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Physicochemical and botanical characterization of honey from stingless bees (Meliponini), occurring in the Taquari Valley – RS, for RTIQ edition

Caracterização físico-química e botânica do mel de abelhas sem ferrão (Meliponini), de ocorrência no Vale do Taquari - RS, objetivando edição de RTIQ

Caracterización fisicoquímica y botânica de la miel de abejas sin aguijón (Meliponini), em el Valle de Taquari – RS, para la edición RTIQ

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Abstract
Rio Grande do Sul does not have a Technical Regulation of Identity and Quality (RTIQ) for honey from Stingless Bees (ASF), consequently the commercialization of this food happens informally and without hygienic-sanitary inspection to guarantee its food safety. Therefore, the present study aimed to generate subsidies for the elaboration of an RTIQ for the ASF Gaucho honey. For this purpose, the physical-chemical characterization of 24 samples of ASF honey, donated by 9 members of the Association of the Meliponicultores (ASF beekeepers) of the Taquari Valley, was carried out. The collections were carried out between February 25th and March 5th, 2018, according to the availability of excess honey in each meliponary. Physical chemical analysis was carried out for the following parameters: Acidity, Reducing Sugars, Hydroxymethylfurfural (HMF), Mineral Residues, Diastasis, Insoluble Solids, and Humidity, in addition to the botanical predominance identification. The results of this study were compared with the RTIQ`s of ASF honey from the states of BA, AM, PR and SP and with national legislation. We ca conclude that in relation to the parameters of Reducing Sugars, Moisture, Insoluble Solids, Ashes and Acidity, they all comply with the regulations mentioned. While for the parameters Diastasis and HMF the framing was partial. Therefore, it is possible keeping the particularities of each region and the diversity of ASF species, to use the existing RTIQ`s as a parameter for the elaboration of a document for RS.
Keywords: Honey; ASF; Technical regulation.

Resumo
O Rio Grande do Sul não possui um Regulamento Técnico de Identidade de Qualidade (RTIQ) para o mel de abelhas Sem Ferrão (ASF), assim sendo, a comercialização desse alimento ocorre informalmente e sem inspeção higiênico-Sanitária que garanta sua inocuidade alimentar. Sendo assim, o presente estudo teve como objetivo gerar subsídios para a elaboração de um RTIQ para o mel gaúcho de ASF. Para tanto, foi realizada a caracterização físico-química de 24 amostras de mel de ASF, doadas por 9 associados da Associação de Meliponicultores do Vale do Alto Taquari. As coletas foram realizadas entre os dias 25 de fevereiro e 05 de março de 2018, de acordo com a disponibilidade de mel excedente em cada meliponário. Realizaram-se análises físico-químicas para os seguintes parâmetros: Acidez, Açúcares Redutores, Hidroximetilfurfural (HMF), Resíduos Minerais, Diastase, Sólidos Insolúveis e Umidade, além da identificação de predominância botânica. Os resultados deste estudo foram comparados com os RTIQ’s de mel de ASF dos estados da BA, AM, PR e SP e com a legislação nacional. Podemos concluir que, em relação aos parâmetros Açúcares Redutores, Umidade, Sólidos Insolúveis, Cinzas e Acidez todos enquadram-se nos regulamentos citados.

Enquanto para os parâmetros Diastase e HMF o enquadramento foi parcial. Sendo assim, é possível, guardadas as particularidades de cada região e a diversidade de espécies de ASF, utilizar os RTIQ's já existentes como parâmetro para a elaboração do documento para o RS.
Palavras-chave: Mel; Abelhas sem ferrão; Regulamento técnico

Resumen
Rio Grande do Sul no tiene Reglamento Técnico de Identidad y Calidad (RTIQ) para la miel de Abejas sin Aguijón (ASF). Por lo tanto, el presente estudio tuvo como objetivo generar subsidios para la elaboración de un RTIQ para la miel ASF Gaucho. Para ello, se realizó la caracterización fisicoquímica de 24 muestras de miel de PPA, donadas por 9 miembros de la Asociación de Meliponicultores do Vale do Alto Taquari. Las colectas se realizaron entre el 25 de febrero y el 5 de marzo de 2018, de acuerdo con la disponibilidad de miel excedente en cada meliponario. Se realizaron análisis fisicoquímicos para los siguientes parámetros: Acidez, Azúcares Reductores, Hidroximetilfurfural (HMF), Residuos Minerales, Diástasis, Sólidos Insolubles y Humedad, además de identificar predominio botánico. Los resultados de este estudio fueron comparados con los RTIQ's de miel de ASF de los estados de BA, AM, PR y SP y con la legislación nacional. Podemos concluir que, con relación a los parámetros Azúcares Reductores, Humedad, Sólidos Insolubles, Ceniza y Acidez, todos cumplen con la normatividad mencionada. Mientras que para los parámetros Diastase y HMF el encuadre fue parcial. Por lo tanto, es posible, manteniendo las particularidades de cada región y la diversidad de especies de ASF, utilizar los RTIQ's existentes como parámetro para la elaboración del documento para la RS.
Palabras clave: Miel; Abejas sin aguijón; Reglamento tecnico.

1. Introduction

The ASF are social bees, distributed in tropical regions, occupying almost all of Latin America and Africa, as well as Southeast Asia and northern Australia. These bees have an atrophied stinger and are therefore called stingless bees (Aguiar, et al., 2016). The meliponines play a very important role in the pollination of many botanical species native to Brazil, as they are distributed in the tropical and subtropical regions of the entire globe.

The breeding of ASF has been known since the year 1500; however, only in the last two decades has it begun to attract greater interest, aiming to add value to the product within a sustainable business purpose (Fonseca, 2006). According to Villas-Bôas (2012), the late prominence that stingless bees have been receiving in Brazil is mainly due to a cultural gap heavily influenced by the keeping of honeybees of the genus Apis, which are the major producers of commercially available honey. Another reason for the delay in the advancement of management techniques and the commercialization of ASF honey is the distance from the initially more productive regions, such as the communities in the northern and northeastern parts of Brazil.

Although ASF honey is known and used by traditional Brazilian populations, its formal commercialization in many states is hindered by the lack of a quality and identity standard that serves as a reference for producers and regulatory bodies (Fonseca, 2006). However, the breeding of stingless bees (ASF) and the possibility of commercializing the honey from these species seem to be an income alternative for family farming in the southern part of the country, as it already is in other Brazilian states, especially those with approved regulations, such as the states of Bahia, Amazonas, Paraná, and São Paulo.

The composition of honeys depends on the nectar of the plant species visited by the bees, which imparts characteristics
distinct and peculiar in each region and depending on each species. Among the physicochemical parameters, humidity is the highest differential to be observed among the honeys, being much higher in the stingless bee honeys, which requires greater care in handling and its storage (Anacleto et al, 2009).

Another important characteristic to highlight is related to the way honey is stored in the nests. The honey, after being partially dehydrated by stingless bees, it is stored in cerumen pots, a mixture of wax and propolis, a factor that helps in the preservation of the product and influences its color and flavor. By themselves, these factors mentioned already contribute to the honey of stingless bees having different characteristics from the honey of A. mellifera and, for this reason, would require its own regulation for inspection and commercialization (Venturieri et al., 2007).

The physicochemical characteristics and botanical analysis of these honeys are still not well known (Sodré, 2005). However, it is known that the composition of honey is highly variable, being influenced by various factors such as the stage of maturation, bee species, climatic conditions, geographical region, and origin (Silva et al., 2004). Generally, the difference between the honey of A. mellifera and the meliponines lies in the levels of compounds, particularly water, or moisture, which has a higher concentration in the meliponines, making the honey less dense and more susceptible to fermentation, posing challenges regarding its stability and longevity (Villas-Bôas, 2012).

Although Brazil presents a great potential for the economic exploitation of ASF honey due to the biodiversity of plant species and favorable climate, not all states have their production regulated. The regulations that govern the production and commercialization of honey (Normative Instruction No. 11 of October 20, 2000) establish standards of identity and quality for Brazilian honeys originating from Apis mellifera.

Due to the particularities existing among these bees, such legislation would not be suitable for ASF honeys. Moreover, the lack of national legislation for the regulation of ASF honeys becomes an obstacle to their commercialization. Thus, states like Bahia, Amazonas, Paraná, and São Paulo sought the regulation of their products by having specific legislation for stingless bee honey (meliponines).

The State of Rio Grande do Sul, despite being a potential producer of stingless bee honey, does not have a Technical Regulation of Identity and Quality (RTIQ) for this product. As a result, the commercialization of this food occurs in a completely informal manner and without hygienic-sanitary inspection to ensure its food safety. Thus, the objective of the present study is to provide support for the development of a Technical Regulation of Identity and Quality (TRIQ) for ASF honey, in accordance with the interest of the State Department of Agriculture in drafting the document.

2. Methodology

A descriptive study was conducted to determine the physicochemical characteristics of ASF honey from the regions of Vale do Taquari and Vale do Rio Pardo in the state of RS. The meliponaries participating in the study were selected for convenience, considering the adherence of the members of the Association of Meliponiculturists of the Vale do Alto Taquari who, in a previous meeting, agreed to donate honey samples for analysis upon signing an Informed Consent Form. The collections were carried out between February 25 and March 5, 2018, randomly with respect to the bee species, according to the availability of surplus honey in each meliponary. Each sample consisted of 350 g of honey.

The study was conducted in six municipalities in two regions of RS, according to the coverage area of the Regional Development Councils of the State of Rio Grande do Sul – COREDES 2019 (Figure 1). In the Vale do Taquari region, five municipalities were selected: Arroio do Meio (29°24'03"S, 51°56'42"W, altitude: 54m), Cruzeiro do Sul (29°30'46"S, 51°59'06"W, altitude: 37m), Forquetinha (29°22'55"S, 52°05'27"W, altitude: 56m), Imigrante (29°21'18"S, 51°46'37"W, altitude: 100m), and Travesseiro (29°17'38"S, 52°03'18"W, altitude: 86m), while in the Vale do Rio Pardo region, samples were collected from meliponaries in the municipality of Santa Cruz do Sul (29°43'04"S, 52°25'33"W, altitude: 122m).

Figure 1 - Map of Rio Grande do Sul showing the location of the municipalities where the honey samples were collected.

Source: Authors (2023).

Physical-chemical analyses

Table 1 – Description of the parameters analyzed for the physicochemical determination of ASF honeys, with the method used for the analysis and the corresponding unit.

Parameter analyzed	Method	Result unit
Reducing sugars	Glucose and fructose levels AOAC 977.20	"mEq/kg"
Diastasis (Diastatic activity)	*Determination of Diastase activity after Schade da International Honey Commission*	Gothe scale
Hydroxymethylfurfural (HMF)	AOAC 980.23	mg of HMF/kg
Humidity	B Method standard AOAC 969.38	g/100 g
Insoluble solids (purity grade)	NBR 15714-5	g/100 g
Mineral waste (Ashes)	NBR 15714-3	g/100 g

Source: Authors (2023).

The physicochemical analyses of the honey samples were carried out at the National Agricultural Laboratory in RS, LANAGRO-RS, according to the technical specifications of the Official Methods Manual for the Analysis of Animal Origin Foods (Brazil, 2018), as presented in Table 1.

Botanical analyses

The botanical analysis was conducted at the Beekeeping Laboratory of UFRGS (LabApis - UFRGS), in conjunction with the Department of Plant Health of the Faculty of Agronomy. This laboratory has a Palynotheca, with a collection of pollen grains from the main botanical species of Rio Grande do Sul, collected and identified over the past 30 years from flowers of apicultural interest, according to the method proposed by Maurizio and Louveaux (1965). This reference collection is used, whenever necessary, to resolve doubts regarding the pollen types of each honey sample analyzed.

Statistical analyses

Descriptive and inferential statistical analyses were performed using the MINITAB software. The georeferencing was carried out using the Tableau software.

The Analysis of Variance (ANOVA) test was applied to verify the comparison between bee species, considering a significance level of 5%. The comparison between medians was conducted using the Mood Median test, assuming a significance level of 5% and 95% confidence intervals.

3. Results and Discussion

The physicochemical characteristics of ASF honey are different from A. mellifera honey, therefore, it requires specific legislation, as it is not covered by the Industrial and Sanitary Inspection Regulation of Animal Origin Products (RIISPOA) or by Normative Instruction No. 11 of October 2000, which regulate honey for commercial purposes. This regulation meets the characteristics of A. mellifera honey (Brazil, 2000). Only a few states in Brazil have regulations for stingless bee honey (meliponines), namely: the states of Bahia (2014), Amazonas (2016), Paraná (2017), and São Paulo (2017).

Thus, despite the different characteristics between ASF honey and A. mellifera honey, the presentation of the results will follow what is prescribed by IN No. 11 of 2000, from MAPA, which describes the parameters as follows: Maturity: Reducing sugars and Moisture; Purity: Insoluble solids and Minerals (ashes), Pollen; Deterioration: Acidity, Diastatic activity (diastase) and Hydroxymethylfurfural (HMF), in addition to the specific RTIQs for ASF honey already existing in Brazil.

Twenty-four (24) honey samples were collected from five different ASF species: Jataí, Mandaçaia, Manduri, Tubuna, and Mandaguari, from nine (9) distinct beekeepers in the Vale do Taquari region, in the municipalities of Arroio do Meio (three), Cruzeiro do Sul (five), Forquetinha (five), Imigrantes (three), and Travesseiro (four). In the municipality of Santa Cruz do Sul, which belongs to the Vale do Rio Pardo region, four samples were collected.

The stingless bee species with potential for meliponiculture in Rio Grande do Sul and most commonly used are Meliponas and Scaptotrigonas because they are easy to manage, have low defensiveness, and good honey production throughout the national territory (Jaffé, 2015; Halinski et al, 2018). Among these, the ones that stand out the most are: i) Jataí – Tetragonisca fiebrigi (Schwarz, 1938 apud Moure; Urban; Melo, 2007) – a small bee with a golden-yellow color, popularly known as Jataí, little German, golden bees, or true little mosquito; ii) Mandaçaia – Melipona quadrifasciata (Lepeletier, 1836 apud Moure; Urban; Melo, 2007) – a large bee with a black color and an abdomen featuring 3 to 5 continuous yellow stripes, transverse to the body axis; iii) Manduri – Melipona torrida (Friese, 1916 apud Moure; Urban; Melo, 2007) – medium-sized bees, dark in color, with faces having very visible yellow spots and an abdomen with thin yellow stripes, transverse to the body axis; iv) Tubuna – Scaptotrigona bipunctata (Lepeletier, 1836 apud Moure; Urban; Melo, 2007) and Mandaguari - Scaptotrigona sp. (Lepeletier, 1836 apud Moure; Urban; Melo, 2007) – medium-sized bees with a dark color, these are two species with very similar habits and appearance. All these species are included in this study.

The evaluation of the physicochemical characteristics of honeys aims to ensure that the consumer obtains a product that meets the predetermined quality standards, which, in the case of honey, are maturity, purity, and deterioration. The evaluation of honey maturity is determined by measuring reducing sugars and moisture.

Table 2 – Average reducing sugars in 24 samples of honey collected in municipalities in Vale do Taquari – RS, distributed according to stingless bee species.

BEE	N	Average g/100g	StandDeviation	CI of 95%
Jataí	7	62.329	0.981	(61.375; 63.283)
Mandaçaia	5	60.640	1.905	(59.511; 61.769)
Manduri	4	61.700	0.408	(60.438; 62.962)
Mandaguari	4	60.825	1.292	(59.563; 62.087)
Tubuna	4	61.500	0.783	(60.238; 62.762)

Source: Authors (2023).

Regarding the parameter reducing sugars (g/100g), the averages varied among the honeys according to the bee species from 60.6 to 61.7 (Table 2). However, there was no significant difference between the means of this parameter among the species (p = 0.170). Similarly, when comparing this parameter in relation to the meliponists, there was also no significant difference between the means of reducing sugars per meliponist (p = 0.171), demonstrating the low variation in the means of this parameter in the analyzed samples.

According to Venturieri (2006), the honeys of meliponine bees present reducing sugar levels without significant variations, but not exceeding those of A. mellifera, which according to IN nº11, must have at least 65g/100g. The ASF honeys have a lower sugar content, but a sweeter taste, with glucose and fructose being the main sugars found, in almost equal proportions.

High levels of reducing sugars usually mean that the honey was recently produced, probably from the previous bloom, that is, a product in which sucrose has not yet been fully converted into glucose and fructose by the action of invertase (Sodré et al., 2007).

This parameter can vary according to the bee species and region, Pereira (2010) analyzed 10 honey samples from the Jataí species and 10 samples from Mandaçaia. The Jataí honey samples from Bahia showed a reducing sugars parameter with an average of 54.71g/100g, while the samples from the State of São Paulo showed an average of 55.05g/100g. For the Mandaçaia honey samples, those from Bahia showed reducing sugars with an average of 63.80g/100g, and those from São Paulo showed an average of 72.03g/100g, differing from the results obtained in the present study, and also showed a visible variation between the different species and states.

Borsato (2013), analyzing 21 honey samples from eight species of stingless bees in the State of Paraná, found results of reducing sugar content in the honeys that ranged between 43.35 and 48.52 g/100g, values lower than those obtained in the present study for all the stingless bee species evaluated and also lower than the results of Pereira (2010), which leads us to suggest that possibly the climate, soil, species, and especially the flora may have an influence on this indicator.

Considering that sugars are the main components of honey, we can infer that the results of the present study are within a standard range for the reducing sugars parameter, as the twenty-four (24) honey samples from the five (5) species studied showed an average higher than 60g/100g (values accepted by the regulations of the states that already have RTIQ for ASF honey).

Regarding the maturity of the honey, the moisture parameter (g/100g) per bee species in the present study was 16.74g/100g for Jataí honey; 15.56g/100g for Mandaçaia honey; 17.02g/100g for Manduri honey; 17.07g/100g for Mandaguari honey; and 15.45g/100g for Tubuna honey, with a total average of 16.71g/100g, revealing no significant difference between the moisture averages among species (p = 0.528). Similarly, when comparing the results by meliponiculturists, no significant difference was observed between the average moisture levels (p = 0.140).

According to Venturieri et al. (2007), the honey from meliponine species is characterized primarily by the differentiation in water content, possessing higher humidity, which makes it more liquid, that is, less viscous than bee honey Africanized. The authors also state that the water present in honey is directly related to the floral origin, geographical location, climatic conditions (temperature and humidity), edaphic conditions (soils), season of the year, original nectar moisture, and the degree of maturation of the hive.

Pinheiro (2016) analyzed the honey from Melipona subnitida originating from 35 beekeeping facilities and obtained an average result of 24.4 g/100g moisture, while Araújo (2014), in 18 samples of honey from the same species, found an average of 26.2 g/100g. In the study by Demeterco (2016), higher moisture was found in samples of honey from Melipona seminigra (average value of 32.3 g/100g). Stramm (2010), analyzing 24 samples of honey from Melipona subnitida – Jandaíra, obtained an average moisture content of 24.80 g/100g. Similarly, Carvalho et al. (2013), in a similar study, obtained an average moisture content of 28.84g/100g for Melipona scutellaris.

The average moisture in the present study showed results lower than those presented in the studies above. In addition to the relationships with climate, soil, and vegetation, the degree of hive maturation may have interfered with this parameter. However, this variable was not analyzed in the present study and may be the subject of future research to confirm the hypothesis that this condition was responsible for the reduction of the parameter in the analyzed samples. Furthermore, if we consider that moisture is the most significant difference to be observed among honeys, being much higher in meliponine honeys, which requires greater care in handling and storage (Anacleto et al, 2009), the reduction of this parameter in the analyzed honeys brings benefits to the honey produced in the region.

The purity of honey is evaluated based on the determination of insoluble solids and minerals (ash) and pollen. The parameter "insoluble solids" (g/100g) showed minimal variability among the species, ranging from 0.0 to 0.1 g/100g. Rosa (2014) analyzed Jataí honey and obtained an average of insoluble solids of 3.65 g/100g of honey, while Lopes (2015) found an average of 0.46 g/100g of honey for the same bee species. Silva (2015) analyzed four samples of honey from Melipona subnitida – Jandaíra, finding values with an average of 1.05g/100g. Evangelista-Rodrigues et al. (2005) found an average of 0.01 g/100g of insoluble solids for samples of Melipona scutellaris - Uruçu honey.

Values of insoluble solids higher than those determined by legislation are generally related to failures during collection, the processing during filtration and/or decantation of the honey, as well as the habits of the bees that store them (Silva, 2007). Although there is no RTIQ for ASF honey, the results obtained are in accordance with IN No. 11 of 2000 from MAPA.

The ash content, or mineral residues, is represented by all the inorganic residue remaining from the burning of organic matter and is therefore an indicator of the mineral content of honey (Saxena et al., 2010), being present in concentrations that vary from 0.02 to 1 g/100g of the total weight of the honey (Camargo, 2002). It is considered a purity parameter because if some stages of honey processing are conducted improperly, inorganic material, such as impurities, can be added to the product. Moreover, environmental pollution can also elevate the levels of this parameter (Anklam, 1998), which makes the location of the stingless bee farms a determinant for this parameter.

Such a parameter correlates with the color of the honey, as the darker the honey, the more ash it contains, consequently a higher amount of minerals (Ortiz-Valbuena, 1988). Furthermore, Bogdanov et al. (1999) add that the content of mineral residues (ash) is a quality criterion and is influenced by its botanical origin. Thus, floral-origin honey typically has a lighter color with a lower concentration of minerals and, consequently, fewer ashes than, for example, "honeydew honey," which has a darker color.

In the same way as the results for the insoluble solids indicator, the "mineral residues" indicator also showed very close results with only one decimal place, which prevented statistical analysis from detecting a significant difference. For this parameter, the present study identified results with values ranging from 0.1 g/100g to 0.3 g/100g in the analyzed samples.

Pereira (2010) in his study with Mandaçaia honey obtained 0.114 g/100g of mineral residues, while Batiston (2017) obtained an average of 1.42g/100g in his study with samples from the same bee species. Referring to IN nº11 of 2000 from MAPA, which stipulates a maximum of 0.6 g/100 g in honeydew or honeydew honey and its mixtures with floral honey, a tolerance of up to 1.2 g/100 g is allowed, the analyzed samples would be in compliance with the existing legislation.

Considering the results indicated in the study, it can be stated that the indicator Mineral residues (ashes) of the tested samples are within the values accepted by the four existing regulations (BA, AM, PR, and SP). Observing the general data, we found that the parameters of insoluble solids and mineral residue/ashes showed measurements with low discrimination, meaning they do not adequately distinguish the samples, which is why they were not used for comparative tests.

Within the characteristics of honey, pollen is also an indicator of purity; therefore, honey must necessarily contain pollen grains. All the analyzed samples were characterized as multifloral, meaning they contain more than four (4) different types of pollen, reaching up to eight (8). The description of the pollen content present in apicultural products is capable of determining the apicultural products of a region and identifying the floral sources visited by the bees (Forcone & Ruppel, 2012; Luz & Barth, 2012).

Regarding deterioration parameters, acidity is an important component of honey, as it contributes to its stability against the development of microorganisms. The acids in honey are dissolved in an aqueous solution and produce hydrogen ions that promote its active acidity, thus allowing the indication of storage conditions and the fermentation process (Cornejo, 1988) used as one of the parameters to verify honey deterioration.

Checking the acidity (mEq/kg) of the honey produced by the different bee species studied, the values vary as follows: Jataí with 22.94 mEq/kg; Mandaçaia with 27.12 mEq/kg; Mandaguari with 21.32 mEq/kg; Manduri with 18.35 mEq/kg and Tubuna with 19.63 mEq/kg. In the statistical analysis of this parameter, no significant differences were found between the acidity averages by bee species ($p = 0.469$).

In the State of São Paulo, studies conducted with Meliponas by Stramm (2011) obtained an average of 28.51 mEq/kg for the acidity parameter, while Fernandes (2017) in São José do Rio Preto, also in the State of SP, found an average of 29.03 mEq/kg of Tiúba honey for the same parameter. Silva (2015), in Mossoró – RN, found an average of 66.18 mEq/kg in Jandaíra honey. In Manaus, Demeterco (2016) found an average of 57.34 mEq/kg in Melipona seminigra. Friese and Lima (2017) found 39.78 mEq/kg in Melipona compressipes Fabricius in Chapadinha – MA.

Honey contains acids that contribute to its stability against the development of microorganisms, therefore if the honey is more acidic, there will be an even greater reduction in the possibility of microorganism development. This increase in stability is important to ensure food safety. On the other hand, the higher acidity found in ASF honeys makes them more attractive for use in gastronomy and cuisine due to the harmony between sweetness and acidity, a characteristic not found in Apis mellifera honey (Carvalho et al., 2013).

Considering that acidity is an indicator of deterioration, that is, it directly indicates the quality of the honey, we can consider that the values obtained in the present study can classify these honeys as of good quality. Furthermore, when we compare our results with the existing regulations (BA, AM, PR, and SP) for the acidity parameter, all the analyzed samples comply with the mentioned RTIQs and are below the limit set by IN nº 11 of 2000 from MAPA, where the maximum permitted acidity is 50 mEq/kg. Comparing the diastatic activity indicator by bee species, there was no significant difference ($p = 0.065$) between the means, with values for Jataí 16.571Gothe; Mandaçaia 13.40Gothe; Mandaguari 13.250Gothe; Manduri 16.75Gothe; and Tubuna 15.750Gothe. Similarly, we cannot assert that there is a significant difference between the mean diastase values by meliponist ($p = 0.387$).

Diastase (alpha-amylase) is one of the enzymes present in honey, primarily formed by the hypopharyngeal glands of bees (Pamplona, 1989). The main relevance of the diastatic activity indicator lies in the fact that it shows greater sensitivity to heat than the enzyme invertase (responsible for the transformation of sucrose into glucose and fructose), making it recommended for evaluating honey quality. Thus, its activity serves as an indicator of the degree of preservation and possible overheating of the honey (Soloveve, 1971).

Lower enzymatic levels are found in honeys from rapid nectar flows, as there will be an accumulation of nectar to be processed within the colony. In situations of not-so-fast nectar flow, the enzymatic levels of alpha-amylase will generally be higher, as the bees will have time to intensely process the material (Carvalho et al, 2005). It is observed that nectar with a high sugar content requires less manipulation by the bees to be converted into honey, thus presenting lower levels of invertase and diastase. This parameter is directly related to the availability (quantity) of nectar to be processed within the colony.

Regarding the hydroxymethylfurfural (HMF) indicator, in the statistical analysis comparing the honeys by bee species, no significant difference was found between the HMF averages by species ($p = 0.569$), yielding the following results: Jataí 12.14 mg/kg; Mandaçaia 16.60 mg/kg; Mandaguari 14.50 mg/kg; Manduri 20.00 mg/kg; and Tubuna 21.25 mg/kg.

Lima (2017) analyzed eight (8) honey samples of the species Melipona fasciculata and found HMF with an average of 16.08 mg/kg, a result similar to that obtained for Mandaçaia in this study. According to the literature, lower HMF results were found in Melipona seminigra (9.75 mg/kg) and in Melipona subnitida, 7.56 and 12.42 mg/kg (Stramm, 2011; Reges, 2014; Demeterco, 2016).

According to Gonzales (2002), HMF is a cyclic aldehyde that primarily originates from the dehydration of fructose in an acidic medium, a process that is closely linked to the degree of aging or processing that involves an increase in temperature. It is therefore a parameter that supports the verification of overheating, inadequate storage, and adulteration with commercial sugar (corn or beet syrup), altering the nutritional value of honey, which may result in the loss of some enzymes, such as glucose oxidase. Moreover, this compound is a constituent that, in addition to overheating, can indicate the age of the honey, with its content potentially increasing with the addition of inverted sugar, with the storage time, and also being affected by the acidity, pH, water, and minerals present in the honey (Seeman & Neira; 1988).

This indicator, which also undergoes changes due to various factors, can present quite different results. Even so, the results obtained in this study are within the guidelines set forth in the RTIQ of the States of Paraná and Amazonas and partially in the regulations of São Paulo and Bahia.

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Table 3 – Presentation of the results obtained from the analysis of PSA honey samples with bee species, analysed periods, pollen profile and number of floral species.

Sample	Entomological origin	Bee Name	Acidity (mEq/kg)	Reducing Sugars (%)	Diastasis (Göthe Scale)	HMF Index (mg/kg)	Insoluble (g/100g)	Mineral Waste (%)	Umidity (%)	Pollen Profile	Nº of floral species
1	*Scaptotrigona sp* (Moure, 1942)	Mandaguari	12.8	60.6	12	4	0	0.1	20.5	Multifloral	6
2	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	22.6	62	20	2	0	0.1	20.4	Multifloral	5
3	*Tetragonisca fiebrigi (*Schwarz, 1938)	Jataí	159	64.4	16	4	0	0.2	16.4	Multifloral	5
4	*Melipona quadrifasciata*, (Lepeletier, 1836)	Mandaçaia	49.1	63.8	14	9	0	0.1	13.9	Multifloral	5
5	*Melipona obscurior*(Moure, 1971)	Manduri	11.3	62	21	17	0	0.2	16.5	Multifloral	6
6	*Scaptotrigona bipunctata* (Lepeletier, 1836)	Tubuna	13.4	61.7	14	37	0	0.3	14	Multifloral	5
7	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	27.5	61.9	14	18	0	0.2	15.7	Multifloral	4
8	*Melipona obscurior* (Moure, 1971)	Manduri	26.7	61.3	16	23	0	0.3	16.4	Multifloral	4
9	*Melipona quadrifasciata*, (Lepeletier, 1836)	Mandaçaia	22.3	59.3	16	22	0.1	0.2	15	Multifloral	6
10	*Scaptotrigona sp* (Moure, 1942)	Mandaguari	26.7	59.5	13	38	0	0.2	14.3	Multifloral	6
11	*Scaptotrigona bipunctata* (Lepeletier, 1836)	Tubuna	18.3	61.1	17	22	0.1	0.2	18.2	Multifloral	5
12	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	29.8	61.3	20	17	0.1	0.2	16.4	Multifloral	4
13	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	15.9	62.1	17	19	0.1	0.3	16	Multifloral	4
14	*Scaptotrigona bipunctata* (Lepeletier, 1836)	Tubuna	23.6	62.5	15	17	0.1	0.3	13.3	Multifloral	5
15	*Scaptotrigona sp* (Moure, 1942)	Mandaguari	23.5	62.6	13	5	0.1	0.1	17.1	Multifloral	4
16	*Scaptotrigona bipunctata*	Tubuna	23.2	60.7	17	9	0.1	0.2	16.3	Multifloral	5

	(Lepeletier, 1836)										
17	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	26.6	62.5	14	7	0	0.2	15.7	Multifloral	6
18	*Tetragonisca fiebrigi* (Schwarz, 1938)	Jataí	22.3	62.1	15	18	0.1	0.1	16.4	Multifloral	5
19	*Melipona obscurior* (Moure, 1971)	Manduri	17.7	61.4	16	21	0.1	0.2	16.8	Multifloral	7
20	*Melipona quadrifasciata*, (Lepeletier, 1836)	Mandaçaia	24	60.9	11	9	0.1	0.2	16.4	Multifloral	8
21	*Melipona quadrifasciata*, (Lepeletier, 1836)	Mandaçaia	21.4	59.1	11	15	0.1	0.2	14.5	Multifloral	7
22	*Scaptotrigona sp* (Moure, 1942)	Mandaguari	22.3	60.6	15	11	0.1	0.2	16.4	Multifloral	5
23	*Melipona quadrifasciata*, (Lepeletier, 1836)	Mandaçaia	18.8	60.1	15	28	0.1	0.2	18	Multifloral	5

Source: Authors (2023).

Based on the analyses conducted in the present study, it was possible to collect data regarding ASF honey from two regions in Rio Grande do Sul. In Table 3, the results of the analyses per sample are presented, including bee species, analyzed parameters, pollen profile, and the number of floral species. It is observed that, regardless of the bee species, the honey is classified as multifloral, indicating that the bees visited more than four distinct botanical species. Furthermore, it is possible to observe the parameters evaluated for each sample.

Table 4 – Summary of the results obtained in the analyzes carried out on 24 honey samples from nine beekeeping farmes in the Vale do Taquari/RS Regions, according to the parameter evaluated.

Variável	N	Average	Stand Deviaation	CoefVar	Minimum	Q1	Median	Q3	Maximum
REDUCING SUGARS	24	61.483	±1,287	2.09	59.100	60.625	61.550	62.100	64.400
DIASTASIS	24	15.250	±2.592	17.00	11.000	14.000	15.000	16.750	21.000
HMF	24	16.29	±9.56	58.67	2.00	9.00	17.00	21.75	38.00
INSOLUBLE	24	0.058	±0.050	86.33	0.000	0.000	0.100	0.100	0.100
MINERAL WASTE	24	0.196	±0.062	31.87	0.100	0.200	0.200	0.200	0.300
UMIDITY	24	16.375	±1.818	11.10	13.300	15.175	16.400	17.025	20.500

Source: Authors (2023).

Given the information provided by the physicochemical analyses (Table 4), it is possible to infer that, in general, the samples met the requirements of the four existing RTIQs in Brazil, with the standard deviation being acceptable for the parameters. Therefore, such analyses may assist in the development of the RTIQ for ASF honey produced in the State.

4. Conclusion

Based on the results presented, showing that the averages of the seven analyzed parameters did not exhibit significant differences by bee species, we can infer that certain factors contributed to keeping the range of results between species narrow, such as a certain similarity in the botanical diversity of the regions where the beekeepers are located, the management of beekeepers with similar standards of box handling, including good practices in honey harvesting and similar stages of honey maturity among the beekeepers.

It should be noted that this study, encompassing five species of bees in a specific region of the State, is the first conducted in the State for the purpose of product regulation. In order to effectively edit an RTIQ for the State of Rio Grande do Sul, further studies are needed in these and other regions of the State. Furthermore, the drafting of legislation will have to address other requirements such as methods of conservation, collection, storage, labeling, classification, sensory and microbiological characteristics, among others.

It is suggested to conduct further studies in the different ASF-producing regions of Rio Grande do Sul so that the regulation to be issued addresses the diversity of characteristics of stingless bee honey produced in the State. Furthermore, it is necessary to conduct microbiological tests on these honeys to determine and ensure the safety of the product.

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