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Physicochemical characterization and determination of pesticides in *Apis mellifera* honey from southern Brazil

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Abstract

Honey is a natural food derived from nectar and other natural plant secretions, collected, and processed by bees. To be safely consumed, it must be free of chemical contamination and have quality and identity parameters following established standards. The continuous search for agricultural productivity has increased the use of pesticides, widely used in crops. Residues of these compounds have been found in honey samples. This work aimed to determine the physicochemical parameters and verify the presence of pesticides in *Apis mellifera* bee honey sold in the regions of Pelotas and Rio Grande/in Rio Grande do Sul state (RS)/ Brazil. The honey samples presented values for the physicochemical parameters within the acceptable limits established by Brazilian legislation. Among the 100 pesticides investigated, the insecticide λ-cyhalothrin was detected in 50% of the samples in concentrations between 5.95 and 25.30 µg/kg. The calculated estimated daily intake was 1.0×10-4 µg/kg bw/day, below the maximum limits Brazilian and European legislation recommended. The results contribute to the generation of data on the quality of honey produced in the region, thus ensuring safe consumption, however, it causes an alert about the environmental contamination by the pollinators bee's exposure to pesticides in regions with intense agricultural activity.

Keywords: *Agrochemicals*; *Beekeeping*; Environmental contamination; Honey quality; QuEChERS; Physicochemical parameters.

Highlights

- Quality parameters of *Apis mellifera* honey were measured
- Most evaluated parameters were in line with the standard recommended by legislation
- λ-cyhalothrin was detected in 50% of samples

1 Introduction

Apiculture is an activity of great social, economic, and ecological impact since it is mainly developed by small producers, which favors the development of family farming. The beekeeping (*Apis mellifera*) is an economical option to the producer due to the products that are obtained, especially honey (Klosowski et al., 2020). Brazil is highlighted in honey production due to its biodiversity, large territory, and climate variability. In this way, about 55.8 thousand tons of honey were produced in 2021, with the Rio Grande do Sul state in the first position (Instituto Brasileiro de Geografia e Estatística, 2023).

Honey is characterized as a natural food product, which is produced by honeybees, is composed of a complex mixture of glucose, fructose, water, organic acids, minerals, aromatic substances, vitamins, and pollen grains, among others (Sousa et al., 2016; Pereira et al., 2020). Usually, honey is used as a sweetener and for therapeutic purposes, thus, the choice for a specific type and its beneficial effects on health are dependent on the composition and the quality of the product (Seraglio et al., 2021), which are strictly related to different factors, such as floral species, geographical origin, bee species, as well as the environmental conditions, processing, and storage (Sousa et al., 2016; Pereira et al., 2020; Seraglio et al., 2021). Therefore, aiming the standardization of the food products of animal origin, the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura e Pecuária - MAPA) and the Codex Alimentarius established standards of identity and quality of honey by the evaluation of its physicochemical parameters (Brasil, 2000; Food and Agriculture Organization, 2019).

The bees, the main pollinator agents, are of great importance to agriculture and to the economy since about 70% of the crops produced for human consumption are pollination-dependent (Klosowski et al., 2020). However, the population of bees has decreased due to climate changes, alterations in their habitat and its biological characteristics, environmental pollution, expansion of agriculture, and by the use of pesticides (Marcolin et al., 2021; Orso et al., 2016; Wang et al., 2022). In this way, it is of great importance to perform the monitoring of residues of pesticides in honey to verify the risks to human health and the level of environmental contamination in different regions where honey is produced (Marcolin et al., 2021; Orso et al., 2016). In Brazil, maximum residue levels (MRLs) ranging from 0.3 to 50 µg/kg are established by the National Program for the Control of Residues and Contaminants (Plano Nacional de Controle de Resíduos e Contaminantes - PNCRC) (Brasil, 2013), which agrees with those applied in the European Union (EU) (European Union, 2022).

By the factors discussed that influence both in quality and safety of honey for consumption, this study aimed to perform a physicochemical characterization and pesticide determination in *Apis mellifera* honey marketed in Pelotas and Rio Grande, Rio Grande do Sul state, Brazil. Therefore, this study stands out due to the lack of data about the quality of honey produced and marketed in Southern Brazil, highly needed to maintenance of family farming, food quality, and safety.

2 Material and methods

2.1 Reagents

Ultrapure water was directly obtained from a Milli-Q Direct-Q UV3® (Millipore, Bedford, MA, EUA). Sulfuric acid (purity 97.0%), High-Performance Liquid Chromatography (HPLC) grade acetonitrile, magnesium sulfate (purity 99.8%), and sodium chloride were bought from JT Baker (Mallinckrodt, NJ, EUA). The 5-hidroxymethylfurfural (5-HMF) standard (purity 99.0%), and the citrate salts, trisodium citrate dehydrate and disodium hydrogen citrate sesquihydrate, were bought from Sigma Aldrich (St. Louis, MO, EUA). The primary and secondary amine (PSA-Bondesil) used as clean-up sorbent was bought from Varian (Harbour City, CA, EUA). The sodium hydroxide was bought from Merck (São Paulo, SP, Brasil). Finally, analytical standards of pesticides with purity ≥ 95% were used (Sigma Aldrich, St. Louis, MO, EUA).

2.2 Honey samples

Eight honey samples were purchased in the local market (bakeries, grocery stores, and fairs) in November 2021, in Pelotas and Rio Grande cities, in the Rio Grande do Sul state. Samples were taken to the laboratory, being identified and classified according to their origin place, supplier, floral origin, and harvest (Table 1). The honey samples were stored at room temperature (20 °C) up to the analysis.

Table 1. Commercial honey classification.

Honey sample	Origin place	Acquisition place	Floral origin	Harvest
1	Monte Bonito (9° District of Pelotas)	Pelotas	Eucalyptus and wildflowers	Aug/21
2	Pelotas	Pelotas	Native flowers	Jul/21
3	Taim	Rio Grande	Native flowers	n.i.
4	Bagé	Rio Grande	Native flowers	Apr/21
5	Bolaxa	Rio Grande	Eucalyptus	n.i.
6	Canguçu	Rio Grande	Native flowers	Aug/21
7	Taim	Rio Grande	Eucalyptus	Aug/21
8	Pelotas	Pelotas	n.i.	Aug/21

n.i.: not informed.

2.3 Physicochemical characterization

The content of moisture and soluble solids (°Brix) of honey samples was determined at 20 °C by an Abbe refractometer (Edutec, model N.S. A0002906). The Chataway table was used for the conversion of the refraction index in ° Brix to g/100 g (Association of Official Analytical Chemists, 2005).

Ash content was determined by the calcination of the sample in a muffle furnace (Quimis, model Q318 M) at 500 °C for 6 h. The results were expressed in g/100 g (Association of Official Analytical Chemists, 2005).

In addition, pH was measured in a calibrated pH meter (Hanna, model pH21). For this measurement, honey samples were diluted in ultrapure water (1:10, m/v) and kept under magnetic stirring (Association of Official Analytical Chemists, 2005).

Electrical conductivity was measured in samples diluted in ultrapure water (1:5, m/v) in a calibrated conductivity meter (Hanna, model EC215) (Bogdanov et al., 1999).

For the determination of free acidity, 1 g sample was solubilized in 70 mL ultrapure water. The solution was titled with 0.1 mol/L sodium hydroxide until pH 8.5. The results were expressed in mEq/kg (Association of Official Analytical Chemists, 2005).

Reducing sugars and total reducing sugars were determined by the 3,5-dinitrosalicylic acid method described by Miller (1959) and miniaturized by Borba et al. (2022). Before the colorimetric reaction, each honey sample was diluted in ultrapure water (1:50, m/v). The quantification was carried out in an Ultraviolet-visible (UV-Vis) spectrophotometer (Quimis, model Q898U2M5) at 546 nm using an analytical curve prepared with glucose (0.02 to 0.12 mg/mL).

2.4 5-HMF determination

The determination of 5-HMF was performed as described by Marcolin et al. (2021), by the solubilization of 1 g honey sample in 10 mL of ultrapure water in a 15 mL polypropylene tube. A Liquid Chromatograph fitted with a quaternary pump (model 600, Waters), a Photodiode Array detector (model 2996, Waters), and a manual sample injector (Rheodyne) were used. Data acquisition and processing was performed using the software Empower® PDA. The chromatographic separation was performed in an analytical column Synergi C18 (Phenomenex, 250 mm × 4.6 mm i.d., 4 µm, 80 Å), using acetonitrile: ultrapure water (10:90, v/v) as mobile-phase in the isocratic

mode, at a flow rate of 1 mL/min, a total run of 13 min. The wavelength of 284 nm was selected for the quantification of 5-HMF. For each sample, 20 µL was injected. The identification was based on the retention time and the absorption spectra of the 5-HMF in the standard solution and the sample. The limit of detection (LOD) was 0.02 mg/L, while the limit of quantification (LOQ) was 0.05 mg/L. The quantification of 5-HMF was carried out using an analytical curve ranging between 0.05 and 10 mg/L, with a correlation coefficient > 0.99.

2.5 Pesticides determination

The citrate QuEChERS (Anastassiades et al., 2007) was carried out for the extraction of 100 pesticides in honey samples, in conditions previously published (Marcolin et al., 2021). Initially, 10 g honey sample was weighed in a 50 mL polypropylene tube and the atrazine-d5 standard was added at the final concentration of 50 µg/kg as a surrogate. Afterward, 10 mL ultrapure water was added to the sample, followed by the addition of 10 mL acetonitrile, being manually shaken for 20 s and vortexed for 1 min. Then, 4 g $MgSO_4$, 1 g NaCl, 1 g $C_6H_5Na_3O_7 \cdot 2H_2O$ and 0.5. g $C_6H_6Na_2O_7 \cdot 1{,}5H_2O$ was added to the tube to promote partition of the solvent by the salting-out effect and buffering, followed by another agitation step and centrifugation for 10 min at 15.904 g. The clean-up step was carried out by transferring 1 mL of the upper acetonitrile layer to another 15 mL polypropylene tube containing 150 mg $MgSO_4$ and 25 mg PSA, followed by vortexing for 1 min and centrifugation for 5 min at 15.904 g. Finally, 1 mL of the final extract was transferred to a vial for further determination by Gas Chromatography-Mass Spectrometry (GC-MS/MS).

The determination of pesticides was carried out in a GC model TQ 8050 (Shimadzu, Japan), fitted with a Combipal AOC 6000 autosampler, and a MS detector with a triple quadrupole type mass analyzer. The chromatographic conditions were as follows: helium (99.999% purity) was used as the carrier gas at a constant flow rate of 2.21 mL/min. The temperature of the injector was 250 °C, and 2 µL sample was injected in the splitless mode at 150 kPa. The separation was carried out in a capillary column Rtx®-5MS (30 m × 0.25 mm × 0.25 µm) (Restek, EUA) using a temperature ramp starting at 90 °C for 1 min, which was raised to 130 °C at 30 °C/min. Finally, the temperature was raised to 320 °C at 10 °C/min, remaining at this temperature for 3 min, which totaled 24.33 min of analysis. The ionization was performed in electron impact (EI) mode with a collision energy of 70 eV. The interface and ion source temperatures were 290 and 230 °C, respectively. The selected reaction monitoring (SRM) mode was carried out with argon (99.9999% purity) as collision gas. Manipulation of the equipment, data collection, and treatment were performed by the software GCMSsolution, version 4.45 SP1 (Shimadzu, Japão). The whole method was previously validated for the determination of 100 pesticides by Marcolin et al. (2021) in agreement with the Instituto Nacional de Metrologia, Qualidade e Tecnologia (2020) and European Union (2019) guidelines. Accuracy between 59 and 99%, with precision expressed in Relative Standard Deviation (RSD) < 20% was achieved. The Limit of Quantification (LOQ) values ranged between 1 and 10 µg/kg. Retention times and SRM conditions are presented in Table S1 (Supplementary Material).

2.6 Estimative of daily exposure

The estimative of daily intake (EDI) of pesticides in honey was calculated using Equation 1 considering a body weight of 60 kg for an adult. According to the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, 2011), honey ingestion is 0.9 g per day.

$$\text{EDI (µg/kg bw/day)} = \frac{\text{Pesticide concentration (µg/kg) x Honey ingestion (kg/day)}}{\text{Body mass (kg)}} \quad (1)$$

2.7 Statistical analysis

The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation. The analysis of variance (ANOVA) and Tukey's test were carried out to check the statistical difference between honey samples at a significance level of 5%, using the software *Statistic 7.0* (Statsoft Inc, Tulsa, OK, USA).

3 Results and discussion

3.1 Physicochemical characterization

The pH of the samples ranged from 4.34 to 4.76 (Table 2). The statistical differences observed ($p \leq 0.05$) could be due to the different acids in the honey samples, geographical origin, floral source, bee species, and mineral content (Pereira et al., 2020; Sant'ana et al., 2020; Vanhanen et al., 2011; Yücel & Sultanog, 2013). This variability has been reported for Brazilian kinds of honey. For *Apis mellifera* honey, Brugnerotto et al. (2021) reported pH values from 3.51 to 4.26 in samples collected in Santa Catarina state, and Pereira et al. (2020) measured pH values from 3.86 to 4.50 in honey from different floral sources commercialized in Minas Gerais state. For *Meliponinae* honeys, Sant'ana et al. (2020) evaluated honey from *Melipona subnitida* and *M. fasciculata* species in Piauí state and pH values were between 3.3 and 3.9. Thus, although pH is not included as a parameter in the investigation of honey quality, its determination is important because in addition to influencing the texture, stability, and shelf life of the product, along with other components of the matrix and storage conditions, pH can contribute to the rate of formation of 5-HMF (Pereira et al., 2020; Sant'ana et al., 2020) and in the microbial growth (Silva et al., 2016; Marcolin et al., 2021).

Free acidity indicates the amount of organic acids in equilibrium with their inorganic esters and ions present in honey (Finola et al., 2007; Pereira et al., 2020). In the analyzed samples, free acidity varied from 29.42 to 43.72 mEq/kg, indicating the freshness of the samples, since values were following the one established by the legislation, which allows a maximum of 50 mEq/kg (Brasil, 2000; Food and Agriculture Organization, 2019). Statistical significance differences ($p \leq 0.05$) occur due to the different floral sources, weather conditions, amount of minerals and gluconic acid resulting from enzymatic action (Sousa et al., 2016; Estevinho et al., 2012; Pereira et al., 2020). High values of acidity (> 50 mEq/kg) are indicative of sugar fermentation, honey deterioration, and organoleptic characteristics alteration (Bogdanov et al., 1999; Pereira et al., 2020). The acidity of the samples under investigation is in accordance with previously published studies about Brazilian honey (Brugnerotto et al., 2021; Marcolin et al., 2021; Pereira et al., 2020).

The maximum moisture allowed in *Apis mellifera* honey is 20 g/100 g (Brasil, 2000; Food and Agriculture Organization, 2019). Just one sample from the evaluated samples showed results higher than the maximum established (sample number 1, 21.53 g/100 g). High values of moisture can lead to fermentation during storage, reduce the shelf life, and alter the sensory properties such as aroma, color, taste, and texture (Kadri et al., 2016; Pereira et al., 2020; Seraglio et al., 2021). The statistical significance differences among the samples ($p \leq 0.05$) can be related to the geographical origin, beekeepers' manipulation during the honey sampling, and processing and storage conditions (Karabagias et al., 2014b; Seraglio et al., 2021).

Ash content varied from 0.36 to 0.65 g/100 g in the analyzed samples. This variability could be related to the soil mineral content in which the nectar-producing floral species was planted. High mineral content led to a darker and stronger flavor honey (Escuredo et al., 2013). One sample (number 6) presented ash content above the maximum value established (0.60 g/100 g) (Brasil, 2000; Food and Agriculture Organization, 2019). High values could be related to a lack of hygiene and/or irregularities in the decantation or filtration processes (Karabagias et al., 2014a; Pereira et al., 2020).

Electrical conductivity (EC) is associated with the ash content and acidity of the honey (Silva et al., 2016; Seraglio et al., 2021; Yücel & Sultanog, 2013). The Codex Alimentarius (Food and Agriculture Organization, 2019) recommends a maximum EC value of 0.80 ms/cm. Samples number 2, 5 and 7 showed values higher than the established suggesting high mineral content and/or honey's adulteration with darker honeys, which can occur naturally by bees or fraudulent during honey processing (Seraglio et al., 2021). Besides, the EC could be used to classify the samples according to the floral source (Pereira et al., 2020; Terrab et al., 2004). Samples 2 and 8 did not present a statistical significance difference for EC ($p > 0.05$) (Table 2), therefore, although the floral source of sample 8 has not been related, both are from the same geographical place (Pelotas), suggesting that sample 2 and 8 can have the same floral source (native flowers).

Values of soluble solids (SS) varied from 76.53 to 80.25 °Brix. These values are in agreement with previously published studies (Sousa et al., 2016; Marcolin et al., 2021; Pereira et al., 2020). This parameter is not established in the legislation, but its evaluation is important as an indicator of adulteration since it has relation with sugar content and moisture (Marcolin et al., 2021; Terrab et al., 2004). This relation could be observed in sample 1, which presented the lower SS (76.53) and the higher moisture (21.53 g/100 g).

Sugar content can indicate early harvest if high values of sucrose are quantified since this indicates that the invertase enzyme had not enough time to convert sucrose into fructose and glucose; or nectar with high levels of sucrose, or bee feeding with sugar syrups or adulterations (Silva et al., 2016; Pereira et al., 2020). The concentration of reducing sugars (RS), used as an indicative of fructose and glucose, varied between 49.07 and 60.25 g /100 g. The total reducing sugars (TRS) content varied from 57.80 to 65.64 g/100 g. The minimum value of reducing sugars established by the Brazilian legislation is 65 g/100 g (Brasil, 2000). Only one sample (number 1) showed values above the recommendation, indicating that other samples could have been collected before the appropriate time. Besides, sample 7 and 8 presented similar values for RS and TRS, indicating that these sample does not present sucrose in their composition. Therefore, by estimating apparent sucrose by the difference between TRS and RS, samples 1, 2, 3, and 5 may have sucrose content higher than 6 g/100 g, that is the maximum established by Brazilian legislation. In general, the sugars content found in this study is in accordance with what has been reported (Marcolin et al., 2021; Sant'ana et al., 2020).

5-HMF quantification is used to evaluate the honey deterioration by overheating or inadequate storage conditions. Thus, low concentrations indicate fresh samples with good quality (Silva et al., 2016; Pereira et al., 2020). According to Brazilian legislation, the maximum concentration of 5-HMF is 60 mg/kg (Brasil, 2000), and the Codex Alimentarius (Food and Agriculture Organization, 2019) recommends a maximum value of 40 mg/kg for honey in general and 80 mg/kg for honey from countries with temperate climate. All samples analyzed in this study presented 5-HMF values lower than legislation (0.14 – 17.88 mg/kg), indicating that samples are fresh and of high quality. Results from the literature showed a high variation in 5-HMF concentrations (Maeda et al., 2023; Pereira et al., 2020). Maeda et al. (2023) found values of 0.61 and 32.52 mg/kg in honey bees by different spectrophotometric methods. Pereira et al. (2020) found values between 4 and 58 mg/kg samples purchased directly from producers located in Minas Gerais.

Table 2. Physicochemical parameters of commercial honey samples.

Honey	pH	Free Acidity (mEq/kg)	Moisture (g/100 g)	Ashes (g/100 g)	EC (ms/m)	SS (°Brix)	RS (g/100 g)	TRS (g/100 g)	5-HMF (mg/kg)
1	4.34 ± 0.01^{e}	40.85 ± 0.22^{bc}	21.53 ± 0.12^{a}	0.46 ± 0.06^{bc}	0.76 ± 0.01^{d}	76.53 ± 0.42^{d}	56.46 ± 0.81^{b}	65.64 ± 1.77^{a}	3.00 ± 0.47^{c}
2	4.52 ± 0.02^{cd}	37.08 ± 0.33^{d}	18.40 ± 0.20^{bc}	0.41 ± 0.00^{bc}	0.81 ± 0.00^{c}	79.92 ± 0.14^{ab}	54.95 ± 0.17^{bc}	64.39 ± 2.21^{ab}	2.60 ± 0.33^{cd}
3	4.34 ± 0.02^{e}	43.14 ± 0.82^{ab}	19.53 ± 0.76^{c}	0.52 ± 0.08^{abc}	0.68 ± 0.00^{f}	79.17 ± 0.29^{c}	55.40 ± 1.33^{bc}	62.58 ± 2.20^{abcd}	17.88 ± 1.12^{a}
4	4.53 ± 0.02^{cd}	29.42 ± 0.68^{e}	18.67 ± 0.46^{bc}	0.59 ± 0.00^{ab}	0.54 ± 0.00^{g}	79.25 ± 0.25^{bc}	54.99 ± 1.58^{c}	58.47 ± 2.28^{cd}	0.14 ± 0.03^{e}
5	4.46 ± 0.01^{d}	36.44 ± 0.82^{d}	18.20 ± 0.00^{bc}	0.57 ± 0.09^{ab}	0.98 ± 0.01^{a}	80.25 ± 0.00^{a}	49.07 ± 0.69^{d}	57.80 ± 1.19^{d}	7.29 ± 0.54^{b}
6	4.76 ± 0.06^{a}	38.42 ± 0.73^{cd}	19.07 ± 0.31^{b}	0.65 ± 0.13^{a}	0.73 ± 0.01^{e}	79.42 ± 0.14^{bc}	60.22 ± 0.05^{a}	64.29 ± 1.78^{abc}	2.29 ± 0.36^{cd}
7	4.62 ± 0.01^{b}	33.76 ± 0.60^{f}	17.49 ± 1.02^{c}	0.43 ± 0.02^{bc}	0.90 ± 0.02^{b}	79.67 ± 0.14^{abc}	60.04 ± 0.02^{a}	60.62 ± 3.05^{abcd}	1.12 ± 0.22^{de}
8	4.46 ± 0.01^{d}	43.72 ± 1.88^{a}	17.60 ± 1.73^{c}	0.36 ± 0.01^{c}	0.80 ± 0.01^{c}	79.17 ± 0.38^{c}	60.25 ± 0.00^{a}	59.50 ± 1.48^{bcd}	6.51 ± 1.18^{b}

Data expressed at mean ± standard deviation. Different lowercase letters in the same column indicate a significant difference by the Tukey's test ($p \leq 0.05$) between commercial honey samples. EC: Electrical conductivity; SS: Total Soluble Solids; RS: Reducing sugars; TRS: Total reducing sugars; 5-HMF: 5-hydroxymethylfurfural.

3.2 Evaluation of pesticides in commercial honeys

The presence of pesticides in honey is considered as an indicator of indirect environmental contamination, mainly from agriculture activities, since pollinating insects such as bees are highly vulnerable to the risks caused by exposure to chemical products often used in fields. Therefore, it is important to monitor these

compounds to verify the profile and/or level of environmental contamination in the region as well as to assess the risk to consumer health (Makni et al., 2023; Marcolin et al., 2021).

One hundred pesticides from different classes, physicochemical properties, and uses were evaluated in the samples using the method proposed by Marcolin et al. (2021). The region where the samples were produced is characterized by high agriculture activities, mainly rice, corn, peach, nectarine, strawberry, among others (Empresa Brasileira de Pesquisa Agropecuária, 2023). The insecticide λ-cyhalothrin was detected in 50% of samples in concentrations from 5.95 to 25.30 µg/kg (Table 3). Brazil and EU established a MRL of 50 µg/kg for this compound (Brasil, 2013; European Union, 2022). Although concentrations were lower than the MRL, results indicated environmental contamination and indirect food exposure. Figure 1 shows a chromatogram of a sample contaminated with λ-cyhalothrin.

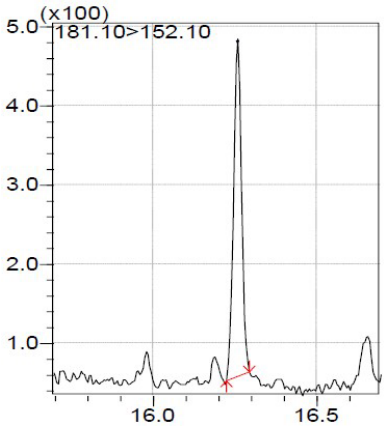

Figure 1. Chromatogram for honey sample (sample 7) contaminated with λ-cyhalothrin.

Concentrations of different pesticides lower than the MRL have been also found in other studies. Wang et al. (2022) detected chlorantraniliprole and thiamethoxam in concentrations from 16.7 to 31.9 µg/kg in honey from China. Orso et al. (2016) detected pesticide residues in 50% from 42 honey samples from different regions of the South of Brazil, all in concentrations lower than MRLs.

According to the concentration detected and data about honey consumption, the EDI of λ-cyhalothrin by consuming honey presented a medium of 1.0×10^{-4} µg/kg bw/day (Table 3); about 500 times lower than the acceptable daily intake of this pesticide (0.05 mg/kg bw/day) (Brasil, 2023). Thus, honey samples evaluated in this study are considered safe for human consumption. Wang et al. (2022) also related that evaluated honey samples were considered safe for adult and child consumption, since EDI values ranged from 5.3×10^{-6} to 1.1×10^{-5} µg/kg bw/day.

Table 3. Concentrations and estimated daily intake (EDI) of λ-cyhalothrin in commercial honeys.

Honey sample	Concentration (µg/kg)	EDI (µg/kg bw/day)
1	nd	-
2	nd	-
3	5.95 ± 0.08	8.9×10^{-5}
4	nd	-
5	17.48 ± 1.09	2.6×10^{-4}
6	6.43 ± 0.38	9.6×10^{-5}
7	25.30 ± 1.04	3.8×10^{-4}
8	nd	-
Mean of total honey samples (n=8)	6.90	1.0×10^{-4}

nd: not detected.

4 Conclusions

Samples of commercial honey from the southern region of Rio Grande do Sul state showed variations in their physicochemical characteristics, probably due to factors such as geographical origin, floral source, processing, and storage conditions, among others. However, most evaluated parameters were in line with the standard recommended by Brazilian legislation. Besides, 5-HMF levels demonstrated the freshness and quality of the honeys.

From 100 pesticides investigated in samples, only one pesticide residue was found, and in concentrations below the maximum limits of Brazilian and European legislation (< 50 μg/kg), resulting in a low risk of exposure (1.0×10^{-4} μg/kg bw/day), which characterizes the honey samples as safe for human consumption. Therefore, the presence of chemical contaminants in this product served as an indication of environmental contamination and corroborates with the need for pesticide monitoring, especially when honey is cultivated near regions of intense agricultural activity to reduce the exposure of pollinating bees to pesticides.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Retention time (RT), Selected Reaction Monitoring (SRM) transitions and collision energy (CE) for the monitored pesticides.

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