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Meads with Brazilian honey from different botanical origins

Abstract – The objective of this work was to evaluate the quality of meads prepared with multifloral honey, unifloral orange blossom honey, and a mixture of both. To prepare the meads, multifloral honey and orange blossom honey from Southern and Southeastern Brazil, respectively, were used. The physicochemical properties, total phenolic content, and antioxidant capacity of the meads were determined. The phenolic compounds were identified by mass spectrometry, and sensory tests were carried out. Multifloral honey showed higher levels of ash content, reducing sugars, and total phenolic compounds than orange blossom honey. The multifloral and mixed meads presented the highest levels of total phenolics, total polyphenol index, and antioxidant capacity. The orange blossom mead showed the highest scores in the sensory analysis for color, aroma, flavor, and global acceptance. The phenolic compounds identified in the meads were the chlorogenic, protocatechuic, syringic, and *p*-coumaric acids, as well as naringenin and quercetin. The physicochemical, functional, and sensory characteristics of the meads are affected by the honey used in their preparation.

Index terms: multifloral honey, orange blossom honey, phenolic compounds, sensory analysis.

Hidroméis com méis brasileiros de diferentes origens botânicas

Resumo – O objetivo deste trabalho foi avaliar a qualidade dos hidroméis preparados com mel multifloral, mel unifloral de flor de laranjeira e uma mistura de ambos. Na preparação dos hidroméis, foram utilizados mel multifloral e mel de flor de laranjeira das regiões Sul e Sudeste do Brasil, respectivamente. Determinaram-se as propriedades físico-químicas, o teor de fenólicos totais e a capacidade antioxidante dos hidroméis. Os compostos fenólicos foram identificados por espectrometria de massas, e testes sensoriais foram realizados. O mel multifloral apresentou maiores teores de cinzas, açúcares redutores e compostos fenólicos totais do que o mel de flor de laranjeira. Os hidroméis multifloral e misto apresentaram os maiores teores de fenólicos totais, índice de polifenóis totais e capacidade antioxidante. Já o hidromel de flor de laranjeira apresentou as maiores notas na análise sensorial para cor, aroma, sabor e aceitação global. Os compostos fenólicos identificados nos hidroméis foram os ácidos clorogênico, protocatecuico, siríngico e *p*-cumárico, bem como naringenina e quercetina. As características físico-químicas, funcionais e sensoriais dos hidroméis são afetadas pelos méis utilizados em sua preparação.

Termos para indexação: mel multifloral, mel de flor de laranjeira, compostos fenólicos, análise sensorial.

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Introduction

Honey is a food rich in nutrients and contains main compounds such as carbohydrates, minerals, proteins, vitamins, lipids, organic and amino acids, enzymes, and other phytochemical compounds, as well as a wide range of phenolic acids and flavonoids responsible for its antioxidant potential (Khalil et al., 2011; Seraglio et al., 2016, 2021). The flavor, color, and other physicochemical properties that determine honey quality come from non-volatile compounds, including minerals, sugars, and phenolic compounds, whose quantities may vary with the floral and geographic origin of the honey (Khalil et al., 2011; Cianciosi et al., 2018; Vasić et al., 2019). Given its high availability, multifloral honey, derived from different types of flowers, has a great commercial prominence (Seraglio et al., 2016; Becerril-Sánchez et al., 2021), while orange blossom honey is among the most important unifloral honeys in the world due to its differentiated sensory characteristics, such as color, aroma, and flavor (Gao et al., 2020; Seraglio et al., 2021).

For honey producers, an economical alternative is the production of mead, a product with high added value and commercial potential (Pereira et al., 2015). Mead, considered the first fermented beverage discovered by man, with an alcoholic strength of 4–14%, is obtained by fermenting honey, water, and yeast, with or without nutrient salt addition (Kahoun et al., 2008; Adamenko et al., 2018; Peepall et al., 2019). Its composition, comprising sugars, vitamins, organic acids, minerals, and phenolic compounds (Švecová et al., 2015; Akalin et al., 2017), varies and is directly related to the type of honey used and the technological processes to which it is subjected, including fermentation, maturation, storage, and consumption (Kahoun et al., 2008, 2017; Švecová et al., 2015; Akalin et al., 2017).

Among the phenolic compounds in meads, the main ones are gallic, caffeic, chlorogenic, ferulic, *p*-coumaric, and syringic acids, as well as flavonoids such as chrysin, galangin, hesperidin, kaempferol, quercetin, and naringenin (Švecová et al., 2015; Akalin et al., 2017; Starowicz & Granvogl, 2020). These compounds in meads are directly affected by the honey's floral and geographical origin, as well as seasonality (Švecová et al., 2015; Akalin et al., 2017).

The objective of this work was to evaluate the quality of meads prepared with multifloral honey, unifloral orange blossom honey, and a mixture of both.

Materials and Methods

The multifloral honey (10 kg) was collected in an apiary located in the municipality of Santiago, in the state of Rio Grande do Sul, in Southern Brazil (29°11'8.7"S, 54°52'20.496"W, at an altitude of 467 m), where the climate is classified as humid subtropical with an annual average temperature of 18–20ºC and an average annual rainfall of 359 mm (IBGE, 2017). The orange blossom honey (10 kg) was purchased from an apiary in the municipality of Rio Claro, in the state of São Paulo, in Southeastern Brazil (22º24'39"S, 47º33'39"W, at an altitude of 617 m), where the climate is classified as Cwa, high altitude tropical, with an annual average temperature of 20.3ºC and an average annual rainfall of 1,294 mm (IBGE, 2022). The samples were kept frozen, at -20ºC, until analysis or use in mead production.

The experimental design was completely randomized with six replicates of three mead treatments (100% multifloral honey, 100% orange blossom honey, and a 50:50% mixture of multifloral and orange blossom honey). The must was prepared with multifloral honey and water until it reached 21 ºBrix, then sulfited at 50 ppm and inoculated with 200 mg L^{-1} SafCider *Saccharomyces bayanus* (Fermentis, Marquette-lez-Lille, France) and 300 mg L^{-1} of the Nutristart fermentation activator (Laffort, Bordeaux, France). Fermentation was carried out in a 5.0 L polyethylene fermenter, and a water seal, at a constant temperature of 20ºC, was used to maintain the system under anaerobic conditions. Fermentation was monitored daily by measuring the total soluble solids content with a refractometer, with results expressed in °Brix (Figure 1). The fermentation process was ended at 18 days by interrupting carbon dioxide evolution and stabilizing total soluble solids, with a final value of 7.75±0.05 °Brix for the multifloral mead, 8.08±0.08 °Brix for the orange blossom mead, and 7.17±0.16 °Brix for the mixed mead. The meads were then stabilized for 25 days at 16°C (Fortes et al., 2023). The total time between fermentation and stabilization was 43 days. In the last step, the meads were sulfited at 50 ppm and bottled in 700 mL bottles.

For the used honey (n = 4 per group), physicochemical analyses were carried out to determine moisture content, ash content, insoluble solids, hydroxymethylfurfural content, pH, total acidity, and reducing and non-reducing sugars. For the meads (n = 6 per group),

analyses were conducted to determine pH, total acidity, total sugars, and alcohol content. To obtain moisture content, the refractive index of honey, at 20ºC, was calculated and later converted into moisture by the table of Chataway, using method 969.38b of Association of Official Analytical Chemists – AOAC (Cunniff, 1995). Insoluble solids contents were determined by the gravimetric method, in which honey is diluted with distilled water, at 80°C, and filtered in a porous crucible according to method 923.03 of AOAC (Cunniff, 1995). Hydroxymethylfurfural was obtained quantitatively by the method of White Jr (1979), whereas pH was determined by the potentiometric method using the DM-22 pH meter (Digimed, São Paulo, SP, Brazil). Ash content and total acidity were obtained by the method described by Instituto Adolfo Lutz – IAL (Zenebon et al., 2008). Total, reducing, and non-reducing sugars were determined according to the method of Lane & Eynon (1923), whereas alcohol content was obtained by distillation in an electronic distiller.

The total phenolic compounds in each honey and mead sample were quantified through spectrophotometry using reduction-oxidation reactions with the Folin-Ciocalteu reagent (Singleton & Rossi, 1965). After the addition of the reagent, the samples were left to rest for 2 hours at room temperature. The absorbances of the samples were read in triplicate using the 600 Plus ultraviolet-visible spectrophotometer (Femto, São Paulo, SP, Brazil) at a wavelength of 765 nm. Phenolic compound contents were calculated by interpolating a calibration curve constituted of 0–80 mg L^{-1} gallic acid, and the results were expressed in milligrams of gallic acid equivalent (GAE) per liter.

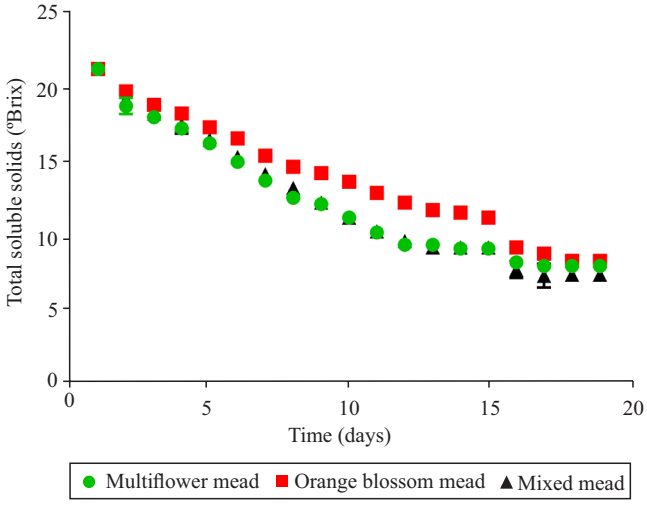

Figure 1. Levels of total soluble solids during the fermentation period of different meads made from multifloral honey, orange blossom honey, and a mixture of both.

Mead antioxidant capacity was determined using the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and the method of Re et al. (1999). The absorbance readings of the samples were taken 6 min after the reaction in the 600 Plus ultraviolet-visible spectrophotometer (Femto, São Paulo, Brazil) at 750 nm. ABTS concentration was calculated from a calibration curve using 0–0.2 mmol L^{-1} Trolox as a standard, with readings performed in triplicate and results expressed as mmol L^{-1} of Trolox equivalent antioxidant capacity (TEAC) per liter.

Mead samples were purified before liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry, following the method of Rodriguez-Saona & Wrolstad (2001) with modifications by Bochi et al. (2015). For this, 6.0 mL of the mead samples were placed in a rotary evaporator, at 35ºC, for 5 min to remove alcohol contents. Afterwards, the samples were loaded into C-18 solid phase extraction (SPE) cartridges (Phenomenex, Torrance, CA, USA), which had been previously activated with methanol and conditioned with acidified water (0.1% v/v formic acid). The polar compounds were washed with two volumes of aqueous formic acid solution (0.1% v/v), and fewer polar phenolic compounds were eluted with two volumes of 3.0 mL ethyl acetate. The ethyl acetate fraction was dried in a rotary evaporator and made up to a known volume of 1.0 mL with 200 µL acidified methanol (0.1% v/v formic acid) and 800 µL acidified water (0.1% v/v formic acid). All fractions were analyzed directly as purified fractions in a chromatograph.

Phenolic compounds were identified based on Quatrin et al. (2019). A liquid-chromatography equipment was connected to the SPD-M20A diode array detector (Shimadzu Corporation, Kyoto, Japan) and a mass spectrometer with a quadrupole-time-of-flight analyzer and an electrospray ionization source (ESI). A 20 µL sample was injected into the C-18 Hypersil Gold reversed-phase column, with 5.0 µm

particle size, 150 mm length, and 4.6 mm diameter (Thermo Fisher Scientific, Waltham, CA, USA). The mobile phase A for this method consisted of ultrapure water:formic acid:methanol (95:5:0.1 v/v), whereas mobile phase B was acetonitrile:formic acid (99.9:0.1 v/v) according to Quatrin et al. (2019). The ESI conditions were a capillary voltage of -4,500 V (negative), nebulizer gas pressure of 30 psi, dry gas at 11 mL min^{-1}, and gas temperature of 310ºC. The tandem mass spectrum experiments were performed in a full-scan range of 100–1800 m/z for all fragments formed from three major parent ions per second. The LC Solutions software, version 3.0 (Shimadzu Corporation, Kyoto, Japan), was used to process the obtained data. Compound identification was based on the combined information of elution order, ultraviolet-visible spectra, and mass spectrometry fragmentation patterns.

The meads were subjected to affective acceptance and ordering tests to measure preference (Zenebon et al., 2008; Balogu & Towobola, 2017). The tasters were recruited locally, completely voluntarily, and verbally informed about the study, its objectives, the risks and benefits of participating, and data confidentiality; this information was also provided in the informed consent form. A 30 mL beverage sample was offered at a temperature of 4ºC (± 2.0) in transparent 50 mL plastic cups coded with three digits in a random order. A seven-point hedonic scale from 1 (really disliked it) to 7 (really liked it) was used to evaluate acceptance attributes, including color, aroma, flavor, and overall acceptance. For the ordering test, the tasters ordered the samples from most to least preferred. The sensory analyses were conducted in a laboratory suitable for this type of analysis, in individual booths, with adequate lighting and free from odors and noise. The tests were carried out with 102 untrained adult tasters of both sexes. The project was approved by the national research ethics committee, under number CAAE 58889316.3.0000.5346, following the guidelines of Resolução CNS. 466 of Conselho Nacional de Saúde (Brasil, 2013).

Statistical analyses of honey were performed using Student's t-test, and mead data were analyzed using the one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The results were expressed as mean ± standard error of the mean, and differences were considered statistically significant when $p \leq 0.05$. The data were analyzed using the Statistica, version 9.0, software (TIBCO Software Inc., Palo Alto, CA, USA). The result of the sensory analysis was subjected to the Friedman test using the table of Newel-MacFarlane (1987) and to acceptability by ANOVA, at 5% probability.

Results and Discussion

The honeys used to produce the meads had the quality required by the Brazilian legislation (Brasil, 2000), as shown in Table 1. The multifloral honey had higher ash and reducing sugar contents, as well as a lower total acidy and hydroxymethylfurfural content, in comparison with the orange blossom honey, indicating its better physicochemical quality. As for the functional proprieties of the honeys, the multifloral honey had a higher total phenolic content, possibly related to its multiple floral origin that may have increased the amount and variety of its phenolic compounds (Becerril-Sánchez et al., 2021). The phenolic compound content in honeys is also related to the geographic region where the bees visit, representing the characteristics of the flora and climate of each region (Becerril-Sánchez et al., 2021). In the present study, the used honeys were from Southern and Southeastern Brazil, so they were expected to have a different total phenolic content.

All meads had the same alcohol content and pH values lower than those obtained for the honeys (Table 2), an expected decrease since mead is a fermented beverage (Starowicz & Granvogl, 2020). The meads produced with multifloral honey, alone or mixed, had a higher total polyphenol content, which was also expected since this honey had greater quantities of these bioactive compounds than orange blossom honey (Table 1). By collecting pollen, bees transfer many of the phenolic compounds of the secondary metabolism of the plant to the honey, which is why these phytochemicals are present in the mead, even if in smaller amounts (Akalin et al., 2017; Cianciosi et al., 2018).

The mixed mead showed the highest values for antioxidant capacity, which depends on the chemical composition of the raw material, environmental factors that directly affect the honey production process, and the technologies used to process the honey (Akalin et al., 2017; Starowicz & Granvogl, 2020). However, the main factor that affects mead antioxidant capacity

is related to the presence of phenolic compounds, whose diversity is directly linked to the used honey (Starowicz & Granvogl, 2020), which, in this case, is rich in phenolic compounds, such as phenolic acids and flavonoids, due to the vast Brazilian flora (Seraglio et al., 2016).

Six compounds belonging to the classes of phenolic acids and flavonoids were identified in the meads under study (Table 3). The diversity of these compounds depends on the composition of the meads, being lower for those made only with honey and water and higher for those produced with fruits, juices, or herbal extracts (Švecová et al., 2015). In the present work, among the identified phenolic compounds, chlorogenic acid and quercetin were found in all samples. Syringic acid was only present in the mead with multifloral honey, whereas protocatechuic acid was found in the multifloral and mixed meads, indicating its presence in multifloral honey. However, the phenolic compounds *p*-coumaric acid and naringenin were only observed in the mead with orange blossom honey. Therefore, the differentiated chemical composition of the meads due to the different honeys used, results in distinct functional and sensory qualities. Since naringenin is one of the main phenolic compounds in citrus fruits, such as oranges, its presence in orange blossom mead confirms the origin of the honey used in preparing this beverage.

In the literature, meads elaborated with multifloral honey and aged for 360 days showed 18 phenolic compounds in their composition (Fortes et al., 2023), including the six found here. Kahoun et al. (2017) identified protocatechuic, syringic, and *p*-coumaric acids in traditional meads, made only with honey and

Table 1. Physicochemical characterization and phenolic compound contents in the honeys used for mead production[(1)].

Physicochemical attribute	Multifloral honey	Orange blossom honey	Brazilian legislation[(2)]
Moisture content (g 100 g^{-1})	19.93±1.33a	19.80±1.20a	Maximum 20
Ash content (g 100 g^{-1})	0.40±0.06a	0.15±0.02b	Maximum 0.6
Reducing sugars (g 100 g^{-1})	72.49±0.91a	66.67±0.45b	Minimum 65
Non-reducing sugars (g 100 g^{-1})	3.39±1.02a	3.91±0.60a	Maximum 6
Total acidity (meq kg^{-1})	3.66±0.01b	4.08±0.03a	Maximum 50
Insoluble solids (g 100 g^{-1})	0.06±0.00a	0.05±0.00a	Maximum 0.5
Hydroxymethylfurfural content (g kg^{-1})	7.10±0.35b	8.38±0.30a	Maximum 60
pH	3.94±0.00a	3.98±0.01a	-
Total polyphenols (mg GAE kg^{-1})	484.90±1.23a	471.30±1.63b	-

[(1)]Means followed by different letters differ by Student's t-test, at 5% probability. Results were expressed as mean ± standard error of the mean (n = 4). [(2)]Values established by the Brazilian legislation (Brasil, 2000) for *Apis mellifera* honey. GAE, gallic acid equivalent.

Table 2. Physicochemical characterization, total phenolic compounds, and antioxidant activity of the meads made from multifloral honey, orange blossom honey, and a mixture of both[(1)].

Physicochemical attribute	Multifloral mead	Orange blossom mead	Mixed mead
Total sugars (g L^{-1})	29.72±0.92b	36.33±0.32a	29.70±0.91b
Total acidity (meq L^{-1})	54.55±1.02b	54.00±0.82b	59.67±1.41a
Residual sugars (g L^{-1})	20.18±0.74b	25.86±0.81a	23.35±0.99a
pH	3.57±0.02a	3.26±0.00c	3.43±0.00b
Alcohol content (°GL)	10.60±0.00a	10.60±0.00a	10.60±0.00a
Total phenolic content (mg GAE L^{-1})	218.30±3.56ab	210.50±7.62b	227.70±4.55a
Total polyphenol index (mg L^{-1})	4.43±0.07a	4.13±0.03b	4.51±0.07a
Antioxidant capacity (mmol L^{-1} TEAC L^{-1})	2.04±0.13ab	1.80±0.04b	2.15±0.07a

[(1)]Means followed by different letters differ by Tukey's test, at 5% probability. Results were expressed as mean ± standard error of the mean (n = 6). GAE, gallic acid equivalent; and TEAC, Trolox equivalent antioxidant capacity.

water, while Adamenko et al. (2018) found *p*-coumaric and chlorogenic acids in mead samples. These findings suggest that phenolic compounds are stable during the mead fermentation process (Švecová et al., 2015; Akalin et al., 2017; Kahoun et al., 2017), which may be due to the acidic nature of the mead and the presence of alcohol, favorable for the solubilization and preservation of these compounds.

All meads differed for the color, aroma, and flavor attributes. The average scores were between 4 and 6, corresponding to "indifferent" and "moderately liked it" (Table 4). The multifloral mead was the least appreciated among tasters, whereas the orange mead was the most appreciated regarding all attributes. Orange blossom honey, considered one of the best unifloral honeys in the world, has a light color, intense aroma, mild flavor, and creaminess due to its unique and striking flora, which conferred the mead more defined characteristics in terms of color, aroma, and flavor (Tette et al., 2017). In contrast, the attributes of the two other meads (multifloral and mixed) may have been affected by the flowering of the multifloral honey, which has a diverse flora in its composition. The orange blossom and the mixed meads presented the highest scores for global acceptance, not differing from each other, which is probably attributed to the fact that these two meads have orange blossom honey in their composition.

Considering the number of samples tested (n = 3) and the number of tests applied (n = 102), according to the Newel-McFarlane table, at a significance level of 95%, the critical difference between the sum of the ordering totals must be 34 (Table 5). No significant differences were found for the sum of the samples. Given this result, the tasters may not have actually preferred one mead over the others, which could be explained by the fact that this beverage is not commonly consumed by them, meaning that their affective memory for this type of analysis is less impactful.

Table 3. Phenolic compounds identified in meads made from multifloral honey, orange blossom honey, and a mixture of both[(1)].

RT (min)	Phenolic compound	Molecular formula	Monoisotopic mass	m/z [M-H] – experimental	m/z [M-H] – correction	Error (ppm)	MS^2 product ions (-) (m/z)	Multifloral mead	Orange blossom mead	Mixed mead
9.1	Chlorogenic acid	$C_{16}H_{17}O_9$	354.0951	353.0877	354.0950	0.3	191.0574	X	X	X
9.3	Protocatechuic acid	$C_7H_5O_4$	154.0266	153.0193	154.0266	0.2	109.0376	X		X
10.8	Syringic acid	$C_9H_9O_5$	198.0528	197.0455	198.0528	0.2	111.0182/125.0360/140.0247	X		
11.1	*p*-coumaric acid	$C_9H_8O_3$	164.0473	163.0400	164.0473	0.4	119.0518		X	
18.4	Naringenin	$C_{15}H_{12}O_5$	272.0685	271.0612	272.0685	0.0	125.0266/197.0639/225.0540 /253.0480		X	
18.7	Quercetin	$C_{15}H_9O_7$	302.0427	301.0353	302.0426	0.2	151.0175/107.0253/116.0828 /121.0426	X	X	X

[(1)]RT, retention time; m/z, mass-charge ratio; MS^2, ion from the mass spectra; and X, presence of the compound in the sample.

Table 4. Mean scores of the sensory attributes evaluated in the acceptance test of meads made from multifloral honey, orange blossom honey, and a mixture of both[(1)].

Sensory attributes	Multifloral mead	Orange blossom mead	Mixed mead
Color	5.36c	5.42a	5.51b
Aroma	4.97c	5.54a	5.07b
Flavor	4.76c	5.35a	5.04b
Global acceptance	5.12b	5.41a	5.30a

[(1)]Means followed by different letters differ by Tukey's test, at 5% probability. Acceptance attributes were evaluated using the following seven-point hedonic scale: 1, really disliked it; 2, moderately disliked it; 3, slightly disliked it; 4, neither liked nor disliked it/indifferent; 5, slightly liked it; 6, moderately liked it; and 7, really liked it.

Table 5. Differences between total sum pair of the preference ordering test for meads made from multifloral honey, orange blossom honey, and a mixture of both [(1)].

	Multifloral mead (I)	Orange blossom mead (II)	Mixed mead (III)
Total sum	127a	148a	135a
Difference vs. I[(2)]		21	8
Difference vs. II[(3)]			13

[(1)]Means followed by different letters differ by the Newel-McFarlane test, at 5% probability. [(2)]Sum difference compared to multifloral honey. [(3)]Sum difference compared to orange blossom honey.

Conclusions

1. The mead made from multifloral honey presents higher levels of phenolic compounds in its composition than that made from orange blossom honey.

2. The meads have different chemical compositions due to the different geographical and floral origins of the honeys used in their production.

3. *p*-coumaric acid and naringenin are only present in the mead produced from orange blossom honey.

4. The mead made from orange blossom honey shows a higher global acceptance than that made from multifloral honey.

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