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Typification and adulteration analysis of Brazilian honeys: a systematic review from 2010 to 2020

Análise de tipificação e adulteração de méis brasileiros: uma revisão sistemática de 2010 a 2020

Análisis de tipificación y adulteración de mieles brasileñas: una revisión sistemática de 2010 a 2020

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Abstract
Honey is the most consumed bee product worldwide, due to its characteristic flavor and the nutritional and therapeutic properties. Brazil is among the largest honey-producing countries, however, due to its high demand, both in the national and international markets, more forms of adulteration have been registered, resulting in economic losses to beekeepers and risks to consumers' health. Thus, this research carried out a systematic review through the CAPES journal portal in order to detect the use of metabolomics analysis based on chromatographic and spectroscopic techniques for typification and detection of fraud and contaminants in honey, over the 2010-2020 years. Of the total articles recovered from the platform (n = 184) using the keywords "honey", "Brazil", "gas chromatography", "liquid chromatography", "nuclear magnetic resonance", and "spectrometry”, 25 studies met the parameters of interest. Chromatographic techniques were mainly used for pesticide detection and spectroscopy for metal detection, while the only NMR-based study found unnatural substances in honey. In addition to the importance of adopting these modern analytical techniques in conjunction with chemometrics, it is necessary to create databases for continuously record the evolution of cases and types of frauds, as already done in other countries, corroborating to jeopardize the occurrence of recursive types of frauds and supporting policy-makers to update the current Brazilian legislation that covers this matter, which dates from 2000.
Keywords: Chromatography; Nuclear magnetic resonance; Spectroscopy.

Resumo
O mel é o produto apícola mais consumido em todo o mundo, devido ao seu sabor característico e às propriedades nutricionais e terapêuticas. O Brasil está entre os maiores países produtores de mel, porém, devido à alta demanda, tanto no mercado nacional quanto no internacional, tem sido registradas mais formas de adulteração, resultando em prejuízos econômicos aos apicultores e riscos à saúde dos consumidores. Assim, esta pesquisa realizou uma revisão sistemática através do portal de periódicos da CAPES a fim de detectar o uso de análises metabolômicas baseadas em técnicas cromatográficas e espectroscópicas para tipificação, detecção de fraudes e contaminantes em mel, no período 2010-2020. Do total de artigos recuperados da plataforma (n = 184) usando as palavras-chave "mel", "Brasil", "cromatografia gasosa", "cromatografia líquida", "ressonância magnética nuclear" e "espectrometria", 25 estudos atenderam aos parâmetros de interesse. As técnicas cromatográficas foram usadas principalmente para detecção de pesticidas e espectroscopia para detecção de metais, enquanto o único estudo baseado em RMN encontrou substâncias

não naturais. Além da importância de adotar essas modernas técnicas analíticas em conjunto com quimiometria, é necessário criar um banco de dados para registrar continuamente a evolução dos casos e os diversos tipos de fraudes, como já é feito em outros países, corroborando para comprometer a ocorrência de tipos recursivos de fraudes e apoiando os formuladores de políticas na atualização da legislação brasileira vigente que trata do assunto, que data dos anos 2000.
Palavras-chave: Cromatografia; Ressonância magnética nuclear; Espectroscopia.

Resumen
La miel es el producto apícola más consumido en el mundo, debido a su característico sabor y propiedades nutritivas y terapéuticas. Brasil se encuentra entre los mayores países productores de miel, sin embargo, debido a la alta demanda, tanto en el mercado nacional como internacional, se han registrado más formas de adulteración, lo que genera pérdidas económicas para los apicultores y riesgos para la salud de los consumidores. Así, esta investigación realizó una revisión sistemática a través del portal de revistas CAPES con el fin de detectar el uso de análisis metabolómicos basados en técnicas cromatográficas y espectroscópicas para la tipificación, detección de fraudes y contaminantes en la miel, en el período 2010-2020. Del número total de artículos recuperados de la plataforma (n = 184) utilizando las palabras clave "miel", "Brasil", "cromatografía de gases", "cromatografía líquida", "resonancia magnética nuclear" y "espectrometría", 25 estudios cumplieron con los parámetros de interés. Las técnicas cromatográficas se utilizaron principalmente para la detección de pesticidas y la espectroscopia para la detección de metales, mientras que el único estudio basado en RMN encontró sustancias no naturales. Además de la importancia de adoptar estas técnicas analíticas modernas en conjunto con la quimiometria, es necesario crear una base de datos para registrar continuamente la evolución de los casos y los diferentes tipos de fraude, como ya se hace en otros países, corroborando para comprometer la ocurrencia de tipos de fraude y apoyando a los hacedores de políticas en la actualización de la legislación brasileña vigente en la materia, que data de la década de 2000.
Palabras clave: Cromatografía; Resonancia magnética nuclear; Espectroscopia.

1. Introduction

Honey, considered one of the purest foods in nature, has a remarkable flavor and high nutritional value. It provides nutrients such as water, sugars, minerals, vitamins, enzymes, proteins, organic acids, and amino acids (De-Melo et al., 2018). Honey also contains phenolic substances, such as phenolic acids, flavonoids, flavones, flavanones, flavanols, anthocyanidins, and isoflavones, which are antioxidants responsible for scavenging free radicals in the human body (Nascimento et al., 2018). Besides, researchers have shown that honey contains antimicrobial compounds capable of inhibiting the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* (Valdés-Silverio et al., 2018), *Salmonella,* the fungus *Candida albicans* (Kakengi & Idani, 2018), and of *Haemophilus influenzae*, a biofilm-producing species (Newby et al., 2018). Thus, this natural product, which has been widely used, acts against several pathogens and has been shown to reduce treatment costs for diseases in underdeveloped countries (Scepankova et al., 2017).

Other bioactivities have been highlighted nowadays, such as anticancer (Aishwarya et al., 2019; Cianciosi et al., 2020; Ghramh et al., 2020), antiviral (Shahzad & Cohrs, 2012), and regenerative wound recovery diabetic people (Sarkar et al., 2017). Yaghoobi et al. (2008) describe that honey intake reduces cardiovascular risk factors, decreases total cholesterol, low-density lipoprotein cholesterol (LDL-C), triacylglycerol, fasting blood glucose (FBG), and C-reactive protein (CRP). Interestingly, the authors have indicated that consuming the product does not increase body weight in overweight or obese individuals.

Honey is a food that presents variations in its physicochemical composition depending on climatic factors, maturation stage, bee species, and type of flowering, for instance (Pavlova et al., 2018). The floral origin of honey is associated with its organoleptic aspects, such as color and flavor (Marcazzan et al., 2017), as well as its nutritional value (Al-Mosa et al., 2019). However, some standards must be observed to guarantee the quality of honey. To this end, Codex Alimentarius, a program of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) establishes some parameters (moisture, fructose and glucose, sucrose, water insoluble solids, heavy metals, residues of pesticides and veterinary drugs) that must be maintained for the commercialization of this bee product (Codex Alimentarius, 2019).

Considering the mentioned benefits of honey to human health, it has become an asset economically important worldwide (Khan & Khan, 2018) and, in return, frequently found on the list of the most fraudulent foods currently. According to the American Bee Journal, between 2007 and 2013, for example, there was a 61% increase in amount of exported honey, dramatically differing from the number of hives that do not exceed 8% growth, showing a strong indication that adulterate honey continues being marketed (Phipps, 2017). A study by Moore et al. (2012), comparing adulteration in different foods from 1980 to 2010 around the world, describes that honey is among the products with the highest report of adulteration. Adulterations in honey can pose a serious risk to consumers' health, due to the presence of illegal antibiotics (chloramphenicol, streptomycin, sulfonamides, and tetracyclines), high levels of pesticides (bactericides, fungicides, herbicides, and insecticides), bacteria, heavy metals, and radioactive materials due to environmental pollution or misuse in beekeeping practices (Marquele-Oliveira et al., 2017; Ritten et al., 2019).

Over the years, the types of honey adulteration have been improved, often making it impossible to prove these frauds through commonly used analytical techniques, such as physicochemical analyses. For this reason, it is essential to improve detection techniques for unnatural constituents. Likewise, the classification of honey can facilitate the identification of eventual problems that may interfere with the final quality of the product. Therefore, methods based on metabolomic analysis coupled to chemometrics using liquid chromatography (LC), gas chromatography (GC), and spectroscopic techniques (nuclear magnetic resonance - NMR, RAMAN, and mid/near infrared – FTIR/NIR), among other, can serve in more sensitive and robust analysis of honey, as already used in the food science field worldwide (Consonni & Cagliani, 2015; Gan et al., 2016; Siddiqui et al., 2017; Liu et al., 2018).

Brazil figures as one of the main honey producers in the world and where many cases of fraud have been reported. Due to its botanical diversity and tropical climate, Brazil is able to produce honey throughout the year, being an important exporter to the rest of the world. According to the Brazilian Association of Honey Exporters (ABEMEL, in Portuguese) only in 2017, the country exported about 27 10^3 tons of honey, occupying the 9th place in the global market of the product (ABEMEL, 2018). The Agricultural Census carried out in 2017, describes over 100 thousand agricultural establishments that produce honey in Brazil, with more than 2 million hives spread across the country (IBGE, 2017). In a more recent scenario, in 2021 Brazil exported 40.5 10^3 tons, mostly for USA, reaching US$ 138.639 10^6 (ABEMEL, 2021). Thus, one should keep in mind that Brazil has an important internal market for honey, as well as it is a common player in the exportation of that food, turning relevant the issues regarding the frauds that might affect it, jeopardizing the quality of the Brazilian honeys worldwide.

Due to the extensive diversity of bee flora available to bees, different types of honey are found in Brazil, mostly polyfloral ones, what makes it even more difficult to characterize their chemical profiles and to detect adulterations by commonly used techniques (Marquele-Oliveira et al., 2017). Thus, this systematic review aims at to identify which are the metabolomics and chemometrics analysis that have been applied worldwide in food science and more specifically in the typification and detection of adulteration of Brazilian honeys.

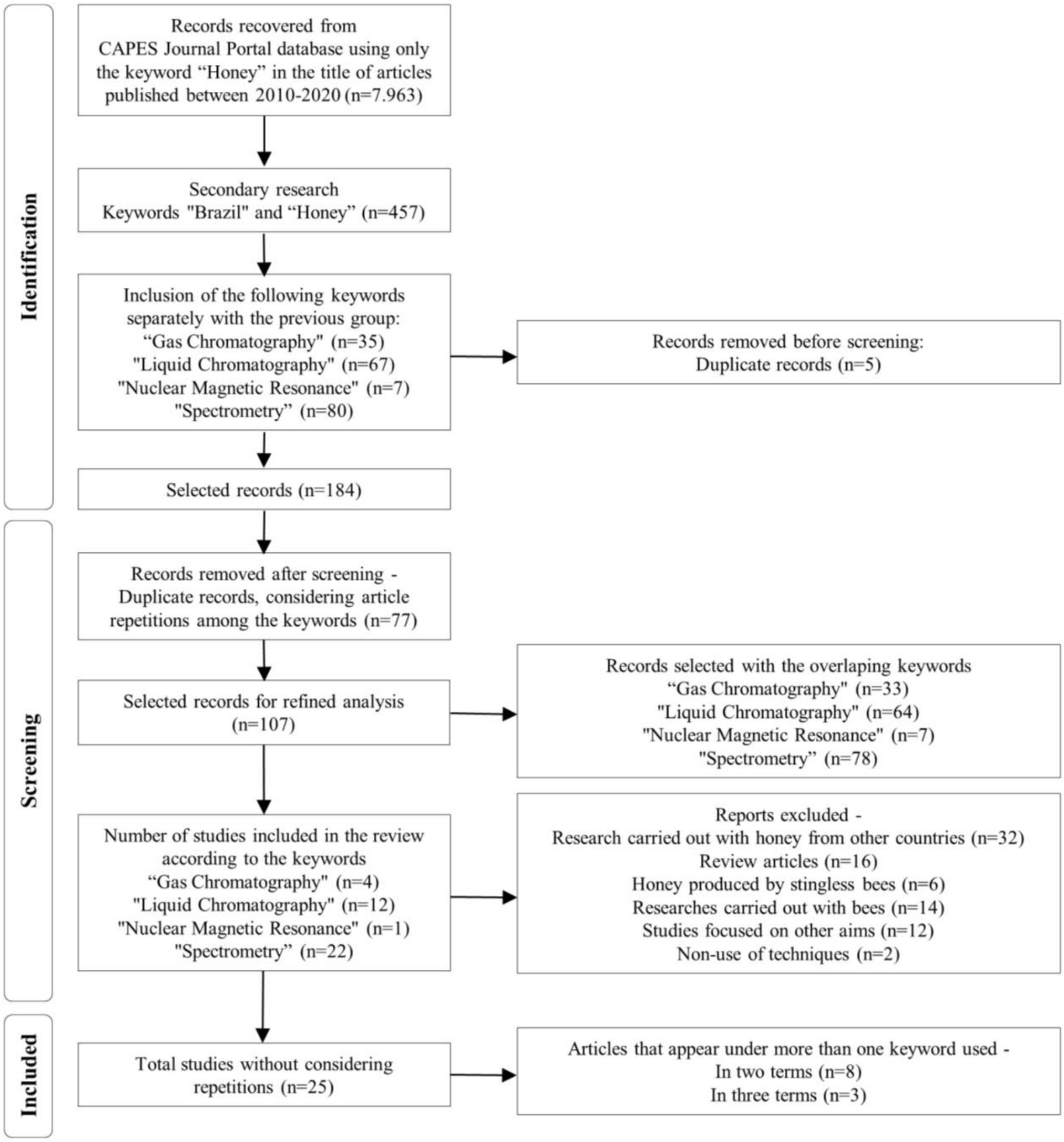
2. Methodology

The systematic review was carried out following the criteria established by Page et al. (2020). The systematic review commonly used for medical research aims to provide a comprehensive and unbiased synthesis of various studies performed. This can be applied to any area, allowing one to check the remaining gaps and questions that must be answered in future researches by the audience (Robinson et al., 2013; Page et al., 2020).

The systematic review was carried out based on data provided by the CAPES Journal Portal (https://www-periodicos-capes-gov-br.ezl.periodicos.capes.gov.br/), which encompasses 294 databases (June 2021) for public access. As initial inclusion criterion, the keyword "honey" was used, considering peer-reviewed articles published between 2010-2020. As a second criterion, the keyword "Brazil" was used, followed by "gas chromatography", "liquid chromatography", "nuclear magnetic resonance" and "spectrometry", which were included in the research separately (Figure 1). As much as nuclear magnetic resonance is a form of spectrometry, many articles may not include the term and, therefore, it was decided to carry out a specific search for it, separately. Likewise, the words typification and adulteration were not considered, as the initial searches with both terms showed a reduction in the number of researches carried out in Brazil, since many articles did not include these words. These delimitations were made by considering more sophisticated and robust techniques for analyzing food and beverages, which have also been applied to honey samples (Consonni & Cagliani, 2015; Ling Chin & Sowndhararajan, 2020).

As the exclusion criteria, the following specifications were adopted: repeated articles within the CAPES Portal for each keyword, outputs on honey studies from other countries, review articles, honey produced by stingless bees, researches carried out with bees, investigations focused on other aims, and non-use of the analytical techniques of interest. From this more refined delimitation, repeated articles that transited between the used keywords were excluded (Figure 1). Articles that fit the established objective were computed and analyzed using tables and bar graphs in the Microsoft Excel electronic sheet (Version 2019), containing all the main data recovered (e.g., types of analytical techniques used for data analysis performed, sample origins, results found, and authors' names).

Figure 1. Flowchart of the results recorded from the CAPES Journal Portal database regarding the use of metabolomic and chemometric techniques for characterization and analysis of honey samples, according to the criteria described in the Material and methods section.



Source: Authors (2022).

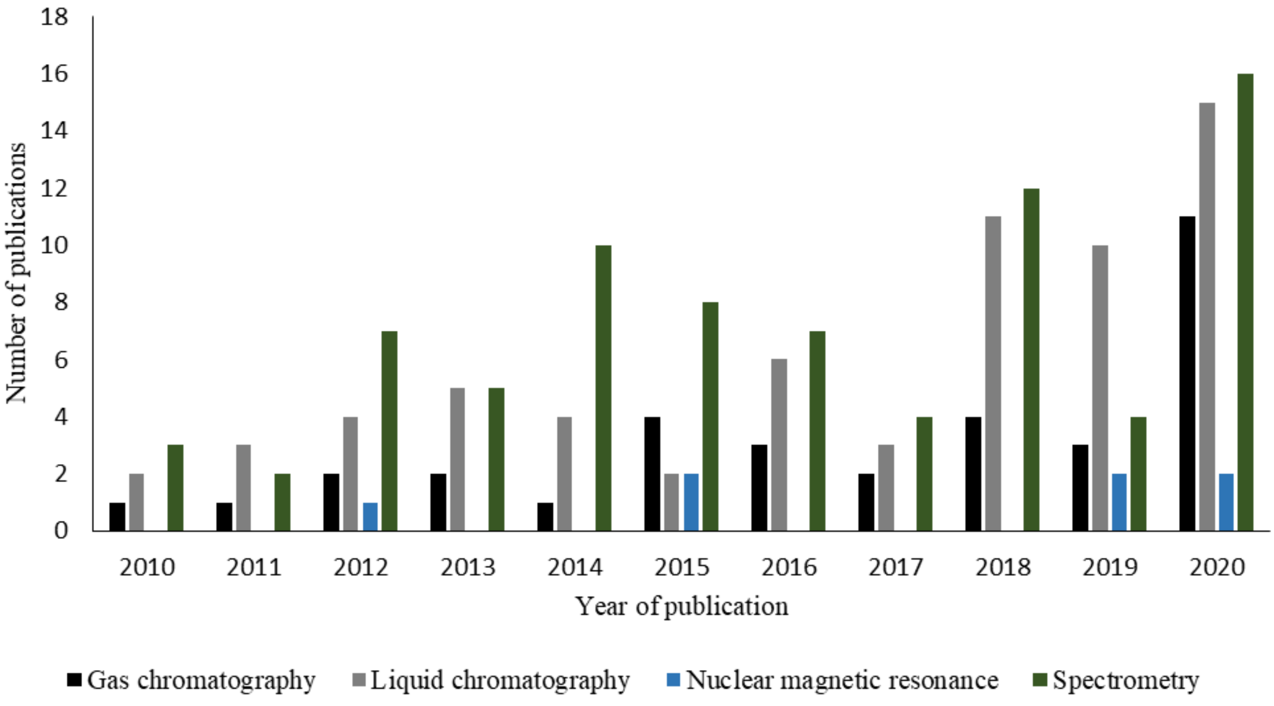
3. Results and Discussion

In recent past, Brazil has been worldwide recognized for producing high quality honeys, as recognized several times in events such as Apimondia where the country has been awarded (APACAME, 2021). This results from several factors, e.g., the rustic characteristics of Africanized bees, the richness of the Brazilian flora, soil and climate conditions, and also the technologies adopted for producing that food. Indeed, Brazilian honey is sought internationally, mainly because of its high quality, being exported mainly with organic certification. To maintain that claimed quality, in light of the ongoing knowledge available on the quality control processes abroad, it is urgent and necessary to improve the adoption and use of modern analytical techniques to identify adulterated honey. According to Drivelos et al. (2020), the classification of honey through physical-chemical parameters can be considered quite questionable, since the parameters can vary considerably depending on the type of honey. In addition to the inclusion of more robust analyzes in Brazilian honey, it will be necessary to review the legislation in force in Brazil, which was last updated in the 2000s (Brazil, 2000).

In the country, numerous cases of honey fraud have been reported over the years, resulting in serious economic losses for the beekeeping industry. According to the United States Pharmacopoeia (USP, 2016), geographic regions that develop with political and social instability and with a large and growing population tend to be highly vulnerable to food fraud and adulteration. As Brazil meets these criteria, mostly in recent past, many frauds have been recorded in the country (Tibola et al., 2018). Considering the increase in honey adulteration cases in recent years, the adoption of modern detection techniques is a great demand in the country. In this sense, metabolomics and chemometrics have been used to detect fraud in different foods and can be applied to honey authentication.

The search on the CAPES Journal Portal identified a total of 189 scientific articles, 35 associated to the keyword “gas chromatography”, 67 to “liquid chromatography”, 7 to “nuclear magnetic resonance”, and 80 to “spectrometry”. However, 1 repeated article was detected for the term “gas chromatography”, 2 for “liquid chromatography” and 2 for “spectrometry”, resulting in 184 records (Figure 2). Among the keywords "gas chromatography", "liquid chromatography" and "spectrometry" which resulted in a greater number of articles compared to "nuclear magnetic resonance", the largest number of publications in the last ten years occurred over 2020. Carrying out the NMR article search separately from spectrometry was essential, since of the 7 articles found, 2 did not appear indexed as a spectroscopic technique.

Figure 2. Number of original and peer-reviewed articles published per year (2010-2020) on Brazilian honey chemical analysis, according to the indexing terms “gas chromatography”, “liquid chromatography”, “nuclear magnetic resonance”, and “spectrometry”. The data were recovered from the CAPES Journal Portal as described in Material and methods section.



Source: Authors (2022).

From the 184 studies recorded, 77 appeared in more than one keyword, i.e., there was repetition within the CAPES Journal Portal database, being further excluded upon a more refined analysis. Thus, initially 107 articles were carefully checked, resulting 25 studies that met the proposed objective in the end. In these, it was found that 8 articles appeared in 2 of the terms, and 3 were identified in 3 terms, demonstrating that the authors choose to apply more than one technique to the same samples, e.g., chromatography and spectroscopy. Thus, as a result, 22 manuscripts were computed for the keyword "spectrometry", followed by 12 for "liquid chromatography", 4 for "gas chromatography", and only 1 for “nuclear magnetic resonance”. For the description of the articles, it was decided to distribute the articles firstly by the keywords gas chromatography (GC), liquid chromatography (LC), nuclear magnetic resonance (NMR), and, finally, spectrometry, where the repetitions of the articles that were present in the same term were removed (Table 1).

Table 1. Determination of adulteration and typification of Brazilian honeys, using metabolomic and chemometric techniques.

Analytical technique	Aim	Location of samples (Region, State)	Result	References
Gas chromatography (GC)	Multiresidue analysis of 22 pesticides	Southeast - São Paulo	The MEPS-GC methodology proved to be efficient for the determination of pesticides in honey. The samples used to assess the applicability of the method did not show concentrations higher than the LOQ values, as established with the MRL values	Salami and Queiroz (2013)
	Determination of sugars and cyclitols and their correlation with physicochemical properties	Not determined	Correlations have been identified between the physicochemical properties and carbohydrate profiles determined by GC for 18 types of honeys from different countries. The year of harvest influenced the honey composition and also the correlation coefficients between chemical profiles of samples from different countries	Ratiu et al. (2019)
	Identification of volatile compounds and monitoring the stability thereof over 540 days	Southern - Santa Catarina	32 volatile compounds were detected, 24 of which were evaluated for stability. The volatile compounds *cis*- and *trans*-linalool oxide and hotrienol showed increased contents in 7 samples and considered possible indicators of honey degradation	Silva et al. (2019)
	Identification and quantification of pesticides by GC-MS/MS and LC-MS/MS	Midwest - Distrito Federal and Goiás; Southeast - Minas Gerais and São Paulo; Northeast - Rio Grande do Norte; Southern - Rio Grande do Sul	Fifteen analytes were identified in 31 honey samples (93.9% of the samples). The method showed to be very sensitive to detect a wide range of pesticides	Almeida et al. (2020)
Liquid chromatography (LC)	Development of HFRLM-LC-MS/MS methodology for determination of five sulfonamides	Not determined	The method proved to be efficient for extracting the target compounds, providing low LOQ, with good recovery, precision, and linearity	Bedendo et al. (2010)
	Detection of the insecticide fipronil through liquid chromatography, using DLLME and QuEChERS	Southern - Not determined	The DDLME technique compared to QuEChERS allowed to use less solvent and showed a lower limit of quantification, as QuEChERS proved to be more robust for honey analysis, given the complexity of the matrix	Tomasini et al. (2011)
	Determination of chloramphenicol antibiotic	Different geographical origins	The method proved to be efficient by CCα (0.05 mg kg^{-1}) and CCβ (0.09 mg kg^{-1}), confirming the presence of CAP in honey	Barreto et al. (2012)
	The QuEChERS method was used to determine pesticides and 5-hydroxymethylfurfural (HMF) as quality indicators	Southern - Not determined	The method showed robustness with recoveries in the range of 70-120% for all compounds (fipronil, imidacloprid, thiamethoxam, dimethoate, carbendazim, tebuconazole, amitraz, τ-fluvalinate, and HMF) in samples from different floral origins	Tomasini et al. (2012)
	Development of method for screening and confirming the antibiotic chloramphenicol	Four geographic regions - Not determined	Tandem mass spectrometry using MRM transitions provided a very selective, sensitive, and robust method for detecting chloramphenicol with values of 0.08 μg kg^{-1} for CCα and 0.12 μg kg^{-1} for CCβ	Taka, Baras and Chaudhry (2012)
	Development of a quick and simple method for determination of eight pyrrolizidine alkaloids (PAs) using LC-ESI-MS/MS	Southern - Santa Catarina, Paraná and Rio Grande do Sul Southeast - São Paulo, Rio de Janeiro, Minas Gerais, and Espírito Santo; Midwest - Mato Grosso do Sul; Northeast - Bahia, Ceará, and Piauí; Northern - Pará	The method proved to be efficient for detecting PAs, with LOD = 0.1-1.0 μg kg^{-1} and LOQ = 0.2-1.5 μg kg^{-1}. In 99.1% of the honey samples analyzed, at least three PAs were detected	Valese et al. (2016)
	Determination of 79 pesticides and 13	Southwen - Rio Grande do Sul	The UHPLC-MS/MS method showed selectivity and sensitivity	Orso et al.

	antibiotics in monofloral and multifloral honey		adequate for routine analysis	(2016)
	Method for detection of 16 phenolic acids and 14 flavonoids in honeys from different countries	Not determined	The most dominant compounds were gallic acid and chrysin. The phenolic profiles were influenced by geographic and floral origin, and their classification was determined by multivariate statistical method, i.e., LDA	Cheung et al. (2019)
	Development of method for determination of neonicotinoid residues using QuEChERS	Not determined	The method proved to be sensitive for the determination of neonicotinoids in the range of 60.80 to 80.98 ng/g (LOD) and 184.26 to 245.40 ng/g (LOQ). In a Brazilian honey sample, a high amount of the systemic insecticide acetamiprid (1340.33 ± 27.50) was detected	Ligor et al. (2020)
Nuclear Magnetic Resonance (RMN)	Identification of unnatural substances	Southeast - São Paulo	In adulterated honeys, signs of 5-hydroxymethylfurfural, citric acid, and ethanol were found. Chemometrics based on KNN, SIMCA, PLS-DA models classified correctly 66.7%; 22.2%, and 72.2% of the samples, respectively	Boffo et al. (2012)
Spectrometry	Development of a method for mercury determination through CPE and CV-ICP OES for detection	Southern - Not determined	The method proved to be efficient for the detection of Hg in honey, presenting a very low and viable LOD (1.25 ng g^{-1}), in addition to being relatively simple, cheap, and with minimal waste generation	Depoi, Bentlin and Pozebon (2010)
	Development of a rapid method for determination of arsenate using HGAAS	Southeast - Minas Gerais	The method was validated and served to quantify arsenate in 27 honey samples, all of which showed concentrations below the LOD (20 ng g^{-1})	Vieira et al. (2012)
	Determination of chromium (Cr), lead (Pb), and cadmium (Cd) through electrothermal atomic absorption spectrometry	Southern - Paraná	Analytical protocols provided accurate data. The LOQ values for Cd, Pb, and Cr were 2.0, 5.4, and 9.4 ng g^{-1}, respectively. The contents of the analyzed samples ranged from 141 to 228 ng g^{-1} for Pb, < 2.0 to 8 ng g^{-1} for Cd, and 83 to 94 ng g^{-1} for Cr	Andrade et al. (2014a)
	Determination of traces of lead (Pb), cadmium (Cd), and chromium (Cr) using GFAAS	Southern - Paraná	Mean values were 200 ± 76 ng g^{-1} for Pb, 88 ± 14 ng g^{-1} for Cr, 4.1 ± 4 ng g^{-1} for Cd. Honey samples were geographically separated, mainly in terms of Pb and Cd, according to multivariate statistical analysis, i.e., PCA.	Andrade et al. (2014b)
	Method for determination of copper (Cu), lead (Pb), cadmium (Cd) and zinc (Zn) through DPASV in BDD, Hg-GC, and GFAAS	Not determined	All proposed methodologies were efficient in determining the elements. The use of chemometric tools allowed to build prediction models with accurate measurements and good linearity. It was possible to establish a simultaneous analysis of Cu, Pb, and Cd	Honório et al. (2014)
	Development of method for the determination of inorganic constituents using ICP-MS and two methods for the preparation of samples (acid mineralization in a block digester and microwave-assisted acid digestion)	Midwest, North, Northeast, South, Southeast	Microwave radiation mineralization proved to be the most suitable method for sample preparation. Through ICP-MS eleven elements (Se, Mg, Ca, Al, P, Mn, Fe, Cu, Zn, Ba and Pb) in 60 honey samples have been determined	Leme et al. (2014)
	Development of a method for determination of mercury (Hg) through CVAAS and DMA	Southeast - Minas Gerais	Both methods proved to be fast and simple for detecting Hg in honey. The 35 honey samples revealed concentrations < 2.5 ng/g Hg	Vieira, Nascentes and Windmöller (2014)

Development of method for ultra-trace determination of halogens using ICP-MS after decomposition by MIC	Southern - Rio Grande do Sul; Southeast - Minas Gerais	The LODs captured by MIC were 34 ng g^{-1} for Br and 6.0 ng g^{-1}, greatly improving the method when compared to data obtained using MA-AD. The MIC method proved to be efficient for the determination of Br in honey	Costa et al. (2015)
Evaluation of seasonal patterns of potassium (K), calcium (Ca), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), (bromine) Br, and strontium (Sr) from four seasons for two years through TXRF	Southeast - Rio de Janeiro	Seasonal changes significantly affected the elemental concentration, especially K and Ca, with higher values in spring and summer. No seasonal influence was detected for the contents of Cr, Ni, Se, and Ti in honey samples, suggesting a low level of contamination in the regions studied at any time of the year	Ribeiro et al. (2015)
Development of sample preparation method using H_20_2 in a single reaction chamber system for honey digestion and subsequent determination of bromine (Br), chlorine (Cl), and iodine (I) amounts by ICP-OES and ICP-MS	South - Rio Grande do Sul	The LOD by the proposed method was 10 µg g^{-1} for Cl using ICP-OES and 0.03 and 0.005 µg g^{-1} for Br and I using ICP-MS	Muller et al. (2017)
Establishment of method for honey decomposition, halogens and sulfur (S) analysis using MIC combined with IC-CD-MS	Not determined	The MIC method allowed to efficiently track honey decomposition, as well as the determination halogen and S by IC-CD-MS. The methodology proved to be innovative adequate, and reliable for performing routine analyses	Mesko et al. (2020)

Metabolomics and chemometric abbreviations: MEPS-GC - Microextraction Packed Sorbent and Gas Chromatography; LOQ – Limit of Quantification; LOD – Limit of Detection; MRLS – Maximum Residue Limits; GC-MS/MS – Gas Chromatography-Tandem Mass Spectrometry; LC-MS/MS – Liquid Chromatography-Tandem Mass Spectrometry; HFRLM-LC-MS/MS - Hollow Fiber Renewal Liquid Membrane with High Performance Liquid Chromatography–Tandem Mass Spectrometry; CCα – Decision Limit; CCβ – Detection Capability; DLLME - Dispersive Liquid-Liquid Microextraction; QuEChERS - Quick, Easy, Cheap, Effective, Rugged, and Safe; MRM – Multiple-Reaction Monitoring; LC–ESI-MS/MS – Liquid Chromatography–Electrospray Ionization-Tandem Mass Spectrometry; UHPLC-MS/MS – Ultra-High Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry Detection; LDA – Linear Discriminant Analysis; KNN - *K* Nearest Neighbor; SIMCA - Smooth Independent Modeling by Class Analogy; PLS-DA - Discriminant Analysis by Partial Least Squares; CPE – Cloud Point Extraction; CV-ICP OES – Cold Vapor-Optical Emission Spectrometry; HGAAS – Hydride Generation Atomic Absorption Spectroscopy; ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometry; ICP-MS – Inductively Coupled Plasma Mass Spectrometry; GFAAS – Graphite Furnace Atomic Absorption Spectrometry; DPASV – Differential Pulse Anodic Stripping Voltammetry; BDD – Boron-Doped Diamond Electrode; Hg-GC – Hg-Coated Glassy Carbon; CVAAS – Cold Vapor Atomic Absorption Spectrometry; DMA – Direct Mercury Analyzer; MIC – Microwave Induced Combustion; MA-AD – Microwave-Assisted Alkaline Dissolution; TXRF – Total Reflection X-ray Fluorescence Spectroscopy; IC-CD-MS – Ion Chromatography with Conductimetric and Mass Detection. Source: Authors.

Source: Authors (2022).

As shown in Table 1, metabolomics techniques together with chemometrics have successfully been used in Brazil, mainly in recent years, for detecting pesticides, antibiotics, minerals, and other unnatural substances in honey samples. Besides, a series of methods have been developed, allowing the characterization of sugars, volatile compounds, phenolic acids, and flavonoids. For this reason, there is a substantial bulk of knowledge in the country and, of course, abroad, supporting the adoption of those sensitive and accurate techniques in improved and updated honey quality control pipelines. If so, it is expected to result in an increasing level of product safety and quality, strengthening small beekeepers and beekeeping industries that maintain quality criteria (Consonni & Cagliani, 2015). In addition to promoting food safety through metabolomic analysis, the beekeeper can eventually use fingerprints and biochemical markers of certain types of honeys associated to their geographical/floral origin and/or biological activities to achieve higher incomes with the marketed products (García, 2018; Machado et al., 2020).

Studies carried out in other countries demonstrate that techniques such as near-infrared spectroscopy (NIR) and RAMAN in combination with chemometrics serve to discriminate between authentic honey and imported and/or adulterated honey (Guelpa et al., 2017; Oroian et al., 2018). Likewise, NMR can be useful to verify the botanical origin and detect added sugars (Kuballa et al., 2018), while GC-MS and LC-MS can help to distinguish between mature honey and immature honey, which significantly alters its profile and, therefore, its final quality (Sun et al., 2021).

Among the selected articles, a second approach was adopted regarding the analysis of the geographic origin of the honey samples. The southern region encompassed 33% of the outputs, followed by the southeast (25%), midwest (8%), northeast (6%), and northern (6%) regions. However, 17% of the articles did not indicate where honey sample have been collected in Brazil. Importantly, the southern region encompasses the states of Santa Catarina, Paraná, and Rio Grande do Sul and account for 41.7% of the Brazilian honey production, being an important exporter of the product (IBGE, 2017). Finally, GC was used in two studies with honey samples from the southern region, as LC and spectrometry were found in four and six investigations, respecitvely.

Even with the growing use of modern analytical techniques for characterization and detection of adulteration in Brazilian honeys, it is worth mentioning that a series of honey producer states in the country have not been investigated so far, as herein shown. Indeed, Brazil has 26 states and the Federal District, but only for a few (i.e., 15, Table 1) metabolomic analysis of honey samples were found. In general, spectroscopy techniques have been mostly applied for metal detection, while gas and liquid chromatography stand out in the detection of pesticides. Interestingly, Brazil is the largest consumer of pesticides worldwide since 2008, what has led not only to an increase in bee mortality, but also to the contamination of bee products (Nunes et al., 2021).

Aiming at to promote food safety to consumers, currently, some government programs have been performed to prevent product fraud in Brazil. The sanitary control of food in the market is carried out by the National Health Surveillance Agency (ANVISA, in Portuguese), anchored in the Ministry of Health, as well as by the Ministry of Agriculture, Livestock, and Supply (MAPA, in Portuguese). The National Plan for the Control of Residues and Contaminants (PNCRC, in Portuguese) is a risk management tool adopted by MAPA, with the aim of promoting the chemical safety of foods of animal origin. In it, residues of antimicrobial drugs, pesticides, inorganic contaminants, antiparasitic agents, dyes, and others have been ordinarily analyzed (Brazil, 1999; Brazil, 2019a). Another action is the Program for the Analysis of Pesticide Residues in Food (PARA), created in 2001, due to the increasing use of pesticides in the country (Brazil, 2019b).

The mentioned programs use chromatographic and spectroscopic techniques, being carried out through the Federal Agricultural Defense Laboratories, where various foods are monitored. The same techniques are used in the subprogram established in Brazil from the PNCRC, the Honey Residue Control Program (PCRM, in Portuguese). The purpose of this is to investigate infractions resulting from the misuse of veterinary drugs or environmental contaminants, not taking into account the typification or analysis of adulterants, such as extenders or sugars. Currently, PCRM determines some antimicrobial analytes, halogenated and organochlorine compounds, carbamates and pyrethroids, organophosphates, and inorganic contaminants (Brazil, 1999; Brazil, 2014). However, considering the excessive use of pesticides and the rampant release, especially in the last 4 years in the country, the number of analyzed compounds has been lower than the annual release of new molecules (Nunes et al., 2021; Brazil, 2022).

As far as it is known, Brazil still does not have a food fraud and adulteration database, and it is possible to obtain records only in scientific research and in national program reports, focused only on some foods and in certain periods, with no analysis of bee products. Databases available in the European Union or in the United States have proven to be efficient in recording the history of adulteration, enabling support preventive measures and improve detection techniques (Tibola et al., 2018). The improvement of these detections should help in the release of norms and legislation because, in some cases, the current analyzes are not efficient in detecting all the means used for adulteration of the product. Ritten et al. (2019) report that the lack of databases can become a major problem even for the traceability of a fraudulent product, leading to continuous recurrence and with the inclusion of many prohibited additives, chemical substitutes, microorganisms, and high rates of pesticides.

As described above, modern analytical techniques based on chromatographic and spectroscopic methods have been adopted over the world to improve the quality control pipelines of honeys, mainly when combined with chemometrics. However, it is noticed that such techniques are still little used in Brazil for discrimination and fraud detection in honeys. In light of this, with the continuous improvement of adulteration techniques and stricter food safety standards adopted in other countries, it is expected that Brazil will be induced to establish and adopt new fraud detection methods, eventually based on metabolomics, and criteria, i.e., legislation, to keep being a major exporter of this bee product (Marquele-Oliveira et al., 2017; García, 2018).

4. Conclusion

The legislation in force in Brazil that determines the physicochemical parameters in honey is considerably out of date (the 2000s) and recognized as unable to detect all forms of adulteration and, accordingly, to protect consumer health and beekeepers as expected. Besides, it is noticeable that there are few researches carried out between 2010-2020 with Brazilian honeys using more robust techniques, considering the social and economic importance of beekeeping for the country. In fact, chromatographic and spectroscopic techniques have provided greater food security and can even revert financially to beekeepers through the identification of *fingerprints* and biochemical markers that allow adding value to the products. Thus, metabolomics in conjunction with chemometrics has been proposed as an efficient and updated approach for the characterization and detection of honey adulterations in the country, as already noted in many developed countries. Finally, in addition to the adoption of modern analytical techniques for the quality control of Brazilian honey, the lack of databases continuously added of records on the chemical and microbiological quality of that food makes it difficult to block its illegal entry in the country and its internal tracking, setting precedents for new means of adulteration to be carried out. It is suggested that to increase monitoring of the quality of Brazilian honey, more research is carried out focusing on the typification and analysis of adulterants.

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