contains sugars, water, mineral salts, vitamins, phenolic compounds, and other nutrients (Uran, Aksu, & Dülger Altiner, 2017). In Brazil, honey production and commercialization are regulated according to the Normative Instruction No. 11 of October 20, 2000 (Brasil, 2000) and Ordinance No. 6 of July 25, 1985, documented by the

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Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA, 1985). These documents establish the standards of the identity and minimum quality requirements of honey intended for human consumption, as well as the hygienic, sanitary, and technological standards for honey, beeswax, and their derivatives (Basilio et al., 2020; Ribeiro & Starikoff, 2019). The physicochemical properties of honey depend on its floral source, climatic conditions, stage of maturation, bee species, processing, and storage, rendering it important to characterize the honey in this aspect. A physicochemical analysis of honey facilitates validating its quality, identifying fraud, and assisting in inspection (Nunes et al., 2022; Vieira et al., 2014).

'Local Production Arrangement' (LPA) is a form of economic and geographical organization, in which small companies and other institutions with common commercial purposes collectively leverage their businesses (Ultramari & Duarte, 2012). The Bois River Valley Beekeeping LPA, headquartered in Jandaia, is one of the important LPAs in Goiás. According to *Observatório Brasileiro Arranjos Produtivos Locais* (Observatório, 2022), 65 companies and/or producers are involved in the Bois River Valley Beekeeping LPA. These companies prioritize honey production while also working in the fields of propolis and beeswax production. The Bois River Valley Beekeeping LPA was established in 2018, and even though it is important from an economic perspective, initiatives that promote the structuring of the Bois River Valley Beekeeping LPA are lacking, which could compromise the competitiveness of this arrangement and might be related to the development of public investment policies that strengthen the sector (Faria et al., 2022).

Goiás has a climate, relief, and vegetation suitable for beekeeping, although honey production is not the highlight in the national scenario currently (Nascimento et al., 2021). A physicochemical analysis of the honey produced in the Bois River Valley Beekeeping LPA might contribute to validating the quality standard of this honey and encourage beekeeping activities, in addition to facilitating inspection and obtaining inspection seals. Such analyses are also advantageous for the consumer, who is protected from purchasing adulterated/counterfeit products (Mendes et al., 2009; Nunes et al., 2022). In this context, the present study aimed to evaluate the quality of the honey produced in the Bois River Valley Beekeeping LPA through physicochemical analyses and then compare it to the standards listed in the Brazilian legislation.

Material and Methods

The honey collected in the Bois River Valley Beekeeping LPA was evaluated for its physicochemical characteristics, and the determined values of different parameters were compared to the corresponding values established by the Technical Regulation of Honey Identity and Quality (*Regulamento Técnico de Identidade e Qualidade do Mel* - RTIQM) documented in Normative Instruction No. 11 of October 10, 2000 (Brasil, 2000).

The quantity of 32 honey samples collected from the different municipalities (as depicted in Figure 1) in the Bois River Valley Beekeeping LPA was evaluated. The respective municipalities of this beekeeping LPA and the number of samples per municipality were as follows: Abadia de Goiás (0), Acreúna (2), Anicuns (1), Aurilândia (0), Cezarina (0), Cromínia (0), Edealina (2), Edéia (2), Firminópolis (1), Indiara (1), Jandaia (13), Mairipotaba (0), Palmeiras de Goiás (0), Palminópolis (1), Paraúna (0), Pontalina (5), São João da Paraúna (0), São Luís de Montes Belos (2), and Turvânia (2).

Samples of potted honey from *Apis mellifera* bees comprised the honey harvested in the 2021 and 2022 seasons. The collected samples were sent to the Federal Institute Goiano (IF Goiano) – Iporá Campus, located in the municipality of Iporá (GO), for analyses to determine the characteristics of the honey, which were conducted in collaboration with the Laboratory of Quality Control of Bee Products of Embrapa Mid-North, Teresina (PI).

Physicochemical analysis of honey samples

The total soluble solids (°Brix) and moisture content were determined using refractometry according to method no. 969.38b (Association of Official Agricultural Chemists - AOAC, 1998). In order to determine the moisture content, the refractive index of the honey sample at 20 °C was determined, and for each degree of difference, a correction of 0.00023 was performed. The refractive index value was then converted to the moisture content percentage using a reference table. The insoluble solids content in the honey sample was determined using gravimetry according to the method described in item 7.4 of the *Codex Alimentarius* (Codex Alimentarius, 1990). Minerals (ashes) in the honey sample were characterized by incinerating the samples, as described in item 7.5 of *Codex Alimentarius* (Codex Alimentarius, 1990), in a muffle furnace heated to 600 ºC.

The content of reducing sugars was determined using the method described in item 7.1 of *Codex Alimentarius* (Codex Alimentarius, 1990). This method includes a modification of the Lane and Eynon procedure. Fehling's solution modified by Soxhlet during titration at the boiling point is reduced using a solution of reducing sugars present in honey, and the reduction is quantified using methylene blue as an indicator. Apparent sucrose was determined using the method described in item 7.2 of the *Codex Alimentarius* (Codex Alimentarius, 1990), in which apparent sucrose is determined after inversion through acid hydrolysis. Free acidity and pH were determined using method No. 962.19 (AOAC, 1998). The honey samples were titrated with 0.05 N NaOH solution until pH 8.5 was reached.

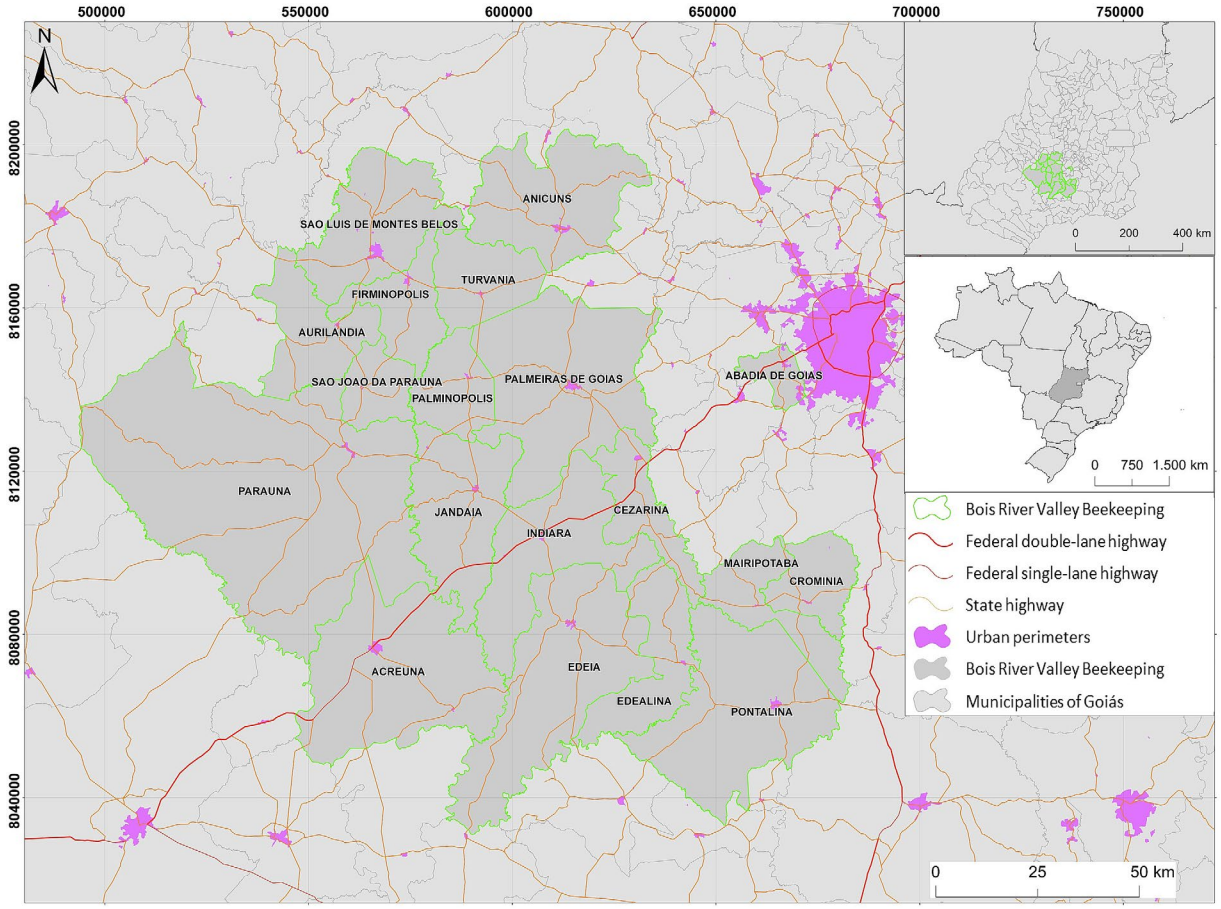

Figure 1: Map of the study region, depicting the municipalities in the Bois River Valley Beekeeping LPA.
Source: João Vítor Silva Costa (2023).

Diastatic activity (*Göthe scale*) was determined using method 7.7 described in *Codex Alimentarius* (Codex Alimentarius, 1990), in which a buffered starch-honey solution placed in a water bath is used. A *Göthe* unit is defined as the amount of enzyme capable of converting 0.01 g of starch in one hour at 40 °C. The hydroxymethylfurfural (HMF) content was determined using the quantitative method no. 980.23 (AOAC, 1998), which is a spectrophotometric method in which absorbance is determined at 284 nm and 336 nm.

The color of the honey samples was evaluated spectrophotometrically by determining the absorbance at 560 nm (Brasil, 1981). Pfund scale was used for color quantification as follows: values from 0 to 34 mm for white color (absorbance < 0.120), 34 to 50 mm for extra light amber (absorbance 0.120 to 0.188), 51 to 85 mm for light amber (absorbance 0.188 to 0.440), and >86 mm for dark amber (absorbance > 0.440).

Statistical analysis

All analyses, except pH determination, were performed in triplicate, and the results were expressed as means ± standard deviations (SD). Each of the analyses performed constituted a variable, and the set of results obtained for these variables was subjected to a linear regression analysis using Statistica 7.0 software to obtain the correlation matrix with a significance level of 5%.

Results and Discussion

The evaluated samples were obtained from different floral sources (Silvestre, cipó-uva [*Serjania lethalis*], Assa-Peixe [*Vernonia polysphaera*], Aroeira [*Schinus terebinthifolia*], and Eucalyptus [*Eucalyptus* sp.]). The type of floral source has a strong influence on the physical and chemical composition of honey. However, in the present study, no direct relationship was observed between the results obtained and the floral sources of the respective samples. This might have occurred because other factors, such as climatic conditions, stage of maturation, bee species, processing, and storage, also influence the characteristics of honey (Vieira et al., 2014).

Moisture, insoluble solids, minerals, and total soluble solids

Water ranks second among all components of honey in terms of quantity, which ranges from 15% and 21% (Mendes et al., 2009). This water quantity is determined based on the moisture content and is one of the most important characteristics of honey, as it affects the viscosity, specific weight, maturity, crystallization, flavor, preservation, and palatability of honey (Piana et al., 2004). In the present study, the moisture content values for all samples (Table 1) were below the maximum value established by the Brazilian legislation and the *Codex Alimentarius* (20 g/100 g). The moisture content values ranged from approximately 13 g/100 g to 17 g/100 g. This variation was expected, as moisture is affected by climatic conditions, storage, relative humidity, degree of maturity of the honey in the hive, and the botanical origin of the honey (Fátima et al., 2010). It is noteworthy that all samples evaluated in the present study had moisture contents below 18 g/100 g. This is an important factor in preventing fermentation due to osmophilic yeasts, which could compromise the quality of the final product (Camargo, 2002; De Queiroz Rolim et al., 2018).

Analyses of the insoluble solids and minerals (ashes) in honey samples did not reveal the presence of impurities in honey, indicating that hygienic control measures were followed along with good processing. Insoluble solids are a result of the presence of particles larger than 15.4 μm, which are insoluble in water at 80 °C. These particles could be derived from wax, bee legs, and wings, as well as other components, such as plant remains, wood, etc. (De Queiroz Rolim et al., 2018). On the other hand, the mineral content indicates the botanical quality of the honey or processing (filtration/decantation) irregularities (Evangelista-Rodrigues et al., 2005). All analyzed samples presented parameter values within the established limits for insoluble solids and ashes (Table 1). These data suggested that the analyzed honey samples were obtained by following good processing and hygienic control. The total soluble solids content reflects the amount of total sugars present in the honey, and even though the legislation does not require this analysis to be conducted necessarily, the analysis does contribute to the determination of honey quality (Santos et al., 2021). In the present study, the evaluated samples presented values between 80.50 and 84.60 °Brix, similar to those reported in the literature (Meireles & Cançado, 2013; Silva et al., 2003).

Contents of reducing sugars and apparent sucrose

The contents of reducing sugars may cause physical changes in the viscosity, density, hygroscopicity, and crystallization of honey. The reducing sugars present in honey are glucose and fructose, which are monosaccharides with characteristic free carbonyl and ketone groups that are oxidized in the presence of oxidizing agents present in alkaline solutions (Fernandes, Silva, & Rosa, 2020; Silva et al., 2003).

Brazilian legislation (Brasil, 2000) has established that the minimum content of reducing sugars must be 65 g/100 g in honey. Among the samples analyzed in the present study (Figure 2A), just one (sample 6) presented reducing sugar content below the level recommended by the legislation (57.55 g/100 g), indicating a premature harvest of this honey sample. Honey production involves the action of enzyme invertase on sucrose, transforming the latter into glucose and fructose. Sucrose is a disaccharide characterized as a non-reducing sugar, and its content in honey confirms that the honey sample was prematurely harvested (Bertoldi et al., 2010). In the present study, sample (6) had a sucrose content of 8.17 g/100 g, which is above the level established by the legislation (max. 6 g/100 g), confirming that this honey sample was harvested prematurely.

According to *Codex Alimentarius* (Codex Alimentarius, 2001), glucose and fructose contents should be at least 60 g/100 g, while the maximum sucrose content should be 5 g/100 g. The comparison of sugar contents in honey samples from the Bois River Valley Beekeeping LPA (Figure 2A) to the corresponding values recommended by the legislation of other nations, such as *Codex Alimentarius*, revealed that most analyzed samples met the international standards (Codex Alimentarius, 2001).

Acidity and pH

The levels of organic acids, particularly gluconic acid, in honey are important indicators of honey quality. Acidity interferes with the organoleptic characteristics of the product. In addition, acidity determination contributes to the identification of deterioration that might occur due to the action of the glucose-peroxidase enzyme, which remains active in honey during its storage. The deterioration may also occur due to the fermentation of sugars in the presence of xerotolerant yeasts (Finola, Silva, & Rosa, 2007). It is important to highlight that the presence of acids at certain concentrations may also contribute to honey preservation, conferring greater stability to the honey against microorganisms (Camargo, 2002). Among the samples evaluated in the present study (Figure 2B), sample 28 presented the highest acidity value (56.04 Meq/kg), which is above the recommended value documented in Normative Instruction No. 11 of October 20, 2000 (50.00 Meq/kg).

In regard to the pH of honey, the legislation has not established standard values. However, this complementary analysis of the acidity of honey could reveal deterioration and fermentation, and is, therefore, important. The pH of a honey sample varies with the pH of the nectar, soil, association of plants, and mandibular substances of the bee (Mendes et al., 2009). The pH values of the honey samples analyzed in the present study (Figure 2B) ranged from 3.8 to 4.6, which are similar to the values reported by Grando et al. (2023), who reported pH values between 3.2 and 4.5 for honey samples from stingless bees collected from the state of Paraná.

Table 1: Standard values of different parameters established by the Brazilian legislation[1] and the result values obtained in the present study for moisture, insoluble solids, minerals, and total soluble solids in the honey samples from the Bois River Valley Beekeeping LPA.

Sample	Floral source	Moisture (g/100 g)	Insoluble solids (g/100 g)	Minerals (g/100 g)	Total soluble solids (°Brix)
Reference values according to Brazilian legislation[1]	-	Maximum 20	Maximum 0.1	Maximum 0.6	-
1	Silvestre	14.78 ± 0.00	0.08 ± 0.00	0.10 ± 0.02	83.30 ± 0.00
3	Silvestre	15.90 ± 0.03	0.08 ± 0.01	0.08 ± 0.02	82.23 ± 0.05
4	Silvestre	15.04 ± 0.02	0.07 ± 0.00	0.08 ± 0.02	83.07 ± 0.05
5	Silvestre	15.14 ± 0.00	0.08 ± 0.01	0.12 ± 0.03	83.00 ± 0.00
6	Silvestre	15.08 ± 0.02	0.07 ± 0.00	0.57 ± 0.10	83.00 ± 0.00
7	Silvestre	13.97 ± 0.02	0.03 ± 0.00	0.34 ± 0.08	84.03 ± 0.05
10	Silvestre	16.06 ± 0.00	0.06 ± 0.01	0.24 ± 0.10	82.10 ± 0.00
14	Silvestre	14.51 ± 0.04	0.04 ± 0.01	0.26 ± 0.03	83.53 ± 0.05
21	Silvestre	14.19 ± 0.05	0.05 ± 0.01	0.09 ± 0.07	83.87 ± 0.05
26	Silvestre	15.40 ± 0.02	0.02 ± 0.01	0.15 ± 0.12	82.70 ± 0.00
2	Cipó-Uva	13.47 ± 0.00	0.09 ± 0.01	0.07 ± 0.02	84.60 ± 0.00
11	Cipó-Uva	15.06 ± 0.00	0.05 ± 0.03	0.06 ± 0.01	83.00 ± 0.00
17	Cipó-Uva	13.65 ± 0.02	0.07 ± 0.01	0.20 ± 0.01	84.30 ± 0.00
19	Cipó-Uva	14.49 ± 0.04	0.06 ± 0.02	0.04 ± 0.01	83.57 ± 0.05
20	Cipó-Uva	14.77 ± 0.02	0.04 ± 0.01	0.06 ± 0.04	83.30 ± 0.00
24	Cipó-Uva	14.54 ± 0.00	0.03 ± 0.01	0.15 ± 0.12	83.50 ± 0.00
27	Cipó-Uva	14.79 ± 0.04	0.02 ± 0.01	0.17 ± 0.01	83.30 ± 0.00
31	Cipó-Uva	14.17 ± 0.05	0.08 ± 0.02	0.08 ± 0.02	83.83 ± 0.05
28	Assa-Peixe	16.39 ± 0.02	0.14 ± 0.03	0.28 ± 0.11	81.80 ± 0.00
32	Assa-Peixe	17.76 ± 0.02	0.09 ± 0.00	0.05 ± 0.02	80.50 ± 0.00
9	Assa-Peixe and Eucalyptus	17.74 ± 0.04	0.04 ± 0.01	0.44 ± 0.05	80.50 ± 0.00
12	Assa-Peixe and Aroeira	15.43 ± 0.02	0.07 ± 0.00	0.47 ± 0.07	82.70 ± 0.00
16	Assa-Peixe and Aroeira	14.42 ± 0.00	0.03 ± 0.00	0.16 ± 0.01	83.60 ± 0.00
22	Assa-Peixe and Aroeira	15.26 ± 0.00	0.02 ± 0.00	0.27 ± 0.09	82.90 ± 0.00
23	Aroeira	16.02 ± 0.00	0.04 ± 0.01	0.26 ± 0.11	82.10 ± 0.00
30	Aroeira	15.64 ± 0.02	0.06 ± 0.00	0.06 ± 0.04	82.50 ± 0.00
18	Angico	16.40 ± 0.02	0.05 ± 0.02	0.15 ± 0.01	81.80 ± 0.00
25	Aroeira/Angico	14.62 ± 0.00	0.02 ± 0.00	0.26 ± 0.06	83.40 ± 0.00
8	--	14.59 ± 0.02	0.02 ± 0.00	0.05 ± 0.00	83.50 ± 0.00
13	--	14.54 ± 0.00	0.05 ± 0.02	0.05 ± 0.03	83.50 ± 0.00
15	--	15.47 ± 0.02	0.03 ± 0.01	0.07 ± 0.01	82.60 ± 0.00
29	--	15.63 ± 0.02	0.09 ± 0.01	0.09 ± 0.04	82.50 ± 0.00

[1] Normative Instruction No. 11 of October 20, 2000.
Values presented are means ± standard deviations (n = 3 ± SD).
--: Unidentified samples. Cipó-Uva (*Serjania lethalis*); Assa-Peixe (*Vernonia polysphaera*); Aroeira (*Schinus terebinthifolia*); Eucalyptus (*Eucalyptus* sp.).

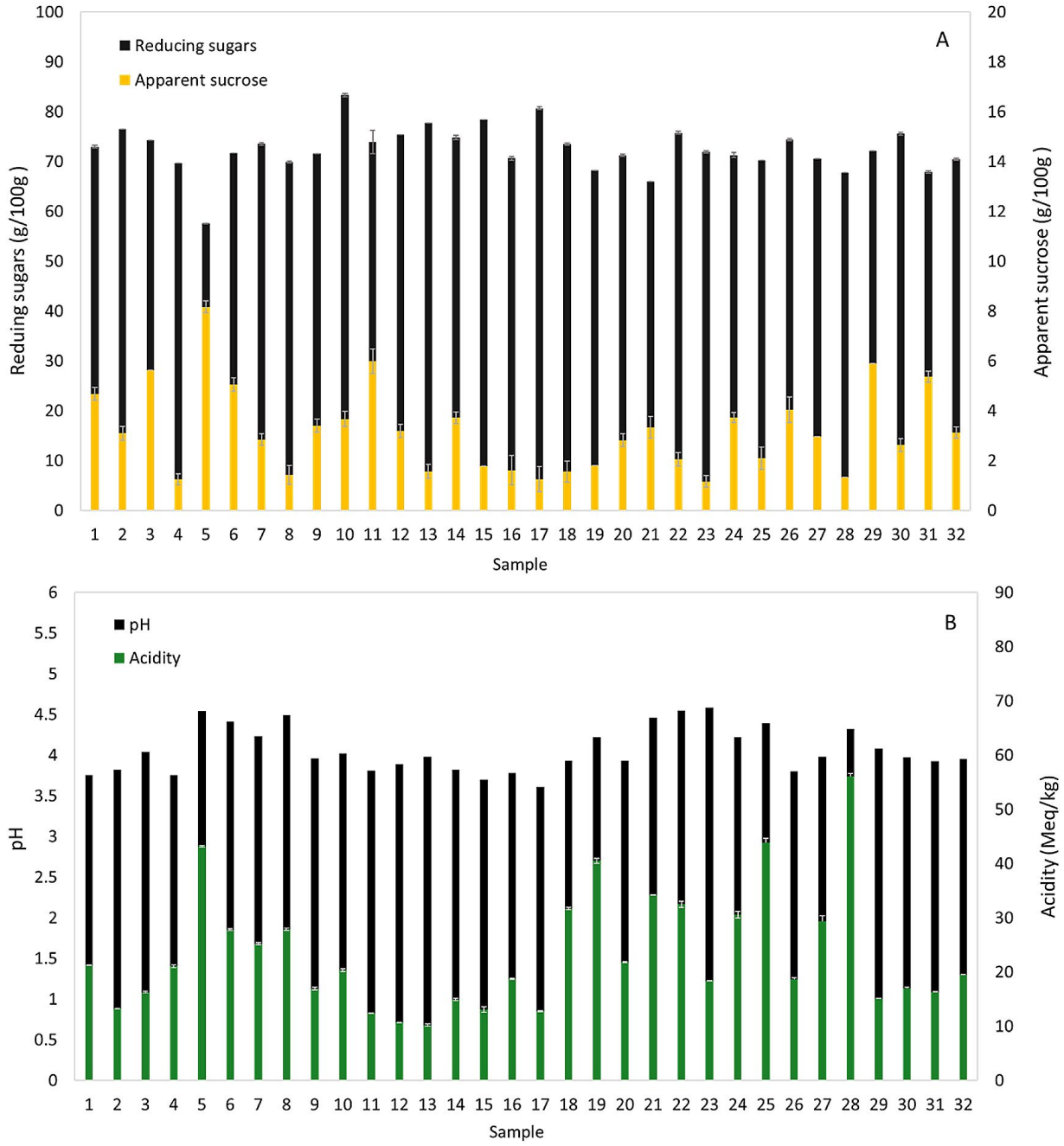

Figure 2: (A) Contents of reducing sugars and apparent sucrose and (B) pH and acidity in the honey samples from the Bois River Valley Beekeeping LPA. All values, except for pH values, are means ± standard deviations (n = 3 ± SD).

Diastatic activity and hydroxymethylfurfural (HMF)

Diastatic activity indicates the presence of the diastase (α-amylase) enzyme, which digests the starch molecule and is used as an indicator of honey quality in international standards. This enzyme is highly sensitive to heat and, therefore, indicates the degree of conservation and overheating of the product (Mendes et al., 2009). According to the Normative Instruction No. 11 of October 20, 2000 (Brasil, 2000), the diastatic activity must be at least 8 units on the Göthe scale. In the present study, a variation between 8.20 and 22.54 was observed among the evaluated samples (Table 2), although all honey samples presented results above the minimum value established by the legislation. Therefore, all samples were in accordance with the necessary standards, including those for export.

Table 2: Parameters established by the Brazilian legislation[1] and the results of the diastatic activity and hydroxymethylfurfural analyses for the honey samples collected from the Bois River Valley Beekeeping LPA.

Sample	Floral source	Diastatic activity	Hydroxymethylfurfural (mg/kg)
Reference values according to Brazilian legislation[1]	-	Minimum of 8 on *Göthe scale*	Maximum of 60
1	Silvestre	15.82 ± 0.67	69.71 ± 1.07
3	Silvestre	12.91 ± 0.15	29.32 ± 1.13
4	Silvestre	15.35 ± 1.59	45.34 ± 0.62
5	Silvestre	11.42 ± 0.63	80.00 ± 0.23
6	Silvestre	12.48 ± 0.16	114.45 ± 5.39
7	Silvestre	11.49 ± 0.27	61.59 ± 1.05
10	Silvestre	22.37 ± 1.87	39.54 ± 0.61
14	Silvestre	10.60 ± 0.68	94.50 ± 1.34
21	Silvestre	15.86 ± 0.32	27.99 ± 0.86
26	Silvestre	11.53 ± 0.17	52.98 ± 1.96
2	Cipó-Uva	9.61 ± 0.27	38.60 ± 0.99
11	Cipó-Uva	11.37 ± 1.00	16.36 ± 0.11
17	Cipó-Uva	8.20 ± 0.25	42.97 ± 0.71
19	Cipó-Uva	11.61 ± 0.26	28.58 ± 0.31
20	Cipó-Uva	14.53 ± 0.04	27.64 ± 0.70
24	Cipó-Uva	13.83 ± 0.26	27.65 ± 2.25
27	Cipó-Uva	15.63 ± 0.45	29.38 ± 1.14
31	Cipó-Uva	20.49 ± 1.61	127.12 ± 2.00
28	Assa-Peixe	15.37 ± 0.55	108.27 ± 4.68
32	Assa-Peixe	16.34 ± 0.23	43.60 ± 0.32
9	Assa-Peixe and Eucalyptus	16.92 ± 0.34	38.27 ± 0.45
12	Assa-Peixe and Aroeira	20.41 ± 0.45	37.61 ± 0.44
16	Assa-Peixe and Aroeira	22.54 ± 2.00	30.29 ± 0.29
22	Assa-Peixe and Aroeira	15.91 ± 0.21	63.05 ± 1.71
23	Aroeira	18.89 ± 0.31	58.17 ± 2.35
30	Aroeira	14.50 ± 0.21	36.60 ± 0.18
18	Angico	14.35 ± 1.19	87.90 ± 1.82
25	Aroeira/Angico	19.51 ± 0.77	92.29 ± 2.83
8	--	8.89 ± 0.65	40.41 ± 0.56
13	--	13.18 ± 0.38	102.17 ± 1.06
15	--	13.08 ± 0.63	38.03 ± 0.42
29	--	15.68 ± 0.64	50.71 ± 1.47

[1] Normative Instruction No. 11 of October 20, 2000.
Values represent means ± standard deviations (n=3 ± SD).
--: Unidentified samples. Cipó-Uva (*Serjania lethalis*); Assa-Peixe (*Vernonia polysphaera*); Aroeira (*Schinus terebinthifolia*); Eucalyptus (*Eucalyptus* sp.).

The hydroxymethylfurfural analysis reveals the freshness of honey and also indicates important changes due to prolonged storage at high ambient temperature and/or overheating or a possible adulteration with commercial sugar (Evangelista-Rodrigues et al., 2005; Mendes et al., 2009). Hydroxymethylfurfural is cytotoxic and is, therefore,

associated with harmful effects on the health of consumers when present at high concentrations in the honey. These effects include irritation in the eyes, upper respiratory tract, skin, and mucous membranes, with these effects having a genotoxic potential (Maeda et al., 2023; Ribeiro et al., 2012). In Brazil, which is a tropical country, the legislation has established a maximum limit of 60 mg/kg for HMF. *Codex Alimentarius* has established that the value of HMF in honey after processing should not exceed 40 mg/kg. However, for the honey samples of declared origin from countries or regions with tropical ambient temperatures, as well as for the mixtures of these honey samples, *Codex Alimentarius* has established that the HMF content should not exceed 80 mg/kg. Among the 32 samples evaluated in the present study, 11 samples presented hydroxymethylfurfural content above 60 mg/kg. These samples were, therefore, considered outside the standards established by Normative Instruction No. 11 of October 20, 2000 (Brasil, 2000). The apparent sucrose contents in these samples were within the limits established by the legislation, and the values above 60 mg/kg were attributed to the aging of honey or heating of the honey samples. Honey is mostly stored at ambient temperatures, while the Bois River Valley region is characterized by high temperatures in certain months of the year, which could explain the above results. In addition, prolonged storage might have contributed to such results. Nonetheless, these observations suggest the importance of working with beekeepers to improve the quality of the products they offer to consumers. Those involved in the beekeeping production chain and commercialization of honey could benefit from obtaining a qualification in Good Beekeeping Practices. It is noteworthy that guidance from retailers is necessary as storage of honey at high temperatures could also contribute to the loss of honey quality.

Color

The color of honey varies with the bee species, climatic conditions, ash content, floral source, and storage. In certain cases, honey could be dark in color due to the Maillard reaction that results from factors such as storage at high temperatures or for prolonged periods (Santos et al., 2021). Figure 3 depicts samples with different colors collected from the Bois River Valley Beekeeping LPA, presenting a visual representation of the classification of these honey samples according to the Pfund scale. According to this classification, the different colors include white, extra light amber, light amber, amber, dark amber, and extra dark amber.

Variations in the color of honey from white to dark amber were observed across the analyzed samples (Table 3), similar to the findings of Grando et al. (2023). According to Vieira et al. (2014), consumers present a greater acceptance of lighter-colored honey. Only 8 samples in the present study were amber, while 5 samples were dark amber. Most of the evaluated samples had lighter colors, thereby having the potential for a higher market price and good acceptance among consumers. However, the samples with a darker color were also consistent with the Normative Instruction No. 11 of October 20, 2000 (Brasil, 2000), which establishes that honey color varies from almost colorless to dark brown.

Next, it was investigated whether the darkening of honey had occurred due to prolonged storage or because of storage at high temperatures, for which the color and HMF results were analyzed. It was observed that most samples with higher levels of HMF were amber, while just three samples were dark amber, and two were light amber. This association could be linked to the Maillard reaction, although it is important to consider that other factors could also have interfered with the honey color, as stated earlier. In addition, the presence of phenolic compounds and flavonoids could have contributed to the darker colors of certain samples (Aguiar et al., 2016).

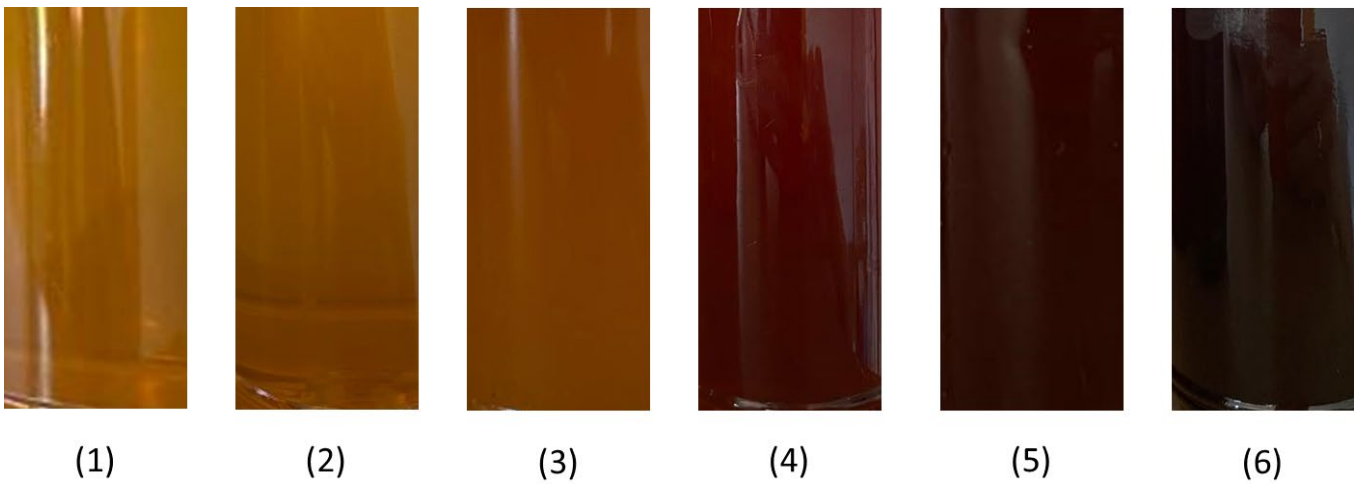

Figure 3: Classification of honey samples from the Bois River Valley Beekeeping LPA according to their color: (1) white, (2) extra light amber, (3) light amber, (4) amber, (5) dark amber, and (6) extra dark amber.

Table 3: Results of the color analysis of honey samples from the Bois River Valley Beekeeping LPA.

Sample	Floral source	Absorbance	Color
1	Silvestre	0.296 ± 0.008	Light amber
3	Silvestre	0.189 ± 0.003	Light amber
4	Silvestre	0.379 ± 0.002	Light amber
5	Silvestre	0.666 ± 0.003	Amber
6	Silvestre	1.295 ± 0.002	Dark amber
7	Silvestre	0.816 ± 0.008	Amber
10	Silvestre	0.384 ± 0.005	Light amber
14	Silvestre	0.908 ± 0.005	Amber
21	Silvestre	0.324 ± 0.002	Light amber
26	Silvestre	0.318 ± 0.003	Light amber
2	Cipó-Uva	0.147 ± 0.001	Extra light amber
11	Cipó-Uva	0.111 ± 0.004	White
17	Cipó-Uva	0.139 ± 0.002	Extra light amber
19	Cipó-Uva	0.161 ± 0.002	Extra light amber
20	Cipó-Uva	0.117 ± 0.003	White
24	Cipó-Uva	0.183 ± 0.001	Extra light amber
27	Cipó-Uva	0.094 ± 0.001	White
31	Cipó-Uva	0.535 ± 0.003	Amber
28	Assa-Peixe	0.882 ± 0.006	Amber
32	Assa-Peixe	0.413 ± 0.002	Light amber
9	Assa-Peixe and Eucalyptus	1.010 ± 0.004	Dark amber
12	Assa-Peixe and Aroeira	0.676 ± 0.002	Amber
16	Assa-Peixe and Aroeira	0.592 ± 0.003	Amber
22	Assa-Peixe and Aroeira	0.630 ± 0.007	Amber
23	Aroeira	0.987 ± 0.003	Dark amber
30	Aroeira	0.363 ± 0.010	Light amber
18	Angico	1.218 ± 0.015	Dark amber
25	Aroeira/Angico	1.717 ± 0.007	Dark amber
8	--	0.161 ± 0.002	Extra light amber
13	--	0.257 ± 0.003	Light amber
15	--	0.262 ± 0.000	Light amber
29	--	0.444 ± 0.004	Amber

Values represent means ± standard deviations (n=3 ± SD).
--: Unidentified samples. Cipó-Uva (*Serjania lethalis*); Assa-Peixe (*Vernonia polysphaera*); Aroeira (*Schinus terebinthifolia*); Eucalyptus (*Eucalyptus* sp.).

Correlation analysis

The correlation analysis of the different parameters evaluated to determine the quality of honey samples from Bois River Valley Beekeeping LPA revealed significant ($p < 0.05$) positive and negative correlations (Table 4). The negative correlations indicated that the variables were inversely proportional, as in the case of the correlation between minerals and reducing sugars ($r = –0.57$), reducing sugars and pH ($r = –0.53$), and reducing sugars and HMF ($r = –0.54$). The highest degree of correlation was observed between moisture and total soluble solids (TSS) ($r = –1.00$), acidity and absorbance ($r = 0.91$), minerals and pH ($r = 0.79$), and reducing sugars and absorbance ($r = –0.72$). The strong correlation between moisture and TSS

Table 4: Linear correlation between the different parameters of honey samples evaluated in the present study.

Variables	1	2	3	4	5	6	7	8	9	10	11
1. Moisture	1.00	0.20	0.19	-1.00*	-0.23	-0.05	0.29	0.16	0.30	0.01	0.27
2. Insoluble solids		1.00	-0.10	-0.20	-0.17	0.15	-0.02	-0.16	-0.10	0.29	-0.10
3. Minerals			1.00	-0.20	-0.57*	0.19	0.67*	0.79*	0.17	0.35	0.64*
4. TSS				1.00	0.23	0.06	-0.30	-0.17	-0.31	-0.01	-0.27
5. Reducing sugars					1.00	-0.30	-0.66*	-0.53*	-0.12	-0.54*	-0.72*
6. Apparent sucrose						1.00	-0.01	0.07	-0.31	0.05	0.02
7. Acidity							1.00	0.66*	0.43*	0.68*	0.91*
8. pH								1.00	0.37	0.31	0.68*
9. Diastatic activity									1.00	0.06	0.28
10. HMF										1.00	0.67*
11. Abs											1.00

* Significant within the 5% confidence interval. TSS: total soluble solids; HMF: hydroxymethylfurfural; Abs: absorbance.

was expected as a higher water content (moisture) is associated with a lower content of total soluble solids (Shamsudin et al., 2019). The color parameter was directly proportional to acidity and inversely proportional to the content of reducing sugars. Strong correlations were also observed between minerals and acidity (r = 0.67), reducing sugars and acidity (r = –0.66), acidity and pH (r = 0.66), acidity and HMF (r = 0.68), pH and absorbance (r = 0.68), HMF and absorbance (r = 0.67), and minerals and absorbance (r = 0.64). A few of these correlations were expected due to the intrinsic relationship between acidity and pH, acidity and sugars, and moisture and TSS, for example. It is also important to highlight that the absorbance values, which indicated the color of honey samples, exhibited several significant correlations, consistent with the previous reports on the different variables that interfere with honey color.

Conclusions

The physicochemical analyses of honey samples from the Bois River Valley Beekeeping LPA revealed a satisfactory honey quality. Honey samples are generally obtained after good processing and hygienic control, and most of the honey samples evaluated, therefore, presented good conservation. However, certain samples were probably stored at high temperatures. It is important that those involved in honey production and commercialization are qualified in Good Beekeeping Practices. This would ensure improved honey quality, consumer safety, greater income for beekeepers, and a strengthened regional economy.

Author Contributions

Conceptual idea: Furquim, M.G.D.; Sousa Júnior, J.C.; Oliveira, S.S.C.; Cruz, S.J.C.; Methodology design: Romani, V.P.; Oliveira, D.E.C.; Furquim, M.G.D.; Sousa Júnior, J.C.; Data collection: Romani, V.P.; Santos, A.B.; Lima, F.F.; Data analysis and interpretation: Romani, V.P.; Oliveira, S.S.C.; Cruz, S.J.C.; Writing and editing: Romani, V.P.; Oliveira, D.E.C.

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