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Parâmetros de qualidade, perfil mineral e correlação da origem e parâmetros físico-químicos me méis de *Apis mellifera* produzidos no sul do Brasil

Quality parameters, mineral profile and correlation of origin and physical-chemical parameters of *Apis mellifera* honey produced in southern Brazil

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RESUMO

Produtos da colmeia, incluindo mel, são valiosos indicadores ambientais, uma vez que as abelhas transferem compostos do ambiente para a colmeia. Poluentes ambientais podem reduzir a qualidade do mel e representam um risco para os consumidores. Neste trabalho, amostras de mel de *Apis mellifera* provenientes de diferentes cidades do Rio Grande do Sul e vendidas sem qualquer inspeção foram caracterizadas. Um total de 23 amostras de mel foram coletadas de 11 cidades do Rio Grande do Sul. Foram realizados Testes de Lund e de Lugol, além da determinação da condutividade elétrica (EC), pH, acidez, umidade, teor de cinzas, teor de açúcares redutores, sacarose aparente, teor de hidroximetilfurfural (HMF) e atividade diastásica. Foram realizadas analises por espectroscopia de fluorescência de raios-X por energia dispersiva a fim de determinar o perfil mineral das amostras. Análise de componentes principais (PCA) foi aplicada aos dados físico-químicos para diferenciar as amostras de mel baseadas em seus locais de origem. A análise dos parâmetros físico-químicos indicou que sete amostras (30,4%) apresentaram inconformidade com relação aos valores de referência. O PCA representou 80,83% da variância com os quatro primeiros componentes principais. A maioria das amostras foram separadas pela cidade de origem, sustentando a possibilidade de uso do mel como um marcador ambiental.

Palavras-chave: Controle de Qualidade; Marcador Ambiental; Rio Grande do Sul

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ABSTRACT

Beehive products, including honey, are valuable environmental indicators, as bees transport compounds in the environment by transferring them to the hive. Environmental pollutants can reduce the quality of honey and represent a risk for consumers. In this work, we characterised honey samples of Apis mellifera from different cities of the Rio Grande do Sul state (southern Brazil), sold without any inspection. A total of 23 honey samples were collected from 11 cities of Rio Grande do Sul. We carried out Lugol's and Lund's tests, analysed the electrical conductivity (EC), pH, and acidity of the honey samples, and determined their moisture, ash, reducing sugar, apparent sucrose, hydroxymethylfurfural (HMF), and diastase contents. Energy-dispersive X-ray fluorescence analysis was performed to determinate the mineral profile of the samples. Principal component analysis (PCA) was applied to the physicochemical data in order to differentiate the honey samples based on their origin. The analysis of the physicochemical parameters indicated that seven samples (30.4%) showed deviations from the reference values. PCA accounted for 80.83% of the variance with the first four principal component variables. Most samples were separated by the city of origin, supporting the possibility of using honey as an environmental marker

Keywords: Quality Control; Environmental marker; Rio Grande do Sul

1 INTRODUCTION

Honey is a sweet, viscous, and highly nutritive substance produced by honey bees from plant nectar, secretions of living parts of plants, or excretions of plant-sucking insects (KIRS et al., 2011; KARABAGIAS et al., 2014). Bees collect nectar, transform it by combining it with their own specific substances, then deposit, dehydrate, store, and leave it in the honeycomb to ripen and mature (KIRS et al., 2011).

Honey is a concentrated aqueous solution of sugars containing about 200 different substances (FERREIRA et al., 2009; HABIB et al., 2014; MANZANARES et al., 2014). The main components are carbohydrates, especially fructose and glucose; however, other minor components including oligosaccharides, polysaccharides, enzymes, amino acids, organic acids, minerals, flavonoids, vitamins, pollen grains, waxes, and other phytochemicals are also present (YÜCEL and SULTANOĞLU, 2013; HABIB et al., 2014, MANZANARES et al., 2014, PONTIS et al., 2014). The chemical

composition of honey depends on its botanical origin, the nectar chemical composition of honey-producing plants or secretions, the beehive type, and the climate (MANZANARES et al., 2014; ESTEVINHO et al., 2012).

The quality of natural honey samples can be evaluated by measuring physicochemical parameters such as moisture, sucrose, hydroxymethylfurfural, protein, insoluble matter, and diastase contents, as well as acidity and specific conductivity (YÜCEL and SULTANOĞLU, 2013). The quality of a honey sample determines its nutritional properties and possible therapeutic uses (MANYI-LOH et al., 2010; ESTEVINHO et al., 2012).

Bees and their products are excellent biological indicators of the environmental conditions. The presence of toxic metals, pesticides, radioisotopes, and antibiotics endangers various bee families (MUJIĆ et al., 2011). On the other hand, marked deficiencies of any particular element in soil, rocks, or water are reflected in the mineral composition of plants, and hence in that of nectar and pollen. Such deficiencies will obviously be reflected in the mineral profile of honey, despite its rather low mineral content (HERNANDÉZ et al., 2005).

It is clear that honey for therapeutic purposes should be harvested in areas with no contamination sources (FEÁS and ESTEVINHO, 2011). The therapeutic applications of honey include wound and burn healing (MOLAN, 2001), oncology care (BARDY et al., 2008), as well as antioxidant and antimicrobial uses (THEUNISSEN et al., 2001, AKBULUT et al., 2009; GOMES et al., 2010).

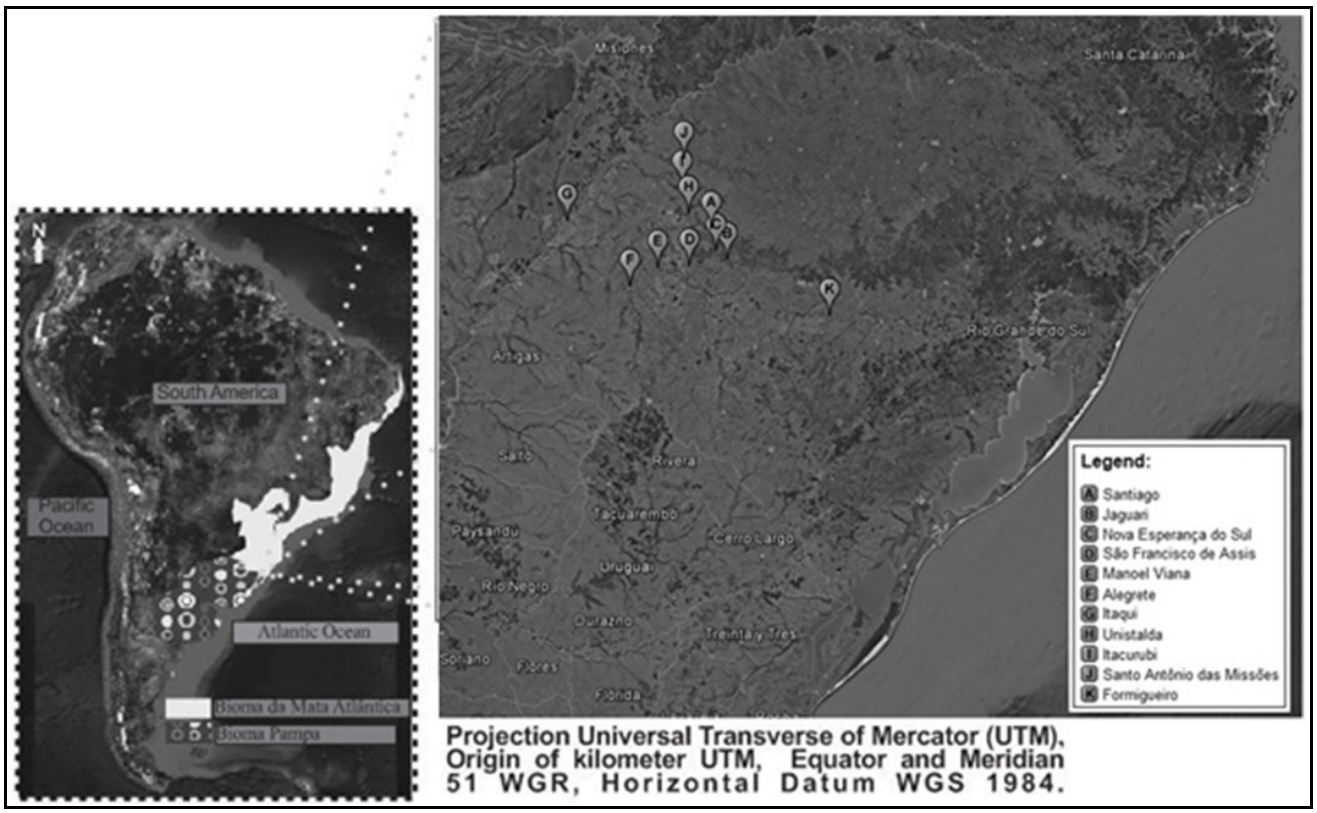
This work aimed to perform a physicochemical characterisation of honey samples produced by *Apis mellifera* in Rio Grande do Sul (southern Brazil), evaluating their physicochemical characteristics and mineral profile, as well as detecting the presence of gross adulterations and incorrect handling that could cause health problems to consumers. In addition, the work aimed to differentiate the honey samples according to their origin and evaluate the presence of heavy metals, which can reflect the environmental conditions of the locations where the samples were collected.

2 MATERIALS AND METHODS

2.1 Samples

The present study was carried out using 23 honey samples of various floral origins (one sample per beekeeper) from different cities of Rio Grande do Sul, Brazil: Nova Esperança do Sul (NE1, NE2, and NE3), Santiago (S1, S2, and S3), Jaguari (J1, J2, and J3), Itacurubi (I1, I2, and I3), São Francisco de Assis (SF1, SF3, and SF4), Itaqui (Iq1 and Iq2), Manoel Viana (MV1 and MV2), Alegrete (A1), Formigueiro (F1), Santo Antônio das Missões (SA1), and Unistalda (U1). Figure 1 shows the location of the cities involved in this study.

Figure 1 - Geographical origin of the honey samples collected from different regions in Rio Grande do Sul, Brazil



The honey samples were purchased in January 2014 (however, the processing date of the samples is unknown) from informal markets or directly from professional beekeepers, transferred to the Laboratory of Chemistry of Universidade Regional

Integrada do Alto Uruguai e das Missões and kept refrigerated (4 ± 2 °C) in dark conditions until analysis. The obtained samples were packed in different vessels, generally reusing vessels for other foods and beverages (such as plastic soda and glass pickle vessels).

2.2 Moisture

Moisture measurements were carried out using an Abbe refractometer (Biocotek WYA). The analyses performed at temperatures different from 20 °C were corrected by adding +0.00023 for each degree higher than 20° C and subtracting the same value for each degree lower than 20 °C. The moisture content was determined in triplicate and the values corresponding to the corrected refractive indexes were calculated using Wedmore's table (Bogdanov et al., 1997).

2.3 Colour

The colour of samples was determined by spectrophotometric measurements (Biospectro SP220) of the absorbance of a 50% honey solution (w/v) at 635 nm (White Junior, 1984; Ferreira et al., 2009). The honey samples were classified according to the Pfund scale after conversion of the absorbance values, using the equation 1:

$$\text{mm Pfund} = -38.70 + 371.39 \times \text{absorbance} \quad (1)$$

2.4 pH and free acidity

The pH values were measured in a solution of 10 g of honey in 75 mL ultrapure water (CO2-free) using a DIGIMED DM-22 pH meter. Afterward, to determine the free acidity, the solution was titrated with a 0.1 mol/L NaOH solution to pH 8.3 (Bogdanov et al., 1997). The free acidity was calculated using the equation 2:

$$\text{acidity} = 10.V_{NaOH} \quad (2)$$

where V_{NaOH} is the volume of 0.1 mol/L NaOH solution added.

2.5 Reducing sugars and sucrose contents

The content of reducing sugars was estimated using the Layne-Enyon method (Bogdanov et al., 1997). About 2.0 g of honey was weighed and transferred to a 200 mL volumetric flask (honey solution). An aliquot of 100 mL of the honey solution was transferred to a 200 mL volumetric flask and then brought to volume with water (diluted honey). Five millilitres of standardised Fehling's solutions A and B were transferred to a 250 mL Erlenmeyer flask containing water. The Erlenmeyer flask was then heated and 1.0 mL of methylene blue (0.2%) was added to it. Titration was carried out by adding the diluted honey solution until the indicator was decolorised.

To determinate the apparent sucrose content, a 50 mL aliquot of water was added to 100 mL of honey solution. The resulting solution was heated at 65 °C in a water bath, followed by addition of 20 mL of 5 mol/L HCl. The solution was then neutralised using 5 mol/L NaOH solution, cooled, transferred to a 200 mL volumetric flask, and diluted to the mark. Finally, the Layne-Enyon method was applied and the sucrose content was obtained by difference (Bogdanov et al., 1997).

2.6 Lugol's test

After adding 20 mL of water to 10 g of honey, the solution was kept in a water bath for 1 h and then cooled to room temperature, followed by addition of 0.5 mL of Lugol solution. The colour of the resulting solution was monitored (INSTITUTO ADOLFO LUTZ, 2008).

2.7 Lund's test

Two grams of each sample were weighed and transferred to a volumetric flask (50 mL), followed by the addition of 20 mL of water and 5 mL of tannic acid (0.5%). After adding water to bring the volume to 40 mL, the mixture was shaken and kept at rest for 24 h. Then, the volume of precipitate was measured (INSTITUTO ADOLFO LUTZ, 2008).

2.8 Ash content

The ash content was determined by placing 3 g of honey samples in a crucible set in a muffle furnace and heating at 640 °C for 6 h (INSTITUTO ADOLFO LUTZ, 2008).

2.9 Electric conductivity

The electrical conductivity was measured at 20 °C in a 200 g/L solution of honey in ultrapure water, using an Analyser 650 conductivity meter; the results were expressed in mS/cm (BOGDANOV et al., 1997).

2.10 Hidroxymethylfurfural content

The HMF content was determined after clarifying the samples with Carrez I (potassium ferrocyanide trihydrate) and Carrez II (zinc acetate dehydrate) solutions and adding sodium bisulphate. The absorbance was measured at 284 and 336 nm on a Biospectro SP220 spectrophotometer, and the results were expressed in mg/kg (BOGDANOV et al., 1997).

2.11 Diastase activity

The diastase activity was determined using a buffered solution of soluble starch and honey, incubated in a thermostatic bath at 40 °C. Thereafter, a 1 mL aliquot of this mixture was periodically removed and the absorption of the sample at 660 nm was followed on a Biospectro SP220 spectrophotometer. The diastase number was calculated from the time required for the absorbance to reach 0.235, and the results were expressed in Gothe degrees (BOGDANOV et al., 1997).

2.12 Mineral content

About 10 g of honey were dissolved in 0.1 mol/L HNO_3 and diluted to 100 mL with additional nitric acid in a calibrated flask. The Na contents were determined directly in the solution by atomic emission spectroscopy, using a flame photometer (DIGIMED DM-62).

To determine the other elements present in the samples, energy-dispersive X-ray fluorescence (EDXRF) measurements were carried out on an X-ray spectrometer

(Shimadzu EDX8000) using a semiquantitative method. A spectral resolution of 132.58 eV was achieved at the Mn K_α line, with a maximum count of 1088.5417 cps/μA.

2.13 Statistical analysis

The generated data were examined by the analysis of variance (ANOVA) technique using the Assistat (version 7.7) software. Tukey's test was used to separate means when ANOVA revealed a significant difference between mean values, and significant differences were reported at 5% level of probability. Principal component analysis was performed with the SAS University Edition software, using the PROC FACTOR procedure to evaluate the differences between the 23 honey samples.

3 RESULTS AND DISCUSSION

Table 1 shows the obtained parameters of the honey samples. The colour of a specimen can be associated to its floral origin, mineral content, handling, and storage conditions (MENDES et al., 2009). The colours of the honey samples ranged from extra light amber to dark amber. Two samples (8.7%) were classified as extra light amber, 10 (43.5%) as light amber, 5 (21.7%) as amber, and 6 (26.1%) as dark amber. Moreover, 12 samples (52.2%) were classified as light (sum of extra light and light amber samples) indicating a predominance of light honeys. This is in agreement with the results of Moreti et al. (2006), who studied 346 honey samples from different Brazilian states and observed a predominance of light honeys. Other studies also indicate the predominance of light honeys in different regions of Brazil (ANACLETO and MARCHINI, 2004; ARRUDA et al., 2004).

Moisture is one of the factors that determine the shelf life of honey during storage, because a high moisture favours the growth of microorganisms and results in the degradation of honey by fermentation (WHITE JÚNIOR, 1979; PEŔEZ-ARQILLUE et al., 1994). The moisture levels of the analysed honey samples were in the range of 15.5% (sample S1) to 21.3% (sample SA1). The Codex Alimentarius Commission (2001) recommended a maximum moisture of 20%. Therefore, the I2, I3, MV2, and

SA1 samples did not meet the standard requirements because they showed moisture levels higher than 20%. These high values may be due the floral origin of the samples, their harvesting before complete dehydration, as well as to inadequate handling and/or storage (MENDES et al., 2009).

Table 1 – Physicochemical parameters of the honey samples analysed in this study.

sample	colour (mmPfund)	Moisture (%)	pH	acidity (mEq/kg)	reducing sugars (%)
A1	88.0	17.3±0.1^{l}	4.15±0.00^{def}	37.7±2.1^{d}	75.2±0.3^{g}
F1	73.7	17.5±0.3^{jl}	4.23±0.04^{cde}	25.0±1.0^{mn}	780±0.3^{bcd}
I1	50.3	18.2±0.0^{ghi}	4.36±0.07^{b}	26.3±1.1^{lmn}	75.2±0.8^{gh}
I2	168.4	20.7±0.3^{b}	4.20±0.00^{cde}	42.0±1.7^{bc}	72.4±0.3^{i}
I3	136.6	20.7±0.1^{b}	4.06±0.03^{fg}	44.3±1.2^{b}	73.7±0.3^{hi}
Iq1	97.7	18.0±0.0^{hij}	3.93±0.09^{hi}	38.7±1.1^{cd}	75.9±0.3^{efg}
Iq2	58.3	19.6±0.4^{cd}	3.99±0.05^{gh}	51.0±1.0^{a}	78.1±0.6^{bc}
J1	93.7	18.7±0.1^{efgh}	4.15±0.01^{ef}	32.0±1.0^{fghij}	72.9±0.6^{i}
J2	121.0	19.0±0.3^{def}	3.99±0.03^{gh}	30.3±2.5^{ijl}	77.1±0.7^{cdef}
J3	156.2	17.6±0.2^{ijl}	4.29±0.02^{bc}	22.7±1.5^{n}	75.3±0.3^{g}
MV1	148.3	17.6±0.2^{ijl}	4.22±0.01^{cde}	33.3±1.5^{efghi}	75.6±0.5^{fg}
MV2	75.3	21.3±0.1^{ab}	4.18±0.05^{cde}	35.7±0.6^{defg}	80.7±0.3^{a}
NE1	96.0	19.9±0.1^{c}	3.91±0.00^{hij}	43.0±1.7^{b}	76.3±0.5^{efg}
NE2	35.3	18.6±0.0^{efg}	3.84±0.01^{ij}	31.3±1.1^{hij}	76.0±0.3^{efg}
NE3	43.0	19.3±0.2^{cde}	3.89±0.04^{hij}	36.0±2.0^{def}	77.3±0.3^{cde}
S1	106.7	15.5±0.1^{m}	4.19±0.01^{cde}	26.3±1.5^{lmn}	79.1±0.3^{b}
S2	68.0	19.3±0.1^{cde}	4.07±0.02^{fg}	34.7±0.6^{defgh}	76.9±06^{cdef}
S3	67.3	18.0±0.0^{hij}	4.13±0.05^{ef}	26.3±1.1^{lmn}	78.2±0.9^{bc}
SA1	76.0	21.5±0.2^{a}	3.81±0.03^{j}	29.0±0.0^{jlm}	76.5±0.3^{defg}
SF1	69.3	18.8±0.2^{efg}	4.37±0.04^{b}	26.0±1.7^{mn}	77.4±0.6^{cde}
SF3	50.5	18.6±0.0^{efgh}	4.26±0.01^{bcd}	35.3±0.6^{defgh}	78.4±0.3^{bc}
SF4	50.7	18.9±0.1^{efg}	4.27±0.01^{bc}	36.3±1.1^{de}	79.4±0.3^{ab}
U1	140.7	18.4±0.2^{fgh}	4.50±0.03^{a}	31.7±0.6^{ghij}	73.3±0.3^{i}

Values presented are mean ± SD of three determinations. Mean values with different superscript along a row are significantly different ($p < 0.05$).

The pH of honey is an important parameter because it influences the growth of microorganisms and the rate of formation of hydroxymethylfurfural. However, the pH value is not regarded as a quality parameter. The pH of honey can be affected by that of nectar and soil, as well as by the combination of plants used in

the honey production or the mandibular substances added by the bees (KESIĆ et al., 2014; NASCIMENTO et al., 2015). The measured pH values were in the range of 3.81 (sample SA1) to 4.50 (sample U1). Studies carried out in other countries recorded pH values similar to those obtained in our work: 3.19–3.49 (Hatay region, Turkey) (YÜCEL and SULTANOĞLU 2013), 2.25–4.71 (Morocco) (TERRAB et al., 2002), 3.7–4.3 (Portugal) (GOMES et al., 2010), and 3.7–4.4 (Índia) (SAXENA et al., 2010).

The acidity of honey is due the presence of organic acids in equilibrium with their lactones or internal esters and of some inorganic ions such as phosphate and sulphate (TERRAB et al., 2002). The acidity affects the flavour and antimicrobial properties of honey (ALEMU et al., 2013). The main acid formed is gluconic acid, which is the product generated during maturation by the combined action of glucose-oxidase enzymes and some bacteria. Other acids that can be found in honey are acetic, benzoic, citric, butyric, formic, lactic, phenylacetic, isovaleric, maleic, oxalic, propionic, pyroglutamic, succinic, and valeric acid (WHITE JUNIOR, 1957, STINSON et al., 1960; SILVA et al., 2016). The values of acidity found in the honey samples analysed in this work were in the range of 22.7 (sample J3) to 51.0 (sample Iq2). The acidity of sample Iq2 was slightly higher than 50 meq/kg of honey; hence, this sample did not comply with the established standard (CODEX ALIMENTARIUS COMMISSION, 2001). The obtained acidities were in the same range of values found in other studies: 18.06–34.88 meq/kg (Hatay region, Turkey) (YÜCEL and SULTANOĞLU 2013), 10.31–102.0 meq/kg (Morocco) (TERRAB et al., 2002), 9.9–36.8 meq/kg (Argentina) (ISLA et al., 2011), 16.0–32.0 meq/kg (Portugal) (GOMES et al., 2010), and 17.988–58.823 mEq/kg (Algeria) (REBIAI and LANEZ, 2014).

As mentioned above, honey is mainly composed of water and sugars, and the latter may make up as much as 99% of the solids (WHITE JUNIOR, 1957, FERREIRA et al., 2009; HABIB et al., 2014; MANZANARES et al., 2014). The reducing sugar contents found in this study ranged between 72.4% (sample I2) and 80.7% (sample MV2). All honey samples showed contents in agreement with the standard values, because the recommended minimum amount of reducing sugars for floral honey is 60% (SILVA et al., 2016). The reducing sugar contents were also comparable to those

obtained in other studies: 67.7–71.5% (Northwest Argentina) (ISLA et al., 2011), 60.19–67.7% (Algeria) (KHALIL et al., 2012), 67.7–73.7% (Portugal) (GOMES et al., 2010), 43.3– 65.5% (India) (SAXENA et al., 2010), and 49.14–76.34% (Nigeria) (ELEAZU et al., 2013).

Table 2 shows the results of the analysis carried out to evaluate gross adulterations and incorrect handling of honey samples. The Lugol's test evaluates the fraudulent addition of starch or dextrins to the honey (Alves et al., 2015). This determination is based on the reaction between iodine and potassium iodide in the presence of glucose, which results in a stained solution (red-purple to blue). The intensity of the colour depends on the amount of glucose dextrins or starch. The test is considered positive when the colour of the stained solution is blue (ALMEIDA-MURADIAN et al., 2013). The Lugol's test was negative for all analysed honey samples, indicating the absence of adulteration by addition of starch or dextrins.

The Lund's test reaction is based on the precipitation of naturally occurring honey proteins by tannic acid. The test is considered positive, indicating the presence of pure honey, when the amount of precipitate varies from 0.6 to 3.0 mL (Almeida-Muradian et al., 2013). For all honey samples, the values found for the protein deposits after Lund's test were within the range established by the Instituto Adolfo Lutz, Brazil (INSTITUTO ADOLFO LUTZ, 2008).

The ash content and consequently the electrical conductivity of a honey sample are associated to its mineral composition. The latter is related to the geographic and botanical origins of the sample (ALMEIDA-MURADIAN et al., 2013; YÜCEL and SULTANOĞLU, 2013). Some studies associated the ash content with lack of hygiene and absence of decantation and/or filtration in the final process of honey collection, as well as with honey adulteration with molasses (MENDES et al., 1998; SEREIA et al., 2011). The measured ash contents were in the range of 0.090% (sample SA1) to 0.478% (sample U1). The average ash content was 0.295%. Similar studies involving honey samples from other countries reported similar average ash contents: 0.28% and 0.23% in Turkey (KAHRAMAN et al., 2010; YÜCEL and

SULTANOĞLU, 2013), 0.21% in Portugal (GOMES et. al., 2010), and 0.20% in India (SAXENA et al., 2010).

Table 2 – Tests to identify adulterations or incorrect handling in the analysed honey samples.

sample	Lugol's test	Lund's test (mL)	ash (%)	Electrical conductivity (mEq/kg)	sucrose (%)	HMF (mg/kg)	Diastase (Göethe/g)
A1	negative	2.0	0.380±0.044	0.84±0.00	0.7±0.1	14.6±1.8	23.4±0.8
F1	negative	2.3	0.258±0.040	0.58±0.00	1.5±0.3	37.8±0.2	8.9±0.3
I1	negative	1.8	0.243±0.051	0.61±0.01	1.0±0.3	20.6±2.1	14.9±1.0
I2	negative	1.3	0.349±0.016	0.72±0.00	1.0±0.3	11.3±0.9	20.4±0.7
I3	negative	2.3	0.216±0.043	0.57±0.00	1.0±0.2	16.0±0.9	16.0±0.3
Iq1	negative	2.2	0.347±0.029	0.68±0.01	1.1±0.3	27.5±0.4	19.7±0.9
Iq2	negative	2.2	0.308±0.050	0.85±0.01	1.3±0.3	1.1±0.3	11.8±0.5
J1	negative	1.7	0.263±0.032	0.59±0.00	1.3±0.0	29.9±1.7	20.3±0.9
J2	negative	2.2	0.279±0.020	0.47±0.00	0.5±0.3	5.4±0.2	10.0±0.6
J3	negative	2.0	0.363±0.020	0.54±0.00	1.6±0.2	2.9±0.1	12.9±0.8
MV1	negative	2.0	0.370±0.026	0.77±0.00	0.8±0.3	5.6±1.1	22.0±0.5
MV2	negative	1.5	0.341±0.088	0.90±0.00	0.1±,0.0	2.9±0.6	5.7±0.2
NE1	negative	2.0	0.220±0.000	0.57±0.01	0.9±0.3	12.4±0.4	20.7±0.3
NE2	negative	1.2	0.187±0.015	0.42±0.00	2.0±0.3	16.9±0.6	13.8±0.3
NE3	negative	2.0	0.200±0.010	0.49±0.01	1.4±0.0	13.9±1.1	16.5±0.4
S1	negative	1.3	0.332±0.016	0.51±0.01	1.9±0.4	110.0±6.0	0.0±0.0
S2	negative	1.8	0.327±0.016	0.57±0.00	0.9±0.3	29.0±0.9	19.6±0.8
S3	negative	1.7	0.200±0.026	0.53±0.00	0.3±0.1	26.9±1.6	15.5±0.4
SA1	negative	1.7	0.090±0.017	0.24±0.00	2.4±0.0	6.6±0.8	12.9±0.8
SF1	negative	1.8	0.387±0.028	0.89±0.01	0.1±0.0	2.8±0.2	14.6±1.1
SF3	negative	1.8	0.332±0.005	0.70±0.01	1.5±0.3	2.0±0.0	17.5±0.3
SF4	negative	1.8	0.348±0.044	0.67±0.00	0.4±0.2	1.5±0.1	16.7±0.7
U1	negative	2.0	0.478±0.030	0.96±0.00	0.6±0.3	10.8±1.4	28.9±0.5

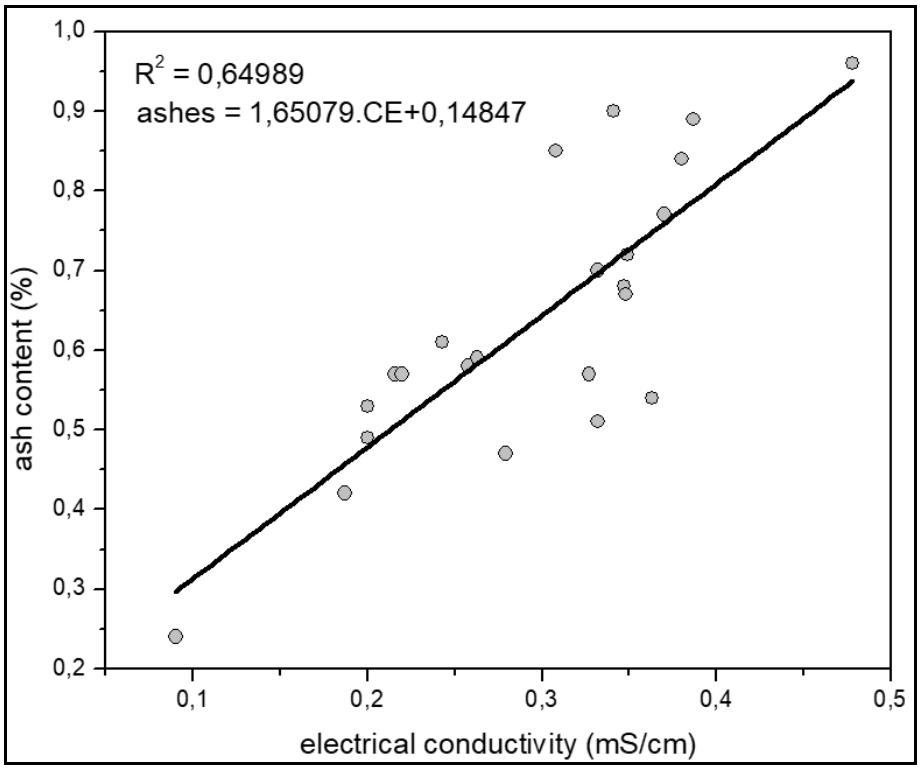
Values presented are mean ± SD of three determinations.

The electrical conductivity is associated to the mineral composition (ash content), organic acids (pH and acidity), and proteins present in the honey (YÜCEL and SULTANOĞLU, 2013; CORBELLA and COZZOLINO, 2006). According to the Codex Alimentarius Commission (2001) the electrical conductivity of blossom honeys should not be higher than 0.8 mS/cm. The electrical conductivity values found in this study were in the range of 0.24 (sample SA1) to 0.96 (sample U1) mS/cm. Therefore,

four (17.4%) honey samples (Iq2, MV2, SF1, and U1) presented electrical conductivity above the standard values for blossom honeys, indicating a possible blend.

The electrical conductivity and ash content data are plotted in Figure 2; the linear correlation coefficient (R2) between electrical conductivity and ash content was 0.64989, and the following regression equation was obtained: ash content = 1.65079·EC + 0.14487. The linear correlation coefficient was much lower than those found in studies of honeys from Algeria (0.92) (OUCHEMOUKH et al., 2007), India (0.98) (SAXENA et al., 2010), and Uruguay (0.997) (CORBELLA and COZZOLINO, 2006).

Figure 2 – Linear regression analysis of ash content vs. electrical conductivity data of the investigated honey samples.



Besides the reducing sugars, the amount of sucrose is another very important parameter for evaluating the honey maturity. The sucrose content in honey is analysed with the purpose of identifying any improper manipulation; high sucrose levels may indicate a variety of adulterations (such as addition of cane or refined beet sugar), early harvesting (indicating that the sucrose was not completely transformed into glucose and fructose), or prolonged artificial feeding of honeybees

with sucrose syrups for commercial profit (ESCURERO et al., 2013; PUSCAS et al., 2013; TORMUK et al., 2013; SILVA et al., 2016). The apparent sucrose contents determined for the present samples ranged between 0.1% (samples MV2 and SF1) and 2.4% (sample SA1). The Codex Alimentarius Commission (2001) stipulates a maximum value of 5% of sucrose in blossom honey; therefore, all samples had sucrose contents within the standard limits.

Hydroxymethylfurfural is a toxic and carcinogenic compound, whose content increases as a result of adulterations and overheating of honey. In high quantity, hydroxymethylfurfural causes the deterioration of honey enzymes, resulting in flavour and colour changes. Diastase is an enzyme present in honey, responsible for the transformation of starch in glucose; its content is another important parameter to assess the quality of a honey sample. This enzyme is sensitive to heat and adulterations: therefore, the absence of diastase indicates frauds and/or overheating (White Junior, 1992; White Junior, 1994). The HMF contents found in our samples were in the range of 1.1 (sample Iq2) to 110.1 (sample S1) mg/kg of honey. The diastase contents were in the range of 0.0 (sample S1) to 28.9 (sample U1) Gothe/g of honey. According to the Codex Alimentarius Commission (2001), in the case of honey samples of declared origin from countries or regions with tropical ambient temperatures, as well as blends of these honeys, the HMF content should be no more than 80 mg/kg. Thus, sample S1 did not conform to this standard. The diastase activity of honey, determined after processing and/or blending, should in general be higher than 8 Gothe units; therefore, the MV2 and S1 samples did not meet the standard requirements (CODEX ALIMENTARIUS COMMISSION, 2001).

Table 3 shows the mineral contents of the honey samples obtained by EDXRF and flame photometry. The samples were found to contain mainly K, Na, Cl, Cu, Zn, and Rb elements. As expected, potassium was the dominant element in all samples, followed by chlorine, as reported in other studies (HERNÁNDEZ et al., 2005). The possible sources of other elements are variable. Besides their natural occurrence, Cu and Zn (considered as heavy metals) can be present as impurities in fertilisers, pesticides, manure, waste, and other sources (HASSAN et al., 2016).

The SA1 sample was the only one that did not contain Cl and Rb, while the compositions of the SA1 and S3 samples did not include Zn. Eleven samples (47.8%) did not contain Ca (I2, I3, J1, J3, MV1, NE1, S1, S2, S3, and U1). Nine samples (39.1%) contained Br (A1, I3, Iq1, Iq2, MV1, MV2, SF1, SF3, and SF4), while five samples (21.7%) contained Fe (A1, I2, I3, MV2, and S1); finally, Mn (15 ppm) was only present in the Iq2 sample

Table 3 – Mineral content of honey samples determined by atomic emission spectroscopy and EDXRF.

sample	K (ppm)	Na (ppm)	Cl (ppm)	Cu (ppm)	Rb (ppm)	Zn (ppm)	Ca (ppm)	Br (ppm)	Fe (ppm)
A1	1967	22	650	41	13	6	182	2	8
F1	1214	66	395	42	6	4	131	-	-
I1	1745	12	280	44	10	3	135	-	-
I2	1714	216	383	44	8	5	-	-	14
I3	1245	52	306	42	6	8	-	2	20
Iq1	1711	26	578	43	9	4	147	4	-
Iq2	1563	148	720	41	10	11	300	12	-
J1	1511	16	262	42	10	4	-	-	-
J2	1202	54	288	37	5	6	109	-	-
J3	1431	22	183	40	5	3	-	-	-
MV1	2173	68	481	40	8	4	-	2	-
MV2	1891	102	656	44	15	6	209	16	8
NE1	1194	14	333	41	4	5	-	-	-
NE2	829	26	255	44	5	4	71	-	-
NE3	1031	116	242	40	4	3	75	-	-
S1	1532	11	206	41	5	10	-	-	18
S2	1380	13	269	41	7	3	-	-	-
S3	1221	20	297	41	5	-	-	-	-
SA1	350	12	-	42	-	-	54	-	-
SF1	1780	214	602	40	13	3	221	5	-
SF3	1667	42	439	41	11	6	234	2	-
SF4	1722	38	422	43	11	5	227	2	-
U1	2667	12	204	42	10	5	-	-	-

The potassium contents were in the range of 350 to 2667 ppm, with samples SA1 and the U1 showing the lowest and highest values, respectively. The Na contents were in the range of 12 to 216 ppm, corresponding to samples SA1 and I2, respectively. Turning to chlorine, the contents ranged between 183 and 720 ppm,

with the J3 and Iq2 samples showing the lowest and highest values, respectively. The Cu contents were in the range of 37 to 44 ppm, with the lower value corresponding to J2 and the higher value shared by samples I1, J2, MV2, and NE2. The Rb contents varied from 4 ppm for samples NE1 and NE3 to 15 ppm for MV2, while the Zn contents ranged from 3 ppm (samples I1, J3, NE3, S2, SF1) to 11 ppm (sample Iq2). The Ca contents were in the range of 54 to 300 ppm, with the lowest and highest content corresponding to the SA1 and Iq2 samples, respectively. Moreover, the samples presented Br levels between 2 and 16 ppm: the A1, I3, MV1, SF3, and SF4 samples presented lower Br contents, while the MV2 sample showed the highest content. The Fe contents were in the range of 8 to 20 ppm, with the lower value corresponding to the A1 and MV2 samples and the higher value to sample I3.

Fermo et al. (2013) reported that honeys produced by *Apis mellifera* tend to present higher contents of K, Ca, Na, and Mg, due the geological characteristics of the place of production. The present results are in agreement with those of studies carried out in other countries, such as India (NANDA et al. 2003), Saudi Arabia (ABU-TARBOUSH et al., 1993), Egypt (RASHED AND SOLTAN, 2004), Ireland (DOWNEY et al., 2005), France (DEVILLERS et al., 2002), Spain (GONZÁLEZ et al., 2000; TERRAB et al., 2004, TERRAB et al., 2005; GARCÍA et al., 2006), Italy (CONTI, 2000), Poland (PONIKVAR et al., 2005), Czech Republic (LACHMAN et al., 2007), Turkey (YILMAZ and YAVUZ, 1999), Israel (DAG et al., 2006), Slovenia (GOLOB et al., 2005), Macedonia (STANKOVSKA et al., 2008), and Brazil (FRANCHINI et al., 2007).

According to Mujić et al. (2010), the contents of Zn, Cu, and Fe cannot be higher than 10, 1, and 20 ppm, respectively. Therefore, all samples presented Fe concentrations lower than the above limit. On the other hand, all samples showed Cu contents about four times higher than the corresponding limit, whereas sample Iq2 presented a slightly higher content of Zn (11 ppm). Brazilian law sets maximum contents only for As, Pb, and Cd elements; however, none of the present samples contained such elements (MINISTÉRIO DA SAÚDE, 2013).

The physicochemical parameters less affected by the beekeeper handling (colour, reducing sugar content, ash content, electrical conductivity, and K, Na, Cl,

Cu, Rb, and Zn contents) were used to perform principal component analysis (PCA), in order to reveal differences among the evaluated honey samples. The eigenvalues and percentage of variance explained by each principal component are shown in Table 4.

Table 4 – Eigenvalues and variances of principal components.

Number of PC	Eigenvalue	Variance	Cumulative
1	4.32141840	0.4321	0.4321
2	1.56485181	0.1565	0.5886
3	1.26772105	0.1268	0.7154
4	0.92917824	0.0929	0.8083
5	0.76693080	0.0767	0.8850
6	0.51885367	0.0519	0.9369
7	0.35086375	0.0351	0.9720
8	0.17414537	0.0174	0.9894
9	0.08493513	0.0085	0.9979
10	0.02110178	0.0021	1.0000

Principal component analysis is a method used to search for data trends. By combining the original variables, this technique provides a partial view of the data in a space with a reduced number of dimensions while preserving most of their variability. The first four components accounted for 80.83% of the total variance, which showed that the honey samples were well differentiated by their physicochemical parameters, similar to the data obtained by similar studies (HERNANDÉZ et al., 2005; YÜCEL and SULTANOĞLU, 2013). Although PC1 and PC2 explain less than 70% of the variance of the original data (similar to the observed in the cited studies (HERNANDÉZ et al., 2005; YÜCEL and SULTANOĞLU, 2013), a relatively small number of components were extracted (CP1 and CP2) with the ability to explain the greatest variability in the original data (58,86%).

The first principal component (PC1) represented 43.21% of the total variance, while the next principal components accounted for 15.65%, 12.68%, and 9.29%. Table 5 shows the loadings of the variables for the four chosen principal components. The main PC1 component was strongly associated with the K, Cl, Rb,

and ash contents, as well as with the electrical conductivity. The reducing sugar and colour were the dominant variables for PC2, while the Cu and Zn contents presented high correlation with PC3, and the Na content was the dominant variable for PC4.

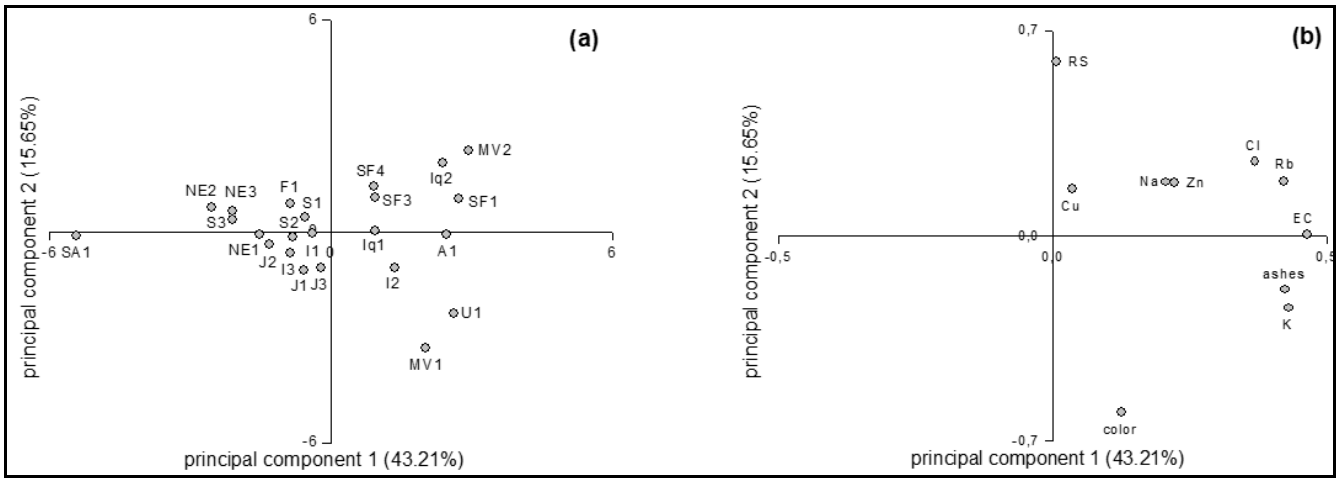
Table 5 – Loadings of the variables for the four principal components

Name	PC1	PC2	PC3	PC4
K	**0.429828**	-0.246516	-0.124416	-0.216287
Na	0.204891	0.182388	0.179286	**0.847874**
Cl	**0.368182**	0.253623	0.182309	0.141657
Cu	0.034905	0.160350	**-0.783212**	0.111084
Rb	**0.420582**	0.184276	-0.227853	-0.059783
Zn	0.221328	0.180749	**0.241600**	-0.240335
reducing sugar	0.006925	**0.591976**	0.327241	-0.293274
electrical conductivity	**0.464122**	0.003502	-0.077371	0.029609
ashes	**0.423498**	-0.183313	0.040714	-0.200283
colour	0.125730	**-0.604357**	0.284079	0.116571

Figure 3 shows the score and loading plots of PC1 and PC2, used for the discrimination of honey samples according to their origin. Most samples were separated by their city of origin, except for the samples produced in Manoel Viana (samples MV1 and MV2), one sample from Itacurubi (sample I2), one sample from Nova Esperança do Sul (sample NE1) and one sample from Santiago (sample S2). The samples from São Francisco de Assis, Itaqui and the sample MV2 were characterized by positive values of PC1 and PC2, associated to high contents of reducing sugars, Cu, Na, Zn, Cl and Rb. The samples A1, I2, MV1 and U1 presented positive values of PC1 and negative values of PC2, ascribed to color (darker samples) and high contents of ashes and K. The samples from Jaguari, together with the samples I1, I3, NE1, S2 and SA1 were characterized by negative values of PC1 and PC2, while the

samples F1, S1, NE2, NE3 and S3 showed negative values of PC1 and positive values of PC2.

Figure 3 – Principal component analysis. (a) Distribution of honey samples on scores plot and (b) distribution of variables on loadings plot.



It is possible that the similarity between the biomes of cities, prevented a better grouping of the samples to be achieved. These data support the possibility of using honey as an environmental marker. Probably, in addition, the mineral profile can be used as an origin indicator similar to the related by Hernandéz et al. (2005) in study involving honeys from Canary Islands.

4 CONCLUSION

This work reports the analysis of the physicochemical parameters of 23 honey samples from Rio Grande do Sul, southern Brazil. The honey samples were not subject to any inspection and were acquired directly from beekeepers and informal markets. No gross adulterations were detected in the analysed honey samples. The analysis of the physicochemical properties indicated that seven samples (30.4%) had parameters incompatible with reference values: four (I2, I3, MV2 and SA1) had moisture levels higher than the reference value, one (Iq1) exhibited acidity superior to the recommended level, another one (S1) contained a higher hydroxymethylfurfural

amount than that allowed, and two samples (S1 and MV2) presented diastase numbers lower than the recommended value. Honey samples not meeting established identity and quality standards can cause health problems to the consumers; this highlights the need of more thorough and careful inspections, in addition to providing technical guidance and support to beekeepers. The analysed samples mainly contained K, Na, Cl, Cu, Zn, and Rb elements, and potassium was the dominant element in all samples, followed by chlorine. In addition to these elements, some samples also contained Ca, Br, Fe, and Mn. Four components accounted for 80.83% of the total variance, indicating that the honeys were well differentiated by their physicochemical parameters. Most samples were separated by the city of origin, supporting the possibility of using honey as an environmental marker.

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