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 from 11 distinct regions of the State of Santa Catarina (Brazil) as to the profile of amino acids and biogenic amines by HPLC. The highest L-tryptophan (Trp), 5-hydroxytryptophan (5-OH-Trp), and tryptamine (Tryp) levels were detected in Cfb climate and harvested in 2019. Although we have found high content of serotonin, dopamine, and L-dopa in Cfb climate, the highest values occurred in honey produced during the summer 2018 and at altitudes above 900 m. 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

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Introduction

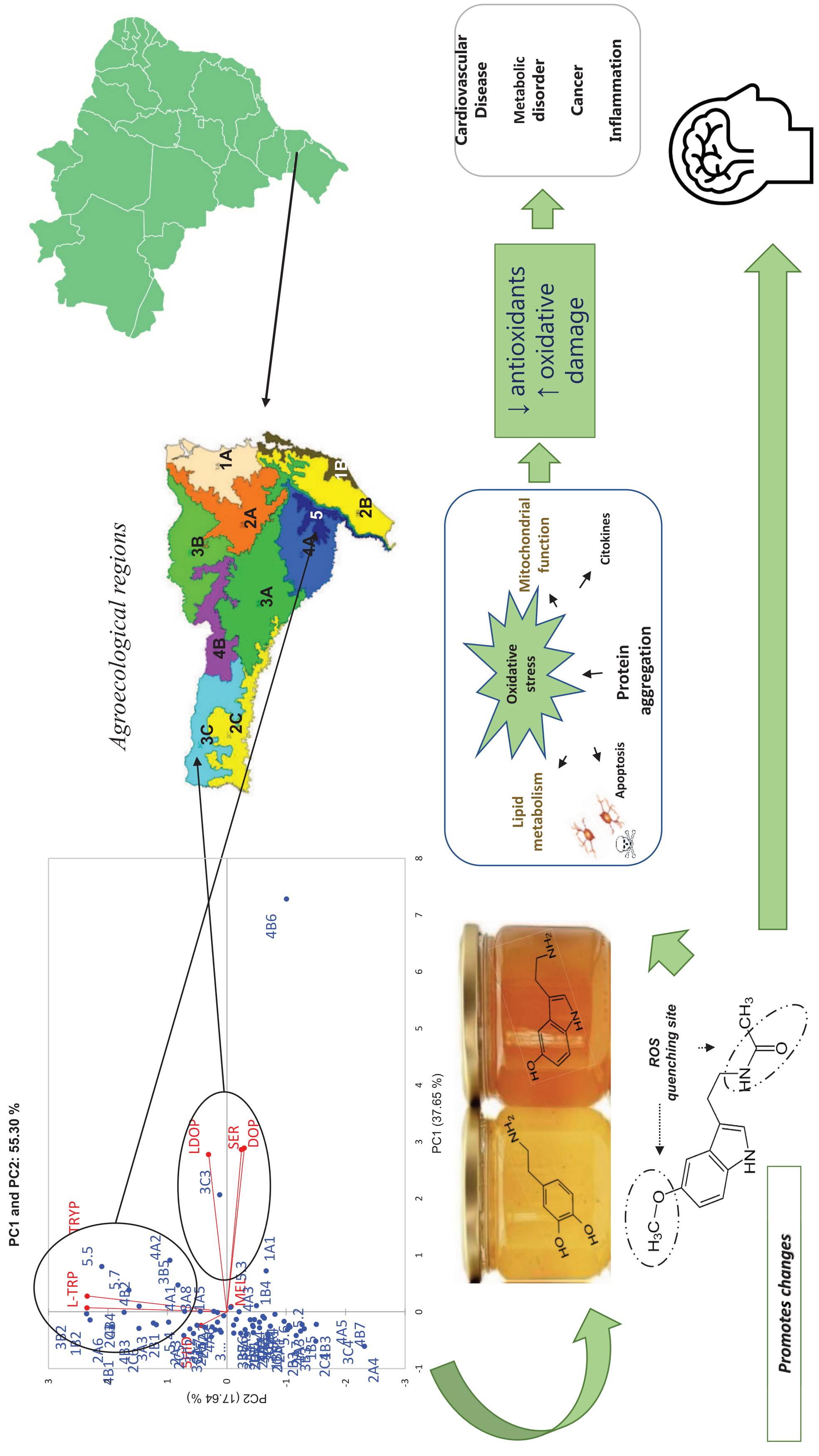
Honey is known for its antimicrobial, anti-inflammatory, anticancer, antiatherogenic, antithrombotic, and antioxidant effects, in addition to its analgesic activity in the human body.[1] Conscious consumption and the search for foods with good nutritional quality, aiming at the prevention of diseases, 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.[2] Phenolic compounds seem to be the most important constituents of honey, responsible for its antioxidant activity.[3] 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,[4] such as serotonin and dopamine.

Some studies report the presence of biogenic amines in samples of honey and other bee-derived products (ie, 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,[5] 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[6] 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.[7] 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.[8] 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.[9]

Honey and its derivatives have been increasingly valued natural substances; however, their physicochemical and

GRAPHICAL ABSTRACT



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.[10] 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 (ie, Trp and 5-OH-Trp) in honeys from different agro-ecological regions.

Methods

Honey collection places

Samples of honey (65) harvested in 2018 to 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.[11] Honey samples (1 g) were mixed with 3 mL of perchloric acid (5%, v/v), homogenized (vortex, 1 minute) and incubated in a cold ultrasonic bath (30 minutes), followed by centrifugation (10 minutes, 6000*g*, 5°C). The supernatant containing the free amines and amino acids was collected and subjected to derivatization using supernatant, 4.5 mol/L Na_2CO_3 and 18.5 mmol/L dansyl-chloride in acetone. The solution was kept at 60°C for 1 hour and, in sequence, proline (1 mg/mL) was added and the mixture kept in the dark for 1 hour and homogenized by vortexing every 15 minutes. After this interval, toluene (HPLC grade) was added, the mixture was vortexed (1 minute) 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 minute) 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 et al[12] 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 to 2 minutes, 40% *A* + 60% B; 2 to 4 minutes, 60% *A* + 40% B; 4 to 8 minutes, 65% *A* + 35% B; 8 to 12 minutes, 85% *A* + 15% B; 12 to 15 minutes, 95% *A* + 5% B; 15 to 21 minutes, 85% *A* + 15% B; 21 to 22 minutes, 75% *A* + 25% B; 22 to 25 minutes, 40% *A* + 60% B. Identification of biogenic amine and amino acids of interest (eg, L-Trp, 5-OH-Trp, Tryp, 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 6 replicates/honey samples chosen at random. Mean recovery (%, n = 6) was tested with 5 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 ($P < .05$). The ANOVA and the mean comparison test were performed using SISVAR statistical software.[13] 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.[10]

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 coast of Florianópolis and Laguna (1B2) (Cfa climate, humid

Table 1. Location and predominant flowering of honey from Santa Catarina (flowering year/collection and season).

SAMPLE	REGION	CITY	ALTITUDE (M)	CLIMATE (KOPPEN-GEIGER)	PREDOMINANT FLOWERING	YEAR OF COLLECTION	SEASON
1A1	Coast, Itajaí, and Tijucas river valleys	Benedito Novo	683	Humid subtropical climate (Cfa)	Cinnamon and *Piptocarpha angustifolia*	2019	Summer
1A2		Itapoá	457		Wild flowers	2018	Spring
1A3		Itapoá			Wild flowers	2019	Summer
1A4		Nova Trento	156		Wild flowers	2018	Spring
1A5		Nova Trento			Eucalyptus	2019	Autumn
1B1		Jaguaruna	92		Eucalyptus	2019	Autumn
1B2	Coast of Florianópolis and Laguna	Balneário Gaivota	9	Humid subtropical climate (Cfa)	Wild flowers	2019	Spring
1B3		Jaguaruna	92		Wild flowers	2018	Autumn
1B4		Jaguaruna			Eucalyptus	2019	Spring
1B5		Major Gercino	42		Wild flowers	2018	Autumn
1B6		Major Gercino			Eucalyptus	2019	Autumn
2A1	Alto Vale do Itajaí	José Boiteux	726	Humid subtropical climate (Cfa)	*Baccharis dracunculifolia*	2018	Spring
2A2		José Boiteux	491		Dracena *frangans* and *Holvenia dulcis*	2018	Spring
2A3		José Boiteux	717		Eucalyptus	2019	Autumn
2A4		Salete	593		*Holvenia dulcis*	2018	Spring
2A5		Vidal Ramos	763		*Baccharis dracunculifolia*	2019	Summer
2A6		Vidal Ramos	493		*Holvenia dulcis*	2019	Summer
2A7		Vidal Ramos	761		*Baccharis dracunculifolia*	2019	Summer
2B1	Carboníferous, Extreme South, and Colonial Serrana	Anitápolis	760	Humid subtropical climate (Cfa)	*Baccharis dracunculifolia, Piptocarpha angustifolia, trimera and Holvenia dulcis*	2018	Spring
2B2		Anitápolis			*Eugenia sp., Holvenia dulcis*, Clethra scabra, and Dracena *fragans*	2019	Summer
2B3		Orleans	600		Wild flowers	2018	Spring
2C1	Uruguai River Valley	Descanso	272	Humid subtropical climate (Cfa)	*Holvenia dulcis*	2018	Spring
2C2		Descanso			Eucalyptus	2019	Autumn
2C3		Itapiranga	300		Eucalyptus	2019	Autumn
2C4		Itapiranga			*Holvenia dulcis*	2019	
2C5		Saudades	364		*Holvenia dulcis*	2018	Spring
2C6		Saudades			Eucalyptus	2019	Autumn
3A1	Alto Vale do Rio do Peixe and Central Plateau	Curitibanos	995	Humid temperate climate (Cfb)	Wild flowers	2019	Summer
3A2		Curitibanos			Wild flowers	2019	Summer
3A3		Erval Velho	699		Native fruit tree	2018	Spring
3A4		Erval Velho			*Holvenia dulcis*	2018	Spring

(Continued)

Table 1. (Continued)

SAMPLE	REGION	CITY	ALTITUDE (M)	CLIMATE (KOPPEN-GEIGER)	PREDOMINANT FLOWERING	YEAR OF COLLECTION	SEASON
3A5		Erval Velho			*Baccharis dracunculifolia* and *Holvenia dulcis*	2019	Summer
3A6		Luzerna	725		Cinnamomum verum	2018	Spring
3A7		Luzerna			*Holvenia dulcis*	2018	Spring
3A8		Luzerna			Wild flowers	2019	Summer
3B2	Northern Santa Catarina Plateau	Bela Vista do Toldo	793	Humid temperate climate (Cfb)	*Sebastiania commersoniana* and *Campomanesia xanthocarpa*	2018	Spring
3B3		Três Barras	766		*Sebastiania commersoniana*	2018	Spring
3B5		Rio Negrinho	982		Wild flowers	2018	Spring
3B6		Rio Negrinho	982		Wild flowers	2019	Summer
3B7		Santa Terezinha	625		*Holvenia dulcis*	2018	Spring
3C1	Santa Catarina Northwest	Campo Erê	894	Humid temperate climate (Cfb)	*Holvenia dulcis*	2019	Summer
3C2		Campo Erê	650		Eucalyptus	2019	Autumn
3C3		Dionísio Cerqueira	856		*Holvenia dulcis*	2018	Spring
3C4		Dionísio Cerqueira			Eucalyptus	2019	Autumn
3C5		Xaxim	598		*Holvenia dulcis*	2018	Spring
4A1	Lages Field	Bocaina do Sul	980	Humid temperate climate (Cfb)	*Campomanesia xanthocarpa, Piptocarpha angustifolia, Eugenia* sp., and *Vochysia tucanorum*	2018	Spring
4A2		Bocaina do Sul			*Clethra scabra*	2019	Summer
4A3		Bom Retiro	522		*Holvenia dulcis*	2019	Summer
4A4		Bom Retiro	944		*Clethra scabra*	2019	Summer
4A5		Capão Alto	1007		Native plants	2018	Spring
4A6		Capão Alto			Wild flowers	2019	Summer
4B1	Alto Vale do Rio do Peixe and Alto Irani	Água Doce	1203	Humid temperate climate (Cfb)	*Piptocarpha tomentosa*	2018	Spring
4B2		Água Doce	1203		*Ocotea porosa*	2018	Spring
4B3		Água Doce	1203		*Baccharis trimera*	2019	Summer
4B4		Lebon Régis	1280		Cinnamon, *Piptocarpha angustifolia, Clethra scabra, Baccharis uncinella*, and *Cupania vernalis*	2018	Spring
4B5		Lebon Régis	1280		*Vernonia polysphaera* and *Baccharis trimera*	2019	Autumn
4B6		Matos Costa	973		*Astronium fraxinifolium, Lithrea brasiliensis*, and *Ilex theezans*	2018	Spring
4B7		Matos Costa	1156		*Clethra scabra*	2019	Autumn

(Continued)

Table 1. (Continued)

SAMPLE	REGION	CITY	ALTITUDE (M)	CLIMATE (KOPPEN-GEIGER)	PREDOMINANT FLOWERING	YEAR OF COLLECTION	SEASON
5(1)	Planalto Serrano de São Joaquim	Bom Jardim da Serra	1272	Humid temperate climate (Cfb)	*Piptocarpha angustifolia, Lithraea molleoides, Astronium fraxinifolium*, *Baccharis trimera*, and *Brosimum gaudichaudii*	2018	Spring
5(2)		Bom Jardim da Serra	1247		*Senna bicapsularis, Struthanthus flexicaulis*, and *Baccharis trimera*	2019	Autumn
5(3)		São Joaquim	1276		*Piptocarpha angustifolia*, *Lithrea brasiliiensis*, *Astronium fraxinifolium*, and *Baccharis trimera*	2018	Spring
5(4)		São Joaquim	1309		*Eupatorium* sp., *Struthanthus flexicaulis*, *Baccharis trimera*, and *Strychnos pseudoquina*	2019	Autumn
5(5)		São Joaquim	1148		*Piptocarpha angustifolia* and *Mimosa scabrella*	2018	Spring
5(6)		São Joaquim	1051		*Senna bicapsularis*	2019	Summer
5(7)		São Joaquim	1055		Wild flowers	2019	Summer

Table 2. LOD, LOQ, linear regression calibration curves, regression coefficient (*R*2), recovery, and repeatability of biogenic amines, by the analytical method in samples of honey.

AMINOACIDS/BIOGENIC AMINES	LOD[A] (MG/L)	LOQ (MG/L)	REGRESSION EQUATION	*R*2	RECOVERY[B] VALUES (%)	REPEATABILITY[C]
Tryptophan	0.019	0.104	$y = 0.3614x + 0.1297$	.99	96.5	3.3
5-OH-tryptophan	0.026	0.102	$y = 0.2574x + 0.0561$	.99	97.3	3.1
Tryptamine	0.049	0.106	$y = 4.232x + 42.342$	.99	94.8	4.1
Serotonin	0.013	0.085	$y = 4.0423x + 19.911$	.99	96.7	3.2
Melatonin	0.013	0.102	$y = 1.3749x + 0.9985$	.99	92.3	3.9
L-dopa	0.018	0.110	$y = 205.1x - 0.012$	.99	91.9	3.5
Dopamine	0.029	0.099	$y = 3.822x + 71.767$	.98	101.2	4.1

[a]Range for amino acids and biogenic amines 12.5 to 200 mg/L.
[b]n = 6.
[c]n = 6.

subtropical climate – harvested in spring 2019), from the Northern Plateau of Santa Catarina (3B2, harvested in spring 2018), and from the agroecological region of the Planalto Serrano de São Joaquim (Cfb climate – temperate oceanic climate) (5.5 and 5.7, harvested in summer 2018 and 2019) stood out for their L-Trp content (PC1+ and PC2+). Honeys from Cfa climate (Alto Vale do Itajaí—2A6 and Uruguai River Valley—2C3) harvested in 2019 (summer and autumn, respectively) are distinguished from the others by having the highest tryptamine content (35.01 and 35.43 µg/100 g, respectively). Both L-Trp and Tryp are precursors of Ser and their presence may be important for the control of some physiological disorders, such as obsessive-compulsive disorder.[14] Ser and Dop ($r = .89$, $P < .05$), as well as the amino acid L-dopa with these amines, showed a significant and strong correlation (Ser: $r = .76$ and Dop: .79, $P < .05$). Honey from the agroecological

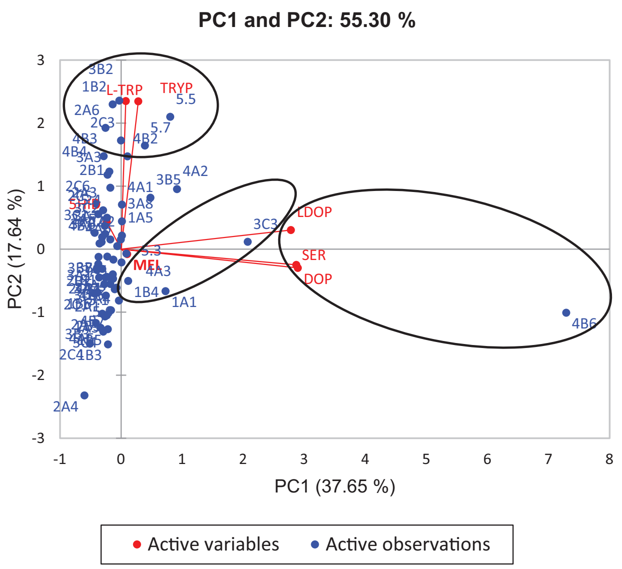


Figure 1. Two-dimensional projection and scores of profile of precursor amino acids and biogenic amines of floral honeys from the agroecological regions of the State of Santa Catarina. Honey samples are represented by blue points (see Table 1) and amino acids and biogenic amines by red points. Abbreviations: 5-OH-TRP, 5-hydroxy-tryptophan; L-DOPA, DOP, dopamine; L-TRP, L-tryptophan; MEL, melatonin; SER, serotonin; TRYP, tryptamine.

regions Alto Vale do Rio do Peixe and Alto Irani (4B6), harvested in Spring 2018, showed the highest levels of these amines (Ser: 495.15 µg/100 g; Dop: 33.98 µg/100 g) and the amino acid L-dopa (0.72 µg/100 g) (PC1+ and PC2−) (Figures 1 and 2B; Table 3).

L-Trp levels ranged from 263.31 µg/100 g (2A4, Spring 2018) to 3358.65 µg/100 g (1B2, Spring 2019) (Figure 2A; 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/season of harvest. Other studies highlight L-Trp values greater than 14 mg/kg in rosemary honeys,[15] while levels reached 1.9 mg/100 g in sunflower honeys[16] and 1.10 mg/100 g in lavender honey.[17] 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 and harvested in Spring 2018 and grouped in PC1− and PC2− (Figure 1). It is worth mentioning that, at the same time of harvest, Mel was not detected. These results also demonstrate that the year of harvest and season affects the levels of biogenic amines (Figure 2A and B). 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 (polyfloral honey) and harvested in spring 2019 (Table 1; Figure 2A and B). Some articles describe that L-Trp content varies depending on the predominant flora. Hermosín et al[17] 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 and sample harvested in spring showed the highest level of this amino acid; however, it is worth noting that the botanical origin, as well as the climate, region, and season, can affect the level of metabolites, including amino acids. The levels of metabolites formed from tryptophan are not correlated with the results of L-Trp content.

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[18,19] or much higher, such as described in *Rhododendron ponticum* pollen (8053.00 µg/g).[20]

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.[21,22] Intake of L-Trp has been linked to decreased levels of psychosis in both animal and human studies and sleep deprivation.[22] Other studies have demonstrated that ingestion of honey with milk before bedtime may decrease hypoglycemic effects in diabetic patients,[23] or improve the sleep quality of healthy people or those with coronary heart problems.[24] 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 (eg, tyrosine, phenylalanine, leucine, isoleucine, and valine) called "large neutral amino acids" (LNAA).[2] The L-Trp::p:LNAA ratio determines the flux of this amino acid and, consequently, the biosynthesiSer in the brain.[25] 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.[2] Furthermore, excess L-Trp may cause adverse reactions, such as gastric problems and dizziness, among others.[21]

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[26]) or those related to neuroimmunological signaling, such as Ser and Mel. About 1% of dietary L-Trp is used for Ser synthesis,[26,27] 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

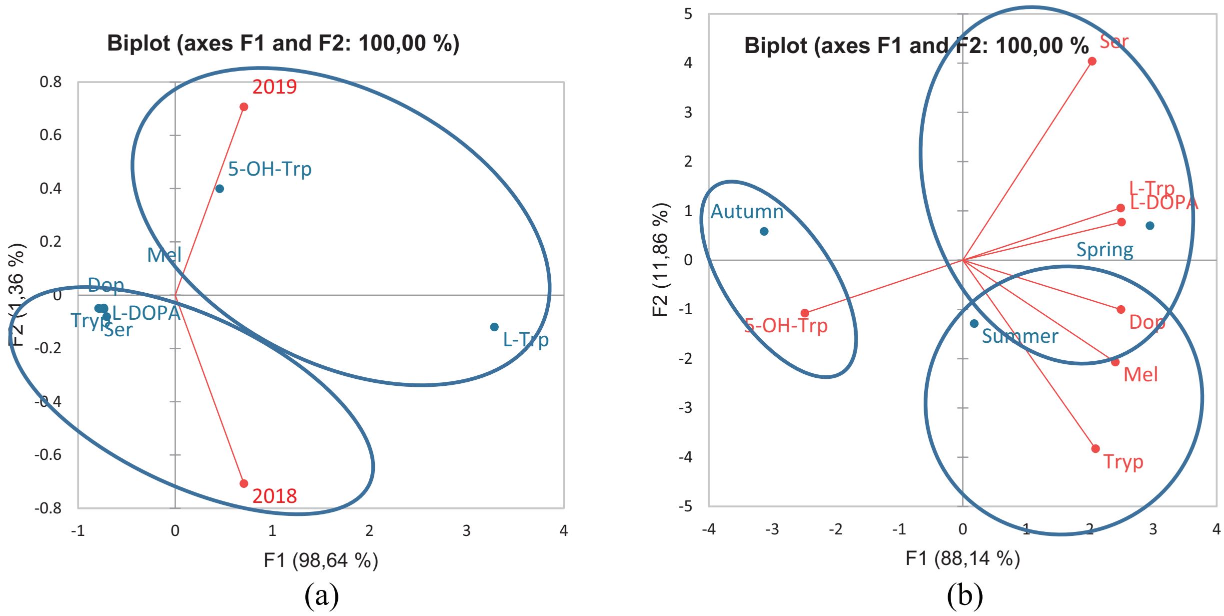


Figure 2. Two-dimensional projection and scores of profile of precursor amino acids and biogenic amines of floral honeys from the agroecological regions of the State of Santa Catarina analyzed as to the time of harvest (A) and season (B). Honey samples are represented by blue points (see Table 1) and amino acids and biogenic amines by red points.
Abbreviations: 5-OH-TRP, 5-hydroxy-tryptophan; L-DOPA, DOP, dopamine; L-TRP, L-tryptophan; MEL, melatonin; SER, serotonin; TRYP, tryptamine.

nicotinamide adenine dinucleotide (NAD^+).[27] In this pathway, 60 mg of Trp produces 1 mg of nicotinic acid or niacin.[28] The usual intake of Trp is approximately 900 to 1000 mg per day,[26] and the recommended daily dose is around 4 mg/kg body weight per day for adults and 12 mg/kg body weight for children.[7] 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.[27] In children and adolescents with autism spectrum disorder, the intake of L-Trp may decrease symptoms of irritability and mild depression due to increased Ser levels.[29]

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 as 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),[27] since both the synthesis and the turnover of Ser depend on the intake of L-Trp and 5-OH-Trp.[30] 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), that is, 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, L-dopa and dopamine (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 biogenic amine can induce vasoactive or psychoactive effects on the human body.[31] According to the authors, the consumption of 25 to 30 mg/kg of Tryp 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.

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,[32] mainly in the process of lipid peroxidation.[14] 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, produced in spring 2018 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, 1B4-93.26 µg/100 g) with a Cfa climate, low altitude (92 m), predominantly flowering of *Eucalyptus* and harvest in Spring 2019. Mel, Ser, and Dop were grouped in PC1− (Figure 2A) according to the year of harvest and in PC1+ in relation to the season (Figure 2B). In these figures and in Table 3 it is possible to verify that the levels of Mel varied

Table 3. Precursor amino acids and biogenic amines (µg/100 g) of floral honeys from the agroecological regions of the State of Santa Catarina (harvest 2018 and 2019).

HONEY	L-TRP[1]	5-OH-TRP[2]	TRYP[3]	SER[4]	MEL[5]	L-DOPA[6]	DOP[7]
1A1	749.08 ± 33.86^{p}	1314.04 ± 1.36^{d}	15.58 ± 2.47^{g}	21.27 ± 1.93^{l}	25.64 ± 3.23^{h}	0.07 ± 0.04^{h}	12.79 ± 0.60^{b}
1A2	930.66 ± 7.18^{o}	nd	22.47 ± 0.20^{d}	55.63 ± 0.79^{c}	nd	0.02 ± 0.00^{j}	1.68 ± 0.22^{j}
1A3	875.64 ± 0.45^{o}	nd	12.36 ± 0.14^{h}	20.68 ± 0.51^{l}	nd	nd	2.62 ± 0.17^{i}
1A4	970.84 ± 9.24^{o}	nd	17.21 ± 0.19^{f}	24.11 ± 0.52^{k}	11.99 ± 0.74^{k}	0.03 ± 0.00^{j}	2.32 ± 0.09^{i}
1A5	1612.14 ± 46.25^{j}	989.14 ± 67.77^{g}	15.26 ± 1.29^{g}	26.32 ± 1.75^{i}	24.54 ± 5.72^{i}	0.05 ± 0.00^{h}	3.93 ± 0.02^{g}
1B1	1408.79 ± 19.32^{k}	708.82 ± 3.43^{j}	12.60 ± 0.18^{h}	23.80 ± 0.14^{k}	nd	0.02 ± 0.00^{j}	1.48 ± 0.05^{j}
1B2	3358.65 ± 13.70^{a}	764.09 ± 0.06^{i}	18.24 ± 1.93^{f}	29.10 ± 0.25^{h}	nd	0.04 ± 0.00^{i}	1.66 ± 0.07^{j}
1B3	387.66 ± 23.42^{r}	1188.36 ± 53.31^{e}	10.63 ± 0.18^{i}	10.21 ± 0.16^{r}	36.16 ± 8.71^{g}	0.02 ± 0.00^{j}	4.08 ± 0.14^{g}
1B4	1113.56 ± 36.04^{n}	864.93 ± 78.17^{h}	13.83 ± 1.94^{h}	18.83 ± 0.73^{m}	93.26 ± 12.95^{a}	0.09 ± 0.04^{g}	3.86 ± 1.03^{g}
1B5	1150.29 ± 19.44^{n}	36.76 ± 0.86^{t}	8.25 ± 0.07^{j}	15.26 ± 0.23o	20.85 ± 3.97^{i}	0.02 ± 0.01^{j}	2.99 ± 0.21^{h}
1B6	1224.87 ± 13.32^{m}	749.16 ± 14.09^{i}	10.88 ± 2.20^{i}	12.01 ± 0.02^{q}	nd	0.01 ± 0.00^{j}	1.96 ± 0.18^{j}
2A1	1226.04 ± 11.92^{m}	112.67 ± 0.06^{s}	11.44 ± 0.00^{i}	13.16 ± 0.21^{p}	nd	0.05 ± 0.00^{h}	1.55 ± 0.00^{j}
2A2	1316.26 ± 142.72^{l}	79.55 ± 30.53^{s}	18.00 ± 0.12^{f}	2.75 ± 0.10^{v}	nd	0.09 ± 0.00^{g}	1.12 ± 0.01^{k}
2A3	1081.90 ± 86.28^{n}	1210.13 ± 90.22^{e}	22.51 ± 0.39^{d}	17.73 ± 0.06^{n}	34.23 ± 1.30^{g}	0.03 ± 0.02^{i}	1.10 ± 0.13^{k}
2A4	263.31 ± 21.91^{s}	22.99 ± 0.44^{t}	6.24 ± 0.07^{k}	7.62 ± 0.21^{s}	nd	nd	Nd
2A5	1032.62 ± 14.80^{o}	90.07 ± 32.13^{s}	21.26 ± 0.22^{d}	6.76 ± 0.12^{t}	nd	0.02 ± 0.00^{j}	1.60 ± 0.17^{j}
2A6	1083.81 ± 35.33^{n}	494.50 ± 102.30^{m}	35.01 ± 0.92^{a}	4.74 ± 0.07^{u}	30.19 ± 4.83^{h}	0.03 ± 0.00^{j}	1.01 ± 0.08^{k}
2A7	944.98 ± 21.23^{o}	34.40 ± 0.82^{t}	11.90 ± 0.06^{i}	13.50 ± 0.05^{p}	19.17 ± 0.63^{i}	0.02 ± 0.00^{j}	1.73 ± 0.09^{j}
2B1	1504.82 ± 4.88^{k}	98.67 ± 13.70^{s}	23.57 ± 0.20^{c}	7.80 ± 0.40^{s}	nd	0.07 ± 0.00^{h}	1.03 ± 0.01^{k}
2B2	576.57 ± 6.24^{q}	48.96 ± 5.73^{t}	20.51 ± 0.10^{e}	2.22 ± 0.15^{v}	nd	0.03 ± 0.01^{j}	0.63 ± 0.07^{k}
2B3	627.68 ± 7.54^{q}	57.28 ± 11.45^{t}	14.58 ± 0.02^{g}	26.92 ± 0.75^{i}	21.19 ± 2.64^{i}	0.01 ± 0.00^{k}	1.90 ± 0.18^{j}
2C1	602.91 ± 6.76^{q}	337.50 ± 81.44o	16.95 ± 0.94^{f}	12.58 ± 0.79^{p}	16.33 ± 1.14^{j}	nd	0.84 ± 0.06^{k}
2C2	1191.99 ± 28.47^{m}	141.90 ± 27.21^{r}	14.47 ± 2.67^{g}	8.02 ± 0.07^{s}	12.21 ± 2.42^{k}	0.01 ± 0.00^{j}	1.75 ± 0.06^{j}
2C3	836.37 ± 11.59^{p}	182.38 ± 80.60^{r}	35.43 ± 0.81^{a}	7.16 ± 0.57^{t}	nd	0.10 ± 0.03^{g}	1.32 ± 0.13^{j}
2C4	377.62 ± 5.99^{r}	111.11 ± 3.78^{s}	12.35 ± 0.15^{h}	5.43 ± 0.07^{u}	14.15 ± 1.56^{k}	nd	0.71 ± 0.22^{k}
2C5	743.89 ± 32.08^{p}	138.26 ± 3.26^{r}	26.97 ± 0.41^{b}	5.94 ± 1.48^{t}	17.43 ± 0.67^{j}	nd	1.13 ± 0.02^{k}
2C6	1764.97 ± 20.53^{i}	1156.40 ± 2.59^{f}	17.62 ± 1.83^{f}	8.24 ± 0.30^{s}	20.01 ± 0.72^{i}	0.01 ± 0.00^{j}	1.44 ± 0.30^{j}
3A1	1666.18 ± 15.98^{j}	343.50 ± 23.86o	11.77 ± 0.15^{i}	15.27 ± 0.99^{o}	17.87 ± 1.27^{j}	0.02 ± 0.01^{j}	0.90 ± 0.02^{k}
3A2	967.73 ± 12.19^{o}	423.85 ± 14.23^{n}	15.39 ± 0.87^{g}	10.84 ± 0.36^{r}	nd	0.06 ± 0.00^{h}	1.23 ± 0.02^{j}
3A3	1515.81 ± 16.03^{k}	624.56 ± 21.23^{k}	24.17 ± 1.99^{c}	7.10 ± 0.61^{t}	nd	0.07 ± 0.00^{h}	0.86 ± 0.02^{k}
3A4	1096.43 ± 47.62^{n}	173.39 ± 5.96^{r}	13.59 ± 0.33^{h}	8.09 ± 0.05^{s}	nd	0.07 ± 0.00^{h}	3.20 ± 0.04^{h}
3A5	1207.37 ± 14.26^{m}	664.09 ± 4.33^{j}	15.31 ± 0.05^{g}	8.80 ± 0.04^{r}	nd	0.08 ± 0.00^{g}	2.34 ± 0.12^{i}
3A6	1381.80 ± 1.09^{k}	156.42 ± 9.71^{r}	17.69 ± 0.96^{f}	11.28 ± 0.03^{r}	27.75 ± 2.76^{h}	0.02 ± 0.00^{j}	1.24 ± 0.01^{j}
3A7	1440.32 ± 17.23^{k}	255.07 ± 16.63^{q}	18.28 ± 0.01^{f}	17.31 ± 0.20^{n}	37.79 ± 0.35^{f}	0.02 ± 0.00^{j}	1.48 ± 0.10^{j}
3A8	1466.00 ± 4.52^{k}	973.13 ± 34.25^{g}	18.31 ± 0.47^{f}	12.98 ± 0.47^{p}	nd	0.05 ± 0.02^{h}	4.86 ± 1.04^{f}

(Continued)

Table 3. (Continued)

HONEY	L-TRP[1]	5-OH-TRP[2]	TRYP[3]	SER[4]	MEL[5]	L-DOPA[6]	DOP[7]
3B2	3158.17 ± 32.94[b]	352.68 ± 9.56o	21.29 ± 0.26[d]	37.01 ± 0.09[e]	nd	0.05 ± 0.00[h]	1.54 ± 0.19[j]
3B3	928.68 ± 1.88o	173.53 ± 9.38[r]	10.30 ± 0.01[i]	13.74 ± 0.01[p]	14.93 ± 2.44[k]	0.02 ± 0.00[j]	0.69 ± 0.04[k]
3B5	1729.82 ± 16.02[i]	777.89 ± 2.44[i]	20.38 ± 0.02[e]	13.97 ± 0.18[p]	nd	0.06 ± 0.01[h]	9.84 ± 0.06[c]
3B6	1228.09 ± 12.22[m]	221.52 ± 15.86[q]	16.89 ± 0.15[f]	8.82 ± 0.01[r]	nd	0.02 ± 0.00[j]	2.92 ± 0.03[h]
3B7	1075.95 ± 14.30[n]	287.19 ± 2.56[p]	17.75 ± 0.16[f]	9.61 ± 0.50[r]	nd	0.02 ± 0.00[j]	1.10 ± 0.04[k]
3C1	934.12 ± 3.76[o]	567.29 ± 13.81[l]	21.67 ± 0.81[d]	4.97 ± 0.00[u]	nd	0.01 ± 0.00[k]	0.93 ± 0.00[k]
3C2	1309.26 ± 25.95[l]	1547.30 ± 55.99[c]	17.76 ± 0.99[f]	21.38 ± 0.13[l]	nd	0.02 ± 0.00[j]	2.39 ± 0.07[i]
3C3	1378.81 ± 13.15[k]	87.45 ± 9.45[s]	16.50 ± 2.51[g]	51.52 ± 1.57[d]	58.63 ± 0.31[d]	0.53 ± 0.01[b]	8.49 ± 1.48[d]
3C4	637.20 ± 8.88[q]	161.84 ± 3.21[r]	11.99 ± 5.05[i]	12.98 ± 0.35[p]	17.60 ± 0.45[j]	0.01 ± 0.00[k]	2.51 ± 0.17[i]
3C5	954.75 ± 2.01[o]	108.54 ± 0.48[s]	16.13 ± 1.05[g]	14.92 ± 0.41o	41.77 ± 0.88[e]	nd	2.36 ± 0.62[i]
4A1	2250.72 ± 2.55[f]	119.28 ± 0.34[s]	15.59 ± 0.63[g]	31.31 ± 1.24[g]	nd	0.04 ± 0.01[i]	3.33 ± 0.37[h]
4A2	944.05 ± 2.95[o]	109.71 ± 1.51[s]	27.22 ± 1.02[b]	11.40 ± 0.52[r]	nd	0.32 ± 0.00[c]	4.68 ± 0.07[f]
4A3	1451.82 ± 59.95[k]	155.58 ± 33.77[r]	14.61 ± 0.47[g]	10.56 ± 2.09[r]	44.49 ± 6.87[e]	0.06 ± 0.00[h]	3.96 ± 0.07[g]
4A4	1452.47 ± 51.42[k]	76.18 ± 7.58[s]	12.37 ± 0.78[h]	24.81 ± 0.74[j]	nd	0.03 ± 0.00[i]	1.54 ± 0.06[j]
4A5	826.83 ± 0.73[p]	71.95 ± 5.36[s]	10.85 ± 0.54[i]	24.71 ± 0.25[j]	nd	0.01 ± 0.00[k]	1.23 ± 0.01[j]
4A6	1291.53 ± 36.42[l]	287.23 ± 11.21[p]	17.77 ± 0.40[f]	37.70 ± 0.44[e]	nd	0.03 ± 0.00[j]	2.40 ± 0.12[i]
4B1	1455.32 ± 30.95[k]	98.47 ± 10.14[s]	17.07 ± 0.33[f]	33.04 ± 0.24[f]	22.05 ± 1.30[i]	0.00 ± 0.00[k]	0.00 ± 0.00[l]
4B2	2412.74 ± 59.28[e]	153.78 ± 2.29[r]	20.26 ± 0.41[e]	26.98 ± 0.54[i]	nd	0.09 ± 0.01[g]	2.48 ± 1.61[i]
4B3	2141.29 ± 10.93[g]	2045.59 ± 72.26[a]	18.65 ± 0.50[f]	12.05 ± 0.05[q]	45.15 ± 0.23[e]	0.09 ± 0.00[g]	Nd
4B4	1951.09 ± 17.79[h]	115.81 ± 7.38[s]	22.13 ± 1.20[d]	20.83 ± 1.09[l]	nd	0.05 ± 0.01[h]	0.68 ± 0.02[k]
4B5	837.81 ± 9.78[p]	76.73 ± 2.56[s]	16.03 ± 0.34[g]	11.82 ± 1.30[r]	nd	0.09 ± 0.02[g]	0.69 ± 0.36[k]
4B6	884.27 ± 25.97[o]	99.36 ± 29.06[s]	17.41 ± 0.86[f]	495.15 ± 0.14[a]	nd	0.72 ± 0.00[a]	33.98 ± 0.20[a]
4B7	786.62 ± 8.64[p]	71.21 ± 1.31[s]	13.62 ± 0.25[h]	33.38 ± 0.44[f]	nd	0.03 ± 0.00[j]	1.74 ± 0.07[j]
5.1	626.98 ± 7.19[q]	1714.61 ± 28.85[b]	14.83 ± 1.48[g]	33.11 ± 0.54[f]	30.03 ± 1.32[h]	0.05 ± 0.00[h]	2.69 ± 0.03[i]
5.2	662.25 ± 7.29[q]	1700.99 ± 10.37[b]	15.95 ± 0.02[g]	23.05 ± 0.24[k]	32.83 ± 0.22[g]	0.05 ± 0.00[h]	2.97 ± 0.10[h]
5.3	1122.22 ± 46.81[n]	80.16 ± 6.03s	19.63 ± 2.77[e]	34.02 ± 3.20[f]	88.08 ± 2.31[b]	0.02 ± 0.00[j]	4.02 ± 0.99[g]
5.4	2024.21 ± 31.36[h]	70.86 ± 0.20[s]	15.89 ± 0.20[g]	16.87 ± 1.19[n]	35.24 ± 3.00[g]	0.02 ± 0.01[j]	1.85 ± 0.01[j]
5.5	2569.65 ± 92.00[d]	87.26 ± 5.95[s]	25.13 ± 0.13[c]	64.13 ± 0.05[b]	44.29 ± 2.48[e]	0.16 ± 0.03[e]	5.02 ± 0.02[f]
5.6	750.03 ± 28.87[p]	115.33 ± 15.91[s]	16.01 ± 0.32[g]	25.57 ± 1.65[j]	66.25 ± 3.76[c]	0.02 ± 0.00[j]	3.67 ± 0.65[g]
5.7	2827.54 ± 41.19[c]	81.84 ± 15.31[s]	18.65 ± 0.09[f]	27.92 ± 0.08[h]	35.53 ± 7.00[g]	0.13 ± 0.03[f]	4.19 ± 0.93[g]
Minimum	263.31	nd	6.24	2.22	nd	nd	Nd
Maximum	3358.65	2045.59	35.43	495.15	93.26	0.72	33.98

Results are given as means ± standard deviation. Means followed by the same letter in the column do not differ statistically from each other by Scott-Knott test ($P \leq .05$).

[1]L-tryptophan.
[2]5-Hydroxy-tryptophan.
[3]Tryptamine.
[4]Serotonin.
[5]Melatonin.
[6]L-DOPA.
[7]Dopamine.

between seasons, year, flowering, and climate and it is not possible to use this compound as a biochemical marker of honey quality. 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.[33] 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.[34] 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.[35,36] 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.[37] In honeys from Turkey, whose predominant flowering was fava beans (*Vicia fava*), Topal et al[39] 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.[39] In addition to acting as a neutrotransmitter, Dop may act as an immunomodulatory regulator, besides being indispensable for neuroimmune communication, i.e., its relationship with alterations in the functions of macrophages, lymphocytes, neutrophils and monocytes, showing that immune cells, in homeostatic and pathological conditions, interact with Dop centrally and peripherally.[40] Some studies have shown a relationship between Dop levels and Dop receptors and diseases such as glaucoma, diabetes and cardiovascular disease.[41] 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 and 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), at higher altitudes and in Spring 2018. A higher content of 5-OH-Trp was also found in samples from a milder climate, harvest in 2019. On the other hand, L-Trp and Tryp, as well as Mel, were found at higher levels in honeys harvested in 2019 during the hottest seasons and in Cfa climate.

Author Contributions

CVB: Formal analyses, statistical analysis, writing (draft preparation and review and 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 and editing), and funding acquisition. GPPL: Conceptualization, formal analysis, supervision and project administration, writing (original draft preparation and review and editing), and funding acquisition.

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