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Profile and levels of bioactive amines in orange juice and orange soft drink

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Abstract

The levels of bioactive amines, pH, soluble solids, acidity, specific gravity, and total sugars were determined in different brands of orange products. Nine amines were detected in orange juice at mean total levels of 53.5 mg l^{-1}. There were significant differences, among orange juice brands, in the levels of spermidine, synephrine, spermine, octopamine, pH and total acidity. Five amines were detected in soft drinks with mean total levels of 3.85 mg l^{-1}. There were significant differences, among orange soft drink brands, in the levels of most amines and the physicochemical characteristics. The predominant amine was putrescine, followed by synephrine and spermidine, in both orange juices and soft drinks. The levels of these amines in the soft drink varied from 5.0% to 7.6% of the mean levels in orange juice, suggesting that less than 10% of orange juice could have been used in the soft drink.

Keywords: Orange; Juice; Soft drink; Bioactive amines; Synephrine; Octopamine

1. Introduction

Certain amines fulfil a number of important physiological functions in living organisms. Amines are present in foods since they are formed during normal metabolic processes (Bardócz, Grant, Brown, Ralph, & Pusztai, 1993). They comprise polyamines and biogenic amines. The polyamines, spermidine and spermine, occur ubiquitously in the plant kingdom, together with their diamine precursor putrescine. Polyamines are required for normal development and can be used as organic nitrogen sources. Polyamines in higher plants play a critical role in several processes, which are, root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development and response to abiotic stress, such as potassium deficiency, osmotic shock, drought and pathogen infection. They are also important in the synthesis of secondary metabolites of biological interest (Adão & Glória, 2005; Lima & Glória, 1999; Smith, 1985; Walters, 2003).

Biogenic amines are generally either psychoactive or vasoactive. Psychoactive amines, such as histamine and serotonin, act on neural transmitters in the central nervous system. Vasoactive amines act directly or indirectly on the vascular system. Pressor amines (tyramine, tryptamine and phenylethylamine) cause a rise in blood pressure by constricting the vascular system and increasing the rate and force of contraction of the heart. Histamine is a strong capillary dilator and can produce hypotensive effects (Lima & Glória, 1999; Smith, 1980–1981). Synephrine is a sympathomimetic amine. It causes vasoconstriction, increased blood pressure and relaxation of the bronchial muscle. It is used pharmacologically as a stimulant, decongestant and in the treatment of hypotension in oral form (Kusu, Matsumoto, Arai, & Takamura, 1996; Stewart, Newhall, & Edwards, 1964).

Several amines are naturally present in foods. Some of them are specific to some plant species and can be used as a chemotaxonomic index (Wheaton & Stewart, 1965).

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Others are produced from the decarboxylase activity of amino acids by microbial enzymes. Microorganisms can be added for the production of fermented products or can be introduced by contamination due to inadequate hygienic conditions (Lima & Glória, 1999).

Amines in foods do not usually cause a health hazard for individuals unless excessive amounts are ingested or the natural mechanism for their catabolism is genetically deficient or impaired by diseases or by pharmacological agents, such as monoaminoxidase inhibitor (MAOI) drugs (Fuzikawa, Hara, Glória, & Rocha, 1999; Lima & Glória, 1999). High levels of histamine and tyramine have been implicated as the causative agent in a number of food poisoning episodes (Shalaby, 2000). Tyramine, tryptamine and phenylethylamine are associated with migraine (Lima & Glória, 1999).

Knowledge of the levels and types of bioactive amines in different food products is necessary, since amines can be used as a chemotaxonomic index and can attest to the authenticity or to the hygienic sanitary quality of the food (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Furthermore, it can provide information for dietitians to prescribe diets for patients with migraine or under treatment with MAOI drugs. However, very few studies have been undertaken to determine the types and levels of bioactive amines in different food products.

Orange (*Citrus sinensis*) was introduced to Brazil in the 1500s. Significant growth of production was observed in 1960, with the expansion of frozen concentrated orange juice in the state of São Paulo. Brazil is today the world largest orange producer and the state of São Paulo is responsible for 70% of the national production (Toledo & Castillo, 2004).

Orange is considered to be an excellent source of vitamin C, thiamin, potassium and other important nutrients. Its consumption in Brazil is mainly in the form of juice or of carbonated soft drink. Orange juice is the liquid obtained by squeezing or pressing the interior of an orange. Orange soft drink is a carbonated orange drink, which includes real orange juice (Nagy, Shaw, & Veldhuis, 1977). According to Brazilian standards for identity and quality, orange juice must have colour and flavour characteristics of the fruit. The levels of soluble solids must be $\geqslant 10.5$ °Brix (at 20 °C), Brix/acidity ratio $\geqslant 7.0$ °Brix/g 100 ml^{-1}, and total sugar content $\leqslant 13$ g 100 g^{-1}. The orange soft drink must contain at least 10% (v/v) of orange juice (Brasil, 1997).

Studies on the types and levels of bioactive amines in orange and products are scarce. Synephrine, octopamine and tyramine were the first amines to be reported in orange (Stewart et al., 1964; Udenfriend, Lovenberg, & Sjoerdsma, 1959). According to Wheaton and Stewart (1965), synephrine and octopamine are formed via tyramine (Fig. 1). Recent studies have also indicated the presence of spermidine, spermine and putrescine in orange (Eliassen, Reistad, Risoen, & Ronning, 2002; Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997; Tassoni, Germana, & Bagni, 2004). There is no information on the overall profile of bioactive amine in orange products. Therefore, the objective of this work was to determine the types and levels of bioactive amines and the physicochemical characteristics, for both orange juice and orange soft drink. The existence of correlation between amines and the physicochemical characteristics, and between juice and soft drink was also investigated.

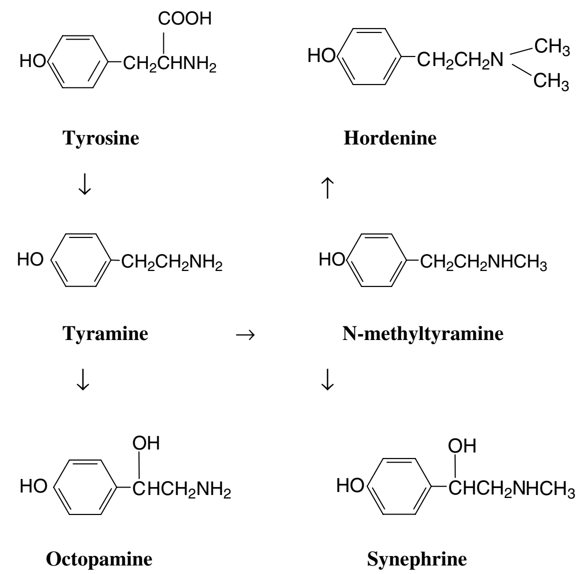

Fig. 1. Synthesis of phenolic amines in citrus (Wheaton and Stewart, 1969).

2. Materials and methods

2.1. Material

Samples of orange juice (3 lots from 7 brands) and orange soft drink (5 lots of 7 brands) available in the market of Belo Horizonte, state of Minas Gerais, Brazil, were purchased during the months of August and September of 2002. The brands were representative of the Brazilian market.

Standards of 12 amines were purchased from Sigma Chemical Co. (St. Louis, MO, EUA). They were putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, agmatine sulfate, cadaverine dihydrochloride, serotonin hydrochloride, histamine dihydrochloride, tyramine, tryptamine, 2-phenylethylamine dihydrochloride, synephrine and octopamine hydrochloride.

Reagents used were of analytical grade, except for HPLC reagents that were of LC grade. Ultrapure water was obtained from Milli-Q (Millipore Corp., Milford, MA, USA). The mobile phases were filtered in HAWP and HVWP membranes, used respectively for aqueous and organic solvents (47 mm diameter and 0.45 μm pore size, Millipore Corp., Milford, MA, USA).

2.2. Analytical methods

2.2.1. General

The samples of orange juice and orange soft drink were analyzed for bioactive amines, pH, total soluble solids, titratable acidity, specific gravity and total sugars.

2.2.2. Bioactive amines

The samples of orange juice and soft drink were centrifuged at 11,180*g* at 4 °C for 20 min and filtered through qualitative filter paper. The soft drink samples were also degassed using a magnetic stirrer. The extracts were filtered through 0.45 μm HAWP membrane.

The separation of the amines was performed by ion pair HPLC using a *μ*Bondapak C18 column, 300 × 3.9 mm i.d., 10 μm (Waters, Milford, Massachusetts, EUA), at 22 ± 2 °C, using two distinct systems. For the determination of spermine, spermidine, putrescine, agmatine, cadaverine, histamine, tyramine, serotonin, tryptamine and phenylethylamine, a gradient elution programme was used (Table 1). Eluent A was 0.1 M acetate buffer containing 10 mM 1-octanesulfonic acid sodium salt, pH adjusted to 4.9 with acetic acid and eluent B was acetonitrile, at a flow rate of 0.7 ml min^{-1}. The chromatograph used consisted of LC-10AD pumps and automatic injector model SIL-10AD VP (Shimadzu, Kyoto, Japan). After separation, the amines were derivatized with *o*-phthalaldehyde (OPA) and detected fluorimetricaly (RF-551, Shimadzu, Kyoto, Japan) at 340 nm excitation and 445 nm emission. The post-column derivatization reagent was delivered at 0.4 ml min^{-1}. It consisted of 1.5 ml Brij-35, 1.5 ml mercaptoethanol and 0.2 g OPA dissolved in a 500-ml solution of 25 g boric acid and 22 g KOH (pH adjusted to 10.5 with 30 g l^{-1} KOH) (Adão & Glória, 2005).

For the determination of octopamine and synephrine, a purification step using C18 Sep pak (Waters, Milford, MA, EUA) was required. The amines were separated using an isocratic elution system (91 parts of 0.1 M acetate buffer containing octanesulfonic acid sodium salt at 10 mM, pH adjusted to 4.9 with acetic acid + 9 parts of acetonitrile) at a flow rate of 0.8 ml min^{-1}. The HPLC system used (Shimadzu, Kyoto, Japan) contained LC-10AD pumps, diode array detector (SPD-M10AVP) at 275 nm, and manual Rheodyne injector with a 20-μl loop (Tavares-Neto, Vieira, & Glória, 2003).

The identification of the amines was performed by comparison of the retention times of peaks in the samples to those of standard solutions and also by addition of the suspected amine to the samples. Quantification was accomplished by direct interpolation in standard curves with $R^2 \geqslant 0.9926$ for each amine (Adão & Glória, 2005; Vale & Glória, 1997).

Table 1
Gradient elution programme used for the separation of 10 bioactive amines by ion pair HPLC

Time (min)	% of Eluent B
0.01	12
20	12
22	13
35	13
43	26
66	26
71	12

Eluent B = acetonitrile.

2.2.3. pH

The pH values were measured with a digital pH meter, Digimed model DM-20, after calibration with pH 4.0 and 7.0 standard buffers (AOAC, 1995).

2.2.4. Soluble solids

The levels of soluble solids, expressed as degrees Brix, were determined using a refractometer, model RL1-PZO (Warszawa, Poland), equipped with a thermometer. The results were corrected for the temperature when the determinations were made at temperatures other than 20 °C (AOAC, 1995).

2.2.5. Titratable acidity

Total acidity was determined by titration with 0.1 N NaOH, adding phenolphthalein indicator (AOAC, 1995).

2.2.6. Specific gravity

The specific gravity was determined by pycnometer at 20 °C (AOAC, 1995).

2.2.7. Total sugars

The reducing and non-reducing sugars were determined by the Lane and Eynon method. The clarified samples, containing equal volumes of Fehling A and B solutions were titrated with 0.5% glucose solution. Inversion for non-reducing sugars was necessary to calculate the total sugars (AOAC, 1995).

2.3. Statistical analysis

The data was submitted to analysis of variance and the means were compared by the Tukey test at 5% probability using SPSS 9.0. The existence of significant correlation between amines and physicochemical characteristics was determined by Pearson correlation at 1% probability (Sampaio, 1998).

3. Results and discussion

3.1. Profile and levels of bioactive amines in orange juice

Nine of the 12 amines were detected in orange juice, spermidine, spermine, putrescine, agmatine, synephrine, tyramine, octopamine, histamine and serotonin. Six of these amines (spermidine, spermine, putrescine, synephrine, tyramine and histamine) were present in every

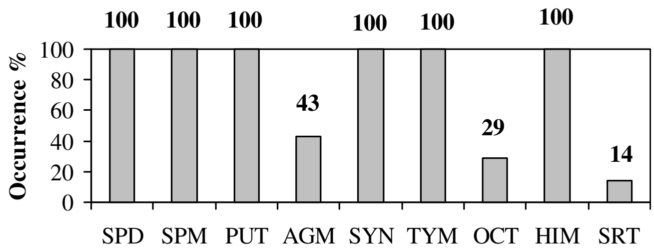

Fig. 2. Occurrence of bioactive amines in orange juice (SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; SYN, synephrine; TYM, tyramine; OCT, octopamine; CAD, cadaverine; HIM, histamine; SRT, serotonin).

sample analyzed (Fig. 2). Agmatine was found in 43% of the samples, octopamine in 29%, and serotonin in 14%.

Some of these amines had already been investigated in previous researches. Studies with oranges indicated the presence of tyramine (Coutts, Glen, & Pasutto, 1986; Udenfriend et al., 1959), synephrine (Coutts et al., 1986; Wheaton & Stewart, 1965), octopamine (Wheaton & Stewart, 1965), putrescine (Bardócz et al., 1993; Eliassen et al., 2002; Okamoto et al., 1997; Tassoni et al., 2004), spermidine (Bardócz et al., 1993; Eliassen et al., 2002; Okamoto et al., 1997), and spermine (Eliassen et al., 2002; Okamoto et al., 1997).

The presence of spermidine was expected since it is the predominant polyamine in vegetables. The presence of putrescine was also expected, as it is an obligate intermediate in the synthesis of spermidine (Bardócz et al., 1993; Flores, Protacio, & Signs, 1989; Walters, 2003). According to Wheaton and Stewart (1965), synephrine is a typical bioactive amine in citrus. Therefore, synephrine, along with its precursor tyramine, are expected to be found in the juice (Fig. 1). The fact that octopamine, another metabolite of tyramine, was not present in 100% of the samples, suggests that the formation of synephrine is predominant over octopamine in orange.

No information was found in the literature regarding the other amines detected in the orange juice samples. The presence of agmatine, an intermediate in the synthesis of polyamines via arginine, suggests that this can be an alternative pathway for the formation of polyamines in orange (Flores et al., 1989; Walters, 2003). Histamine has been observed to be naturally present in some vegetables, e.g., eggplant (Botelho et al., 2004; Starling, 1998). According to Flores et al. (1989), histamine can play an important role in the protection of the plant against predators. However, histamine can also be formed by the decarboxylation of amino acids, by enzymes from contaminating bacteria (Lima & Glória, 1999). Serotonin has been found in some plant tissues, among them, banana, pineapple, peach, avocado, tomato, eggplant, coffee and nuts (Adão & Glória, 2005; Coffin, 1970; Flores et al., 1989; Udenfriend et al., 1959). According to Flores et al. (1989), serotonin also plays an important role in plant protection. Based on the results obtained, it is likely that six amines (SPD, SPM, PUT, SYN, TYM and HIM) are indigenous to orange.

The contributions of each amine to total levels in the different brands of orange juice analyzed are indicated in Fig. 3. In every brand, putrescine was the predominant amine, contributing with 51–63% (average = 57%), followed by synephrine (28–38%, average = 32%), and by spermidine (4–6%, average = 5%). The levels of the remaining amines contributed with less than 2% of total amine levels. The predominance of putrescine was also observed in orange (Bardócz et al., 1993; Eliassen et al., 2002; Okamoto et al., 1997; Tassoni et al., 2004).

According to Table 2, the total amine levels varied from 34.9 to 66.6 mg l^{-1} for the different brands of orange juice available in the market of Belo Horizonte, MG. However, there was no significant difference in total levels among brands (Tukey test, 5% probability).

The mean levels of amines detected in the different brands of orange juice are described in Table 2. Putrescine was detected at higher levels (mean = 33.6 mg l^{-1}), followed by synephrine (mean = 16.01 mg l^{-1}) and by

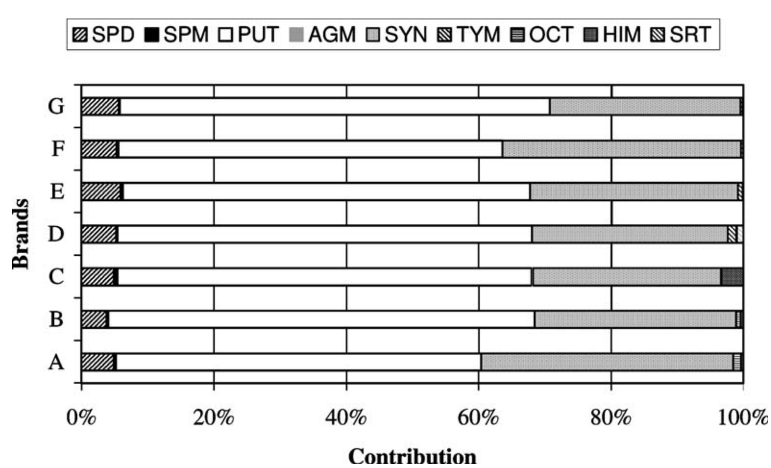

Fig. 3. Contribution of each bioactive amine to total levels in different brands of orange juice (SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; SYN, synephrine; TYM, tyramine; OCT, octopamine; HIM, histamine; SRT, serotonin).

Table 2
Mean levels of bioactive amines (mg l^{-1}) in orange juices of different brands

Amines[a]	Mean amines levels[b] (CV)/brands							
	A	B	C	D	E	F	G	Mean
SPD	3.44^{a} (14)	1.80^{b} (24)	3.05^{a,b} (8)	2.68^{b} (30)	4.22^{a} (11)	2.21^{b} (4)	1.96^{b} (13)	2.77
SPM	0.25^{a,b} (18)	0.08^{b} (36)	0.34^{a} (3)	0.13^{b} (14)	0.34^{a,b} (6)	0.14^{a,b} (7)	0.07^{b} (3)	0.16
PUT	43.6 (36)	30.63 (34)	39.0 (20)	31.3 (14)	43.7 (35)	24.1 (47)	22.6 (44)	33.6
AGM	0.02 (33)	0.01 (33)	0.14 (33)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.02
SYN	16.3^{a,b} (11)	21.8^{a} (1)	15.8^{a,b} (10)	14.7^{b} (28)	17.9^{a,b} (21)	15.5^{a,b} (11)	10.1^{b} (13)	16.0
TYM	0.02 (33)	0.02 (33)	0.04 (33)	0.67 (0)	0.51 (0)	0.13 (0)	0.12 (0)	0.21
OCT	0.88^{b} (24)	1.29^{a} (1)	0.00^{c} (0)	0.00^{c} (0)	0.00^{c} (0)	0.00^{c} (0)	0.00^{c} (0)	0.31
HIM	0.26 (70)	0.19 (46)	0.07 (45)	0.03 (2.5)	0.10 (60)	0.04 (17)	0.04 (58)	0.38
SRT	0.00 (0)	0.00 (0)	0.00 (0)	0.48 (33)	0.00 (0)	0.00 (0)	0.00 (0)	0.07
Total	64.8 (25)	55.9 (19)	60.5 (18)	50.0 (5)	66.6 (24)	42.1 (22)	34.9 (24)	53.5 (28)

[a] SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; SYN, synephrine; TYM, tyramine; OCT, octopamine; CAD, cadaverine; HIM, histamine; SRT, serotonin.
[b] Mean values with different letters in the same row are significantly different (Tukey test, $p < 0.05$).

spermidine (mean = 2.77 mg l^{-1}). The remaining amines were present at concentrations below 1.0 mg l^{-1}. There was significant variation in the levels of amines among samples of different lots from the same brand (numbers in parentheses, in Table 2). These results suggest that there is no standardization of the levels of amines in samples from the processing industries.

When comparing amine levels among juices of different brands, significant differences were observed in the levels of spermidine, spermine, synephrine and octopamine. Agmatine was only detected in samples from brands A, B and C, octopamine in brands A and B, and serotonin only in samples from brand D.

The levels of putrescine, spermidine and spermine detected in this study are lower than levels reported by Bardócz et al. (1993), Okamoto et al. (1997) and Eliassen et al. (2002) for orange. However, these studies did not provide detailed information on sample, variety, or way of preparation and concentration. The levels of synephrine detected were similar to those found by Stewart and Wheaton (1964) for several varieties of oranges (Hamlin, Parson Brown, Pineapple, Valencia, Pope, Navel and Temple), except for Murcott orange, in which synephrine levels were higher. Regarding the levels of octopamine and tyramine, the methodology used in this study had a lower detection limit (0.01 mg l^{-1}) than did the one (1 mg l^{-1}) used by Wheaton and Stewart (1965).

3.2. Physicochemical characteristics of orange juice

The results of the physicochemical characteristics of the orange juice samples are shown in Table 3. Low coefficients of variation (<13%) were observed for different lots of the same brand of orange juice. This suggests that the industries are controlling the quality of the juice with respect to these parameters. The values of pH, acidity and the °Brix/acidity ratio varied significantly among brands (Tukey test, $p < 0.05$). Significantly higher pH values were observed in brands A, C, D and E, and higher acidity in brands A, B, C and E. Significantly higher °Brix/acidity ratios were found in brands D, F and G. No significant difference was observed among brands for soluble solids, total sugars and specific gravity.

Every sample analyzed complied with the Brazilian regulation, which limits soluble solids to a minimum of 10.5 °Brix at 20 °C, the Brix/acidity ratio to a minimum of 7.0 °Brix/g 100 ml^{-1}, and total sugars to a maximum of 13.0 g 100 g^{-1}.

Correlation among the levels of bioactive amines and the physicochemical characteristics of the orange juice, determined by means of Pearson correlation, is shown in Table 4. Synephrine correlated significantly with total levels. The formation of some amines was affected by the same parameters, e.g., histamine and agmatine. There was significant positive correlation between pH and putrescine and

Table 3
Physicochemical characteristics of orange juices from different brands

Parameters	Mean values[a] (CV)/brands							
	A	B	C	D	E	F	G	Mean
pH	3.84[a] (3)	3.50[bc] (2)	3.84[a] (1)	3.79[a] (1)	3.84[a] (1)	3.44[c] (3)	3.43[c] (3)	3.67 (5)
°Brix	11.0 (7)	10.5 (1)	11.1 (5)	11.0 (3)	10.6 (2)	10.5 (2)	10.4 (2)	10.7 (4)
Titratable acidity (g 100 ml^{-1})	0.85[a] (5)	0.73[b] (3)	0.73[c] (2)	0.61[c] (13)	0.75[ab] (2)	0.65[bc] (2)	0.63[bc] (4)	0.71 (12)
Brix/acidity (°Brix/g 100 ml^{-1})	12.9[b] (3)	14.4[b] (3)	15.2[ab] (7)	18.3[a] (14)	14.0[b] (3)	16.0[ab] (2)	16.5[a] (6)	15.3 (13)
Specific gravity (g ml^{-1})	1.047 (0)	1.046 (0)	1.045 (0)	1.046 (0)	1.046 (0)	1.044 (0)	1.044 (0)	1.045 (0)
Total sugars (g 100 g^{-1})	10.1 (3)	–	–	10.4 (1)	8.1 (2)	–	–	9.5 (13)

–, lost samples.
[a] Mean values with different letters in the same row are significantly different (Tukey test, $p < 0.05$).

Table 4
Pearson correlation between the levels of bioactive amines and the physicochemical characteristics of orange juice

Parameters	Correlation coefficient[a]
Histamine × agmatine	0.9975
Synephrine × total amines	0.9153
Putrescine × pH	0.8906
Serotonin × specific gravity	0.8209
Synephrine × titratable acidity	0.8333

[a] Significant at 99% probability.

acidity and synephrine. These results suggest that, the higher the pH, the higher was the putrescine content, whereas the higher the acidity, the higher were the synephrine levels.

3.3. Profile and levels of bioactive amines in orange soft drink

Only five of the 12 amines investigated were found in orange soft drink, which are, spermidine, spermine, putrescine, synephrine and histamine (Fig. 4). Spermidine and putrescine were found in 100% of the samples analyzed. However, spermine and synephrine were detected in 86% while histamine was detected in 14% of the samples. These five amines, found in the soft drink, were detected in 100% of the samples of orange juice analyzed. It might be a fact that some of the amines, which seemed inherent in orange juice, were not detected in the soft drink since a low percentage of juice was added to the soft drink, causing significant dilution.

The total levels of bioactive amines varied significantly among brands of orange soft drinks. Lower levels were found in brand J with 0.80 mg l^{-1} and higher in brand M with 7.53 mg l^{-1}. The contribution of each amine to total amine levels is indicated in Fig. 5. Putrescine was the prevalent amine, contributing with 57–93% (mean = 70%) of total levels. It was followed by synephrine, that represented 0–42% of total levels (mean = 25%), by spermidine (1–5%, mean = 3%), by spermine (0–4%, mean = 1%) and by histamine (0–1%, mean = 0.2%).

According to Table 5, the levels of amines were variable among different lots of the same brand, as indicated by the high coefficients of variation (CV < 57). These results suggest that there is no control or standardization of amine levels in the soft drinks by the industries.

There were significant differences in the levels of spermidine, spermine and putrescine, synephrine among the different brands of orange soft drink analyzed. Significantly higher putrescine levels were found in brands H, I and M. Higher spermidine levels were found in brand M, higher

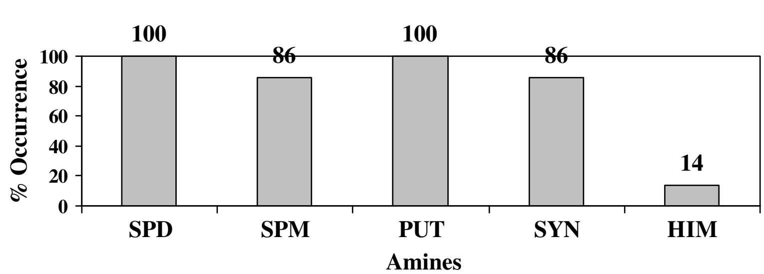

Fig. 4. Occurrence of bioactive amines in orange soft drinks (SPD, spermidine; SPM, spermine; PUT, putrescine; SYN, synephrine; HIM, histamine).

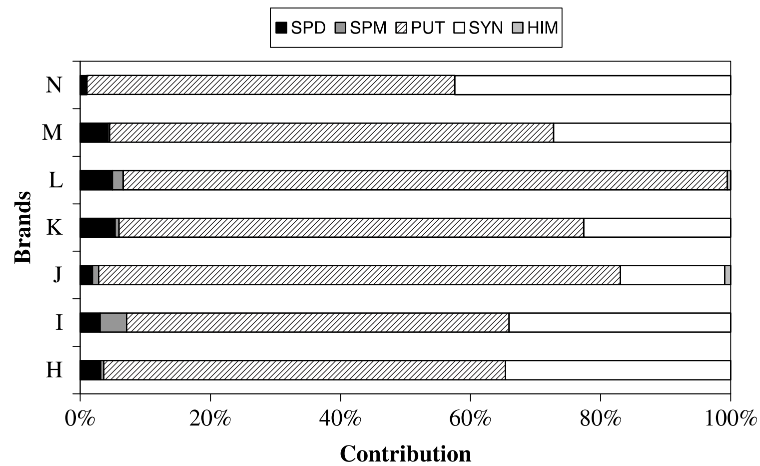

Fig. 5. Contribution of each bioactive amine to total levels in different brands of orange soft drink.

Table 5
Mean levels of bioactive amines (mg l^{-1}) in different brands of orange soft drink

Amines[a]	Mean levels of amines[b] (CV)/brands							
	H	I	J	K	L	M	N	Mean
SPD	0.21[b] (14)	0.19[b] (32)	0.01[c] (5)	0.09[c] (56)	0.09[c] (52)	0.32[a] (3)	0.02[c] (13)	0.13
SPM	0.04[b] (6)	0.25[a] (44)	0.01[b] (56)	0.02[b] (34)	0.03[b] (31)	0.02[b] (29)	0.00[b] (0)	0.05
PUT	4.12[a] (24)	3.64[a] (30)	0.69[b] (18)	1.20[b] (57)	1.69[b] (6)	5.14[a] (20)	1.12[b] (9)	2.54
SYN	2.31[a] (6.5)	2.11[a] (8.7)	0.31[bc] (0)	0.94[cd] (22)	0.00[c] (0)	2.05[a] (13)	0.84[d] (17)	1.12
HIM	0.00 (0)	0.00 (0)	0.03 (34)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00
Total	6.67[a] (13)	6.19[a] (21)	0.80[b] (18)	1.67[b] (71)	1.90[b] (3)	7.53[a] (16)	1.98[b] (2)	3.85

[a] SPD, spermidine; SPM, spermine; PUT, putrescine; SYN, synephrine; HIM, histamine.
[b] Mean values with different letters in the same row are significantly different (Tukey test, $p < 0.05$).

spermine levels in brand I, and higher synephrine levels in brands H, I and M. No information was found in the literature regarding the profile and levels of bioactive amines in orange soft drink.

The levels of spermidine detected in the orange soft drinks corresponded to 0.7–11.5% of the mean level of this amine detected in the orange juices. Spermine corresponded from 6.3% to 15.6%, putrescine from 2.6% to 15.4%, synephrine from 0% to 12.4% and histamine from 0% to 2.6% of levels found in the orange juice. If orange juice were the only source of bioactive amines to the soft drink, there should be 10% of the levels of each amine found in the juice. Since spermine levels in soft drink were higher than levels found in orange juice, there might be another source of this polyamine in orange soft drink. The levels of putrescine, spermidine and synephrine in the soft drink varied from 0% to 15% of levels found in the orange juice. These results suggest that there might be a predominantly lower addition of orange juice to soft drink than is stipulated by Brazilian legislation. Further studies are needed to investigate the veracity of this hypothesis.

3.4. Physicochemical characteristics of orange soft drink

The results of the physicochemical analysis of the orange soft drink are indicated in Table 6. Every sample analyzed was in compliance with Brazilian legislation that limits the acidity to 0.1 g 100 ml^{-1} (Brasil, 1997). There was a small variation in the values of the physicochemical parameters (CV < 32) for samples from different lots of the same brand. These results indicate that the soft drink industries are standardizing the quality of the product with regard to the parameters analyzed.

There were significant differences among brands, in the levels of every physicochemical parameter analyzed.

Table 6
Physicochemical characteristics of different brands of orange soft drinks

Parameters	Mean values[a] (CV)/brands							
	H	I	J	K	L	M	N	Mean
pH	3.75[a] (1)	3.62[b] (1)	3.35[d] (1)	3.18[e] (3)	3.49[c] (1)	3.77[a] (3)	3.32[d] (0)	3.49
°Brix	11.96[a] (1)	12.17[a] (2)	11.10[c] (2)	11.24[c] (2)	0.74[d] (0)	11.66[b] (1)	11.74[ab] (1)	9.88
Titratable acidity (g 100 ml^{-1})	0.18[b] (4)	0.19[ab] (3)	0.15[c] (5)	0.21[a] (5)	0.14[c] (13)	0.19[b] (1)	0.21[a] (2)	0.18
Specific gravity (g ml^{-1})	1.049[a] (0)	1.049[a] (0)	1.044[b] (0)	1.043[b] (0)	1,003[d] (0)	1.050[a] (0)	1.044[bc] (0)	1.040
Total sugars (g 100 ml^{-1})	10.5[c] (6)	11.0[bc] (5)	14.7[bc] (20)	12.9[c] (14)	0.31[d] (32)	18.2[b] (14)	32.0[a] (11)	11.6

[a] Mean values with different letters in the same row are significantly different (Tukey test, $p < 0.05$).

Table 7
Correlation between the levels of bioactive amines and the physicochemical characteristics in orange soft drink

Parameters	Coefficient of correlation
Spermidine × putrescine	0.9752
Synephrine × specific gravity	0.9468
Putrescine × specific gravity	0.9100
Synephrine × putrescine	0.8985

Significant correlation (Pearson, 99% probability).

Samples from brands H and M had significantly higher pH values and brands I, K and N had higher acidity. The brands H and I had higher soluble solids and brands H, I and M had higher specific gravities. With respect to total sugars, brand N had higher levels (32 g 100 ml^{-1}), whereas brand L had the lowest (0.31 g 100 ml^{-1}). Such a low level observed for brand L was confirmed by the low °Brix (0.74). It could be explained by the fact that this soft drink was labelled as a product with reduced calories and a non-caloric sweetener had been added.

Pearson correlation indicated significant correlations, as indicated in Table 7. There was significant positive correlation between spermidine and putrescine and between synephrine and putrescine. These results suggest that similar factors affect the formation of the amines. It was also observed that specific gravity correlated significantly with the levels of synephrine and putrescine.

4. Conclusions

Nine bioactive amines were found in orange juice. Six (spermidine, spermine, putrescine, synephrine, tyramine and octopamine) had been reported previously. However, three were detected for the first time (agmatine, histamine and serotonin). Putrescine was the predominant amine, followed by synephrine and spermidine. There were significant differences, among brands, in the levels of spermidine, spermine, synephrine and octopamine and in the pH, acidity and Brix/acidity ratio.

The profile and levels of bioactive amines in orange soft drink were investigated for the first time. Five amines were found: putrescine was the prevalent amine, followed by synephrine, spermidine, spermine and histamine. There were significant differences, among different brands, in the levels of every amine found, except histamine. There were also significant differences in every physicochemical characteristic. The levels of amines found in the soft drink corresponded to 5%, 31%, 7.6%, and 6.6% of the mean levels of spermidine, spermine, putrescine and synephrine, respectively, detected in the orange juice. Considering the incorporation of 10% of orange juice into the soft drink, determined by the Brazilian legislation, percentages of approximately 10% of each amine should be found in the soft drink, especially those that are specific to citrus, such as synephrine. Lower percentages of amines suggest the incorporation of less than 10% of orange juice during soft drink production. However, higher values observed for spermine could indicate that other ingredients might contribute with this amine in soft drink. Studies are needed in order to determine which ingredients, beside the juice, could contribute bioactive amines to the soft drink.

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