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Physicochemical analysis of floral honeys produced by *Apis mellifera* L. between 2018 and 2020 in the State of Santa Catarina, southern Brazil

Análise físico-química de méis florais produzidos por Apis mellifera L. entre 2018 e 2020 no Estado de Santa Catarina, sul do Brasil

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ABSTRACT

Brazil is a major producer of honey and is internationally recognized for the quality of this product. To assess the quality of the product, physicochemical analyses were carried out. In this sense, samples of honey from the eleven agroecological zones of Santa Catarina, southern Brazil, were analyzed for moisture content, pH, reducing sugars, apparent sucrose, water activity, diastase enzyme, color, and Lund reaction. Sixty-five samples of honey collected in the 2018-2019 harvest and 50 in the 2019-2020 harvest were analyzed. The data were analyzed using analysis of variance and the Scott & Knott test ($p<0.05$) with the aid of scripts written in the R language. Principal component analysis (PCA) was also applied to the physicochemical dataset to verify possible sample groupings between the agroecological zones of the State and interactions between the variables. Moisture and apparent sucrose contents of the honey showed the greatest discrepancies in their values regarding what is permitted by the Brazilian legislation. Three agroecological zones were correlated in terms of the data found. PCA showed that moisture, pH, and water activity were relevant physicochemical parameters for grouping and discriminating samples. From the results herein shown, it is perceived the need for continuous monitoring of honey produced in Santa Catarina State, as well as the qualification of beekeepers for harvesting, processing, and storage.

KEYWORDS: beekeeping; PCA; food safety.

RESUMO

O Brasil é um grande produtor de mel e é reconhecido internacionalmente pela qualidade deste produto. Para avaliar a qualidade do produto foram realizadas análises físico-químicas. Nesse sentido, amostras de mel das onze zonas agroecológicas de Santa Catarina, sul do Brasil, foram analisadas quanto ao teor de umidade, pH, açúcares redutores, sacarose aparente, atividade de água, enzima diastase, cor e reação de Lund. Foram analisadas 65 amostras de mel coletadas na safra 2018-2019 e 50 na safra 2019-2020. Os dados foram submetidos à análise de variância e ao teste de Scott & Knott ($p<0,05$) com auxílio de *scripts* implementados em linguagem R. A análise de componentes principais (ACP) também foi aplicada ao conjunto de dados fisico-químicos para verificar possíveis agrupamentos de amostras entre as zonas agroecológicas do estado de SC e interações entre as variáveis. Os valores de umidade e a sacarose aparente apresentaram as maiores discrepâncias em relação ao permitido pela legislação brasileira. Três zonas agroecológicas foram correlacionadas em termos dos dados encontrados. A ACP mostrou serem a umidade, pH e atividade de água parâmetros relevantes ao agrupamento e discriminação das amostras de méis. A partir dos resultados observados, percebe-se a necessidade de monitoramento contínuo da qualidade daquele alimento, bem como a capacitação dos apicultores para colheita, processamento e armazenamento.

PALAVRAS-CHAVE: apicultura; ACP; segurança alimentar.

INTRODUCTION

Honey is among the most consumed products worldwide, with a production of 1,852,598 tons in 2019, according to the Food and Agriculture Organization (FAO 2021). It has been estimated that between 2020-2025, the beekeeping market will register a Compound Annual Growth Rate (CAGR) of 4.3%, fostered by the increased demand, both for medicinal purposes and for use in cosmetic products (MORDOR INTELLIGENCE 2020).

In Brazil, the production of honey in 2019 reached 45,980,621 tons, which generated approximately R$ 500,000.00. The south of the country produced 17,571,271 tons, with the State of Santa Catarina being responsible for 23.2% of that amount, from 16,838 agricultural establishments (IBGE 2020).

Because of its added value, studies have pointed out honey is among the most adulterated foods worldwidde, being the third "favorite" food for adulteration (GARCÍA 2018, AHMAD & KHAIRATUN 2021). In the Brazil, studies have reported adulterated honey through the addition of commercial sugars, glucose, honeydew, corn syrup, sucrose syrup, sugar cane juice, and inverted sugar solution, among others (ARAÚJO et al. 2006, DIAS et al. 2009, GOMES et al. 2017, ROLIM et al. 2018).

Following the recommendation of the Codex Alimentarius (2001) that establishes standards and guidelines for food safety, Brazil, through the Normative Instruction 11 of 2000 of the Ministry of Agriculture, Livestock and Supply (MAPA, in Portuguese), establishes physicochemical analyzes to determine maturity, purity, and deterioration of honey, in order to ensure food safety to consumers (BRASIL 2000). Presently, physicochemical analyzes are still the most used methods to guarantee the quality of honey, being routine techniques that need to be in accordance with the legislation.

The State of Santa Catarina has the potential to produce different types of honey, with distinct physicochemical traits, depending on factors such as geographic and/or botanical origin, climatic processing, and storage conditions, among others (SILVA et al. 2004, KÜÇÜK et al. 2007). Thus, the aim of the study was to apply physicochemical analyzes to honey samples collected from the eleven agroecological zones of the State of Santa Catarina (southern Brazil), to verify their quality accordance regarding the current legislation in the country.

MATERIAL AND METHODS

Sample collection

Floral honey samples produced by *Apis mellifera* were collected over two harvests (2018-2019 and 2019-2020) in the eleven agroecological subregions of the State of Santa Catarina, southern Brazil (Figure 1).

Sixty-five samples from the 2018-2019 harvest and fifty ones from the 2019-2020 harvest were analyzed. The honeys were kindly provided by Santa Catarina beekeepers to carry out the tests, therefore, it is assumed they would not be delivered tampered with, as personal and the location of the hives data were collected for later feedback to the producers of the results found. Sample collection from beekeepers was carried out with support of the extension workers of the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), who were responsible for publicizing the project and accepting it among beekeepers.

Physicochemical analysis

In the study, the physicochemical variables moisture content, pH, reducing sugars, apparent sucrose, water activity, diastase enzyme, color, and Lund reaction were analyzed. The samples were analyzed at the Laboratory of Microbiology, Immunology, and Molecular Biology (LABMIM) and at the Laboratory of Plant Morphophysiology at the University of the State of Santa Catarina (UDESC – Chapecó, SC). All analyzes were performed in triplicate (n = 3).

Moisture content was determined using a Milwaukee® MA871 refractometer and the refractive index was calculated according to the Chataway Table (CHATAWAY 1935). The pH was measured by the Alfakit® AT-315 pHmeter. For the determination of reducing sugars, the Lane and Eynon procedure was used, involving the Fehling solution, and the analysis of apparent sucrose was performed after inversion by acid hydrolysis. The diastatic activity was performed using the Lugol solution, with adaptations.

Water activity was measured using the Decagon Devices® AquaLab Lite analyzer, using the dew point determination technique, following the procedures indicated by the manufacturer.

The color classification was performed in a 560 nm spectrophotometer (Model UV-2000a Instrutherm®) in a 1 mL cell and pure glycerin as blank. Initially, the honey samples were diluted in distilled

water (1:1), and the absorbance reading was carried out in a quartz cuvette. The interpretation of results was performed using the Pfund color scale (VIDAL & FREGOSI 1984).

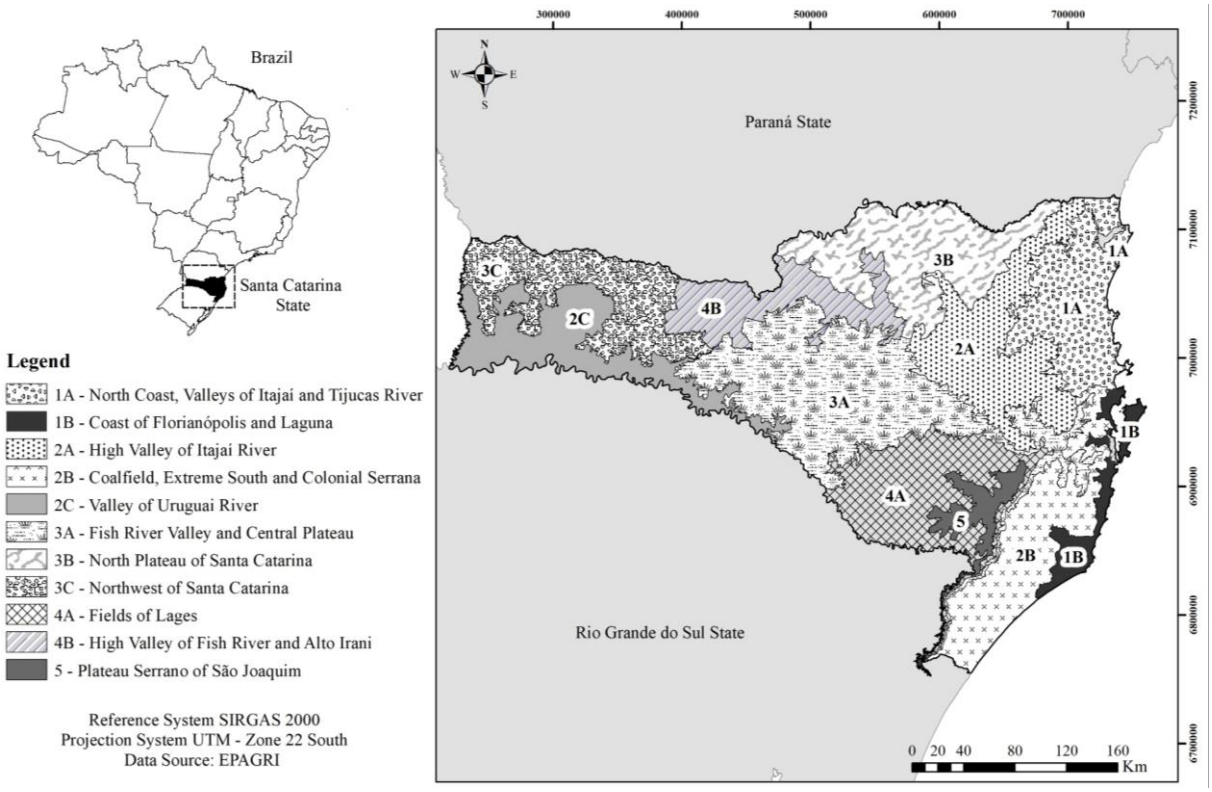

Figure 1. Agroecological subregions of the State of Santa Catarina, Brazil.

Reference data used for physicochemical analysis

As a reference for analyzing the results of moisture, reducing sugars, and apparent sucrose, the Normative Instruction nº 11 (October 20, 2000 - Ministry of Agriculture, Livestock and Supply) was adopted (Table 2).

Table 2. Reference values for moisture content, reducing sugars, and apparent sucrose used in measuring physicochemical characteristics of honey samples produced in Santa Catarina State, southern Brazil.

Analysis	Reference used
Moisture content	≤ 20% (20 g/100 g)
Reducing sugars	≥ 65% (65 g/100 g)
Apparent sucrose	≤ 6% (6 g/100 g)

Source: Adapted from BRASIL (2000).

As a reference to verify the diastase enzyme activity, the protocol described by the INSTITUTO ADOLFO LUTZ (2008) was used, where the unaltered honey samples should present a light brown hue, which is positive for the activity of the diastase enzyme. Water activity and color were also analyzed in samples, also following the protocol described by the INSTITUTO ADOLFO LUTZ (2008) for the Lund reaction. The precipitation noted following the reaction with honey samples in investigation should be in the range of 0.6 to 3.0 mL, as lower or higher values indicate fraud or adulteration of honey.

Statistical analysis

All floral honey samples were analyzed in triplicate (n = 3). Data were separated according to their harvest seasons (i.e., (2018-2019 and 2019-2020), following the analysis of variance (ANOVA) and Scott & Knott test (SCOTT & KNOTT 1974), considering each variable separately, at a 5% probability of error for comparison of means, using scripts written in R language (Version 4.0.2).

The moisture, pH, reducing sugars, apparent sucrose, and water activity data were calculated by the median per agroecological zone and submitted to principal component analysis (PCA) to identify eventual sample grouping regarding the influence of geographic origin e harvest seasons. PCA was calculated using the singular value decomposition (SVD) algorithm for matrix factorization available at The Unscrambler® X (Version 10.4) statistical package.

RESULTS AND DISCUSSION

The physicochemical analysis of the honey revealed that all the studied parameters (e.g., moisture, pH, reducing sugars, and apparent sucrose) varied, with some samples with values above or below the reference data in accordance with Normative Instruction nº 11/2000 of the MAPA. The Scott & Knott's statistical analysis ($p>0.05$) showed a wide distribution in all physicochemical parameters, especially in samples produced in the 2019-2020 season. Interestingly, the parameter apparent sucrose presented great heterogeneity of data (Supplementary material) and, therefore, greater coefficient of variation.

For the 2018-2019 harvest, the moisture content of honey ranged from 17.2-23.8%, with 47.7% of the samples showing values above those allowed by legislation (>20%). In the next harvest season i.e., 2019-2020), similar values of moisture (17.5%-24.1%) were found in honey, as 52.0% of the samples were classified as non-compliance ones to the ongoing legislation (Figure 2). The moisture content in honey is a determining factor for stability against fermentation and granulation. Low contents protect that food from microbiological deterioration, prolonging its shelf life. However, the content will depend on environmental conditions, such as temperature and relative moisture of the air at the honey's geographical origin.

Likewise, the harvesting of immature honey, that is, extracted early before maturation, and/or the incorrect handling of honey by beekeepers can interfere with the moisture levels, eventually compromising the quality of the product (EL SOHAIMY et al. 2015, ZAREI et al. 2019). In the study conducted by MARSARO JÚNIOR et al. (2022) it was found that of 29 samples of honey produced in the state of Rio Grande do Sul (2015-2016), eight samples presented a content higher than that permitted by legislation, ranging from 20.17% to 27.15%. Likewise, RIBEIRO et al. (2022), when analyzing 66 samples of honey from the state of Paraná (2018-2019), found that 4.60% of the samples presented a higher index than that allowed by legislation.

Moisture values recorded in non-compliance may indicate the need for greater qualification of beekeepers to collect honey at an appropriate time, i.e., with low relative humidity and mature honey. As recommended by Embrapa, honey collection should take place between 9 am and 4 pm, on sunny days, and it is necessary to select the supers, not harvesting frames in which young are present at any stage of development, with a large amount of pollen and green honey, i.e., not yet ripe, as these factors directly interfere with the percentage of moisture (EMBRAPA 2023).

According to a study by SILVA et al. (2020) the moisture content, in addition to factors such as weather conditions and honey management by beekeepers, may change during storage. Maintaining moisture at 20% is necessary to prevent yeast growth, for this reason, the storage location is an important factor for the non-development of fermentation (SINGH & SINGH 2018). Moisture contents below 20% are required by legislation in several countries, including Codex Alimentarius standards (THRASYVOULOU et al. 2018).

For the pH parameter, there is no delimitation of allowed limits, not being a variable of mandatory analysis. However, it is an important parameter related to honey storage and the growth of microorganisms, which can modify texture and stability of that food (ZAREI et al. 2019). In the 2018-2019 harvest, the pH ranged from 3.4-4.6, and in the samples colletec in the 2019-2020 production season a wider range of values was found, i.e., 2.9-4.5 (Figure 2). Similar data were found by RATIU et al. (2019), with a variation from 3.20-4.49 in honey samples from several countries, including Brazilian polyfloral honey (4.04).

The reducing sugar content that corresponds to the sum of glucose and fructose present in honey is related to the honey's crystallization capacity (EL SOHAIMY et al. 2015). Honey produced in the 2018-2019 season presented reducing sugars amounts varying from 55.2-99.7% and 20% of the samples were classified as non-compliance (≥ 65%) ones. A narrower amplitude of values for that variable (60.6-94.9%) was detected in 2019-2020 harvested-samples, with 8% of these with contents below the limit indicated by the current legislation (Figure 2).

Similar to moisture, amounts of reducing sugars lower than the reference value established by the ongoing Normative Instruction 11 (MAPA) may indicate the harvesting of immature honey (FERNANDES et

al. 2020). Collecting immature honey is quite common due to the lack of methods to distinguish mature honey from immature ones However, the maturity of that food has a great impact on its quality, which directly affects its market price (ZHANG et al. 2021).

The apparent sucrose contents detected in honey harvested in the 2018-2019 producing season ranged from 0.24-27.81%, with 60% of the samples above the legal limit (≤ 6%) for that parameter. On the other hand, the samples collected in the next season (2019-2020) presented a narrower range of values for that variable (0.67-16.4%), but 66% of the samples were found to be above the permitted level (Figure 2). Non-conforming samples may be linked to the incomplete transformation of sucrose into glucose and fructose by the action of the enzyme invertase secreted by bees (AZEREDO et al. 1999).

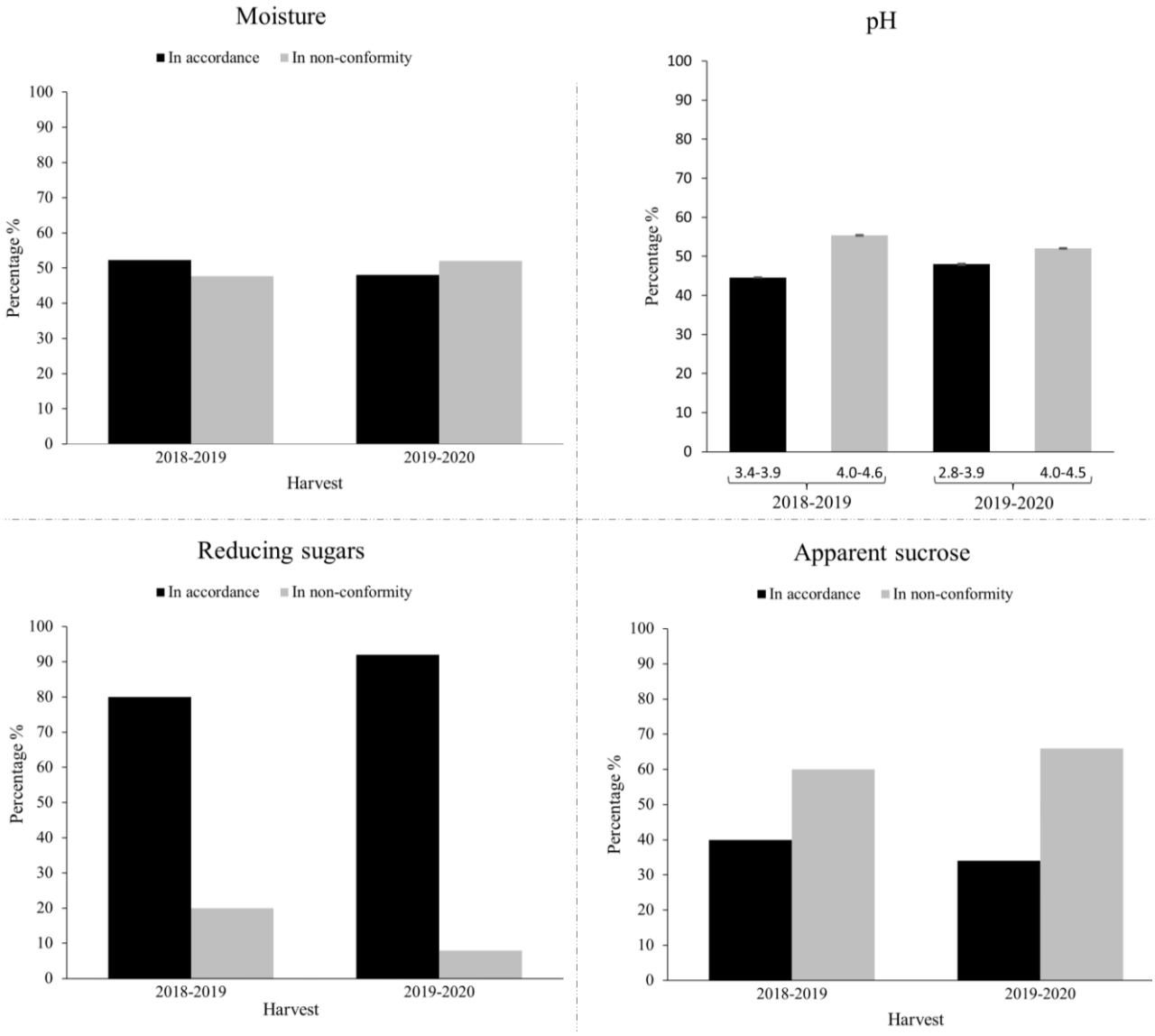

Figure 2. Physicochemical analyzes (moisture, pH, reducing sugars, and apparent sucrose) of floral honeys samples produced in the State of Santa Catarina over the 2018-2019 and 2019-2020 production seasons.

When analyzing the maximum limits allowed in Brazil for apparent sucrose in comparison to other countries, one might find distinct values. For example, Argentina allows up to 8%, as the Czech Republic and Ethiopia up to 10%. In fact, countries' legislation has allowed different reference values to that variable, mainly due to the wide range of honey types found worldwide, resulting from geographical and climatic conditions of the zones of production, for instance (THRASYVOULOU et al. 2018).

Water activity (WA) is a parameter that determines the amount of water available for the proliferation of microorganisms (PASCUAL-MATÉ et al. 2018). Bacterial species can cause food poisoning, so that the analysis of WA is relevant regarding the quality of food products such as honey (SYAMALADEVI et al. 2016).

For example, species such as *Staphylococcus aureus* can proliferate with WA between 0.83-0.86 (MEDVEĎOVÁ et al. 2019) and *Clostridium botulinum* above 0.94 (KANAAN & TAREK 2020).

A water activity lower than 0.60 is considered adequate for product stability (ROPCIUC et al. 2017) and one should keep in mind that moisture content can be directly correlated with WA (PASCUAL-MATÉ et al. 2018). The most of floral honey herein investigated (89.2%) showed WA ranging from 0.51-0.60, when collected in the 2018-2019 season. The remaining samples, i.e., 10.8% presented higher WA values, varying from 0.61 to 0.67. In their turn, the honey harvested in 2019-2020 season revealed lower WA, varying between 0.40-0.50 for 36% of the samples and 0.51-0.63 for the remaining ones (Figure 3). Thus, in the study, the lowest values of WA (i.e., 0.40-0.50) were found in the floral honey collected in the 2019-2020 season.

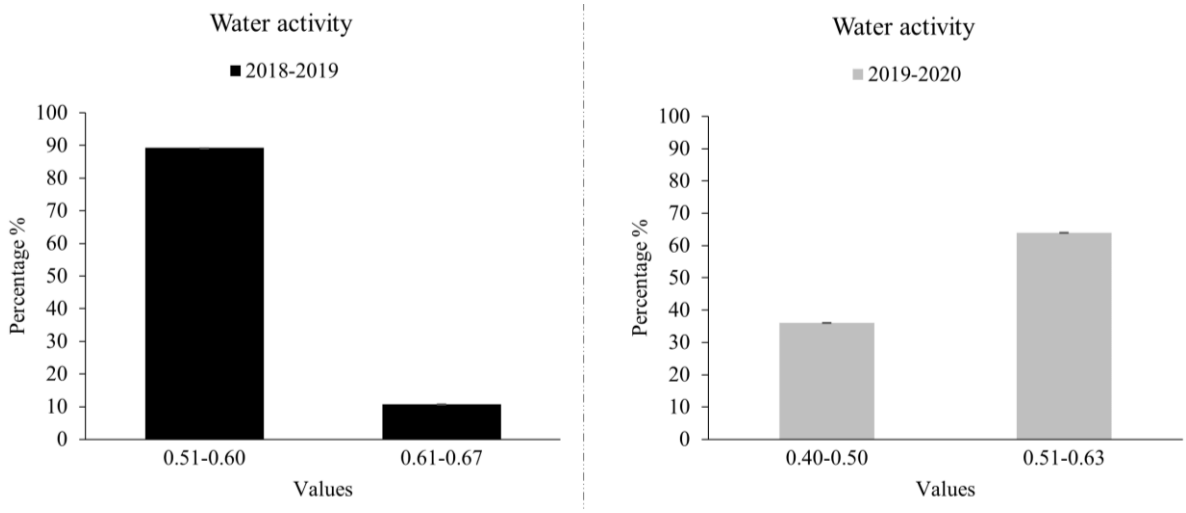

Figure 3. Water activity (WA) of floral honeys produced in the State of Santa Catarina, and harvested in the 2018-2019 and 2019-2020 seasons.

α- and β-amylase (i.e., diastase) are the main enzymes present in honey and the measure of their activities has been used as a proxy of the degree of conservation and overheating of honey, which can compromise its quality (BAGLIO 2018). In this study, all samples investigated were positive for diastase activity, indicating that no sample was adulterated, considering this analysis.

The floral honey studied were also investigated through the Lund reaction and showed for only one sample (1.5%) collected in the 2018-2019 season a negative result, i.e., with possible adulteration. Importantly, all other samples were within the established parameter.

Further analysis of the honey's color was also performed, despite the fact that no reference has been established by law for that parameter. This is because honey can present quite different shades, without interfering with the final quality of the product. Thus, according to the Pfund scale, 35.4% of the 2018-2019 harvested-samples displayed extra light amber shade, as the remaining ones (64.6%) showed light amber shade. For the honey collected in the 2019-2020 season 6% of the samples were classified as extra light amber, as 54% and 40% showed light amber and amber colors, respectively (Figure 4).

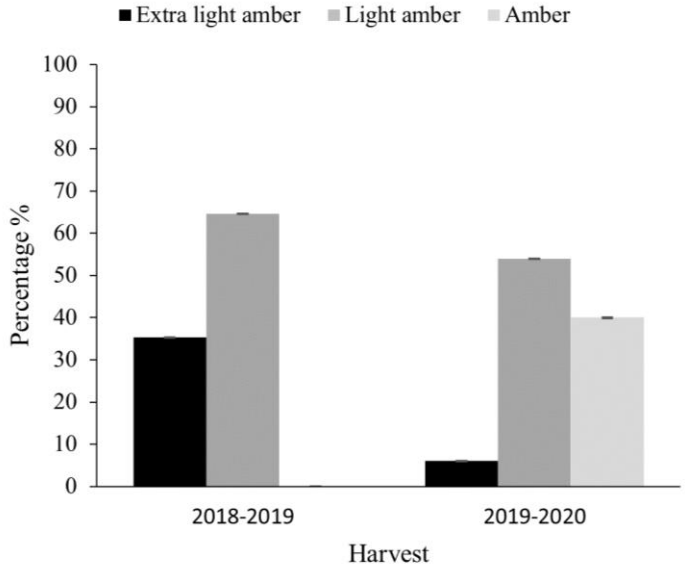

Figure 4. Color of floral honey produced in the Santa Catarina State in the 2018-2019 and 2019-2020 seasons, according to the classification of the Pfund scale.

Because Brazil has a huge botanical diversity and, therefore, a rich flora for bee foraging, thousands of honey types have been produced all over the country, with peculiar chemical compositions according to the zones of production (SOUZA et al. 2019, HAIDAMUS et al. 2019, DRIVELOS et al. 2021). Located into the the Atlantic Forest biome in southern Brazil, Santa Catarina State still has the highest percentage of remaining forest cover its territory, where more than 20,000 plant species might be found (CARDOSO 2016). In this sense, according to EPAGRI (2021), more than 100 types of honey have been produced in that State, with different colors, aromas, flavors, and textures. In addition to the flora diversity, factors such as geographic origin, climatic conditions, maturation stage of honey, bee species, processing and storage conditions, among other, can influence the final quality of that food (SILVA et al. 2004, KÜÇÜK et al. 2007, ESCUREDO & SEIJO 2019).

In order to detect eventual sample clusters resulting from the physicochemical traits of honey and their agroecological zones of origin, principal component analysis was applied to the quantitative dataset (moisture, pH, reducing sugars, apparent sucrose, and water activity) of the 2018-2019 and 2019-2020 harvested-samples. By doing so, a descriptive model was built, where the principal components 1 and 2 explained 64% and 35% of the dataset's variability, respectively. Interestingly, it was possible to identify a grouping of samples for the two harvests investigated in PC1-/PC2-, associated to their geographic origins in the agroecological zones 1A and 1B (Figure 5).

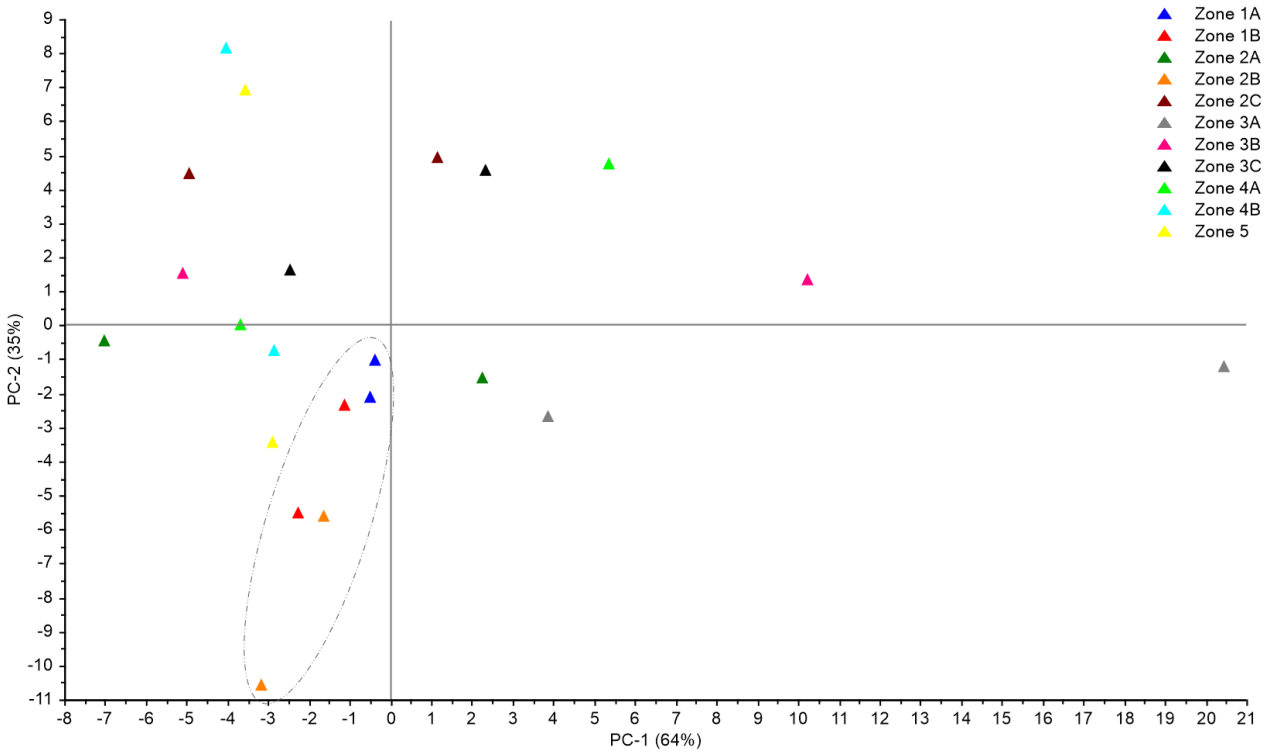

Figure 5. Principal component analysis of the physicochemical dataset of 115 floral honey samples collected in the eleven agroecological zones of the State of Santa Catarina (southern Brazil), during the 2018-2019 and 2019-2020 production seasons.

The sample cluster noted (1A and 1B) may eventually be explained due to the proximity of those agroecological zones (Figure 1), allowing one speculating that similar climatic conditions and apicultural blooms might exist in those regions, being relevant factors for the sample grouping noted. Further PCA calculation was also performed to investigate the seasonality effect (spring, summer, and autumn) in the honey's physicochemical traits, but the descriptive model failed in to discriminate the samples.

Finally, PCA was also applied to the physicochemical dataset (moisture, pH, reducing sugars, apparent sucrose, and water activity) to determine their contribution and eventual association in the grouping samples identified through the descriptive model built. The results showed that moisture, pH, and water activity were correlated, grouping in PC1+/PC2-, and differing from reducing sugars (PC1-/PC2-) and apparent sucrose (PC1+/PC2+) (Figure 6).

The descriptive model resulting from the PCA revealed that the moisture, pH, and water activity parameters seems to be associated, what is relevant for purpose of the analysis of the quality in honey, since it has been shown that those parameters are directly linked to the development and proliferation of microorganisms in foods (TERRA et al. 2007, BELL 2020). On the other hand, the reducing sugars and apparent sucrose were shown to be uncorrelated parameters, with quantitative dimensions quite discrepant in floral honey, as herein shown. However, it should be noted that these are complementary carbohydrate parameters, affording relevant information when one aims to determine the quality of honey.

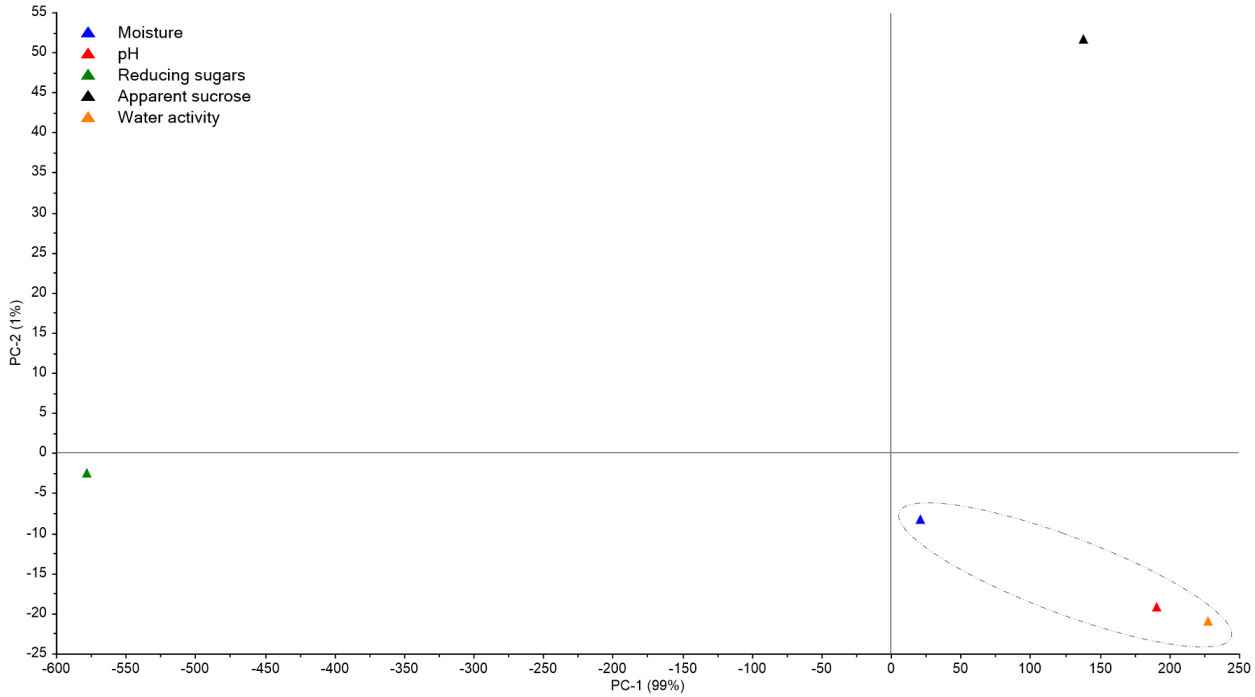

Figure 6. Principal component analysis of the physicochemical dataset of floral honeys produced in the State of Santa Catarina (southern Brazil), in the 2018-2019 and 2019-2020 seasons.

The physicochemical analyzes that were carried out in the study show that there are some irregularities in the honeys, as determined in the legislation in force. Moisture and apparent sucrose were the parameters that showed the greatest variation. In the 2018-2019 harvest, 47.7% of the honeys had a moisture content higher than that allowed by legislation, and in the 2019-2020 harvest it was 52.0%. According to a study by SILVA et al. (2020) the moisture content, in addition to factors such as weather conditions and honey management by beekeepers, may change during storage. Maintaining humidity at 20% is necessary to prevent yeast growth, for this reason, the storage location is an important factor for the non-development of fermentation (SINGH & SINGH 2018). Moisture contents below 20% are required by legislation in several countries, including Codex Alimentarius standards (THRASYVOULOU et al. 2018).

As Brazil is an important country in the honey export sector, it should be relevant to note the permitted limits of parameters associated to the quality of that food, which vary between countries. Such a scenario demonstrates the need to investigate possible implications of the reference data adopted for honey in the country and, according to the findings, eventually update them, without compromising the quality of the food and also avoiding commercial damages to it, both internally and abroad.

CONCLUSION

Most honey samples produced in Santa Catarina, a southern State of Brazil, showed physicochemical parameters that comply with the legislation in force in the country. However, a few samples were classified as non-compliant, especially related to their moisture and apparent sucrose contents, which may be related with immature harvest of that food and in inappropriate periods (i.e., in rainy seasons with high relative humidity of the air).

The monitoring and tracking of honey quality are necessary to guarantee food safety to consumers, as well as to enable beekeepers to be informed regarding the quality of their production in respect to the technical references established by legislation. Honey that presents one or more parameters differing from the quality standard adopted in the country may or may not be linked to fraud and, therefore, it is important to routinely perform physicochemical analysis of that food, also verifying international studies and their established standards.

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