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Bioactive amines in *Passiflora* are affected by species and fruit development

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ABSTRACT

Bioactive amines were determined in selected passion fruit species and throughout fruit development. The same amines (spermine, spermidine, agmatine, putrescine and tryptamine) were found in four *Passiflora* species (2008–2010 growing seasons) at different concentrations: *P. alata* had higher polyamines (spermine + spermidine, 8.41 mg/100 g); *P. setacea* and *P. nitida* had higher putrescine (>7.0 mg/100 g); *and P. setacea* had higher agmatine contents (1.37 mg/100 g) compared to the others. The indolamine tryptamine was present at low concentrations in all species (~0.05 mg/100 g). *P. nitida* and *P. alata* had the highest soluble solids (~18°Brix); *P. edulis* had the lowest pH (2.97) and *P. nitida* the highest pH (4.19). Throughout *P. setacea* fruit development, the concentrations of spermidine, putrescine and agmatine decreased; spermine contents did not change; and pH decreased. Fruit shelf life and some of the health promoting properties of *Passiflora* and their synthesis are modulated by species.

1. Introduction

Passion fruit is valued due to its pleasant sweet-acid taste, intense flavor and also by its health promoting properties. The fruit belongs to the *Passifloraceae* family and, although hundreds of *Passiflora* species can be found worldwide, only some are edible. The most cultivated species are the yellow and purple (*Passiflora edulis* Sims) and the sweet passion fruits (*Passiflora alata*). The yellow species is the most widely grown, however it is susceptible to diseases, among them, anthracnose (Dembitsky et al., 2011; Jiménez et al., 2011; Oliveira, Soares, Barbosa, Santos-Filho, & Jesus, 2013). Passion fruit is widely used fresh and also for juice and jam processing. It has also been utilized as ingredient for desert, ice cream, candy and gourmet preparations. Passion fruit is rich in minerals (calcium and phosphorus) and vitamins, especially A, C, thiamine, riboflavin and niacin. It is also a good source of carotenoids, anthocyanins and alkaloids (Jiménez et al., 2011; Porto-Figueira, Freitas, Cruz, Figueira, & Câmara, 2015).

Several functional properties have been attributed to passion fruit, including: antioxidant, anti-inflammatory, antipyretic, analgesic, sedative and hypotensive activities. This fruit is also neuroactive, demonstrating antianxiety and anticonvulsant effects (Dembitsky et al., 2011; Porto-Figueira et al., 2015). The antioxidant activity has been attributed to commonly known endogenous components that display antioxidant activity, including vitamin A, ascorbic acid and phenolic compounds. However, polyamines have also been suggested to contribute to this activity (Kalac, 2014). The polyamines spermine and spermidine belong to a larger group of biologically active amines – the bioactive amines. They are ubiquitous to living organisms, playing important roles in cell metabolism, growth and differentiation. Due to

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polycationic characteristics, polyamines can scavenge free radicals and thereby prevent oxidation (Bassard, Ullmann, Bernier, & Werck-Reichhart, 2010; Gloria, 2005). In plants, polyamines are also associated with many developmental processes, such as cell division, root formation, embryogenesis, organogenesis, floral initiation, senescence, fruit development and ripening. Furthermore, polyamines are involved in the control and modulation of abiotic stress tolerance in plants (Fariduddin, Varshney, Yusuf, & Ahmad, 2013; Pathak, Teixeira da Silva, & Wani, 2014). Other amines, the biogenic amines, can also be present in fruit. Some are inherent to the plant, whereas others are synthesized in response to stress or as protection against predators. Some amines are precursors of important plant hormones such as indole-3-acetic acid and phenyl acetic acid. Some can be vasoactive and neuroactive, playing relevant roles in vascular and neurological systems. The pharmacological potential of biogenic amines in neurotransmission has also been described (Fujiwara et al., 2010; Gloria, 2005; Sánchez-Jiménez, Ruiz-Pérez, Urdiales, & Medina, 2013). The neuroactivity associated with *Passiflora* could result from the presence of certain biogenic amines. At high concentrations, some amines, e.g., putrescine and cadaverine, are undesirable due to organoleptic issues. In the searched literature, only two studies investigating amines in *Passiflora* have been reported in the literature. Smith (1977) reviewed studies on tryptamines and reported them in different *Passiflora* tissues and Santiago-Silva, Labanca, and Gloria (2011) surveyed passion fruit and four other fruits from the market for bioactive amines. There is thus a lack of studies related to the presence of these amines which have potential impact on health and quality in *Passiflora* species, and there is a need to ascertain their occurrence and role in plant physiology and in health promoting properties of this fruit.

Embrapa Cerrados, affiliated with the Brazilian Ministry of Agriculture, created a program (Passitec) with the purpose of generating information and technologies for different savanna native *Passiflora* species. Another focus of this initiative is to improve functional properties of *Passiflora* fruit by enhancement of cultivation practices, production, processing and storage. Among *Passiflora* species included in the present investigation, *P. nitida* and *P. setacea* have been valued due to their sweet and pleasant sensory characteristics, and resistance to diseases. *P. setacea* has also been highlighted due to its association with sleep modulation; thus, it has been popularly named sleep passion fruit. Information in the literature on the nutritional and functional properties of these species are lacking (Cohen et al., 2008). The objective of this study was to investigate the occurrence of polyamines and other bioactive amines in four different savanna *Passiflora* species and to use this information to distinguish between species. Additionally, changes in bioactive amines concentrations were also investigated during development of a unique species, *Passiflora setacea*, which does not show significant color change during fruit development and also is promising as a disease resistant species.

2. Material and methods

2.1. Material

Passion fruit (fruit and pulp) was obtained from the experimental area of Embrapa Cerrados – 15°37′09″S 47°39′09″W (BR - 020; km 18; Embrapa Planaltina, DF, Brasil) from the 2008–2011 growing seasons.

All reagents were of analytical grade, except HPLC reagents which were chromatographic grade. Bioactive amine standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They included spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, agmatine sulfate, cadaverine dihydrochloride, 5-hydroxytryptamine (serotonin), histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride and tryptamine. *o*-Phthaldehyde was also purchased from Sigma Chemical Co. Ultrapure water was obtained from a Milli-Q System (Millipore Corp., Milford, MA, USA). Mobile phases were filtered through HAWP and HVWP membranes (47 mm diameter and 0.45 μm pore size, Millipore Corp., Milford, MA, USA), used for aqueous and organic solvents, respectively.

2.2. Methods

2.2.1. Comparison of different Passiflora species regarding the profile and concentrations of amines and some physicochemical characteristics

For discrimination among the four different *Passiflora* species, pulps from *Passiflora alata*, *P. edulis*, *P. nitida* and *P. setacea* BRS PC from the 2008–2010 growing seasons (total of 3 different harvest times) were used. The fruit was harvested at physiological maturity and had similar visual quality (color and size). A minimum of 30 fruits of each species was considered for each season. The pulp was obtained by gently grinding in a blender and sieving through 1 mm polyethylene mesh. Samples were homogenized, packed in polyethylene bags and kept at −80 °C until analysis for ten bioactive amines and also for some physicochemical characteristics (total soluble solids and pH).

2.2.2. Investigation of the changes in the profile and concentrations of amines and some physicochemical characteristics during development of Passiflora setacea

To investigate the changes in amines throughout development, *Passiflora setacea* BRS PC fruits from plants from the same experimental area of Embrapa Cerrados from the 2011 season were used. Fruit was collected at different stages of development starting from the 6th day after anthesis. Seven developmental stages were sampled: three growth stages (G0–6 to 8 days, G1–9 to 11 days and G2–12 to 15 days after anthesis); one physiological maturity (PM – 25 to 35 days after anthesis); two ripening stages (R1–40 to 45 days, and R2–43 to 50 days after anthesis); and one senescence stage (S – >50 days after anthesis). A minimum of nine fruits were considered a batch, and three batches of each developmental stage were harvested and used for analysis. The fruit was weighed and longitudinal and transversal diameters were measured (steel caliper, Digimess, Mooca, SP, Brazil). Afterwards, the pulp of the fruit, obtained by gentle blending and sieving, was analyzed for bioactive amines contents and also for total soluble solids, pH and moisture content.

2.2.3. Methods of analysis

2.2.3.1. Determination of bioactive amines. Free bioactive amines were determined by ion-pair reverse phase HPLC, followed by post-column derivatization with *o*-phthaldehyde (OPA) and fluorimetric detection (Santiago-Silva et al., 2011) at a laboratory which is accredited under ISO 17025. Briefly, amines were extracted from 5 g samples with 5% trichloroacetic acid (TCA). Samples were homogenized for 10 min in a shaker (TE Tecnal - 140, Piracicaba, SP, Brazil), centrifuged at 11,180*g* at 4 °C for 21 min, and the supernatant was collected. The solid residue was submitted to two additional extractions with 7 mL TCA and all supernatants were combined, filtered through qualitative filter paper and through a HAWP 0.45 μm pore size membrane (Millipore Corp. Milford, MA, USA) prior to HPLC analysis. The amines were separated and quantified by ion-pair reverse phase HPLC, post-column derivatization with *o*-phthalaldehyde (OPA) and fluorometric detection. Liquid chromatography was carried out in a Shimadzu, Model LC-10 AD HPLC connected to a RF-551 spectrofluorometric detector with 340 and 445 nm of excitation and emission wavelengths, respectively (Shimadzu, Kyoto, Japan). A Novapak C18 column, 300 × 3.9 mm i.d., 10 μm, was used with a Novapak C18 guard-pak insert (Waters, Milford, MA, USA). Two mobile phases were used to generate a gradient elution: A – 0.2 M sodium acetate and 15 mM 1-octanesulfonic acid sodium salt adjusted to pH 4.9 with acetic acid, and B – 100% acetonitrile. The amines were identified by comparison of the retention times of the amines in the sample with those of standard solutions and also confirmed by addition of the suspected amines to the sample. External calibration curves ($r^2 > 0.99$) were used to calculate amines contents from

fluorescence readings and the concentrations were expressed as mg/100 g. In order to assure reproducibility throughout the day and between days, standards were run daily in between every four samples.

2.2.3.2. Determination of selected physicochemical characteristics of the pulp. Fruit pulp was analyzed for selected physicochemical parameters, i.e., total soluble solids, pH, and moisture content (AOAC, 2005). Moisture content was used to report amines on a dry weight basis for samples throughout fruit development.

2.3. Statistical analysis

The results were submitted to analysis of variance and the means were compared by the Tukey test at 5% of probability.

3. Results and discussion

3.1. Occurrence of bioactive amines in different Passiflora species

Among the ten amines investigated only five were detected in passion fruit, i.e., spermine, spermidine, putrescine, agmatine and tryptamine. Cadaverine, histamine, tyramine, serotonin and phenethylamine were not detected in any of the samples. Spermine, spermidine and putrescine were present in 100% of the samples of all the *Passiflora* species; whereas the occurrence of agmatine and tryptamine varied depending on the species (Table 1). The occurrence of agmatine varied from 25% in *P. nitida* up to 100% in both *P. alata* and *P. setacea*; and of tryptamine from 25% in *P. nitida* to 50% in *P. alata* and *P. setacea*. *P. alata* and *P. setacea* showed similar profile of amines.

During investigation of the amine profile in different tropical fruits from the consumer market, Santiago-Silva et al. (2011) observed similar occurrences of spermine, spermidine, putrescine and agmatine in commercial passion fruit. However, they did not detect tryptamine; instead, they detected serotonin in 57% of samples. Both indolamines have been described in some *Passiflora* tissues by Smith (1977): in the tendrils and laminae of *Passiflora quadrangularis* L. and shoots and seedlings of *P. edulis* Sims. According to Fujiwara et al. (2010) and Martins and Gloria (2010), serotonin and tryptamine can be synthesized from tryptophan; the first by a pathway involving two enzymes: tryptophan-5-hydroxylase and aromatic amino acid decarboxylase; and the later by tryptophan-5-decarboxylase. Moreover, tryptamine can be transformed into serotonin by tryptamine-5-hydroxylase. According to these authors, these pathways are followed depending on the species, cultivation practices and stress conditions (Fujiwara et al., 2010; Martins & Gloria, 2010). It is possible that species, cultivation practices and climatic conditions prevalent for savanna *Passiflora* compared to southeastern and northeastern commercially produced *Passiflora*, could influence which indolamine accumulated in *Passiflora*, as depicted in Fig. 1.

The presence of spermidine, spermine and putrescine in fruits has been described in the literature. In fact, the polyamines spermidine and spermine are ubiquitous in plants and other living cells, where they play a role in a wide range of biological processes including plant growth, development and senescence. Polyamines are also important in membrane stabilization by acting as free radical scavengers, inhibiting phospholipids movement and reducing lipoxygenase activity. Moreover, they can increase plant survival under abiotic stresses, such as salt, drought, flooding, chilling, osmotic, acid, nutrient imbalance, radiation-induced oxidative and heavy metal stress, as well as infection by pathogenic fungi and viruses (Bassard et al., 2010; Fariduddin et al., 2013; Harindra Champa, Gill, Mahajan, & Bedi, 2015; Pathak et al., 2014; Sudhakar et al., 2015). Putrescine is a required precursor in the biosynthetic pathway of the polyamines (Gloria, 2005; Kalac, 2014). The presence of agmatine in some of the *Passiflora* species indicates another possible route for the synthesis of polyamines via agmatine, even though the most common pathway is via ornithine, as indicated in Fig. 1. Agmatine has also been reported in fruit, e.g., pineapple and papaya (Gloria, 2005; Santiago-Silva et al., 2011). Tryptamine has been reported in Tahiti lemon and orange (Unpublished data). According to Smith (1977), tryptamine occurs in a variety of plants, especially Gramineae and Leguminosae. It is involved in auxin biosynthesis, e.g., production of the plant growth hormone indole acetic acid. It can also be used as a precursor of a wide variety of alkaloids. Furthermore, it can deter insects (Smith, 1977; Gloria, 2005; Runguphan, Maresh, & O'Connor, 2009).

3.2. Concentration of bioactive amines and physicochemical characteristics in different Passiflora species

Concentrations of the five amines varied among *Passiflora* species and also among samples from the same species (Table 1), which could result from differences among the three different growing seasons (2008–2010) included in this study. However, in general, spermidine and putrescine were present at high concentrations; spermine at intermediate; and agmatine and tryptamine at low concentrations. *P. alata* was characterized by significantly higher spermidine and spermine levels; *P. nitida* had higher putrescine and spermine levels; *P. setacea* had higher putrescine and agmatine levels and *P. edulis* had lower levels of all of the amines. No significant difference among species was observed in tryptamine contents.

When comparing total amines among species, similar levels (~120 g/kg) were found, except for *P. edulis*, which had approximately 30% of the levels of the other species (Table 1). Comparison of the total levels of polyamines (spermine + spermidine), indicated that significantly higher mean levels were found for *P. alata* and *P. nitida* (84.1 and 52.0 g/kg, respectively) compared to *P. edulis* and *P. setacea* (26.3 and 25.9 g/kg, respectively). Based on these results, the polyamines represented approximately 71% of the total amines levels in *P. alata* and *P. edulis*, but only 42% in *P. nitida* and 21% in *P. setacea*.

These results show important trends; for example, *P. alata* is the best source of polyamines; whereas *P. setacea* is the best source of agmatine. Higher availability of polyamines could warrant plant survival under stress. High amounts of putrescine were found in *P. setacea* and *P. nitida*. It is interesting that Rodrigues (2009), when investigating sensory characteristics of the same savanna *Passiflora* species (*P. alata*, *P. nitida*

Table 1
Bioactive amines concentration on a fresh weight basis and percent occurrence in four species of *Passiflora* from the experimental area of Embrapa Cerrados.

	Mean ± standard deviation (mg/kg fresh weight) (occurrence)[a]					
Passiflora species	Spermine	Spermidine	Agmatine	Putrescine	Tryptamine	Total
P. alata	14.3 ± 4.9^{a} (100%)	69.8 ± 19.6^{a} (100%)	1.50 ± 0.3^{b} (100%)	31.6 ± 10.2^{b} (100%)	0.5 ± 0.6^{a} (50%)	117 ± 28^{a}
P. edulis	6.0 ± 1.9^{b} (100%)	20.3 ± 4.4^{b} (100%)	0.70 ± 0.8^{bc} (50%)	9.9 ± 5.5^{c} (100%)	0.6 ± 0.9^{a} (33%)	37.4 ± 9.7^{b}
P. nitida	14.4 ± 12.0^{a} (100%)	37.6 ± 14.7^{ab} (100%)	0.00 ± 0.1^{c} (25%)	70.1 ± 21^{a} (100%)	0.2 ± 0.5^{a} (25%)	122 ± 46^{a}
P. setacea	3.5 ± 2.3^{b} (100%)	22.4 ± 8.6^{b} (100%)	13.7 ± 7.3^{a} (100%)	84.1 ± 37^{a} (100%)	0.6 ± 0.6^{a} (50%)	124 ± 54^{a}

[a] n = 3 different seasons (2008–2010), each replicate consisted of 30 fruits. Means with different letters in the same column are significantly different (Tukey test, $P < 0.05$).

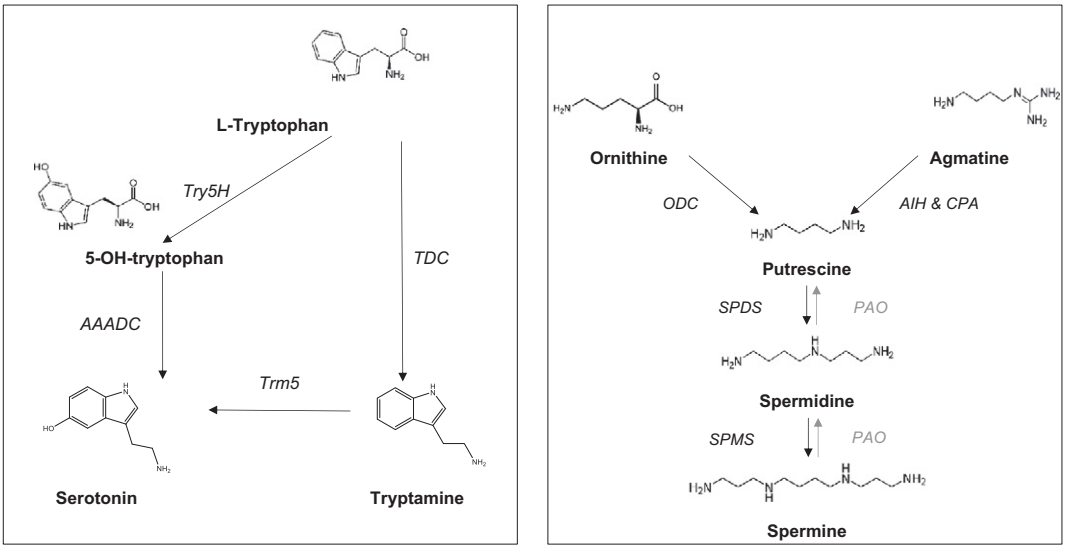

Fig. 1. Potential pathways in *Passiflora* for indolamines [Try5H – tryptophan-5-hydroxylase, TDC – tryptophan decarboxylase, AAADC – aromatic L-amino acid decarboxylase, Trm5H – tryptamine-5-hydroxylase] and polyamines [ODC – ornithine decarboxylase, AIH – agmatine iminohydrolase, CPA – *N*-carbamoylputrescine amidohydrolase, SPDS – spermidine synthase, SPMS – spermine synthase, PAO – polyamine oxidase].

and *P. setacea*), observed a putrid note which they called 'aroma of damaged'. Since putrescine can impart a putrid flavor to food products, its impact in *Passiflora* acceptability should be further investigated. It is possible that by modulating polyamines biosynthesis (Fig. 1), putrescine levels could be minimized, providing fruit with more desirable sensory characteristics.

The levels of amines found in these *Passiflora* species differed from those reported for commercial *P. edulis* (Santiago-Silva et al., 2011). According to Gloria (2005) and Valero, Martínez-Romero, and Serrano (2002), the prevalence of spermidine over spermine is expected in most plants. The prevalence of spermidine over putrescine is also expected in most plants, with few exceptions such as orange and grape (Agudelo-Romero, Bortolloti, Pais, Tiburcio, & Fortes, 2013; Vieira, Silva, & Gloria, 2010). The profile of amines can be inherent to the species. However, under abiotic stress (mineral deficiency, drought stress, and large temperature variations), some plants can accumulate putrescine (Gloria, 2005; Sudhakar et al., 2015). Therefore, the prevalence of putrescine over spermidine for *P. nitida* and *P. setacea*, could indicate a peculiarity of these species or that conditions experienced during growth and cultivation were not completely adequate. Studies are needed to investigate which of these hypotheses is true.

With respect to physicochemical characteristics, total soluble solids contents were 14.4 ± 0.1, 15.2 ± 0.3, 18.7 ± 0.2 and 18.9 ± 1.9°Brix for *P. setacea*, *P. edulis*, *P. nitida* and *P. alata*, respectively; *P. nitida* and *P. alata* had higher sugar contents, being sweeter. pH values also varied significantly among species, with lower values for *P. edulis* (2.97 ± 0.03), higher for *P. nitida* (4.19 ± 0.12) and intermediate for *P. setacea* (3.15 ± 0.03) and *P. alata* (3.73 ± 0.03). Similar pH values were observed by Jiménez et al. (2011) and Santiago-Silva et al. (2011) for commercial *P. edulis* and by Cohen et al. (2008) for *P. alata* and *P. nitida*. Species with different sugar contents and pH values are desirable, depending on consumer's preference and the intended processing and utilization of the fruit.

3.3. Changes in amines and physicochemical characteristics during development of P. setacea

There were visual changes in size and dimensions of the fruits of *P. setacea* during various stages of fruit development; however there was no apparent change in peel color during ripening, which is characteristic of this species (Costa et al., 2009). The dimensions and weights of the fruits (Table 2) are within those reported by Costa et al. (2009) for *P. setacea*. During fruit development, longitudinal and transversal diameters increased up to the physiological maturity – PM (25–35 days after anthesis) and decreased significantly at senescence (>50 days after anthesis). The weight of the fruits reached maximum values on ripening stage R1 (40–45 days after anthesis) and R2 (43–50 days after anthesis), and decreased significantly at senescence.

Higher total soluble solids were obtained at R1 (40–45 days after anthesis) and throughout senescence. pH, which was originally 4.20 (G1),

Table 2
Quality parameters – diameter (cm), mass (g), total soluble solids (SS, °Brix), pH and moisture content (g/100 g) – during development of *Passiflora setacea* fruit.

Stage[a]	Diameter[b] (cm)		Mass	SS	pH	Moisture
	Long.	Transv.	(g)	(°Brix)		(g/100 g)
G0	2.92 ± 0.14^{d}	2.29 ± 0.07^{d}	7.69 ± 0.27^{e}	–[c]	–	–
G1	4.08 ± 0.08^{c}	3.29 ± 0.07^{c}	24.07 ± 2.04^{d}	6.37 ± 0.40^{c}	4.20 ± 0.62^{a}	94.94 ± 0.44^{a}
G2	4.74 ± 0.07^{b}	4.09 ± 0.05^{b}	43.97 ± 3.54^{c}	8.40 ± 2.49^{bc}	3.30 ± 0.54^{ab}	92.06 ± 2.08^{a}
PM	5.54 ± 0.19^{a}	5.13 ± 0.10^{a}	81.68 ± 2.53^{b}	7.70 ± 1.04^{bc}	2.89 ± 0.11^{b}	91.80 ± 1.64^{a}
R1	5.80 ± 0.27^{a}	5.17 ± 0.13^{a}	88.36 ± 0.86^{a}	11.5 ± 0.59^{ab}	2.59 ± 0.05^{c}	87.37 ± 0.73^{b}
R2	5.77 ± 0.17^{a}	5.44 ± 0.04^{a}	91.92 ± 0.12^{a}	13.6 ± 0.44^{a}	2.57 ± 0.01^{c}	85.64 ± 0.80^{b}
S	4.66 ± 0.14^{b}	4.10 ± 0.15^{b}	45.00 ± 5.00^{c}	13.6 ± 0.59^{a}	2.92 ± 0.04^{b}	87.95 ± 1.31^{b}

n = 3 (each replicate consisted of 9 fruits, 2011 season). Means (±standard deviations) with different letters in the same column are significantly different (Tukey test, $P < 0.05$).
[a] G – growth, PM – physiological maturity, R – ripening and S – senescence; G0–6 to 8 days, G1–9 to 11 days, G2–12 to 15 days, PM – 25 to 35 days, R1–40 to 45 days, R2–43 to 50 days, S – >50 days after anthesis).
[b] Diameter – longitudinal and transversal.
[c] – not analyzed, Not enough pulp for analysis.

Table 3
Bioactive amines concentration during development of *Passiflora setacea* fruit on a dry weigh basis.

Stage[a]	Mean levels ± standard deviation (mg/kg dry weight basis)[b]				
	Spermine	Spermidine	Agmatine	Putrescine	Total
G1	36.7 ± 5.6^{a}	373.5 ± 50.2^{a}	289.2 ± 64.2^{a}	1820.1 ± 247^{a}	2519.5 ± 307^{a}
G2	35.7 ± 7.1^{a}	304.6 ± 16.9^{a}	220.4 ± 32.8^{ab}	1296.9 ± 81.9^{b}	1867.6 ± 106^{ab}
PM	32.2 ± 7.2^{a}	205.1 ± 7.8^{b}	208.8 ± 133^{ab}	1111.3 ± 420^{bc}	1557.4 ± 542^{bc}
R1	36.7 ± 5.5^{a}	168.8 ± 47.6^{b}	73.3 ± 26.6^{b}	508.4 ± 216.7^{cd}	787.2 ± 291^{cd}
R2	33.7 ± 2.0^{a}	154.4 ± 21.0^{b}	47.0 ± 20.5^{b}	274.9 ± 164.1^{d}	510.0 ± 204^{d}
S	34.5 ± 3.4^{a}	163.3 ± 33.2^{b}	48.7 ± 10.0^{b}	228.4 ± 110^{d}	474.8 ± 157^{d}

[a] G – growth, PM – physiological maturity, R – ripening and S – senescence; G1–9 to 11 days, G2–12 to 15 days, PM – 25 to 35 days, R1–40 to 45 days, R2–43 to 50 days, S – >50 days after anthesis). There was not enough pulp for analysis in G0.
[b] n = 3 (each replicate consisted of 9 fruits, 2011 season). Means with different letters in the same column are significantly different (Tukey test, $P < 0.05$).

decreased to approximately 2.6 at the ripening stages. Moisture content decreased during development, showing lower values after physiological maturity. Similar changes in pH and moisture content were observed by Jiménez et al. (2011) during ripening of *P. edulis*. The decrease in pH is an unusual behavior during fruit ripening, although it has been observed in a few fruits, like noni, banana and pineapple. Cardenas-Coronel et al. (2016) reasoned that this behavior is typical of some acid tropical fruits, due to the enhanced synthesis of organic acids during ripening. Further studies should investigate the changes taking place during ripening of *P. setacea* which could support such behavior.

Among the ten amines investigated, only four were found in the *Passiflora setacea* fruit: spermidine, spermine, putrescine and agmatine. Tryptamine, which was detected in the previous experiment, was present at trace levels, below the detection limit of the method. This could result from the use of fruits from different growing seasons compared to the previous experiment. Since there were significant differences in moisture content of the pulp throughout fruit development, the concentrations of amines were expressed on a dry-weight basis. There was variability in amines contents among batches from the same stage of development, which could suggest fruit differences resulting from sampling at different parts of the plants or difficulty in being precise during samples collection at each specific development stage. Total amines decreased significantly throughout fruit development, reaching, at the ripening stage, 20% of the initial content (Table 3). Changes in putrescine concentrations were responsible for most of the decline in total amines. Spermidine concentrations were higher during growth compared to ripening and senescence stages. Concentrations of agmatine were lower during ripening compared to initial concentrations. There was no significant difference in spermine concentrations throughout fruit development. The decrease in putrescine, spermidine and agmatine concentrations followed a linear tendency as indicated by their coefficients of linear correlation (R = 0.9414, 0.9058 and 0.9170, respectively). The degradation rate for putrescine (35.2 g/kg per day) was higher compared to those for spermidine and agmatine (4.9 and 5.7 g/kg per day, respectively).

The percent contribution of each amine to total amine levels throughout fruit development is indicated in Fig. 2. Putrescine was the amine which contributed the most to total amines concentration in the fruit throughout development. However, percent contribution decreased from 72.2% in the early stage of growth to approximately 60% in the ripening stage and to 48% during senescence. On the contrary, the contribution of spermine and spermidine to the total concentration increased throughout fruit development from 1.5 to 7.3% for spermine and from 14.8 to 34.4% for spermidine. The contribution of agmatine to total amines remained the same throughout fruit development (9.2–13.4%).

There was no previous information regarding the changes of bioactive amines during passion fruit development. However, these amines follow literature trends reported for the polyamines spermine and spermidine, which are prevalent during fruit growth, mainly characterized by intense cell division, but the concentrations decline during ripening (Gloria, 2005; Valero et al., 2002). According to Mondal et al. (2008) and Valero et al. (2002), this is because the biosynthesis of ethylene, the ripening hormone, and the biosynthesis of

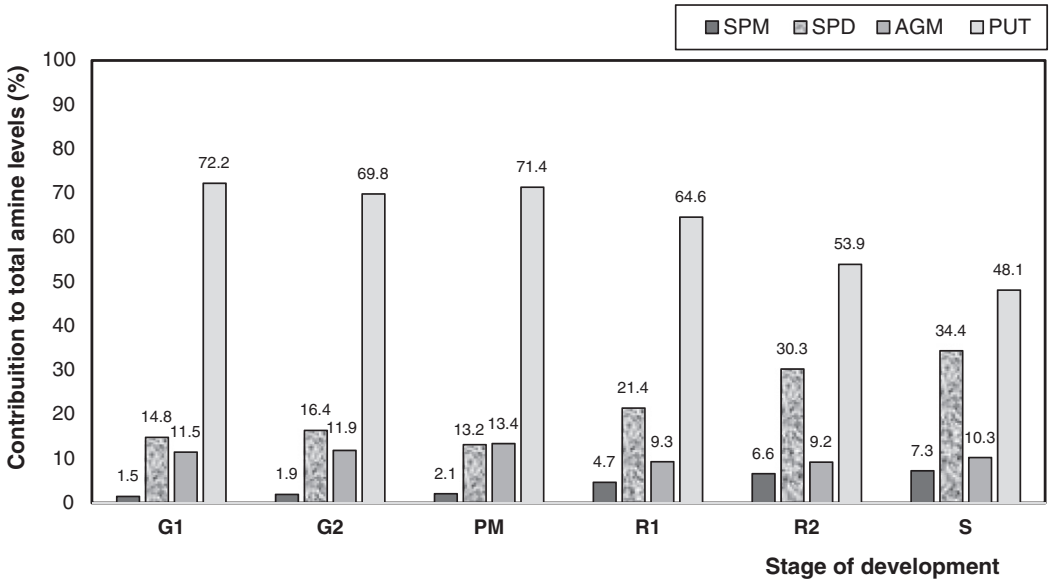

Fig. 2. Percent contribution of each amine to total concentration during the development of *Passiflora setacea* fruits (n = 3, 2011 season; SPM – spermine, SPD – spermidine, AGM – agmatine; PUT – putrescine).

polyamines (spermidine and spermine) share the same precursor S-adenosylmethionine (SAM) and is known to exert opposite effects with respect to fruit ripening and senescence. According to Agudelo-Romero et al. (2013), the decrease in polyamines and putrescine contents may be due to up-regulation of genes coding for diamine and polyamine oxidases and to a significant increase in their enzymatic activity, leading to their rapid metabolization. This was the case in their studies during grape ripening, when significant increases in diamine oxidase and polyamine oxidase activities were observed.

The abundance of polyamines and the occurrence of agmatine in *Passifora* is advantageous for the plant due to their role in fruit development, membrane stability and antioxidant potential. Moreover, the occurrence of tryptamine imparts a role in defense response of the plant. In a similar way, these compounds are relevant to human health due to the potential antioxidant effects of polyamines and to the role of tryptamine as a precursor of the neurotransmitter serotonin.

4. Conclusion

The influence of species and fruit development stages on polyamines, putrescine and the indolamine tryptamine in *Passiflora* was investigated for the first time. *Passiflora* species were significant sources of spermine and spermidine, however *P. setacea* and *P. edulis* had lower concentrations compared to *P. alata* and *P. nitida*. Spermidine was the prevalent amine in *P. alata* and *P. edulis*. *P. nitida* and *P. setacea* contained the highest levels of putrescine, which can affect negatively sensory characteristics. The presence of agmatine in *Passiflora* is evidence of another route for polyamines formation, other than via ornithine. The presence of tryptamine, or serotonin as observed in a previous screening study, and the accumulation of this form of indolamine seems to be modulated by species and also possibly by cultivation practices. The abundance of polyamines and the presence of indolamines could be associated with relevant functional properties for the plant, as well as for human health. The *Passiflora* species differed regarding sugar contents and pH values. *P. nitida* and *P. alata* had higher soluble solids; *P. nitida* showed the highest pH and *P. edulis* the lowest. Differences among species are desirable, depending on consumer's preference and also the technological utilization and shelf life of the passion fruit product.

During development of *P. setacea* fruit, soluble solids, weight and fruit dimensions increased, whereas pH and moisture content decreased. Spermine concentration remained constant, whereas putrescine decreased significantly throughout fruit development. Spermidine and agmatine concentrations also decreased but at a lower rate compared to putrescine. Amines concentrations were similar in ripened and senescent fruits.

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