UNIVERSIDADE ESTADUAL PAULISTA “JULIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS
CÂMPUS DE BOTUCATU

DETERMINAÇÃO DE AMINOÁCIDOS E AMINAS BIOGÊNICAS APLICADA À TIPIFICAÇÃO DE MÉIS FLORAIS PRODUZIDOS NO ESTADO DE SÃO PAULO

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Pós-doutorado: Relatório final
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BOTUCATU – SP
Junho – 2024

UNESP – CAMPUS BOTUCATU
DEPARTAMENTO DE QUÍMICA E BIOQUÍMICA, DO INSTITUTO DE BIOCIÊNCIAS
LABORATÓRIO: BIOQUÍMICA

PÓS-DOUTORADO: RELATÓRIO FINAL

Relatório FINAL: JUNHO de 2024.

Título: Determinação de aminoácidos e aminas biogênicas aplicada à tipificação de méis florais produzidos no Estado de São Paulo.

ESTÁGIO PÓS-DOUTORAL.

Linha de Pesquisa: Bioquímica dos Alimentos.

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Resumo do projeto proposto

O mel e seus produtos derivados estão se tornando substâncias naturais valorizadas pelas suas propriedades biológicas. A composição química do mel é afetada por vários fatores, incluindo espécies de abelhas, a planta a partir da qual foi coletado néctar (específico para cada estação), sazonalidade e a área geográfica. Dada a diversidade de tipos de méis no mercado e à demanda crescente deste alimento, métodos analíticos precisos e robustos são requeridos à padronização de procedimentos e critérios que garantam a autenticidade e a origem dos méis, aspectos de interesse mútuo de produtores idôneos e consumidores. Estas análises químicas de composição, além de serem importantes para a identificação de compostos com atividades farmacológicas, também são fundamentais para uso como marcadores na determinação de origem botânica e verificação de possíveis adulterações. Sendo assim, a presente proposta enfoca a determinação do perfil bioquímico, com ênfase na composição de aminoácidos (i.e., triptofano e 5-hidroxitriptofano) e aminas biogênicas em méis de diferentes regiões e sazonalidades no estado de São Paulo, visando a identificação de compostos com atividades farmacológicas, indicadores de qualidade nutricional e/ou funcional. Além disso, essas análises propiciam a utilização destes compostos supracitados como marcadores bioquímicos para a determinação de origem das amostras analisadas. Dessa forma, compostos biologicamente ativos relevantes foram investigados como possíveis marcadores bioquímicos para determinar a origem geográfica das amostras analisadas. Os resultados da proposta permitiram identificar amostras de mel com base em diversas características de qualidade, incluindo sua região de produção, época de colheita e constituintes biologicamente ativos.

Palavras-chave: época de colheita, antioxidantes, compostos bioativos, indicação geográfica, marcadores bioquímicos.

1. Introdução Geral

O mel é o produto apícola mais consumido em todo o mundo, devido ao seu sabor característico e marcante e, às propriedades nutricionais e terapêuticas. Entre seus efeitos estão os antimicrobianos, anti-inflamatórios, anticancerígenos, antiaterogênicos, antitrombóticos e antioxidantes, além de sua atividade analgésica no corpo humano (Siddiqui et al., 2017). Além disso, é fonte de nutrientes como água, açúcares, minerais, vitaminas, enzimas, proteínas, ácidos orgânicos e aminoácidos (De-Melo et al., 2018).

O Brasil é um dos principais produtores de mel do mundo. Devido, principalmente à sua diversidade botânica, biomas e clima tropical, além de, possibilitar que o Brasil produza mel durante todo o ano, assim, tornando-o um importante exportador para o resto do mundo. De acordo com a Associação Brasileira dos Exportadores de Mel (ABEMEL) em 2017, o país exportou cerca de 27.103 toneladas de mel, ocupando o 9º lugar no mercado global (ABEMEL, 2018). Recentemente, em 2021 o Brasil exportou 40.496 toneladas, como principal destino os EUA, com preço médio de US$ 3,42 por kg, aumento de 33% se comparado com 2017 (ABEMEL, 2022). O estado de São Paulo também tem representatividade nessa produção nacional, produzindo em 2020 em torno de 4.534 toneladas (IEA, 2024).

Portanto, o consumo e produção consciente, além da busca por alimentos de boa qualidade nutricional, visando principalmente a prevenção de doenças, tem sido prioridade para maioria dos consumidores. Além disso, há a busca por consumir alimentos que proporcionem bem-estar, tanto físico quanto emocional (Strasser, Gostner e Fuchs 2016). Os compostos fenólicos (ácidos fenólicos, flavonoides, flavonas, flavanonas, flavanóis, antocianidinas e isoflavonas) parecem ser os constituintes mais importantes do mel, responsáveis por sua atividade antioxidante (Sancho et al., 2016; Nascimento et al., 2018). No entanto, as aminas bioativas, assim como seus aminoácidos precursores, têm sido objeto de muitos estudos, devido ao grande interesse em relação ao paradoxo nutricional e sua possível ação como agente antioxidante e bem-estar. Essas substâncias também estão relacionadas à regulação do ciclo celular e desempenham um papel fundamental na rota de síntese de importantes neurotransmissores (Gomez-Gomez et al., 2018), como serotonina e dopamina.

Alguns estudos relatam a presença de aminas biogênicas em amostras de mel e outros produtos derivados de abelhas, no entanto, a presença de aminoácidos livres em

produtos apícolas pode levar à formação de aminas biogênicas (BA), que podem ser desejáveis ou indesejáveis em produtos alimentícios (Ruiz-Capillas e Herrero, 2019), tornando mais pesquisas essenciais. A ingestão de aminoácidos como o L-triptofano (L-Trp) e 5-hidroxitriptofano (5-OH-Trp), por exemplo, é essencial para a formação de serotonina no cérebro. A serotonina, um neurotransmissor, não atravessa a barreira hematoencefálica (Goihl, 2006), assim, fica dependente da ingestão de aminoácidos que derivam a mesma. Em humanos, devido à sua essencialidade, a dose diária recomendada de L-Trp é de cerca de 4 mg kg^{-1} de peso corporal por dia para adultos e 12 mg kg^{-1} de peso corporal para crianças (Palego et al., 2016). Assim, alimentos contendo níveis mais altos de L-Trp e 5-OH-Trp podem ajudar a equilibrar os níveis de serotonina no cérebro humano. Vale ressaltar que o mel é fonte não só de L-Trp, mas também de outros metabólitos derivados desse aminoácido e importantes para a saúde humana, como nicotinamida (vitamina B6), melatonina, triptamina, quinurenina, 3-hidroxiquinurenina e ácidos quinolínico e xanturênico (Friedman, 2018).

Além disso, entre outros parâmetros, o teor de aminoácidos também foi proposto como um método para determinar a proveniência botânica e/ou geográfica de produtos alimentícios, e o mel tem sido frequentemente o alvo dessa abordagem (Kelly, Blaise e Larroque, 2010). O mel e seus derivados têm sido substâncias naturais cada vez mais valorizadas no mercado, no entanto, suas propriedades físico-químicas e biológicas são dependentes de diferentes fatores, incluindo a espécie de abelha, a espécie de planta com floração e doadora de néctar (específica para cada estação), área geográfica/origem, condições sazonais, estágio de maturação, processamento de colheita e armazenamento e condições climáticas características de cada local (Machado De-Melo et. al., 2018; Pavlova et al., 2018). Um dos principais fatores está a origem floral do mel, que está associada aos seus aspectos organolépticos, como cor e sabor (Marcazzan et al., 2017), bem como ao seu valor nutricional (Al-Mosa et al., 2019). Portanto, é importante ampliar as informações sobre composição química e explorar a atividade biológica de méis de diferentes regiões e métodos de extração, entre outros. Devido à sua alta complexidade, a análise química do mel representa um desafio considerável.

Assim, a determinação identificação compostos da classe das aminas biogênicas, bem como seus aminoácidos precursores (i.e., Trp e 5-OH-Trp) em méis de diferentes regiões agroecológicas, constitui uma abordagem investigatória relevante à identificação de padrões à tipificação. Tal assertiva se traduziu na hipótese de trabalho deste projeto.

Este projeto de pós-doutoramento foi inserido em um projeto maior, o qual foi aceito e financiado pela FAPESC/FAPESP em parceria entre instituições de pesquisa como a UNESP e a UFSC. Os resultados encontrados foram publicados em Anais de Congresso e artigos científicos, os quais serão apresentados neste relatório final.

A apresentação dos resultados finais foi organizada em 3 capítulos. No **primeiro capítulo**, serão apresentados os resumos "Characterization of polyfloral honeys produced in the states of Santa Catarina and São Paulo through uv-vis spectrophotometry, near-infrared spectroscopy, and 1h-nuclear magnetic resonance spectroscopy" e "Bioactive amines and their precursors amino acids in polyfloral honeys", que foram apresentados no 14° SLACA (Simpósio Latino Americano de Ciências dos Alimentos: Impacto na Ciência de Alimentos na Saúde e na Doença). No **segundo capítulo** será apresentado o artigo "Tryptophan and Biogenic Amines in the Differentiation and Quality of Honey", que foi publicado na Revista International Journal Of Tryptophan Research. No **terceiro capítulo** será apresentado o artigo "Biogenic amines and stable isotopes in the quality and authenticity of honeys from Brazil", publicado na Food Chemistry.

CAPÍTULO 1

Resumos apresentados no 14 SLACA, Campinas, SP

Evento promovido pela UNICAMP

Characterization of polyfloral honeys produced in the states of Santa Catarina and São Paulo through uv-vis spectrophotometry, near-infrared spectroscopy, and 1h-nuclear magnetic resonance spectroscopy

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Abstract: Honey is a chemically complex matrix because of the great diversity of forage sources for honey bees in the Brazilian flora. Over the years, efforts have been made to improve the analytical techniques for characterizing honey, further enhancing the value of the product in the market. In this sense, this study aimed to chemically characterize polyfloral honeys originated from the states of Santa Catarina (SC, n = 51) and São Paulo (SP, n = 10) through a typical metabolomic analytical platform, i.e., UV-vis spectrophotometry (200-800 nm), near-infrared (NIR, 800-2500 ηm), and 1H nuclear magnetic resonance (1H-NMR) spectroscopy. Honey samples from the 2019-2020 harvest were analyzed in triplicate and the metabolomic data set was pre-processed (baseline correction, smoothing, and multiplicative dispersion correction), following principal component analysis (PCA). Besides, the total phenolic content (TPC) of the samples was determined (Folin-Ciocalteau reagent) and the five samples with higher contents for each state were further profiled by 1H-NMR (300 MHz). The PCA of the UV-vis data set covered 97% of the sample's total variance and discriminated the SP honeys (PC2+, PC1-) from the SC ones. The NIR spectral profiles revealed prominent bands assigned to water (0-H) and carbohydrates (O-H and C-H), but the description models built did not discriminate samples. The TPC of SC honeys varied between 2.33 and 9.53 mg GAE.g-1, as SP ones showed lower contents (0.76 to 5.83 mg GAE.g-1).

Besides, the five honey samples with the highest TPC revealed distinct 1H-NMR profiles in respect to their geographic origins, i.e., SC and SP. It was found that the analytical techniques employed, especially UV-vis, can help quickly, cheaply, and cleanly characterize the SC and SP polyfloral honeys, revealing different chemical profiles which may be linked to the diversity of flora and Other (a)biotic factors in the production regions.

Funding: Fapesp (2019/27227-1 and 2021/01905-3), Fapesc (2020TR1452), CNPq (425248/2018-6 and 304657/2019-0), and CAPES.

Key-words: metabolomics, chemometrics, pattern recognition techniques.

Bioactive amines and their precursors amino acids in polyfloral honeys

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Bioactive amines (BA) have important neurological and antioxidant functions in the human body, in addition to acting in the prevention and reduction of symptoms caused by COVID-19. This study aimed to evaluate those metabolites and their precursors amino acids in honeys collected in Santa Catarina and São Paulo states (Brazil). Analyzes were performed by HPLC and data were submitted to multivariate analysis. Tryptophan (Trp), L-dopa (L-Dop), and tryptamine (Tryp) stood out for their contents in SC-honeys from agroecological zones of altitude as Campos de Lages (Trp: 9483.94, L-Dop: 0.46, and Tryp: 197.41 µg/100 g), Vale do Rio do Peixe (Trp: 7797.87, L-Dop: 0.93, and Tryp 191.98 µg/100 g), and Planalto Serrano São Joaquim (Trp: 7337.70, L-Dop: 0.91, and Tryp: 159.52 µg/100 g). In contrast, dopamine, serotonin, and melatonin were detected in coastal and valley regions (Cfa). Honeys from the region of Vale do Itajaí, with a predominant cinnamon flowering, presented higher levels of dopamine (39.61 µg/100 g), as those from Litoral de Laguna and Vale do Itajaí showed higher levels of serotonin and melatonin (748.38 and 127.01 µg/100 g, respectively). For the SP honeys, appreciable amounts of Trp (1158.59 µg/100 g) were detected in samples from Avaré (Cfa). Results reveal that the amino acids and BA levels in

honeys vary depending on the region of production, as well as on the nectar donor plants. This analysis, in addition to being important for the identification of compounds with pharmacological activities, can also be used as biochemical markers identification of honey's botanical origin. This is a pioneering study regarding the investigation of BA in honeys from different ecological zones of Brazil. The search for substances with important biological properties creates new opportunities, such as the emergence of market niches for honey, stimulating the beekeeping activity, especially in the context of family farming.

Funding: FAPESP (2019/27227-1 and 2021/01905-3), FAPESC (2020TR1452) and CNPq (307571/2019-0 and 304657/2019-0)

Key-words: tryptophan, dopamine, serotonin.

CAPÍTULO 2

ARTIGO PUBLICADO NA REVISTA

International Journal Of Tryptophan Research

Tryptophan and Biogenic Amines in the Differentiation and Quality of Honey

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ABSTRACT

Honey is a natural product with beneficial properties to health and has different characteristics depending on the region of production and collection, flowering and climate. The presence of precursor amino acids of- and biogenic amines can be important in metabolomic studies of differentiation and quality of honey. We analyzed 65 honeys

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from 11 distinct regions of the State of Santa Catarina (Brazil) as to the profile of amino acids and biogenic amines by HPLC. Altitude and flowering influenced the content of L-tryptophan (Trp), tryptamine (Tryp) and L-dopa (L-Dop), which stood out in regions with Cfb climate and ranged depending on the altitude. In 522 - 1007 m the levels of L-Trp, Tryp and L-Dop were 9483.94, 197.41 and 0.46 µg/100 g, respectively and since 973 to 1280 m, the content of L-Trp was 7797.87, L-Dop was 0.93, and Tryp was 191.98 µg/100 g. In higher altitudes (1051 up to 1309 m – Planalto Serrano de São Joaquim), we detected levels lower than other ones. In contrast, we found high content of serotonin, melatonin and dopamine in coastal and valley regions (Cfa). Honeys from region with a predominant cinnamon flowering, exhibited high dopamine' content. Results indicate that the amino acids and biogenic amine levels in honeys are good indicators of origin. These data warrant further investigation on the honey as source of amino acids precursor of serotonin, melatonin and dopamine, what can guide the choice of food as source of neurotransmitters.

KEYWORDS: L-tryptophan, serotonin, melatonin, L-dopa, dopamine

Introduction

Honey is known for its antimicrobial, anti-inflammatory, anticancer, antiatherogenic, antithrombotic and antioxidant effects, in addition to its analgesic activity in the human body (Siddiqui et al. 2017). Conscious consumption and the search for foods with good nutritional quality, aiming at the prevention of diseases, has been a priority in the human diet. In addition, there is also an interest in eating foods that provide well-being, both physical and emotional (Strasser, Gostner, and Fuchs 2016). Phenolic compounds seem

to be the most important constituents of honey, responsible for its antioxidant activity (Sancho et al. 2016). However, bioactive amines, as well as their precursor amino acids, have been the subject of many studies, due to the great interest regarding the nutritional paradox and their possible action as an antioxidant/well-being agent. These substances are also related to regulation of the cell cycle and play a fundamental role in the synthesis route of important neurotransmitters (Gomez-Gomez et al. 2018), such as serotonin and dopamine.

Some studies report the presence of biogenic amines in samples of honey and other bee-derived products (i.e., propolis), which have biological properties and can be used beneficially in the food and pharmaceutical industry. The presence of free amino acids in bee products can lead to the formation of biogenic amines (BA), which may be desirable or undesirable in food products (Ruiz-Capillas and Herrero 2019), making further research essential.

The ingestion of L-tryptophan (L-Trp) and 5-hydroxytryptophan (5-OH-Trp), for example, is essential for the formation of serotonin in the brain. Serotonin, a neurotransmitter, does not cross the blood–brain barrier (Goihl 2006) and the synthesis and turnover of this monoamine depend on the intake of amino acids. In humans, due to its essentiality, the recommended daily dose of L-Trp is around 4 mg/kg body weight per day for adults and 12 mg/kg body weight for children (Palego et al. 2016). Thus, foods containing higher levels of L-Trp and 5-OH-Trp can help balance serotonin levels. It is noteworthy that honey is a source not only of L-Trp, but also of other metabolites derived from this amino acid and important to human health, such as nicotinamide (vitamin B6), melatonin, tryptamine, kynurenine, 3-hydroxykynurenine, and quinolinic and xanthurenic acids (Friedman 2018). Furthermore, among other parameters, amino acid

content has also been proposed as a method to determine the botanical and/or geographic provenance of food products, and honey has often been the target of this approach (Kelly, Blaise, and Larroque 2010).

Honey and its derivatives have been increasingly valued natural substances; however, their physicochemical and biological properties are determined by many factors, including bee species, the nectar donor plant (specific for each season), geographic area/origin, seasonal conditions, harvesting and storage processes, and climatic conditions (Machado De-Melo et al. 2018). Therefore, it is important to expand the information on chemical composition and explore the biological activity of honeys from different geographic regions and methods of extraction, among other things. Due to its high complexity, the chemical analysis of honey poses a considerable challenge. Therefore, this study aimed to identify compounds from the biogenic amine class, as well as their precursor amino acids (i.e., Trp and 5-OH-Trp) in honeys from different agro-ecological regions.

METHODS

Honey collection places

Samples of honey (65) harvested in 2018–2019 were kindly provided by beekeepers from 11 agroecological regions of Santa Catarina State (Brazil), namely: 1) Planalto Serrano de São Joaquim, 2) coast, Itajaí and Tijucas River Valleys, 3) coast of Florianópolis and Laguna, 4) Alto Vale do Itajaí, 5) Carboniferous, Extreme South and Colonial Serrana, 6) Uruguai River Valley, 7) Alto Vale do Rio do Peixe and Alto Irani, 8) Central Plateau,

9) Northern Santa Catarina Plateau, 10) Santa Catarina Northwest and 11) Campos de Lages (Table 1). All honey samples are polyfloral, because, in addition to presenting the predominant flowering described by beekeepers, they all contain pollen from native species of the regions of origin.

Extraction and chromatographic analysis

Biogenic amines (serotonin – Ser, melatonin – Mel, dopamine – Dop and tryptamine – Tryp), L-Trp, 5-OH-Trp and L-dopa were extracted as described by (Lima et al. 2008). Honey samples (1 g) were mixed with 3 mL of perchloric acid (5%, v/v), homogenized (vortex, 1 min) and incubated in a cold ultrasonic bath (30 min), followed by centrifugation (10 min, 6000 *g*, 5 °C). The supernatant containing the free amines and amino acids was collected and subjected to derivatization using supernatant, sodium carbonate buffer and of dansyl chloride. The solution was kept at 60 °C for 1 h and, in sequence, proline (1 mg/mL) was added and the mixture kept in the dark for 1 h and homogenized by vortexing every 15 min. After this interval, toluene (HPLC grade) was added, the mixture was vortexed (1 min) and the supernatant (hydrophobic part containing the target compounds) was dried in a nitrogen line.

The samples were resuspended in acetonitrile (HPLC grade), homogenized by vortexing and incubated (1 min) in an ultrasonic bath, followed by filtration (0.25 μm) and injection in a high-performance liquid chromatograph (HPLC; Dionex UltiMate 3000; Thermo Fisher Scientific, Bremen, Germany), according to (Dadáková, Křížek, and Pelikánová 2009), with modifications. Briefly, 20 μL of sample was injected into an ACE C18 reverse phase column (4.6 × 250 mm; 5 μm), thermostatted at 25 °C, coupled to a quaternary automatic sampler 3000 pump and diode array detector (DAD-3000RS).

Amines and amino acids were eluted in a gradient system, as follows: 0–2 min, 40% A + 60% B; 2–4 min, 60% A + 40% B; 4–8 min, 65% A + 35% B; 8–12 min, 85% A + 15% B; 12–15 min, 95% A + 5% B; 15–21 min, 85% A + 15% B; 21–22 min, 75% A + 25% B; 22–25 min, 40% A + 60% B. Identification of biogenic amine analytes and amino acids of interest (e.g. L-Trp, 5-OH-Trp, L-dopa, Ser, Mel and Dop) was based on the retention times of analytical standards (Sigma-Aldrich, MO, USA), with detection at λ= 225 nm. For the purposes of compound quantification (μg/100 g), the peak areas were integrated using Chromeleon 7 software (Thermo Fisher Scientific, Bremen, Germany). The limit of detection (LOD), limit of quantification (LOQ), linear regression, regression coefficient (R^2), recovery and repeatability values (n = 6) observed are shown in Table 2. Repeatability (below 5%) was determined using six replicates/honey samples chosen at random. Mean recovery (%, n = 6) was tested with five concentrations (12.5, 50, 100, 150 and 200 mg/L) of analytical standard (Table 2).

Statistical analysis

Data on biogenic amines and their precursors found in honey samples were submitted to analysis of variance (ANOVA), and if the data were significant, they were submitted to the Scott Knott test ($P < 0.05$). The ANOVA and the mean comparison test were performed using SISVAR statistical software (Ferreira, 2011). Principal component analysis (PCA; XLSTAT software, version 2020; Addinsoft, France) was applied to visualize the possible correlation between amino acid content and the different classes of biogenic amines and the agroecological regions where the samples were collected.

Results and discussion

Currently, honey and its products have been valued as natural foods. However, it and its physicochemical and biological properties are affected by several factors of (a)biotic nature. The results clearly reveal that the composition of floral honeys produced in southern Brazil is dependent on several factors, including the geographical area of production, that is, the agroecological region of production and collection, as well as the nectar donor plants (specific for each season), as also verified in other similar studies (Machado De-Melo et al. 2018).

In an attempt to establish a descriptive model for the grouping of precursor amines and amino acids according to the different agroecological regions of collection, as well as flowers from which nectar is collected, it was decided to compare the results obtained by PCA. Honeys from the Northern Plateau of Santa Catarina (3B2), from the coast of Florianópolis and Laguna (1B2), from Alto Vale do Itajaí (Cfa climate) (2A6) and from the agroecological region of the Planalto Serrano de São Joaquim (Cfb climate) (5.5 and 5.7) stood out for their Trp and Tryp content (PC1+ and PC2+). As Trp is a precursor of Ser, its presence may be important for the control of some physiological disorders, such as obsessive-compulsive disorder (Arzoo 2017). Ser and Dop ($r = 0.89$, $p < 0.05$), as well as the amino acid L-dopa with these amines, showed a significant and strong correlation (Ser: $r = 0.76$ and Dop: 0.79, $p < 0.05$). Honey from the agroecological regions Alto Vale do Rio do Peixe and Alto Irani (4B6) showed the highest levels of these amines (Ser: 495.15 µg/100 g; Dop: 33.98 µg/100 g) and the precursor amino acid L-dopa (0.75 µg/100 g) (PC1+ and PC2−) (Figure 1, Table 3).

L-Trp levels ranged from 263.31 µg/100 g (2A4) to 3358.65 µg/100 g (1B2) (Table 3). This difference is attributed to the plant species visited by the bees during nectar collection, as well as the honeys' geographic origin and the time of harvest. Other studies highlight L-Trp values greater than 14 mg/kg in rosemary honeys (Soto et al. 2011), while levels reached 1.9 mg/100 g in sunflower honeys (Qamer et al. 2007) and 1.10 mg/100 g in lavender honey (Hermosín, Chicón, and Cabezudo 2003). Among the samples analyzed, the lowest L-Trp content was found in honey whose main flowering was *Hovenia dulcis* (Japanese grape) (2A4), from the Atlantic Forest region, with a Köppen climate classification of Cfa. On the other hand, the honey with the highest L-Trp content (3358.65 µg/100 g) was produced in the coastal region of Santa Catarina, whose predominant bloom was composed of wild flowers (Table 1). Some articles describe that L-Trp content varies depending on the predominant flora. Hermosín, Chicón, and Cabezudo (2003) detected L-Trp values of between 0.19 and 1.10 mg/100 g and the variation was dependent on the predominant flowering, that is, the lowest content was found in orange blossom honeys and the highest in lavender honeys. The authors claim that amino acid composition does not exactly distinguish the botanical origin of honeys, or their authenticity. In our study, the L-Trp content of most samples is very close to that found in the literature; however, it is worth noting that the botanical origin, as well as the climate and region, can affect the level of metabolites, including amino acids.

L-Trp found in honeys may come from plants (pollen, nectar and resins) or from the metabolism of bees during honey production. In pollen, the contents of this aromatic amino acid are quite variable and may be very low, that is, 0.028 g/kg to 0.197 g/g (Lilek et al. 2015; Vrabie et al. 2019) et al., 2019) or much higher, such as described in *Rhododendron ponticum* pollen (8053.00 µg/g) (Ecem Bayram 2021).

L-Trp in honey has been the subject of several studies related to human health. For example, L-Trp supplementation appears to improve the social behavior of people suffering from disorders due to malfunctioning of the serotonergic system (Steenbergen et al. 2016; Aucoin et al. 2020). Intake of L-Trp has been linked to decreased levels of psychosis in both animal and human studies and sleep deprivation (Aucoin et al. 2020). Other studies have demonstrated that ingestion of honey with milk before bedtime may decrease hypoglycemic effects in diabetic patients (Brod et al. 2013), or improve the sleep quality of healthy people or those with coronary heart problems (Fakhr-Movahedi, Mirmohammadkhani, and Ramezani 2018). Thus, L-Trp supplementation appears to improve control over social behavior in individuals who suffer from disorders or behaviors associated with dysfunctions in serotonergic functioning.

L-Trp is transported into the brain via the leucine-preferring L1 system and may compete with other amino acids (e.g. tyrosine, phenylalanine, leucine, isoleucine and valine) called 'large neutral amino acids' (LNAA) (Strasser, Gostner, and Fuchs 2016). The Trp:LNAA ratio determines the flux of this amino acid and, consequently, the biosynthesis of Ser in the brain (Stone and Darlington 2013). Foods with a high content of L-Trp, such as honey, often also contain other amino acids in varying concentrations. Thus, the net effect of the L-Trp content in honeys is relevant, but it should be considered carefully with regard to possible increases in Ser synthesis, given the competition for a transporter system in the blood–brain barrier between this amino acid and the other LNAA (Strasser, Gostner, and Fuchs 2016). Furthermore, excess Trp may cause adverse reactions, such as gastric problems and dizziness, among others (Steenbergen et al. 2016).

The remainder of the L-Trp that is not used for protein synthesis may be converted to biomolecules such as kynurenine (KYN) (responsible for approximately 90% of L-Trp

catabolism; Richard et al., 2009) or those related to neuroimmunological signaling, such as Ser and Mel. About 1% of dietary Trp is used for Ser synthesis (Comai et al. 2019; Richard et al. 2009), by the conversion of L-Trp to 5-OH-Trp or Tryp, depending on the metabolic pathway. KYN, derived from L-Trp catabolism, originates niacin, precursor of the coenzyme nicotinamide adenine dinucleotide (NAD^+) (Comai et al. 2019). In this pathway, 60 mg of Trp produces 1 mg of nicotinic acid or niacin (Goldsmith 1958). The usual intake of Trp is approximately 900–1000 mg per day (Richard et al. 2009), and the recommended daily dose is around 4 mg/kg body weight per day for adults and 12 mg/kg body weight for children (Palego et al. 2016). According to our results, the consumption of honey as a source of L-Trp may help in several metabolic processes, including psychiatric and neurological disorders and anticancer immunity (Comai et al. 2019). In children and adolescents with autism spectrum disorder, the intake of Trp may decrease symptoms of irritability and mild depression due to increased Ser levels (Kałużna-Czaplińska et al. 2019).

Although there is no consensus on the necessary amount of 5-OH-Trp or Tryp (Ser precursors) to be ingested daily, considerable levels were detected in some honeys: 5-OH-Trp (4B3 – 2045.59 µg/100 g) and Tryp (2A6 – 35.01 µg/100 g and 2C3 – 35.43 µg/100 g) (Table 3). Foods with higher levels of 5-OH-Trp and Tryp may favor the formation of Ser and Mel, due to the ease in crossing the brain–blood barrier (BBB) (Comai et al. 2019), since both the synthesis and the turnover of Ser depend on the intake of L-Trp and 5-OH-Trp (Höglund, Øverli, and Winberg 2019). In this study, we highlighted the maximum content of 5-OH-Trp (2045.59 µg/100 g) in the sample from *Baccharis dracunculifolia* DC (4B3), originating from an altitude of approximately 900 m. The same compound was detected at a lower level (22.99 µg/100 g) in 2A4, from

a lower altitude (Alto Vale do Itajaí), i.e., 593 m, which also showed a lower content of L-Trp (263.31 µg/100 g) and Tryp (6.24 µg/100 g) and no melatonin (Table 3). Tryp has been used as a fermentation marker, along with other amines such as tyramine, cadaverine, putrescine and histamine. This aromatic and heterocyclic diamine can induce vasoactive or psychoactive effects on the human body (Sanlier and Bektesoglu 2021). According to the authors, the consumption of 25–30 mg/kg can cause migraines; however, to reach these values, it would be necessary to consume approximately 900 g of honey from the samples that contain the highest levels of Tryp.

Ser cannot cross the BBB and its intake contributes to a decrease in reactive oxygen species, as described in red blood cells of a mouse model (Amireault et al. 2013), mainly in the process of lipid peroxidation (Azouzi et al. 2017). In the present study, Ser, Dop and the amino acid L-dopa were all detected at higher levels in honeys from the mild Cfb climate. In the present study, Ser was detected in greater amounts (495.15 µg/100 g) in 4B6, a honey from an altitude of 1280 m and Cfb climate (Table 3). In this region, ‘aroeira’ (*Schinus* sp.), ‘aroeira branca’ (*Lithraea molleoides*) and ‘caúna’ (*Ilex theezans* Mart. ex Reissek) are predominant (Table 1). The non-detection of Mel stands out in this sample. The lack of conversion of Ser into Mel could eventually contribute to an increase of Ser in the investigated honey samples. However, this was not observed in samples that did not show detectable levels of Mel. A low Ser content was verified in honeys from 2A2 and 2B2, both from a Cfa climate and from the Atlantic Forest ecosystem, but from different flowerings, regions and altitudes (Table 3).

Higher levels of Mel, unlike Ser and Dop, were detected in honeys from coastal regions (agroecological regions coast of Florianópolis and Laguna, 1B) with a Cfa climate, low altitude (92 m) and predominantly flowering of *Eucalyptus* (1B4). Mel,

unlike Ser, crosses the BBB, in addition to having high antioxidant potential and not being able to be stored in the pineal gland, being released into the bloodstream and rapidly degraded in the liver (Pandi-Perumal et al. 2005). Mel is an essential molecule related to the circadian rhythm; it has immunomodulatory and neuroprotective actions in tumor suppression, in addition to being related to oxidative stress (Vielma et al. 2014). Several studies were carried out during the Covid-19 pandemic using Mel and it has been recommended that the use of this substance may be related to a decrease in side effects due to its anti-inflammatory and antioxidant action (Essa et al. 2020; Kleszczyński et al. 2020). Thus, honeys with higher levels of Mel could be an interesting source of this amine, given its already demonstrated pharmacological effects.

In the studied honeys, the presence of L-dopa and Dop was also evidenced, the levels reaching 0.72 and 33.98 µg/100 g (4B6), respectively (Table 3), in honey from Vale do Rio do Peixe and Alto Irani with an altitude 973 m, Cfb climate and flowering of 'aroeira' (*Schinus* sp.), 'aroeira branca' (*L. molleoides*) and caúna (*I. theezans* Mart. ex Reissek) (Table 1). It is important to mention that the highest Ser content was also detected in these samples.

L-dopa levels in honeys are poorly described in the literature, which makes the data found in this work interesting. L-dopa occurs in plants as it is a precursor of several alkaloids, catecholamines and melanin (Soares et al. 2014). In honeys from Turkey, whose predominant flowering was fava beans (*Vicia fava*), Topal et al. (2020) reported an L-dopa content of 0.0321 mg/10 g, much higher than those found in honeys from Santa Catarina, Brazil. The L-dopa content may be due to the botanical source, as its presence at considerable levels in several plant species has already been described, including in fava bean genotypes, in flowers, leaves and fruits. In humans, L-dopa has been used in

the treatment of Parkinson's disease, characterized by a deficiency in the synthesis of this catecholamine and as Dop cannot cross the BBB, while L-dopa does and is decarboxylated to form Dop in nerve cells (Chagraoui et al. 2020). The analyzed honeys have been demonstrated to be a source of both substances and may be beneficial as adjuvants in therapies aimed at increasing the content of L-dopa and Dop.

Conclusion

The amino acid and biogenic amine content of floral honeys vary depending on several factors, including the agroecological region of production and collection, as well as the nectar donor plants (specific for each season). Ser and Dop, as well as the amino acid L-dopa, showed a significant and strong correlation and were detected in higher levels in honey from agroecological regions with a milder climate (Cfb) and at higher altitudes. A higher content of 5-OH-Trp was also found in samples from a milder climate. On the other hand, Trp and Tryp, as well as Mel, were found at higher levels in honeys from warmer regions (Cfa climate).

Author contribution

CVB: Formal analyses, statistical analysis, writing (draft preparation and review & editing). AN: Methodology and data curation, writing (data preparation). VEC: Methodology. ROO: Methodology and data curation. LSPB: Methodology, data curation and statistical analysis. GCM: Methodology and data curation. MM: Conceptualization, formal analysis, supervision and project administration, writing (original draft preparation and review & editing), and funding acquisition. GPPL: Conceptualization,

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Funding

This work was supported by the São Paulo Research Foundation (FAPESP) (grants 2016/22665-2 and 2019/27227-1); the Foundation for Research Support of Santa Catarina (2020TR1452); and the National Council for Scientific and Technological Development (CNPq) (grants 307571/2019-0, 304657/2019-0 and 304657/2019-0).

REFERENCES

Amireault, Pascal, Elisa Bayard, Jean Marie Launay, David Sibon, Caroline Le Van Kim, Yves Colin, Michel Dy, Olivier Hermine, and Francine Côté. 2013. "Serotonin Is a Key Factor for Mouse Red Blood Cell Survival." *PLoS ONE* 8

(12): 8–13. https://doi.org/10.1371/journal.pone.0083010.

Arzoo, Milind Parle. 2017. “ANTI-OBSESSIVE – COMPULSIVE ACTIVITY OF HONEY” 10 (5): 206–9.

Aucoin, Monique, Laura Lachance, Kieran Cooley, and Sean Kidd. 2020. “Diet and Psychosis: A Scoping Review.” *Neuropsychobiology* 79 (1): 20–42. https://doi.org/10.1159/000493399.

Azouzi, Slim, Hubert Santuz, Sandrine Morandat, Catia Pereira, Francine Côté, Olivier Hermine, Karim El Kirat, et al. 2017. “Antioxidant and Membrane Binding Properties of Serotonin Protect Lipids from Oxidation.” *Biophysical Journal*. https://doi.org/10.1016/j.bpj.2017.03.037.

Brod, Meryl, Betsy Pohlman, Michael Wolden, and Torsten Christensen. 2013. “Non-Severe Nocturnal Hypoglycemic Events: Experience and Impacts on Patient Functioning and Well-Being.” *Quality of Life Research* 22 (5): 997–1004. https://doi.org/10.1007/s11136-012-0234-3.

Chagraoui, Abdeslam, Marie Boulain, Laurent Juvin, Youssef Anouar, Grégory Barrière, and Philippe De Deurwaerdère. 2020. “L-DOPA in Parkinson’s Disease: Looking at the ‘False’ Neurotransmitters and Their Meaning.” *International Journal of Molecular Sciences* 21 (1). https://doi.org/10.3390/ijms21010294.

Comai, S., A. Bertazzo, M. Brughera, and S. Crotti. 2019. “Tryptophan in Health and Disease _ Enhanced Reader.Pdf.” In *Advances in Clinical Chemistry*, 165–217.

Dadáková, Eva, Martin Křížek, and Tamara Pelikánová. 2009. “Determination of Biogenic Amines in Foods Using Ultra-Performance Liquid Chromatography (UPLC).” *Food Chemistry* 116 (1): 365–70. https://doi.org/10.1016/j.foodchem.2009.02.018.

Ecem Bayram, Nesrin. 2021. “Vitamin, Mineral, Polyphenol, Amino Acid Profile of Bee Pollen from Rhododendron Ponticum (Source of ‘Mad Honey’): Nutritional and Palynological Approach.” *Journal of Food Measurement and Characterization* 15 (3): 2659–66. https://doi.org/10.1007/s11694-021-00854-5.

Essa, Musthafa Mohamed, Hamdan Hamdan, Saravana Babu Chidambaram, Buthainah Al-Balushi, Gilles J. Guillemin, David M. Ojcius, and M. Walid Qoronfleh. 2020. “Possible Role of Tryptophan and Melatonin in COVID-19.” *International Journal of Tryptophan Research*. https://doi.org/10.1177/1178646920951832.

Fakhr-Movahedi, Ali, Majid Mirmohammadkhani, and Hossein Ramezani. 2018. “Effect of Milk-Honey Mixture on the Sleep Quality of Coronary Patients: A Clinical Trial Study.” *Clinical Nutrition ESPEN* 28: 132–35. https://doi.org/10.1016/j.clnesp.2018.08.015.

Friedman, Mendel. 2018. “Analysis, Nutrition, and Health Benefits of Tryptophan.” *International Journal of Tryptophan Research* 11. https://doi.org/10.1177/1178646918802282.

Goihl, J. 2006. “Tryptophan Can Lower Aggressive Behavior.” *Feedstuffs* 78: 12–22.

Goldsmith, G.A. 1958. “Niacin-Tryptophan Relationships in Man and Niacin Requirement.” *The American Journal of Clinical Nutrition* 6 (April): 479–86.

Gomez-Gomez, Hector Alonzo, Igor Otavio Minatel, Cristine Vanz Borges, Marcia Ortiz Mayo Marques, Evandro Tadeu da Silva, Gean Charles Monteiro, Marlon Jocimar R. da Silva, Marco Antonio Tecchio, and Giuseppina Pace Pereira Lima. 2018. “Phenolic Compounds and Polyamines in Grape-Derived Beverages.” *Journal of Agricultural Science* 10 (12): 65. https://doi.org/10.5539/jas.v10n12p65.

Hermosín, I., R.M. Chicón, and M.D. Cabezudo. 2003. “Free Amino Acid Composition

and Botanical Origin of Honey." *Food Chemistry* 83: 263–68.

Höglund, Erik, Øyvind Øverli, and Svante Winberg. 2019. "Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review." *Frontiers in Endocrinology* 10 (APR). https://doi.org/10.3389/fendo.2019.00158.

Kałużna-Czaplińska, Joanna, Paulina Gątarek, Salvatore Chirumbolo, Max Stanley Chartrand, and Geir Bjørklund. 2019. "How Important Is Tryptophan in Human Health?" *Critical Reviews in Food Science and Nutrition* 59 (1): 72–88. https://doi.org/10.1080/10408398.2017.1357534.

Kelly, Mary T., Alain Blaise, and Michel Larroque. 2010. "Rapid Automated High Performance Liquid Chromatography Method for Simultaneous Determination of Amino Acids and Biogenic Amines in Wine, Fruit and Honey." *Journal of Chromatography A* 1217 (47): 7385–92. https://doi.org/10.1016/j.chroma.2010.09.047.

Kleszczyński, Konrad, Andrzej T. Slominski, Kerstin Steinbrink, and Russel J. Reiter. 2020. "Clinical Trials for Use of Melatonin to Fight against COVID-19 Are Urgently Needed." *Nutrients*. https://doi.org/10.3390/nu12092561.

Lilek, Nataša, Adriana Pereyra Gonzales, Janko Božič, Andreja Kandolf Borovšak, and Jasna Bertoncelj. 2015. "Chemical Composition and Content of Free Tryptophan in Slovenian Bee Pollen." *Journal of Food and Nutrition Research* 54 (4): 323–33.

Lima, Giuseppina Pace Pereira, Suraya Abdallah da Rocha, Massanori Takaki, Paulo Roberto Rodrigues Ramos, and Elizabeth Orika Ono. 2008. "Comparison of Polyamine, Phenol and Flavonoid Contents in Plants Grown under Conventional and Organic Methods." *International Journal of Food Science & Technology* 43 (10): 1838–43. https://doi.org/10.1111/j.1365-2621.2008.01725.x.

Machado De-Melo, Adriane Alexandre, Ligia Bicudo de Almeida-Muradian, María Teresa Sancho, and Ana Pascual-Maté. 2018. “Composición y Propiedades de La Miel de Apis Mellifera: Una Revisión.” *Journal of Apicultural Research* 57 (1): 5–37. https://doi.org/10.1080/00218839.2017.1338444.

Palego, Lionella, Laura Betti, Alessandra Rossi, and Gino Giannaccini. 2016. “Tryptophan Biochemistry: Structural, Nutritional, Metabolic, and Medical Aspects in Humans.” *Journal of Amino Acids* 2016. https://doi.org/10.1155/2016/8952520.

Pandi-Perumal, S. R., N. Zisapel, V. Srinivasan, and D. P. Cardinali. 2005. “Melatonin and Sleep in Aging Population.” *Experimental Gerontology* 40 (12): 911–25. https://doi.org/10.1016/j.exger.2005.08.009.

Qamer, Samina, Mohammed Ehsan, Shahid Nadeem, and Abdul Rauf Shakoori. 2007. “Free Amino Acids Content of Pakistani Unifloral Honey Produced by Apis Mellifera.” *Pakistan Journal of Zoology* 39 (2): 99–102.

Richard, Dawn M., Michael A. Dawes, Charles W. Mathias, Ashley Acheson, Nathalie Hill-Kapturczak, and Donald M. Dougherty. 2009. “L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications.” *International Journal of Tryptophan Research*. https://doi.org/10.4137/ijtr.s2129.

Ruiz-Capillas, Claudia, and Ana M. Herrero. 2019. “Impact of Biogenic Amines on Food Quality and Safety.” *Foods* 8 (2). https://doi.org/10.3390/foods8020062.

Sancho, M. Teresa, Ana Pascual-Maté, Elena G. Rodríguez-Morales, Sandra M. Osés, Isabel Escriche, Ángela Periche, and Miguel A. Fernández-Muiño. 2016. “Critical Assessment of Antioxidant-Related Parameters of Honey.” *International Journal of Food Science and Technology* 51 (1): 30–36. https://doi.org/10.1111/ijfs.12988.

Sanlier, Nevin, and Melike Bektesoglu. 2021. “Migraine and Biogenic Amines,” 1362–71.

Siddiqui, Amna Jabbar, Syed Ghulam Musharraf, M. Iqbal Choudhary, and Atta ur Rahman. 2017. “Application of Analytical Methods in Authentication and Adulteration of Honey.” *Food Chemistry* 217: 687–98. https://doi.org/10.1016/j.foodchem.2016.09.001.

Soares, Anderson Ricardo, Rogério Marchiosi, Rita de Cássia Siqueira-Soares, Rogério Barbosa de Lima, Wanderley Dantas dos Santos, and Osvaldo Ferrarese-Filho. 2014. “The Role of L-DOPA in Plants.” *Plant Signaling and Behavior* 9 (4). https://doi.org/10.4161/psb.28275.

Soto, María E., Ana M. Ares, José Bernal, María J. Nozal, and José L. Bernal. 2011. “Simultaneous Determination of Tryptophan, Kynurenine, Kynurenic and Xanthurenic Acids in Honey by Liquid Chromatography with Diode Array, Fluorescence and Tandem Mass Spectrometry Detection.” *Journal of Chromatography A* 1218 (42): 7592–7600. https://doi.org/10.1016/j.chroma.2011.06.105.

Steenbergen, Laura, Bryant J. Jongkees, Roberta Sellaro, and Lorenza S. Colzato. 2016. “Tryptophan Supplementation Modulates Social Behavior: A Review.” *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2016.02.022.

Stone, Trevor W., and L. Gail Darlington. 2013. “The Kynurenine Pathway as a Therapeutic Target in Cognitive and Neurodegenerative Disorders.” *British Journal of Pharmacology* 169 (6): 1211–27. https://doi.org/10.1111/bph.12230.

Strasser, Barbara, Johanna M Gostner, and Dietmar Fuchs. 2016. “Mood, Food, and

Cognition: Role of Tryptophan and Serotonin." *Current Opinion in Clinical Nutrition and Metabolic Care* 19 (1): 55–61. https://doi.org/10.1097/MCO.0000000000000237.

Topal, Nurdogan, Ibrahim Bulduk, Zeki Mut, Hatice Bozoğlu, and Yener Kortan Tosun. 2020. "Flowers, Pollen and Honey for Use in the Treatment of Parkinson's Disease." *Revista de Chimie* 70 (9): 308–19. https://doi.org/10.37358/RC.20.9.8341.

Vielma, José Ramón, Ernesto Bonilla, Leonor Chacín-Bonilla, Marylú Mora, Shirley Medina-Leendertz, and Yanauri Bravo. 2014. "Effects of Melatonin on Oxidative Stress, and Resistance to Bacterial, Parasitic, and Viral Infections: A Review." *Acta Tropica*. https://doi.org/10.1016/j.actatropica.2014.04.021.

Vrabie, Valeria, Liudmila Yazlovitska, Valentina Ciochină, and Stela Rotaru. 2019. "Comparative Content of Free Aminoacids in Polen and Honey O." *Buletin Ştiinţific. Revistă de Etnografie, Ştiinţele Naturii Şi Muzeologie* 30: 71–78.

CAPÍTULO 3

ARTIGO PUBLICADO
REVISTA INTERNACIONAL FOOD CHEMISTRY

Biogenic amines and stable isotopes in the quality and authenticity of honeys from Brazil

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Abstract

The identification of biogenic amines and some precursor amino acids and the adulteration through stable isotopes was carried out in 114 honey from different geographic regions in Brazil (states of São Paulo (SP) and Santa Catarina (SC)) as support for evaluating quality control and food safety. Serotonin was detected in all samples, while melatonin was quantified in 92.2% of honey from SP and in 94% of SC. L-Dopa, dopamine and histamine appeared at higher levels in honey from SP. Cadaverine, putrescine, spermidine and spermine, varied little according to botanical source. Three

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honey from the metropolitan region of SP were considered adulterated ($C4_{SUGARS}$ >7%), 92 were authentic samples ($C4_{SUGARS}$ −7 to 7%) and 19 unadulterated ($C4_{SUGARS}$ less than −7%), with isotopic values of $\delta^{13}C_H$ and $\delta^{13}C_P$ > 7%. The data were important for differentiating quality as a function of biogenic amines and stable isotope technique was important in detecting honey adulteration.

Keywords: L-Tryptophan, Food Security, Food Adulteration, Geographical Origins

1. **Introduction**

Honey is one of the most consumed foods by humans due to its chemical composition, such as the content of bioactive compounds, including polyphenols, vitamins, sugars and minerals (Silva et al., 2016), which confer therapeutic properties, such as antimicrobial, anticarcinogenic and anti-inflammatory properties (Siddiqui et al., 2017). The chemical components of honey may be quite different depending on the botanical source, geographical regions, collection period, climate conditions, or even storage/processing methods, which can influence the organoleptic character (Zheng et al., 2016; Trifković et al., 2017).

The difference in chemical composition can make it difficult and requires a comprehensive analysis, such as specific compounds to aid authenticity (Trifković et al., 2017), which can be termed biochemical markers, such as biogenic amines. These substances that also act as bioactive compounds are present in honeys (Kim et al., 2021; Borges et al., 2022) and their action can be beneficial, due to the function of monoamines melatonin, serotonin and dopamine. These amines have been described as antioxidants in

several biological systems, including plants and animals (Jodko-Piórecka & Litwinienko, 2015; Kanazawa & Sakakibara, 2000; Gonçalves et al., 2021), decreasing inflammatory processes by reducing free radicals. Serotonin and melatonin have properties that include improvements in the neurological and immune systems, reduction of sleep disorders and control of irritability and aggressiveness (Gonçalves et al., 2021), in addition to being described as antivirals (Bahrampour Juybari et al., 2020; Pashaei, 2021). However, amines as histamine and tyramine can be harmful depending on the concentration, as they can cause problems with headaches, allergies and rash, among others, and people being treated with antidepressants can have more pronounced symptoms due to monoamine oxidase (MAO) and diamine oxidase activity (DAO). Other amines, such as putrescine and cadaverine, have been used as health markers in foods. Furthermore, histamine, thiamine, putrescine and cadaverine can form carcinogenic nitrosamines (Papageorgiou et al., 2018). The polyamines spermidine and spermine are involved in cell differentiation and immune system development (Muñoz-Esparza et al., 2019). Both polyamines decrease during aging, and the consumption of foods with higher levels of these polyamines can reduce the risk of pathologies arising from age, in addition to stimulating longevity (Soda et al., 2009). Both spermidine and spermine have antioxidant activity due to a decrease in the formation of peroxides and secondary oxidation compounds due to the number of positive charges on the amino groups (Muñoz-Esparza et al., 2019). Therefore, the qualitative and quantitative determination of biogenic amines in honeys can help in authenticity (Trifković et al., 2017), since honeys from different flowering and geographic regions may present variations in the contents of these compounds. In addition to authenticity, few studies have been in relation to the levels of biogenic amines

in honeys, in an attempt to combine the data with food safety (Ruiz-Capillas & Herrero, 2019; Basílio et al., 2022), which is one of the objectives of this study.

Although honey is a food of high nutritional quality and presents in its composition bioactive compounds, it can be adulterated by the addition of syrups (Maione et al., 2019), and in Brazil, sugar from sugar cane, a C4 plant, may be added. To detect adulterations in honey, analytical techniques have been used, including the proportion of stable isotopes ($^{13}C/^{12}C$) (Trifković et al., 2017). According to AOAC method 998.12, honey is considered adulterated when $\delta^{13}C$ is heavier than −23.5‰ (AOAC, 2013). Kawashima et al. (2019) stated that the average value of $\delta^{13}C$ for sugars derived from C4 plants is −9.7‰, and honey is considered adulterated if the C4 sugar content is >7%. In this study, we investigated the profile of biogenic amines in honeys from different geographical origins, collection date and botanical sources from two different states of Brazil and correlated with quality parameters. In addition, we analyze the authenticity of these honeys through of isotopic analysis in order to verify the authenticity and detect possible adulterations.

2. **Material and Methods**

2.1. *Collection location of honey samples*

Honey samples (50) were collected in 11 agroecological regions of the State of Santa Catarina between 2018 and 2020: 1A – Itajaí and Tijucas River Valleys (subtropical climate, with hot summer (Cfa)); 1B – Coast of Florianópolis and Laguna (subtropical climate, with hot summer (Cfa)); 2A – Alto Itajaí Valley (subtropical climate, with hot

summer (Cfa)); 2B – Carboniferous, Extreme South and Colonial Serrana (subtropical climate, with hot summer (Cfa)); 2C – Uruguay River Valley (subtropical climate, with hot summer (Cfa)); 3A – Rio do Peixe Valley and Central Plateau (temperate climate, with mild summer (Cfb)); 3B – Northern Plateau of Santa Catarina (temperate climate, with mild summer (Cfb)); 3C – Northwest of Santa Catarina (temperate climate, with mild summer (Cfb)); 4A – Lages Field (temperate climate, with mild summer (Cfb)); 4B – Alto Rio do Peixe Valley and Alto Irani (temperate climate, with mild summer (Cfb)); and 5 – Plateau Serrano de São Joaquim (temperate climate, with mild summer (Cfb)) (Supplementary material; Table S1). From the State of São Paulo, 64 samples were acquired from beekeepers from 10 agroecological regions in 2020 and 2021: Mesoregion of Assis (R1.2., R1.3, R1.5, R1.8, R1. 9, R1.10, R1.11, Z1 and Z2) (tropical climate, with dry winters (Aw) and dry-winter humid subtropical climate (Cwa)); Mesoregion of Bauru (R1.4, R1.6, R1.7, R1.13, M1, M2, M3, M4, M5, M6, M7, M8, M9, M10 and G10) (tropical climate, with dry winters (Aw) and dry-winter humid subtropical climate (Cwa));, Mesoregion of Piracicaba (R1.12, G18 and G27) (tropical climate, with dry winters (Aw)), Mesoregion of Vale do Paraíba (R8.8, R8.9, R9.0, R9.1, G13. G14. G15, G16, G17, G22 and G25) (temperate climate, with mild summer (Cfb) and subtropical climate, with hot summer (Cfa)), Mesoregion of Marilía (M11) (tropical climate, with dry winters (Aw)), Mesoregion of São José do Rio Preto G2 and G4) (tropical climate, with dry winters (Aw)), Mesoregion of Ribeirão Preto (G21, G29 and G34) (tropical climate, with dry winters (Aw)), Mesoregion of Campinas (G1, G5, G6, G7, G23, G24, G32 and G33) (dry-winter humid subtropical climate (Cwa)), Metropolitan Region of São Paulo (G3, G11, G12, G19, G26, G28, G30, G31 and G35) (dry-winter humid subtropical

climate (Cwa)) and Macro-Metropolitan Region of São Paulo (G8, G9 and G20) (dry-winter humid subtropical climate (Cwa)) (Supplementary material; Table S2).

2.2. *Analysis of amino acids and biogenic amines*

Biogenic amines were extracted as described by Lima et al. (2008). Briefly, the samples were mixed with perchloric acid (5%, v/v) and kept for 1 h in an ice bath; then they were homogenized (vortex, 1 min) and incubated in a cold ultrasonic bath (30 min), followed by centrifugation (10 min, 6000 *g*, 5 °C). To the supernatant were added 4.5 mol/L Na_2CO_3 and 18.5 mmol/L dansyl-chloride for derivatization. After 1 h at 60 °C, proline was added and the mixture kept in the dark for 1 h and homogenized by vortexing every 15 min. Toluene (HPLC grade) was added and after vortexed (1 min.), the supernatant was dried in nitrogen line and resuspended in acetonitrile (HPLC grade), homogenized by vortexing and incubated (1 min) in an ultrasonic bath, followed by filtration (0.25 μm). Samples (20 μL) were injected into a high-performance liquid chromatograph (UHPLC, Dionex UltiMate 3000; Thermo Fisher Scientific, Bremen, Germany) (Dadáková et al., 2009), with modifications. The conditions were as follows: reverse phase ACE C18 column (4.6 × 250 mm; 5 μm), thermostated at 25°C, and coupled to a 3000 autosampler quaternary pump and diode array detector (DAD-3000RS). Amines and amino acids were eluted in a gradient system, as follows: 0–2 min, 40% A + 60% B; 2–4 min, 60% A + 40% B; 4–8 min, 65% A + 35% B; 8–12 min, 85% A + 15% B; 12–15 min, 95% A + 5% B; 15–21 min, 85% A + 15% B; 21–22 min, 75% A + 25% B; 22–25 min, 40% A + 60% B. The identification of the biogenic amines (serotonin, melatonin, dopamine, tryptamine, histamine, tyramine, putrescine, cadaverine, spermidine and

spermine), and amino acid (L-tryptophan, 5-hydroxytryptophan and L-dopa) of interest as based on the retention times of analytical standards (Sigma-Aldrich, MO, USA), with detection at λ= 225 ηm. For the purpose of quantifying the compounds (μg 100 g^{-1}), the areas of the peaks of interest were integrated using Chromeleon 7 software (Thermo Fisher Scientific, Bremen, Germany). The limit of detection (LOD), limit of quantification (LOQ), linear regression calibration curves, regression coefficient (R^2), recovery and repeatability values (n = 6) observed are shown in Table S3. Repeatability (below 5%) was determined using six replicates/honey samples chosen at random. Mean recovery (%, n = 6) was tested with five concentrations (12.5, 50, 100, 150 and 200 mg/L) of analytical standard.

2.3. *Isotopic analysis*

To perform the C4 sugar adulteration test, protein was extracted from each honey sample. Protein extraction followed the procedures described in AOAC 998.12 (AOAC, 2013). Briefly, an aliquot of 10 g of sample was diluted in 4 mL of water in a 50 mL centrifuge tube, and then 2 mL of sodium tungstate solution and 2 mL of sulfuric acid solution were added. The mixture was heated at 80 °C for 4 h to form a precipitate. Water was added up to 50 ml, centrifuged at 1500 rpm for 5 minutes and the supernatant discarded, this procedure was repeated 5 times to obtain the precipitated protein free of soluble solids.

Honey and honey protein samples of approximately 0.1 mg were placed in tin capsules. The analyses of $^{13}C/^{12}C$ isotope ratios ($R(^{13}C/^{12}C)$) were carried out in continuous-flow isotope ratio mass spectrometry (CF-IRMS) constituted by an elemental

combustion analyzer (Flash 2000 – Thermo Scientific) coupled to an IRMS (Delta V – Thermo Scientific) through a gas interface (ConFlo IV – Thermo Scientific). The values R($^{13}C/^{12}C$) were expressed as the relative difference of the isotopic ratio of the Vienna Peedee Belemite (VPDB) pattern according to equation 1 (Coplen, 2011):

$$\delta^{13}C = \frac{R(^{13}C/^{12}C)_{sample}}{R(^{13}C/^{12}C)_{VPDB}} - 1 \quad (1)$$

In accordance with the International System of Units (SI) guidelines, the results were expressed in milli Urey (mUr) as recommended (Brand & Coplen, 2012); thus, the isotope value traditionally written −25.0‰ was expressed as −25.0 mUr. The results were normalized with three-point anchorage using USGS82 ($\delta^{13}C$ = −24.31 mUr), USGS83 ($\delta^{13}C$ = −22.20 mUr) and USGS87 ($\delta^{13}C$ = −15.51 mUr) standards (Paul et al., 2007), and the uncertainty analytical measurement in CF-IRMS was estimated (n = 10) to be ±0.15 mUr.

The $C4_{SUGARS}$ content was calculated according to the AOAC 998.12 method using the values of $\delta^{13}C_H$ (honey) and $\delta^{13}C_P$ (protein) determined in each sample according to equation 2. The method considers pure honey, that is, free of added C4 sugars, when $C4_{SUGARS}$ is ≤7% and/or $\delta^{13}C_H$ value is less than −24.0 mUr (AOAC, 2013).

$$C4_{SUGARS(\%)} = \frac{\delta^{13}C_P - \delta^{13}C_H}{\delta^{13}C_P - (-9.7)} \quad (2)$$

2.4. *Statistical analysis*

Data on biogenic amines and amino acids found in honey samples were submitted to analysis of variance (ANOVA), followed by the Scott–Knott mean grouping test. The analysis of data variance and the mean comparison test were performed using the SISVAR statistical software (Ferreira, 2011). Principal component analysis (PCA; XLSTAT software, version 2017; Addinsoft, France) was applied to visualize the possible correlation between the honey data from the states of Santa Catarina and São Paulo, where the samples were collected.

3. **Results and Discussion**

3.1. *Biogenic amines as biochemical marker*

To elucidate the data referring to the analysis of precursor amino acids and biogenic amines, honey samples from Santa Catarina (Table 1) and São Paulo (Table 2) were used for multivariate analysis (Figure 1). In honey samples from Santa Catarina of 2018, the first two principal components exhibited 100 % of the total variance by principal component analysis (PCA). The highest content of 5-OH-tryptophan (1126.86 µg/100 g, 3C4,1) (Table 1) occurred in honey samples from the predominant flowering of *Holvenia dulcis* in the region located in the northwest of Santa Catarina and climate Cfb and were grouped in PC1- and PC2+ (Figure 1A). No honeys collected in the year 2018 contain melatonin. In relation to honey from Santa Catarina harvest in 2019, PC1 and PC2 accounted for 52.55 % of the data variance. The lowest levels of 5-OH-tryptophan were

detected in honey samples harvested from wildflowers in a region with a Cfa climate (2A2.6, 2019) (PC1- and PC2-). Honey harvested from *Piptocarpha angustifolia* (4A1.5) in a Cfb climate and altitude of approximately 1000 m showed higher L-tryptophan and tryptamine content (9483.94 and 41.5 µg/100 g, respectively), and were grouped in PC1- and PC2+ (Figure 1B). However, honey samples harvested in the same year (2019) from *Holvenia dulcis* (2C1.5, 300 m and Cfa) (PC1+ and PC2-) contain the lowest levels of tryptophan and tryptamine. In 2020, honeys from Santa Catarina showed interesting results when analyzed by PCA (Figure 1C), which explains 45.41 %. Honey collected from regions between 610 - 995 m altitude and humid temperate climate (Cfb) (3A, 3B and 3C) (Table 1) were grouped into PC1- and PC2+, while 3A2.7 (native fruit tree and *Cinnamomum verum*) grouped into PC1+ and PC2+ due to the histamine levels (37.66 µg/100 g). These data showed that the year of harvest did not influence the levels of these amines and amino acids in Santa Catarina honey (Table 1), but species and geographical location seem to contribute more to this differentiation.

In the State of São Paulo, orange honey from the 2020 harvest (M2, Itatinga – 23° 06’ 06” S and 48° 36’ 57” W) showed the lowest level of tryptamine (52.05 µg/100 g) and considerable values of 5-OH-tryptophan (994.99 µg /100 g). However, 5-OH-tryptophan was not detected in honey from *Vernonia polysphaera* (R88, Mesoregion of Vale do Paraíba) harvested in 2020 (Table 2), but it had the highest histamine content and was grouped in PC1+ and PC2- (Figure 1D). In the predominant flowering honey of *Citrus* sp. from 2020 (M3, 23°35’46” S and 48°48’40” W), the lowest levels of L-tryptophan were detected (PC1- and PC2+) (Figure 1D). There was a trend toward a lower content of L-tryptophan and 5-OH-tryptophan in honey samples harvested in 2020 (Table 2). Honey samples harvested in São Paulo showed the highest tryptophan contents

(5127.49 and 4306.88 µg/100 g) occurred in the Mesoregion of Bauru (Avaré 23° 05’ 55” S and 48° 55’ 33” W, 768m, northwest region of São Paulo), collected in 2021 from wildflowers (R1.6) and from *Serjania lethalis* (R1.4) (Table 2) and were grouped in PC1- and PC2-. These honey samples were not grouped next to tryptophan (Figure 1E) due to the content of other amines, such as cadaverine, tryptamine and tyramine (Table 2).

L-tryptophan is not synthesized by humans, and its intake determines its fate in the human body. Approximately 90% of tryptophan is converted to kynurenine and less than 1% is used for serotonin synthesis (Chen et al., 2021). Among the analyzed honey samples, those from Santa Catarina in region 4, whether A or B, regardless of the year, proved to be good sources of tryptophan, since the average value in 100 g comprising these regions is approximately 6619 µg (Table 1). In samples from São Paulo harvest in 2020, honey samples from regions R1.4 and R1.6 (Table 2) also showed good levels of tryptophan, with an average of 4716.57 µg/100 g.

Assuming that the consumption of 1 tablespoon contains approximately 18 g of honey, honey from regions 4A or 4B from Santa Catarina (Table S1) contained approximately 1.19 mg L-tryptophan. An intake of 5 mg/kg of tryptophan is recommended for healthy adults; however, only 1% is for serotonin synthesis; that is, only 0.012 mg would be used for serotonin synthesis (Strasser et al., 2016). According to the systematic review by Kikuchi et al. (2021), the daily recommendation for L-tryptophan is 1–3 g/day, and it has been suggested that the availability of tryptophan can be increased by increasing the carbohydrate intake (Fernstrom & Wurtman, 1972), which helps to decrease the levels of competing amino acids (branched chain amino acids – BCAA, leucine, isoleucine and valine) (Choi et al., 2013) through insulin activation. BCAAs and tryptophan share a common transporter in the blood–brain barrier, regulated

by the free tryptophan content, by the transporter and by the level of ‘large neural amino acids’ (LNAAs), which can compromise L-tryptophan levels for serotonin formation (Pinheiro & Pinheiro, 2016). Carbohydrate consumption increases the plasma ratio of L-tryptophan in relation to other neutral amino acids, and as a consequence, there is an increase in serotonin synthesis (Kikuchi et al., 2021). Carbohydrate intake does not induce changes in circulating L-tryptophan levels; however, it decreases the concentration of ‘competing amino acids’ (BCAA) due to insulin activation, increasing the relative availability of L-tryptophan for transport (Kikuchi et al., 2021). However, when there is a decrease in the glycolytic pathway, lipolysis and BCAA increase, becoming the main source of energy, resulting in an increase in free fatty acids that compete with L-tryptophan for binding to albumin, and leading to an increase in free L-tryptophan (Pinheiro & Pinheiro, 2016). With a high L-tryptophan/BCAA ratio, there was an increase in serotonin levels. Faced with this statement, the ingestion of honey would be recommended due to the content of carbohydrates, amino acids and L-tryptophan, which could favor serotonin formation.

Among the samples from São Paulo in the year 2020, 33.3% showed higher levels of tryptamine (Table 2, Figure 1D), while in 2021 only 1.82% showed tryptamine content higher than 5-OH-tryptophan. In the honey samples from Santa Catarina harvested in 2019, 72.41 % contained higher levels of 5-OH-tryptophan compared to tryptamine, an alternative route of serotonin synthesis, while in 2020, 78.94 % shows similar results. Of the 29 honey samples collected in 2019, 86.2% contain higher levels of melatonin, while from 19 honeys samples from 2020, this percentage drops to 57.9%. It is worth noting that 100% of the 2018 honey samples did not have melatonin. The results indicate that the serotonin or melatonin synthesis pathway from tryptophan is preferentially from 5-

OH-tryptophan, as described in other studies (Strasser et al., 2016; Kałużna-Czaplińska et al., 2019; Kim et al., 2021; Farooqi et al., 2022) and that the year of collection influenced the melatonin content.

Serotonin and melatonin are synthesized in bees (Class Insecta) from the same metabolic route as in vertebrates (Kim et al., 2021). L-tryptophan hydroxylase (TPH) catalyzes the formation of 5-OH-tryptophan from L-tryptophan and T5-HTP decarboxylase catalyzes the reaction to form serotonin from 5-OH-tryptophan. Melatonin is formed from serotonin by the action of serotonin N-acetyltransferase (NAT) and hydroxyindole-O-methyltransfer (HIOMT) enzymes (Farooqi et al., 2022). These biogenic amines and precursors are present in most foods and may play a key role in the human mood and the gut–brain axis (Yılmaz & Gökmen, 2020). In the pineal gland of mammals, L-tryptophan can be hydroxylated to 5-OH-tryptophan via tryptophan hydroxylase, and by the action of L-amino acid decarboxylase, 5-OH-tryptophan is converted into serotonin (Macchi & Bruce, 2004). Thus, foods containing high levels of L-tryptophan may be important for mood regulation and depression in people with depressive disorder (Ruhé et al., 2007).

Dopamine and L-dopa have not been well studied in honey (Borges et al., 2022). In the analyzed honey samples from Santa Catarina, the levels of L-dopa varied between 0.01 and 1.09 µg/100 g (2C01.5 and 2C02.5, respectively) and both were harvested in 2019. The content of dopamine varied between 1.51 and 39.61 µg/100 g (3C02.5 and 1A01.5, respectively and from 2019) (Table 1). In São Paulo, the L-dopa content ranged from not detected (M2 and M5) to 0.22 µg/100 g (G5) regardless of harvest year. Dopamine levels ranged from 0.86 (R1.9, from *Citrus* and 22°54'28" S and 49°37'3" W) to 13.60 µg/100 g and R9.1, from *Vernonia polysphaera* and 23°1′51″S and 45° 32′ 54″

W) both from 2021 (Table 2). The highest levels of L-dopa and dopamine found in São Paulo were grouped in PC1+ (Figures 1D and 1E), regardless of year. The predominant species and geographical origin may have influenced the L-dopa and dopamine content. Dopamine has an impact on cognitive functions, including learning and memory (Monte-Silva et al., 2010). L-dopa, a non-protein amino acid, crosses the blood–brain barrier (BBB) and is decarboxylated in nerve cells forming dopamine (Soares et al., 2014) and a deficiency in dopamine synthesis is observed in patients with Parkinson's disease (Haddad et al., 2018). Thus, food, such as honey, can be a way to increase the intake of L-dopa, and in this study, honey from Santa Catarina contains higher levels of this compound, which may be a possible biochemical marker of differentiation.

Higher levels of histamine in honey samples from Santa Catarina were detected in samples from native fruit trees and *Cinnamomum verum* (3A2.7, 2020, 37.66 µg/100 g) and *Piptocarpha tomentosa* (4B 1.5, 2019, 33.37 µg/100 g) (Table 1). Honey from *Mimosa scabrella* (3B2.1, 2018, 788 m) had the lowest tyramine content (0.42 µg/100 g). In the Saudades region, the highest cadaverine content was detected in 2C3.5 (22.42 µg/100 g, 2019), a honey from the predominant flowering of *Holvenia dulcis,* located in the Uruguay River Valley. However, honey collected in a place with the same predominant flowering, climate and harvest year—that is, 2C.1.5 and 2C2.5—showed the lowest levels of this diamine. This indicates these three factors did not influence cadaverine levels. Honey samples from the 2C.1.5 region stand out for the highest levels of putrescine (188.65 µg/100g), while in 2B1.5 (*Cordia magnifolia*, *Dracena fragans* and *Clethra scabra*, 2020), the lowest content of this amine was detected. Some of the honey samples from São Paulo contained the highest levels of histamine and spermine (R88 and R89, respectively) (Table S2), both from very close regions, with similar climates,

however, with different predominant flowering. In São Paulo, the highest levels of histamine occurred in honey acquired from R88 and R89 (2020), R90 and R91 (2021) (regions located close to each other, approximately 10 km apart) (Table S2), from flowering *Vernonia polysphaera* and *Serjania lethalis*. R90 and R91 had the lowest tyramine values (1.04 and 1.75 µg/100 g) (Table 2). The highest histamine content was in R88, but cadaverine was not detected. Based on the results of the minimum and maximum values for each state, honey from São Paulo contained higher levels of histamine and lower levels of tyramine. The R88 and R89 honey samples harvested in 2020 also showed higher levels of tryptamine, which can be a precursor of serotonin (Kang et al., 2007). M7 had the highest value detected of putrescine (27.03 µg/100 g, wildflowers, 2021) among the samples from São Paulo, and in honey samples produced from *Eucalyptus* spp. (predominant flowering) and in 2020, the lowest content was detected (M4).

Histamine, tyramine and putrescine occur at higher levels in poorly packaged and preserved foods, mainly in fish and beef (Shulpekova et al., 2021) and in some beverages (Gomez et al., 2020). Both cadaverine and putrescine, secondary amines, can react with nitrites and form carcinogenic nitrosamines, in addition to potentiating the effects of histamine and tyramine. Nitrosamines formed from polyamines carry risks due to toxicity after consumption, reaching higher than recommended levels (Papageorgiou et al., 2018). Concentrations of 10–100 mg/100 g of histamine and 100-800 mg/100 g of tyramine are considered toxic (Larqué et al., 2007). However, histamine tolerance is related to the activity of MAO and DAO, which can be altered in people who consume antidepressants (MAO and DAO inhibitors), making it important to know the histamine and tyramine

content in foods. In the analyzed samples, the values of both amines did not exceed levels that can bring health risks unless honey intake is in very high amounts.

Few studies have shown the levels of these four biogenic amines in honey samples, and the histamine, tyramine and cadaverine content in combination with putrescine, spermidine and spermine can be used as a quality index (Diamante et al., 2019; Ruiz-capillas & Herrero, 2019; Basílio et al., 2022). Cipolla et al. (2007) reported 0.7 mg/kg of putrescine, while in our samples, honey samples from Santa Catarina showed a 2-fold higher content (2C2.5, 188.65 µg/100 g) (Table 1) and those from São Paulo showed an approximately 7-fold higher content (M7, 27.03 µg/100 g, 2021) (Table 2). However, these data (2C2.5 and M7) was specific for honey samples. All other honey samples did not show high levels, which suggests a possible storage problem or other factors that may influence putrescine content. Despite few human studies, in an oral toxicity study, the observed adverse effect level (NOAEL) was determined to be of 2000 ppm (180 mg/kg body weight/day) (established in Wistar rats) for putrescine and cadaverine (Til et al., 1997; EFSA, 2011). Ingestion of foods, such as cheese, fermented sausages, fish and fishery products, can be harmful to health, as they may contain levels higher than the lowest adverse effect level (LOAEL) of putrescine or cadaverine (LOAEL/putrescine = 10 mM, equivalent to 881.50 mg/kg and LOAEL/cadaverine = 5 mM, equivalent to 510.89 mg/kg) as described by del Rio et al. (2019) in intestinal cell line HT29 (ECACC 91072201). Thus, to cause an undesirable effect, a person would have to ingest a very large amount of honey, even those samples that contain higher levels of putrescine or cadaverine, in order to show symptoms of toxicity.

Spermidine and spermine are tri- and tetramines that have antioxidant action (Basílio et al., 2022), in addition to being related to cell division, growth and

differentiation (Diamante et al., 2019). Spermidine levels reached values of 23.55 µg/100 g (3B1.1, from *Mimosa scabrella*, 2018) in Santa Catarina (Table 1) and 30.71 µg/100 g (M4, from Eucalytpus sp., 2020) in São Paulo (Table 2). Regarding spermine levels, the highest content detected in Santa Catarina was 31.19 µg/100 g (4A3.7, from wild flower blooms, 2020) (Table 1), and in São Paulo, the highest content did not exceed 34.48 µg/100 g (R89, *Serjania lethalis*, 2020) (Table 2). Cipolla et al. (2007) did not detect spermine but described 0.7 µg/kg spermidine in honey. Due to the relationship between both polyamines and cell division, neoplastic patients should avoid consuming high doses (Papageorgiou et al., 2018). Both spermidine and spermine appear to be involved in tumor cell growth (Gerner & Meyskens, 2004) and their uptake by intestinal cells has been implicated as a regulatory mechanism of intracellular polyamine concentration. These polyamines are preferentially taken up by tumors and tissues with a high demand for polyamines. However, spermidine is an important amine, and moderate consumption (0.05% of total daily nitrogen intake) can enhance trauma treatment by improving the protein absorption rate and decreasing glutamine depletion (Larqué et al., 2007).

3.2. *Stable isotopes – adulteration*

We evaluated the authenticity and dispersion of the $\delta^{13}C_H$ and $\delta^{13}C_P$ values for each of the 114 samples from two Brazilian states. Next, we compared the dispersions of the isotopic values of honey and honey protein with the year of collection and the state.

3.2.1. *Authenticity of the honey samples*

The 114 samples analyzed showed mean $C4_{SUGARS}$ levels of −1.7%, and only 3 samples had a $C4_{SUGARS}$ >7% (Table 3), being considered adulterated by adding C4 sugar according to the official AOAC 998.12 method (AOAC, 2013). The samples adulterated with C4 sugar corresponded to 2.63% of the total and came from the same region of São Paulo—the Microregion of Itapecerica da Serra (Table S2). One sample had a $C4_{SUGARS}$ level of 9.5% in the municipality of Cotia, harvested in 2020 (G3), and two samples had $C4_{SUGARS}$ levels of 27.1% and 28.5% in the municipality of Embu-Guaçu (G11 and G28, harvested in 2021).

Honey from São Paulo were also analyzed by year of harvest and by region (Statte of Santa Catarina e State of São Paulo), using principal component analysis (PCA). The analysis of honey samples from Santa Catarina harvested in the years 2018, 2019 and 2020, the PCAs explain 100, 99.96 and 99.95 % of data variance (Figures 2A, 2B and 2C). Despite clustering of honey samples, none of them showed levels of $\%C4_{SUGARS}$, $\delta^{13}C_P$ or $\delta^{13}C_H$ to identify them as adulterated. Analyzing on honey sample from São Paulo in 2020, PC1 and PC2 explained 100 % (Figure 2D) of the data variance and on 2021 explained 99.96% (Figure 2E). Through PC1 of both PCAs (2020 and 2021) it is possible to verify the grouping of honeys with the highest $\%C4_{SUGARS}$ (PC1-) (G3, G11 and G28).

Figure 3A shows the dispersion of $\delta^{13}C_H$ values for *in natura* honey samples and $\delta^{13}C_P$ for extracted protein with marginal distribution curves for the 114 analyzed samples. Despite method 998.12 recommending that honey samples presenting a negative $C4_{SUGARS}$ content be reported as 0% (AOAC, 2013), Figure 3A highlights three samples adulterated with $C4_{SUGARS}$ >7%, 92 authentic samples with $C4_{SUGARS}$ between −7 and 7%,

and 19 unadulterated samples with $C4_{SUGARS}$ less than −7%. The 19 samples highlighted as unadulterated with C4 sugar showed a $C4_{SUGARS}$ level between −17.1 and −7.3%, which corresponded to 16.6% of the total samples analyzed. These samples came from 11 regions, with 8 samples from 3 regions of Santa Catarina and 11 samples from 8 regions of São Paulo (Table 3).

The limit of 7% for the $C4_{SUGARS}$ content in authentic honey samples defined by the AOAC 998.12 method was established from the natural variations between the values of honey and its protein found in evaluations with a large number of samples of authentic honey (White et al., 1998). It was previously verified that the difference between the isotopic values of $\delta^{13}C_H$ and $\delta^{13}C_P$ of a pure honey (unadulterated) sample does not exceed 1.00 mUr (White & Winters, 1989), corresponding to the limited range between -7% to +7% of $C4_{SUGARS}$ content. The samples unadulterated with C4 sugar in Figure 3A showed differences between the isotopic values of $\delta^{13}C_H$ and $\delta^{13}C_P$ that exceeded this limit, however, with $\delta^{13}C_H$ values lower than the $\delta^{13}C_P$ values resulting in a negative content. Furthermore, the curve of marginal distribution of $\delta^{13}C_P$ values in Figure 3A showed that samples classified as unadulterated with C4 sugar had isotopic values higher than authentic ones, suggesting that a $C4_{SUGARS}$ content less than −7% was due to the higher value of $\delta^{13}C_P$ and not the lowest value of $\delta^{13}C_H$ since the values of $\delta^{13}C_H$ were better distributed for authentic samples not adulterated with C4 (Figure 3A).

The higher values of $\delta^{13}C_P$ in the samples unadulterated with C4 sugar can be attributed to the use of C4 sugars to feed the bees in the apiaries during the off-season (Guler et al., 2014). Thus, bees slowly incorporate the isotopic values of C4 into themselves due to the isotopic turnover of C3 to C4, increasing their $\delta^{13}C_P$ value. When the supply of C4 sugar is interrupted and the bees resume pollination of the C3 plant, they

slowly return to the isotopic values of C3, decreasing its $\delta^{13}C_P$ value. These honey samples classified as unadulterated with C4 sugar were probably collected at the beginning of the honey harvest, when the bees were slowly returning to their isotopic value of C3 due to the isotopic turnover from C4 to C3, consequently decreasing their $\delta^{13}C_P$ isotopic value. Thus, honey harvested at this time is authentic, as its sugars come from the nectars of C3 plants with a lower $\delta^{13}C_H$ value; however, the protein from bees has a slightly higher $\delta^{13}C_P$ value due to the excessive supplementary feeding of the colonies with C4 sugars (Soares et al., 2017).

3.2.2. *Precision of carbon isotope analysis for honey authenticity*

For each of the 114 samples, four consecutive determinations of $\delta^{13}C$ were carried out, two for honey *in natura* and two for the protein extracted from honey, totaling 456 determinations of $\delta^{13}C$. For every two consecutive $\delta^{13}C$ determinations, averages were calculated by obtaining the $\delta^{13}C_H$ or $\delta^{13}C_P$ values of each sample and the differences ($\Delta = \delta^{13}C_{measure2} - \delta^{13}C_{measure1}$) between the two Δ_H or Δ_P determinations of each sample were calculated. Figure 3B shows the Δ_H and Δ_P values. The standard deviations for Δ_H were 0.00 ± 0.09 mUr, with a range of −0.48 to +0.41 mUr, and for Δ_P, they were 0.00 ± 0.17 mUr, with a range of −0.58 to +0.85 mUr. These means equal to zero showed that there was no tendency toward an increase or decrease in $\delta^{13}C$ values during the two consecutive measurements. This also showed that there was no memory effect during the isotopic analyses—that is, the influence of one analysis on another consecutive one.

Means, in modulus (Table 3), and standard deviations were 0.05 ± 0.07 mUr for ΔH and 0.12 ± 0.12 mUr for ΔP. Considering that the CF-IRMS analytical uncertainty

for δ13C determinations was ±0.15 mUr, these mean Δ values were within the expected range both for obtaining $\delta^{13}C_H$ values and for $\delta^{13}C_P$ values. The better accuracy in $\delta^{13}C_H$ determinations evidenced by the lower mean and standard deviation of Δ_H can be justified by the physical presentation of honey in its natural state in liquid and viscous form, unlike the protein that is presented at the time of analysis in solid and dry forms. Considering that only 0.1 mg of sample was used for each $\delta^{13}C$ determination, the possibility of obtaining a more homogeneous aliquot is greater in honey than in protein. These results reinforce the fact that the isotopic methodology is very accurate for assessing the authenticity of honey. The official 998.12 method used in this work did not need to be revised in the methodological procedures for extracting the protein, as it was recently reported that heating is not needed to accelerate the precipitation process of honey proteins (Chowdhury et al., 2021).

3.2.3. *Isotope ratio of carbon in honey and honey protein*

The $\delta^{13}C_H$ values of the 114 samples showed a mean and standard deviation of −26.11 ± 1.18 mUr, with a range of −27.79 to −19.68 mUr and a maximum variation of 8.11 mUr. Excluding the 3 adulterated samples and the 19 not adulterated with C4, the $\delta^{13}C_H$ values of the 92 samples showed a mean and standard deviation of −26.13 ± 0.90 mUr, with a range of −27.79 to −24.17 mUr and a maximum variation of 3.62 mUr. The small difference between the means of $\delta^{13}C_H$ for the 114 samples and for the 92 authentic samples and the decrease in the standard deviation for the authentic samples was due to the fact that only the 3 adulterated samples presented values far from the average, as can

be seen in the decrease of the range and the variation in the case of the values of the 92 authentic samples.

The $\delta^{13}C_P$ values of the 114 samples showed a mean and standard deviation of −25.85 ± 0.94 mUr, with a range of −28.48 to −23.32 mUr and a maximum variation of 5.16 mUr. Excluding the 3 adulterated samples and the 19 not adulterated with C4, the $\delta^{13}C_P$ values of the 92 samples showed a mean and standard deviation of −26.03 ±0.90 mUr, with a range of −28.48 to −23.38 mUr and a maximum variation of 5.10 mUr. In this case, the mean values of $\delta^{13}C_P$ and the standard deviations did not show great differences because the intervals, and maximum variations did not change in either case. No significant difference was observed between mean $\delta^{13}C_H$ and $\delta^{13}C_P$ values in all 114 samples, or excluding the 3 samples adulterated and the 19 unadulterated with C4.

When the 114 samples were grouped by year of collection, as shown in Figure 3C, the results showed that $\delta^{13}C_H$ and $\delta^{13}C_P$ values tended to increase over the years. This may be due to an increased use of C4 sugar in bee feed during the off-season. When the same samples were grouped by state, as shown in Figure 3D, the samples from São Paulo had higher $\delta^{13}C_H$ and $\delta^{13}C_P$ values. This differentiation between local collection times was associated because São Paulo collections only occurred in the last two years (2020 and 2021), while in Santa Catarina, collections were carried out in the first three years (2018, 2019 and 2020).

4. Conclusion

Both the location and year of 114 honey harvest from States of Santa Catarina and São Paulo, regardless of the predominant vegetation/flowering, showed different

characteristics in relation to the levels of biogenic amines and some precursor amino acids. Honeys from Santa Catarina have the highest content of L-tryptophan, tryptamine, serotonin, L-dopa, tyramine and putrescine. In the State of São Paulo honey samples, there was a predominance of 5-OH-tryptophan, melatonin, dopamine, histamine, spermidine and spermine. This differentiation found in relation to the levels of biogenic amines could be used as a possible biochemical marker of differentiation. Despite the detection of undesirable biogenic amines, such as histamine, tyramine and cadaverine, the levels are within the permitted limit in several countries. Regarding adulteration, of the 114 samples analyzed, only three samples had a $C4_{SUGARS}$ >7% and 92 samples were considered authentic ($C4_{SUGARS}$ between -7 and 7%) and 19 were considered unadulterated, that is, with $C4_{SUGARS}$ less than −7%. Some honeys showed high $\delta^{13}C_P$ values, meaning that they may have come from hives that were fed with C4 sugar during the off-season; however, this result did not change your authentication. Comparatively, the state of São Paulo contains higher $\delta^{13}C_H$ and $\delta^{13}C_P$ values, which tended to increase over the harvest years.

Conflict of interest statement

Authors declare no conflict of interest.

Acknowledgment

The authors gratefully acknowledge the support by São Paulo Research Foundation (FAPESP - Brazil) (grants 2016/22665-2 and 2019/27227-1); the Foundation for Research Support of Santa Catarina (2020TR1452); CAPES (Coordination for the Improvement of Higher Education Personnel) for granting the scholarship to A. Nunes

(process n° 88882.438338/2019-01); and the National Council for Scientific and Technological Development (CNPq) (grants 304657/2019-0 and 307571/2019-0).

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References

Bahrampour Juybari, K., Pourhanifeh, M. H., Hosseinzadeh, A., Hemati, K., & Mehrzadi, S. (2020). Melatonin potentials against viral infections including COVID-19: Current evidence and new findings. *Virus Research*, *287*(July). https://doi.org/10.1016/j.virusres.2020.198108

Basílio, L. S. P., Vanz Borges, C., Minatel, I. O., Vargas, P. F., Tecchio, M. A., Vianello, F., & Lima, G. P. P. (2022). New beverage based on grapes and purple-

fleshed sweet potatoes: Use of non-standard tubers. In *Food Bioscience* (Vol. 47). https://doi.org/10.1016/j.fbio.2022.101626

Borges, C. V., Nunes, A., Costa, V. E., Orsi, R. de O., Basilio, L. S. P., Monteiro, G. C., Maraschin, M., & Lima, G. P. P. (2022). Tryptophan and Biogenic Amines in the Differentiation and Quality of Honey. *International Journal of Tryptophan Research*, *15*, 117864692211020. https://doi.org/10.1177/11786469221102098

Brand, W. A., & Coplen, T. B. (2012). Stable isotope deltas: Tiny, yet robust signatures in nature. *Isotopes in Environmental and Health Studies*, *48*(3), 393–409. https://doi.org/10.1080/10256016.2012.666977

Choi, S., Disilvio, B., Fernstrom, M. H., & Fernstrom, J. D. (2013). Oral branched-chain amino acid supplements that reduce brain serotonin during exercise in rats also lower brain catecholamines. *Amino Acids*, *45*(5), 1133–1142. https://doi.org/10.1007/s00726-013-1566-1

Chowdhury, S. A., Carter, J. F., Anuj, S. R., Cozzolino, D., Hungerford, N. L., & Fletcher, M. T. (2021). The Validity of Protein in Australian Honey as an Internal Standard for C4 Sugar Adulteration. *Food Analytical Methods*, *14*(4), 823–833. https://doi.org/10.1007/s12161-020-01938-6

Cipolla, B. G., Havouis, R., & Moulinoux, J. P. (2007). Polyamine contents in current foods: A basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients. *Amino Acids*, *33*(2), 203–212. https://doi.org/10.1007/s00726-007-0524-1

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, *25*(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Dadáková, E., Křížek, M., & Pelikánová, T. (2009). Determination of biogenic amines in foods using ultra-performance liquid chromatography (UPLC). *Food Chemistry*, *116*(1), 365–370. https://doi.org/10.1016/j.foodchem.2009.02.018

del Rio, B., Redruello, B., Linares, D. M., Ladero, V., Ruas-Madiedo, P., Fernandez, M., Martin, M. C., & Alvarez, M. A. (2019). The biogenic amines putrescine and cadaverine show in vitro cytotoxicity at concentrations that can be found in foods. *Scientific Reports*, *9*(1), 1–7. https://doi.org/10.1038/s41598-018-36239-w

Diamante, M. S., Borges, C. V., Bartira, M., Minatel, I. O., Corrêa, C. R., Alonzo, H., Gomez, G., Pace, G., & Lima, P. (2019). *Bioactive Amines Screening in Four Genotypes of Thermally Processed Cauliflower*. 1–16.

EFSA. (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. In *EFSA Journal* (Vol. 9, Issue 10, pp. 2393–2486). https://doi.org/10.2903/j.efsa.2011.2393

Farooqi, M. K., Ali, M., & Amir, M. (2022). Melatonin and Serotonin: Their Synthesis and Effects in Insects. *Chronobiology in Medicine*, *4*(1), 24–28. https://doi.org/10.33069/cim.2022.0003

Fernstrom, J. D., & Wurtman, R. J. (1972). Elevation of plasma tryptophan by insulin in rat. *Metabolism*, *21*, 337–342.

Gerner, E. W., & Meyskens, F. L. (2004). Polyamines and cancer: old molecules, new understanding. *Nature Reviews. Cancer*, *4*(10), 781–792. https://doi.org/10.1038/nrc1454

Gonçalves, A. C., Nunes, A. R., Alves, G., & Silva, L. R. (2021). Serotonin and Melatonin: Plant Sources, Analytical Methods, and Human Health Benefits. *Revista Brasileira de Farmacognosia*, *31*(2), 162–175.

https://doi.org/10.1007/s43450-021-00141-w

Guler, A., Kocaokutgen, H., Garipoglu, A. V., Onder, H., Ekinci, D., & Biyik, S. (2014). Detection of adulterated honey produced by honeybee (Apis mellifera L.) colonies fed with different levels of commercial industrial sugar (C3 and C4 plants) syrups by the carbon isotope ratio analysis. *Food Chemistry*, *155*, 155–160. https://doi.org/10.1016/j.foodchem.2014.01.033

Haddad, F., Sawalha, M., Khawaja, Y., Najjar, A., & Karaman, R. (2018). Dopamine and levodopa prodrugs for the treatment of Parkinson's disease. *Molecules*, *23*(1). https://doi.org/10.3390/molecules23010040

Jodko-Piórecka, K., & Litwinienko, G. (2015). Antioxidant activity of dopamine and L-DOPA in lipid micelles and their cooperation with an analogue of α-tocopherol. *Free Radical Biology and Medicine*, *83*, 1–11. https://doi.org/10.1016/j.freeradbiomed.2015.02.006

Kałużna-Czaplińska, J., Gątarek, P., Chirumbolo, S., Chartrand, M. S., & Bjørklund, G. (2019). How important is tryptophan in human health? *Critical Reviews in Food Science and Nutrition*, *59*(1), 72–88. https://doi.org/10.1080/10408398.2017.1357534

Kanazawa, K., & Sakakibara, H. (2000). High content of dopamine, a strong antioxidant, in cavendish banana. *Journal of Agricultural and Food Chemistry*, *48*(3), 844–848. https://doi.org/10.1021/jf9909860

Kang, S., Kang, K., Lee, K., & Back, K. (2007). Characterization of tryptamine 5-hydroxylase and serotonin synthesis in rice plants. *Plant Cell Reports*, *26*(11), 2009–2015. https://doi.org/10.1007/s00299-007-0405-9

Kawashima, H., Suto, M., & Suto, N. (2019). Stable carbon isotope ratios for organic

acids in commercial honey samples. In *Food Chemistry* (Vol. 289, pp. 49–55). https://doi.org/10.1016/j.foodchem.2019.03.053

Kikuchi, A. M., Tanabe, A., & Iwahori, Y. (2021). A systematic review of the effect of L-tryptophan supplementation on mood and emotional functioning. *Journal of Dietary Supplements*, *18*(3), 316–333. https://doi.org/10.1080/19390211.2020.1746725

Kim, T.-K., Atigadda, V. R., Brzeminski, P., Fabisiak, A., Tang, E. K. Y., Tuckey, R. C., Reiter, R. J., & Slominski, A. T. (2021). Detection of Serotonin, Melatonin, and Their Metabolites in Honey. *ACS Food Science & Technology*, *1*(7), 1228–1235. https://doi.org/10.1021/acsfoodscitech.1c00119

Larqué, E., Sabater-Molina, M., & Zamora, S. (2007). Biological significance of dietary polyamines. *Nutrition (Burbank, Los Angeles County, Calif.)*, *23*(1), 87–95. https://doi.org/10.1016/j.nut.2006.09.006

Lima, G. P. P., da Rocha, S. A., Takaki, M., Ramos, P. R. R., & Ono, E. O. (2008). Comparison of polyamine, phenol and flavonoid contents in plants grown under conventional and organic methods. *International Journal of Food Science & Technology*, *43*(10), 1838–1843. https://doi.org/10.1111/j.1365-2621.2008.01725.x

Macchi, M. M., & Bruce, J. N. (2004). Human pineal physiology and functional significance of melatonin. In *Frontiers in Neuroendocrinology* (Vol. 25, Issues 3–4, pp. 177–195). https://doi.org/10.1016/j.yfrne.2004.08.001

Maione, C., Barbosa, F., & Barbosa, R. M. (2019). Predicting the botanical and geographical origin of honey with multivariate data analysis and machine learning techniques: A review. In *Computers and Electronics in Agriculture* (Vol. 157, pp. 436–446). https://doi.org/10.1016/j.compag.2019.01.020

Muñoz-Esparza, N. C., Latorre-Moratalla, M. L., Comas-Basté, O., Toro-Funes, N., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2019). Polyamines in food. *Frontiers in Nutrition*, *6*(July), 1–11. https://doi.org/10.3389/fnut.2019.00108

Papageorgiou, M., Lambropoulou, D., Morrison, C., Kłodzińska, E., Namieśnik, J., & Płotka-Wasylka, J. (2018). Literature update of analytical methods for biogenic amines determination in food and beverages. In *TrAC - Trends in Analytical Chemistry* (Vol. 98, pp. 128–142). https://doi.org/10.1016/j.trac.2017.11.001

Pashaei, Y. (2021). Drug repurposing of selective serotonin reuptake inhibitors: Could these drugs help fight COVID-19 and save lives? *Journal of Clinical Neuroscience*, *88*, 163–172. https://doi.org/10.1016/j.jocn.2021.03.010

Paul, D., Skrzypek, G., & Fórizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, *21*(18), 3006–3014. https://doi.org/10.1002/rcm.3185

Pinheiro, J. L., & Pinheiro, J. (2016). Aminoácidos de cadeia ramificada e fadiga central. *Rev. Medicina Desportiva*, *7*, 15–18.

Ruhé, H. G., Mason, N. S., & Schene, A. H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: A meta-analysis of monoamine depletion studies. *Molecular Psychiatry*, *12*(4), 331–359. https://doi.org/10.1038/sj.mp.4001949

Ruiz-capillas, C., & Herrero, A. M. (2019). Biogenic Amines on Food Safety. In *Biogenic Amines on Food Safety*. https://doi.org/10.3390/books978-3-03921-055-8

Siddiqui, A. J., Musharraf, S. G., Choudhary, M. I., & Rahman, A. ur. (2017). Application of analytical methods in authentication and adulteration of honey. *Food Chemistry*, *217*, 687–698. https://doi.org/10.1016/j.foodchem.2016.09.001

Silva, P. M., GAuche, C., Gonzaga, L. V., Costa, A. C., & Fett, R. (2016). Honey_ Chemical composition, stability and authenticity _ Elsevier Enhanced Reader.pdf. *Food Chemistry*, *196*, 309–323.

Soares, S., Amaral, J. S., Oliveira, M. B. P. P., & Mafra, I. (2017). A Comprehensive Review on the Main Honey Authentication Issues: Production and Origin. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1072–1100. https://doi.org/10.1111/1541-4337.12278

Soda, K., Dobashi, Y., Kano, Y., Tsujinaka, S., & Konishi, F. (2009). Polyamine-rich food decreases age-associated pathology and mortality in aged mice. In *Experimental Gerontology* (Vol. 44, Issue 11, pp. 727–732). https://doi.org/10.1016/j.exger.2009.08.013

Strasser, B., Gostner, J. M., & Fuchs, D. (2016). Mood, food, and cognition: Role of tryptophan and serotonin. *Current Opinion in Clinical Nutrition and Metabolic Care*, *19*(1), 55–61. https://doi.org/10.1097/MCO.0000000000000237

Til, H. P., Falke, H. E., Prinsen, M. K., & Willems, M. I. (1997). Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *35*(3–4), 337–348.

Trifković, J., Andrić, F., Ristivojević, P., Guzelmeric, E., & Yesilada, E. (2017). Analytical methods in tracing honey authenticity. *Journal of AOAC International*, *100*(4), 827–839. https://doi.org/10.5740/jaoacint.17-0142

White, J. W., & Winters, K. (1989). Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *Journal - Association of Official Analytical Chemists*, *72*(6), 907–911. https://doi.org/10.1093/jaoac/72.6.907

White, J. W., Winters, K., Martin, P., & Rossmann, A. (1998). Stable Carbon Isotope Ratio Analysis of Honey: Validation of Internal Standard Procedure for Worldwide Application. *Journal of AOAC International*, *81*(3), 610–619. https://doi.org/10.1093/jaoac/81.3.610

Yılmaz, C., & Gökmen, V. (2020). Neuroactive compounds in foods: Occurrence, mechanism and potential health effects. In *Food Research International* (Vol. 128). https://doi.org/10.1016/j.foodres.2019.108744

Zheng, X., Zhao, Y., Wu, H., Dong, J., & Feng, J. (2016). Origin Identification and Quantitative Analysis of Honeys by Nuclear Magnetic Resonance and Chemometric Techniques. *Food Analytical Methods*, *9*(6), 1470–1479. https://doi.org/10.1007/s12161-015-0325-1

CONSIDERAÇÕES FINAIS

Durante o meu pós-doutoramento, as parcerias firmadas e a realização de diversas análises investigativas em amostras de méis proporcionaram uma experiência única e extremamente valiosa para a minha formação como pesquisador científico, ampliando significativamente meu conhecimento acadêmico. É importante destacar a colaboração de instituições renomadas na área de alimentos, bem como a participação de importantes produtores de méis do Brasil, o que reforçou a relevância deste projeto para a inovação e o desenvolvimento de tecnologias voltadas à análise de qualidade dos alimentos, com foco no mel.

O setor apícola paulista e o mercado de seus produtos são de grande importância, evidenciando a preocupação com a qualidade e a identidade dos méis comercializados. No entanto, o Brasil ainda não tem dado a devida atenção às pesquisas sobre seus méis. Nesse contexto, as análises realizadas neste projeto, focando na tipificação do mel, abordaram: i. autenticidade, ii. composição química regional associada à sazonalidade e origem floral, iii. atividades biológicas, e iv. indicação de origem geográfica. Esses estudos permitiram o desenvolvimento de processos e produtos com indicadores de alta qualidade e apelo agroecológico, considerando o baixo impacto ambiental da atividade apícola, que se insere em sistemas de produção ambientalmente corretos.

A certificação da origem geográfica do mel com base em seu perfil químico, foco deste trabalho, é uma estratégia de valorização desse produto, fundamentada em técnicas analíticas modernas e amplamente reconhecidas por sua eficiência. Os resultados deste projeto de pós-doutoramento, evidenciados em publicações científicas, abrirão novas oportunidades e nichos de mercado para os méis paulistas, tanto no mercado interno quanto no externo, incentivando a atividade apícola, especialmente na agricultura familiar. Esses impactos positivos se refletirão no valor do mel e de seus derivados, beneficiando o pequeno produtor rural e o setor industrial.