Use of visible spectrophotometric fingerprint and chemometric approaches for the differentiation of *Mimosa scabrella* Benthام honeydew honey

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Abstract Honeydew honeys, including bracatinga (*Mimosa scabrella* Benthام) honeydew honey, are highly appreciated due to its nutritional and sensory properties. Therefore, fast and inexpensive methods must be developed to differentiate this distinct class of honey. Although methods using the visible spectrophotometric fingerprint (VSF) have never been used to differentiate bracatinga honeydew honey and blossom honey, this technique appears to be a viable alternative, successfully used in the differentiation and fraud detection of various foods, including honeys from other botanical sources. In this sense, the present study aimed to verify the existence of a specific VSF for bracatinga honeydew honey and blossom honey and differentiate both type of honeys using the VSF associated with chemometric analysis. The VSF of 30 bracatinga honeydew honeys harvested in three different years (2014, 2016, and 2018) and 21 blossom honeys (harvested in 2016) were evaluated. The existence of a different VSF for bracatinga honeydew honeys and blossom honeys allowed the satisfactory differentiation of both types of honeys. Additionally, bracatinga honeydew honey presented a unique VSF, independent of the year of

harvest, which can be exploited as a fingerprint of this type of honey, contributing to its authenticity.

Keywords Bracatinga · Chemometric analysis · Principal component analysis · Blossom honey

Introduction

Honeydew honey has become a highly appreciated food, mainly in the European market, due to its nutritional, sensorial, and therapeutic properties (Alda-Garcilope et al. 2012; Kaygusuz et al. 2016). In Brazil, the production of honeydew honey from bracatinga (*Mimosa scabrella* Benthام) tree has been gaining international prominence mainly for presenting in its composition essential amino acids (Azevedo et al. 2017) and considerable amounts of minerals and phenolic compounds, both highly bioaccessible (Seraglio et al. 2016, 2017; Bergamo et al. 2018), besides high antioxidant capacity (Seraglio et al. 2017; Bergamo et al. 2019a).

The production of this dark honeydew honey occurs every two years when bracatinga (*Mimosa scabrella* Benthام) trees are infested by plant-sucking insects (*Tachardiella* sp. or *Stigmacoccus paranaensis* F.) which release a saccharine exudate that is collected by *Apis mellifera* bees (Mazuchowski et al. 2014; Wolff et al. 2015). This phenomenon, and consequently the production of bracatinga honeydew honey, occurs mainly in the highland region of the state of Santa Catarina and in the first semester (fall and winter period), when there is a scarcity of floral resources to obtain nectar (Mazuchowski et al. 2014). All these particularities make this dark honeydew honey a very peculiar type of honey, adding value to the product (Bergamo et al. 2019b).

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Due to the increasing consumption of bracatinga honeydew honey, it is important to guarantee its quality and avoid the occurrence of frauds. In this sense, the evaluation of authenticity of honeys, especially focus in the distinction of different honey types (blossom and honeydew) and from different botanical origins, have been generally investigated based on its composition and physicochemical parameters associated with chemometrics (Amiry et al. 2017; Oroian and Ropciuc 2017; Oroian et al. 2017; Bergamo et al. 2018, 2019a). Besides the physicochemical characteristics, the investigation of specific compounds as markers of authenticity, such as carbohydrates, minerals, phenolic compounds, and volatile compounds, are widely performed in honeys. However, many of these analyses are complex, too time-consuming or require specialized personnel to operate sophisticated and expensive equipment, such as liquid chromatography and gas chromatography coupled to different detectors (mass spectrometry, ultraviolet/visible, refractive index), flame atomic absorption spectrometry, and atomic emission spectroscopy (Aliferis et al. 2010; Pita-Calvo and Vázquez 2018; Karabagias 2019; Seraglio et al. 2019; Karabagias et al. 2020).

The spectroscopy techniques, including the ultraviolet, visible, and infrared spectra, have some advantages over other methods, such as low-medium cost, simplicity, and ease in analytical operations (Roshan et al. 2013; Vlaeva et al. 2017). The spectrophotometric fingerprint was investigated in honeys mainly using the ultraviolet and infrared spectra. The potential of these spectra in the authenticity of honeys was exploited in some studies. Fourier transform near-infrared (FT-NIR) was applied in acacia, alpine rose, chestnut, dandelion, lime, rape, polyfloral, and fir honeydew honeys from Switzerland and Germany (Ruoff et al. 2006a); in rape, acacia, lime, heather, cornflower, sunflower, clover, and honeydew honeys from Germany (Etzold and Lichtenberg-Kraag 2008); and in acacia, linden, and honeydew honeys from Bulgaria (Vlaeva et al. 2017). Ultraviolet spectrum was also investigated in acacia, linden, and honeydew honeys from Bulgaria (Vlaeva et al. 2017) and in honeys from distinct botanic origins from Republic of Yemen (Roshan et al. 2013), while attenuated total reflectance mid-infrared (ATR-MIR) was applied in 11 honey types from Switzerland, Germany, Italy, Spain, France, and Denmark (Ruoff et al. 2006b).

In this context, the VSF can also be an interesting analytical technique, since it involves minimum sample preparation, little or no use of reagents, and low cost (Souto et al. 2010). This technique has already been used in food products such as extra virgin olive oils, kiwi, pomelo fruits, and coffees for differentiation and fraud detection (Souto et al. 2010; Sârbu et al. 2012; Pizarro et al. 2013). Although

the VSF has never been used for the differentiation between blossom honeys and bracatinga honeydew honeys, this technique has already been successfully used for differentiation of acacia, linden, and honeydew honeys from Bulgaria (Vlaeva et al. 2017) and used together with ultraviolet or infrared spectra in the investigation of authenticity of honeys from different geographical and botanical origins (Li and Yang 2012; Almaleeh et al. 2017; Frausto-Reyes et al. 2017). However, its potential associated with chemometric analysis for the classification of honeys is still very little studied. VSF associated with chemometric analysis may indicate differences or similarities among samples, allowing to establish a specific standard profile for certain food groups, not needing the determination of specific compounds (Castro-Puyana and Herrero 2013).

In this sense, the present study aimed, for the first time, to verify the existence of a specific VSF for bracatinga (*Mimosa scabrella* Benth) honeydew honey and differentiate this honeydew honey from blossom honeys using the VSF associated with chemometric analysis.

Materials and methods

Honey samples

The honey samples were collected from different geographical localities of the state of Santa Catarina, Brazil, as shown in Table 1.

Bracatinga (*Mimosa scabrella* Benth) honeydew honey samples ($n = 30$) were harvested in three different years (2014, 2016, and 2018) from beekeepers with extensive areas of bracatinga trees. The collection of honey was performed from April to July, when plant-sucking insects infested bracatinga trees and high exudate production was observed, ensuring great purity of the samples. Additionally, no other type of saccharine source was observed near the hives during the bracatinga honeydew honey production.

Blossom honeys ($n = 21$) were harvested in 2016 from geographical regions nearby to the collection area of bracatinga honeydew honeys, but during the months with lower exudate production (January–March, August–November) and of apiaries far from bracatinga tree plantations, to avoid the contamination of blossom honeys with honeydew. The botanical origin of the blossom honey samples was considered according to the declaration of the local beekeepers (Table 1).

All honey samples were provided in the form of honeycombs by beekeepers and immediately transported (5 ± 2 °C) to the laboratory. The honeycombs were manually drained, filtered, placed into a polypropylene

Table 1 Geographical information of bracatinga (*Mimosa scabrella* Bentham) honeydew honey and blossom honey samples

Type of honey	Year of harvest	Botanical origin	Geographical origin	Sample
Honeydew	2014	<i>Mimosa scabrella</i> Bentham	Bocaina do Sul	H1, H2
			Lages	H3, H4
			Urubici	H5, H6
			Urupema	H7, H8
	2016	<i>Mimosa scabrella</i> Bentham	Bom Retiro	H9, H10
			Bocaina do Sul	H11, H12, H13
			Lages	H14
			São Joaquim	H15, H16
			Urubici	H17, H18
			Urupema	H19, H20, H21
			Bom Retiro	H22, H23, H24, H25
	2018	<i>Mimosa scabrella</i> Bentham	Bocaina do Sul	H26
			Lages	H27, H28
			Urubici	H29
			Urupema	H30
Blossom	2016	Multiflower	Bom Retiro	B1, B2, B3
		<i>Sida</i> sp.	Bom Retiro	B4
		Multiflower	São Bonifácio	B5
		<i>Citrus sinensis</i> (L.)	São Bonifácio	B6
		<i>Mimosa scabrella</i> Bentham	Lages	B7
		Multiflower	Lages	B8, B9, B10
		<i>Myrcia multiflora</i>	São Joaquim	B11
		<i>Malus domestica</i>	São Joaquim	B12
		Multiflower	São Joaquim	B13, B14
		Multiflower	Urubici	B15, B16, B17, B18
		<i>Clethra scabra</i> Pers.	Urupema	B19
		<i>Eucalyptus</i> sp.	Urupema	B20
		<i>Sida</i> sp.	Urupema	B21

H: Bracatinga (*Mimosa scabrella* Bentham) honeydew honey. B: Blossom honey

tube with a screw cap, and frozen at -18 ± 2 °C. Before analysis, the honey samples were thawed and manually homogenized.

Visible spectrophotometric fingerprint—VSF

To the VSF analysis, honey samples (1.0 g) were diluted in the proportion 1:4 (w/w) with ultrapure water (Milli-Q Simplicity® UV system, Millipore Corporation, France). An aliquot of 1.5 mL of the diluted sample was vortexed for 5 min and centrifuged (Eppendorf AG, Germany) at 10,000 rpm for 10 min. The supernatants (0.2 mL) were transferred to microwells of the plastic microplate (containing 96 microwells) and the VSF measured at 25 °C in a UV–Visible spectrophotometer SpectraMax® Paradigm® Multi-Mode Microplate Reader (United State of America) equipped with xenon flash lamp, ultra-high power LEDs,

and silicon photodiode detector with photomultiplier tube. The absorbance of the honey samples was determined from 380 to 800 nm (visible spectrum) in intervals of 1 nm, resulting in 420 variables. The data acquisition and processing were performed in the SoftMax® Pro GxP software.

The ideal dilution of the samples was determined by preliminary tests. Blossom honeys ($n = 3$) and bracatinga honeydew honeys ($n = 3$) were diluted with ultrapure water in the proportion of 4:1, 3:2, 2:3, and 1:4 (w/v) honey:water. These diluted samples were submitted to the previously described procedure and the VSF was evaluated. The best signal to noise ratio was observed in the honeys diluted in the proportion of 1:4 (w/v) honey:water (Fig. 1). Thus, this dilution was chosen for the determination of VSF of the blossom honey and bracatinga honeydew honey in this study.

Fig. 1 Visible spectrophotometric fingerprint (380–800 nm) of bracatinga (*Mimosa scabrella* Bentham) honeydew honeys and blossom honeys diluted in the proportion (w/w—honey:water) of: **a** 4:1, **b** 3:2, **c** 2:3, and **d** 1:4

Chemometric analysis

All assays were performed in three independent experiments ($n = 3$) for each sample, resulting in three absorbance values for each selected wavelength. The average of these three values, without pre-processing, was used in the chemometric analysis. The principal component analysis (PCA) was used to evaluate the interdependence of VSF data and the possible grouping of bracatinga honeydew honeys and of blossom honeys. Paleontological Statistics® (Past) software, version 2.16, which was used to perform the statistical analyses.

Results and discussion

The VSF analysis results in a unique profile, without identification of compounds, depending on the intrinsic characteristics of the analyzed sample. Therefore, it may be suggested that each sample will have a specific VSF. But it is also expected that there are similarities between the VSF of samples belonging to the same group, allowing to establish a characteristic VSF of a given food group. In this sense, VSF of bracatinga honeydew honeys and blossom honeys harvested in 2016 were investigated. For each sample, 420 variables were obtained and evaluated from PCA analysis. As shown in Fig. 2, principal component 1 (PC1) explained the highest percentage of data variation (87.68%) while principal component 2 (PC2) explained 10.26%. A satisfactory separation between blossom honeys

● Bracatinga honeydew honey (2016); + Blossom honey (2016).

Fig. 2 Principal component analysis for bracatinga (*Mimosa scabrella* Bentham) honeydew honeys and blossom honeys harvested in 2016 using 420 variables (380–800 nm)

and bracatinga honeydew honeys was verified, suggesting the existence of characteristic VSF for each type of honey (blossom and bracatinga honeydew) investigated.

Although a good separation of data has been achieved, aiming at reducing the number of variables, a new PCA analysis was carried out only with those variables with a coefficient value of correlation higher than 0.9 with PC1 (Online Resource 1). As shown in Fig. 3, only the use of

Fig. 3 Principal component analysis for bracatinga (*Mimosa scabrella* Bentham) honeydew honeys and blossom honeys harvested in 2016 using 105 variables (380–485 nm)

105 variables (measures from 380 to 485 nm) was enough to promote the differentiation of the honey samples studied. Also, the PC1 was responsible for explaining 93.05% of the data variability, increasing the reliability of the results obtained. The smaller number of variables also is advantageous because it would reduce the time of analysis.

The existence of a common VSF for bracatinga honeydew honeys and blossom honeys harvested in 2016 allowed the satisfactory differentiation of both types of honeys, suggesting that this profile can be used as a fingerprint of bracatinga honeydew honey. In this context, the VSF of bracatinga honeydew honey was investigated in samples harvested in 2014, 2016, and 2018, aiming to verify the existence of a similar VSF of these honey samples capable of differentiating them from blossom honeys (harvested in 2016). As shown in Fig. 4, it was possible to verify that bracatinga honeydew honeys from different years of harvest were well differentiated from blossom honeys. In this analysis, the sum of PC1 (94.89%) and PC2 (4.27%) represented 99.16% of the total variability of the data, indicating an excellent explanation of the data. These findings indicate that bracatinga honeydew honey has a unique VSF, independent of the year of harvest, which can be exploited as a fingerprint of this type of honey contributing to its authenticity. Although blossom honeys harvested in 2014 and 2018 were not included in the assessment, the findings of this study are solid indicators suggestive of differentiation between these two types of honey (bracatinga honeydew and blossom) regardless of the year of harvest. The visible spectrum associated with PCA analysis

Fig. 4 Principal component analysis for bracatinga (*Mimosa scabrella* Bentham) honeydew honeys harvested in 2014, 2016 and 2018 and blossom honeys harvested in 2016 using 105 variables (380–485 nm)

was also one of the best techniques for differentiation of acacia, linden, and honeydew honeys from Bulgaria (Vlaeva et al. 2017). In that study, the most intense differences were observed in the bands between 400 and 500 nm. These differences were associated with the color of the honeys, in which honeydew honeys showed the highest absorbance values, followed by linden honeys and acacia honeys. This same tendency was found in the present study, in which the bracatinga honeydew honeys presented the highest absorbance values concerning blossom honeys and their visible spectra differing mainly in the bands between 380 and 500 nm. Therefore, these findings reinforce the strong influence of color components in the visible spectrum of honeys.

It was also observed that honeydew honeys from Bulgaria appear to have a visible spectrum distinct from that found for bracatinga honeydew honeys. An increase in absorbance values between 380 and 425 nm has been reported for undiluted honeydew honeys from Bulgaria, as well as the occurrence of an absorbance peak between 450 and 470 nm for several samples of this type of honey (Vlaeva et al. 2017). This characteristic was not observed for bracatinga honeydew honeys, which showed a continuous decrease in the absorbance values between 380 and 500 nm, in the samples diluted 1.25–5 times (Fig. 1). Therefore, although similar behaviors are found among the visible spectrum of honeydew honeys of different botanical and geographic origins, differences are also observed which allow proposing that the intrinsic chemical

composition of each honeydew honey strongly influences its visible spectrum, resulting in a specific VSF.

The use of the visible spectrum, as a single parameter, is still poorly investigated in the literature for the classification of honeys. However, reinforcing the findings showed in this study, the potential of its use can be observed in other studies, in which the visible spectrum together with the ultraviolet and infrared spectra and chemometric analysis allowed the differentiation of honeys from different origins (Li and Yang 2012; Almaleeh et al. 2017; Frausto-Reyes et al. 2017). This fact becomes important, since this technique can be considered of low complexity, being accessible to most laboratories and of easy reproducibility when compared mainly to the determination of infrared spectrum and other analytical methods that involve for example liquid and gas chromatographies. Therefore, the VSF associated with chemometric analysis seems to be a promising technique for the differentiation of honeys, especially bracatinga honeydew honeys and blossom honeys.

Conclusion

The investigation of the VSF of bracatinga honeydew honey and blossom honey and its potential for honey differentiation was evaluated for the first time in this study.

The VSF associated with chemometric analysis allowed the differentiation of bracatinga honeydew honeys from blossom honeys. It was possible because, regardless of the year of harvest, bracatinga honeydew honey seems to have a unique VSF able to distinguish it from blossom honey, which also presented a characteristic VSF. Therefore, the technique presented in this work associated with chemometric analysis seems to be a viable, quick, and low-cost alternative to the differentiation of bracatinga honeydew honeys from blossom honeys, contributing to its authenticity.

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