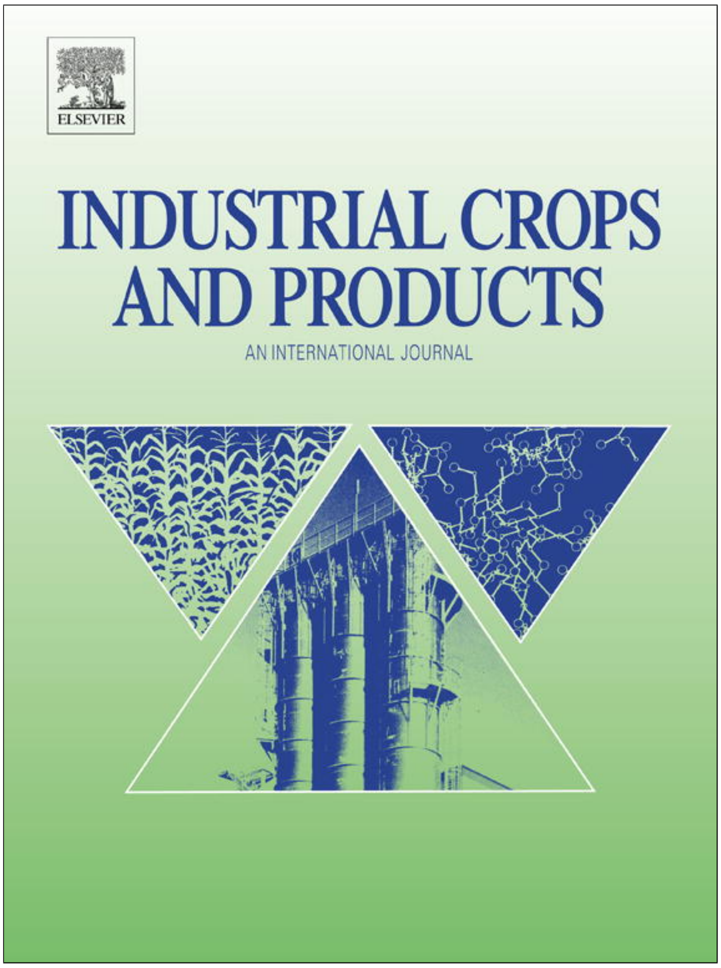
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Free amino acid composition of *Annona* (Annonaceae) fruit species of economic interest

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ABSTRACT

Annona is an economically important genus, with some widely commercialized species. Free amino acid profiles of two cultivars of atemoya (*Annona cherimola* Mill. × *Annona squamosa* L.) and one of cherimoya (*A. cherimola* Mill.) were investigated by reverse HPLC-F. The total yield of free amino acids in these three samples ranged from 5.57 mg/g to 25.66 mg/g dry matter. The *A. cherimola* cv. Madeira presented the highest concentration. A total of 20 free amino acids were detected. Arginine, glutamine and serine were the most abundant. Free amino acid composition points to the nutritional potential of these fruit species, in addition to possible application in quality control tests of derivatives.

1. Introduction

Annonaceae comprises about 128 genera and 2300 species. *Annona* is the largest genus, with approximately 120 species, and is widely distributed in tropical and subtropical regions in world (Maas et al., 2001).

This genus is considered economically important, with some widely commercialized species, like *Annona squamosa* (sugar apple), *A. muricata* (soursop), *A. reticulata* (custard apple), *Annona cherimola* (cherimoya) and *A. cherimola* × *A. squamosa* hybrid (atemoya). Their fruits are used *in natura* or as juices, desserts and ice-creams (Lorenzi and Matos, 2002).

In recent decades, the idea that a balanced diet can prevent the development of some diseases has been supported by several investigations. Within this scenario, the concept of functional food has become very important due the beneficial effects this food class offers concerning human health. This type of food has been associated with reduction of risks of some chronic diseases (Jew et al., 2009). Organic extracts of fruits of *A. cherimola* have been suggested with high activity against lipid peroxidation, powerful antioxidant activity against a wide variety of ROS, and protective activities in isolated human peripheral blood lymphocytes exposed to damaging agents (Barreca et al., 2011; Loizzo et al., 2012). Antioxidant properties may contribute to the prevention of chronic health problems, such as cancer, cardiovascular diseases, inflammatory and neurodegenerative process (Zibadi et al., 2007). Fruit extracts of *A. cherimola* × *A. squamosa* have been related to hypolipidemic effect (Beppu et al., 2009) and reduction of abdominal adipose tissues in rats (Niwano et al., 2009). An acetogenin isolated from fruits of this hybrid induces apoptosis in tumor cells (Balachandran and Govindarajan, 2005).

A wide variety of functional substances, which are known as phytochemicals or phytonutrients, like flavonoids, ω3-fatty acids, glucosinolates, fibers, carotenoids, α-tocopherol, ascorbic acid, among others, has been characterized (Jew et al., 2009).

Free amino acids are an important kind of biologically active compounds. Besides their role as building blocks of proteins and polypeptides, some amino acids have been demonstrated as essential compounds for the maintenance of the metabolism, growth, reproduction, and immunity functions (Cylwik et al., 2005; Morris et al., 2005; Mateo et al., 2007), as antioxidants (Roesler et al., 2007), and also with specific functions in the central nervous system. These compounds, called functional amino acids, include arginine, cysteine, glutamine, leucine, proline, and tryptophan (Wu, 2009).

In addition to nutritional properties, many studies have shown that the free amino acid profile of vegetable foods can be useful in authenticity and quality control strategies (Rohsius et al., 2006; Vasconcelos et al., 2007).

Lisine, methionine and tryptophan have been reported to be the main free amino acids in *Annona muricata* and *A. squamosa*

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fruits (Morton, 1987). Investigations using other plant organs have unveiled arginine, hystidine, lysine and ornithine as important compounds in *Annona crassiflora* (Leboeuf et al., 1982). However, little information has been made available covering species of this genus.

The main purposes of this study were: to characterize free amino acids total concentration and profile of two cultivars of atemoya (*A. cherimola* Mill. × *A. squamosa* L.), 'Pink' Mammonth' and 'Gefner', and one of cherimoya (*A. cherimola* Mill.), 'Madeira' looking forward for potential application of these fruits for disease-preventing food, or as useful parameter for quality control of food-derived products.

2. Materials and methods

Five ripe fruits from three to five specimens of *A. cherimola* × *A. squamosa* cv. Pink' Mammonth (Santa Isabel – São Paulo, Brazil), *A. cherimola* × *A. squamosa* cv. Gefner (Indaiatuba – São Paulo, Brazil) and *A. cherimola* cv. Madeira (Pedra Branca – São Paulo, Brazil) were harvested. Fruits were kept in ice-bath for transportation. In the lab, pulps were manually separated from the seeds, freeze-dried for 120 h (Labconco FreeZone 4,5 Liter Benchtop – Freeze Dry System – 77500 model), and then powdered. Samples for the analysis were composed by pooling the pulp of the five fruits of each specimen.

Amino acid was determined according to Silveira et al. (2004). The samples (200 mg of dry matter) were ground in 6 mL of 80% (v/v) ethanol, concentrated in 'speed vac', re-suspended in 2 mL of water and centrifuged at 15,000 × *g* for 10 min, at 4 °C. The supernatant was strained in a filter (0.2 μm – Millipore).

Amino acid derivatization was done using *o*-phthaldialdehyde (OPA), and the identification performed by high performance liquid chromatography (HPLC) with fluorescence detector (HPLC-F) (Shimadzu), using a C18 reverse phase column (Supelcosil LC-18, 25 cm × 4.6 mm i.d., fused-silica, mesh 5 μm) (Shimadzu Shin-pack CLC ODS).

The gradient was developed by mixing increasing proportions of 65% methanol (solvent 1) to a buffer solution (50 mM sodium acetate, 50 mM sodium phosphate, 20 mL/L methanol, 20 mL/L tetrahydrofuran and pH 8.1 adjusted with acetic acid) (solvent 2), according the following program: 0.01–30 min = 10–22% of solvent 1; 30–40 min = 22–38% of solvent 1; 40–46 min = 38–55% of solvent 1; 46–56 min = 55–60% of solvent 1; 56–59 min = 60–80% of solvent 1; 59–76 min = 80–100% of solvent 1; 76–88 min = isocratic with 100% of solvent 1; 88–90 min = decreasing to 10% of solvent 1. The flow rate was constant at 1 mL/min, and the column temperature was 40 °C. Fluorescence excitation and emission wavelengths of 250 nm and 480 nm, respectively, were used for amino acid detection. The amino acids were identified by comparison of retention times with authentic standards (Sigma).

Free amino acids composition was submitted to One-Way analysis (ANOVA). In the event of significant variations ($p \leq 0.05$), data were submitted to Bonferroni test ($p < 0.05$) (Sokal and Rohlf, 1995). All statistical analysis was performed using software SigmaStat version 2.

3. Results and discussion

Free amino acids in plant foods are generally divided into two classes, essential and nonessential. Essential amino acids are those that cannot be synthesized in vivo by humans; therefore, plants (fruits, nuts, vegetables, etc.) are an important source of these amino acids.

The total yield of free amino acids in pulp of crop *Annona* species ranged from 5.57 ± 1.06 mg/g dry matter to 25.66 ± 1.75 mg/g dry matter. The *A. cherimola* presented the highest concentration (25.66 ± 1.75 mg/g dry matter), followed by *A. cherimola* × *A. squamosa* cv. Gefner (23.15 ± 2.78 mg/g dry matter). *A. cherimola* × *A. squamosa* cv. Pink' Mamonth presented the lowest quantity of free amino acids (5.57 ± 1.06 mg/g dry matter) (Table 1). Previous data for *A. cherimola* showed a total free amino acid amount (1.6 mg/g dry matter) much lower than that obtained at this study (Maldonado et al., 2002). Distinct ripe stage, plant age, crop conditions possible reasons to explain the difference.

A total of 20 amino acids were detected in the pulp of *Annona*: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-cytosine, L-glutamine, L-glycine, L-hystidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-ornithine and L-gama amino butyric acid (Table 1). Lysine, methionine and tryptophan have already been reported as important amino acids in pulp of *A. muricata* and *A. squamosa* (Morton, 1987). Arginine, hystidine, lisine and ornithine were found in stem, bark and root of *A. crassiflora* (Leboeuf et al., 1982).

In comparison to previous data, distinct free amino acids can be pointed out as important components of fruits of *Annona* species. Arginine is the main component of *A. cherimola*, while glutamine appears as major component of *A. cherimola* × *A. squamosa* cv. Gefner, and serine was the most important one of *A. cherimola* × *A. squamosa* cv. Pink' Mamonth (Table 1).

Arginine, glutamine and serine present several benefits for human health. Arginine displays remarkable metabolic and regulatory versatility. The administration of arginine reverses endothelial dysfunction, enhances wound healing, prevents the early stages of development of tumors and improves cardiovascular (Cylwik et al., 2005), reproductive (Mateo et al., 2007), pulmonary (Morris et al., 2005), renal (Rolleman et al., 2003), digestive (Jiménez et al., 2002), and immune functions (Wu et al., 2009). Glutamine is essential for most homeostatic functions (Peng et al., 2004), particularly the immune system. This amino acid has been suggested to improve the efficacy of nutritional supplements, since it stimulates protein synthesis (Massambani and Bazotte, 1998). Serine, a nonessential amino acid, has a central role in cellular proliferation. In recent years, serine and its metabolites have been recognized as crucial for the correct functioning of the central nervous system. Inadequate levels of serine are associated to psychiatric disorders and severe neurological abnormalities (Koning et al., 2003).

Another important property of fruit pulp of these *Annona* species is the presence of essential amino acids. All analyzed samples presented isoleucine, leucine, methionine, phenylalanine, tyrosine and tryptophan. *A. cherimola* presented the highest amount of these amino acids (Table 1). Although these amino acids were not detected at high concentrations, the consumption of these fruits could be included in human diets, since they belong to the group of essential amino acids. Gama amino butyric acid also appears in all samples, mostly in *A. cherimola*.

Gama amino butyric acid is an important neurotransmitter that mediates several functions in the central nervous system. This amino acid is involved in every behavioral functions of the brain, including autonomic nervous system, sexual, growth and motor functions, as well as behavioral functions of anxiety, fear and aggression (Paredes and Agmo, 1992). Tyrosine and phenylalanine are precursors of the neurotransmitter serotonin and the catecholamines (dopamine, norepinephrine, epinephrine) (Fernstrom and Fernstrom, 2007). Dopamine is well known for its involvement in reinforcement, motor control and frontal lobe functions, such as attention and memory (Linssen et al., 2011).

Free amino acid profiles can also be a useful parameter for quality control of fruit-derived products (Silva et al., 2006). Silva et al. (2004) evaluated free amino acid composition of jams and fruit pulp samples of quince (*Cydonia oblonga* – Rosaceae), and observed that profiles were very similar. Since the amino acid profiles of these three species are distinctive, this characteristic could be pointed out

Table 1
Composition (mg/g dry matter) of free amino acids of three cultivated *Annona* species.

Amino acids	Species		
	A. cherimola (*n* = 3)	*A. cherimola* × *A. squamosa* cv. Gefner (*n* = 5)	*A. cherimola* × *A. squamosa* cv. Pink' Mamonth (*n* = 3)
Alanine	0.42 ± 0.018 a	0.05 ± 0.004 c	0.08 ± 0.002 b
Arginine	**18.47 ± 2.232 a**	3.49 ± 0.333 b	0.85 ± 0.669 c
Asparagine	0.30 ± 0.078 a	0.01 ± 0.002 c	0.01 ± 0.005 b
Aspartic acid	0.21 ± 0.056 a	0.02 ± 0.004 c	0.09 ± 0.017 b
γ-Aminobutyric acid	0.43 ± 0.054 a	0.08 ± 0.006 c	0.25 ± 0.017 b
Glutamic acid	0.40 ± 0.066 a	0.09 ± 0.027 b	0.08 ± 0.013 b
Glutamine	2.43 ± 0.172 b	**20.46 ± 3.531 a**	1.21 ± 0.028 c
Glycine	0.10 ± 0.025 a	0.004 ± 0.001 b	0.01 ± 0.003 b
Hystidine	0.29 ± 0.003 a	0	0.06 ± 0.010 b
Isoleucine	0.03 ± 0.003 a	0.004 ± 0.001 b	0.01 ± 0.004 b
Leucine	0.12 ± 0.002 a	0.01 ± 0.002 b	0
Lysine	0.22 ± 0.073 a	0	0.02 ± 0.011 b
Methionine	0.03 ± 0.005 b	0.0016 ± 0.001 c	0.06 ± 0.029 a
Ornithine	0.05 ± 0.011	0	0
Phenylalanine	1.55 ± 0.191 a	0.07 ± 0.012 b	0.01 ± 0.005 c
Serine	0.13 ± 0.024 b	0.02 ± 0.007 c	**2.29 ± 0.859 a**
Threonine	0.38 ± 0.029 a	0	0.06 ± 0.022 b
Tryptophan	0.03 ± 0.001 a	0.02 ± 0.002 b	0.01 ± 0.001 c
Tyrosine	0.17 ± 0.024 a	0.01 ± 0.002 c	0.07 ± 0.011 b
Total	25.66 ± 1.75 a	23.15 ± 2.78 b	5.57 ± 1.064 c

Number between parenthesis indicates the number of individuals sampled of each species. Bold numbers correspond to the main free amino acid for each species. Different lowercase letters following numbers indicate significant difference ($p \leq 0.05$) among species.

as a parameter for quality control. *A. cherimola* is easily differentiated by the highest amount of arginine. *A. cherimola* × *A. squamosa* cv. Gefner presents glutamine as the major component, while serine is the main amino acid of *A. cherimola* × *A. squamosa* cv. Pink' Mamonth (Table 1). Belitz and Grosh (1999) have already suggested that free amino acid profiles could be a useful tool for detection of possible adulteration or falsification of *Annona* derivatives.

Significant difference among the two cultivars of atemoya, an interspecific hybrid resultant from the crossing between the cherimoya (*A. cherimola* Mill.) and sugar apple (*A. squamosa* L.) was noted. The profiles of both *A. cherimola* × *A. squamosa* cv. Gefner and *A. cherimola* × *A. squamosa* cv. Pink' Mamonth are completely different from that of *A. cherimola*, which is rich on arginine (Table 1), and from that of *A. squamosa* in which lysine, methionine and tryptophan are common (Morton, 1987). Neves and Yuhara (2003) suggested that the characteristics of this kind of hybrid may present large variations, mainly among cultivars.

4. Conclusions

1 To our knowledge, this is the first report on the composition of free amino acids in the pulp of these two commercial crops, cherimoya (*A. cherimola*) and atemoya (*A. cherimola* × *A. squamosa*).
2 Arginine, glutamine and serine were the most abundant free amino acids of these species of *Annona*.
3 All analyzed samples presented isoleucine, leucine, methionine, phenylalanine, tyrosine and tryptophan, all of them essential amino acids.
4 Besides the interesting features of their composition concerning human diet, we suggest that the free amino acid profiles of these species should be used as quality control parameters for manufactured products.

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