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Volatile compounds identified in Barbados Cherry 'BRS-366 Jaburú'

Y. M. Garcia, M. N. S. Guedes, J. C. M. Rufini, A. G. Souza, R. Augusti, J. O. F. Melo

Universidade Federal de São João del-Rei

Author for correspondence: onesiomelo@gmail.com)

Abstract. In foods, the flavor and aroma are very important attributes, thus the main objective of this study was to identify the volatile compounds (VC) of the "BRS-366 Jaburú" acerola variety, for which we used the solid phase microextraction method (SPE). The separation and identification of volatile compounds was made using gas chromatography-mass spectrometry (GC-MS). Three fibers were evaluated, Polydimethylsiloxane / Divinylbenzene (PDMS / DVB), 65 micrometres Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB / CAR / PDMS) 50/30 m and polyacrylate (PA) 85 uM to compare the extraction of its components. Thirty-three volatile compounds were identified and classified into eight chemical classes: carboxylic acids, alcohols, aldehydes, ketones, esters, hydrocarbons, phenylpropanoids and terpenoids. The peak areas of each of the extracted compounds were expressed as percentages to indicate the relative concentration of each, of which ethyl acetate is distinguished by being responsible for the fruity aroma notes. Thus, the fiber PDMS / DVB was the best as it enabled to extract a greater amount of volatile compounds.
Keywords: Aroma. Gas chromatography-mass spectrometry. *Headspace. Malpighia emarginata.* solid phase microextraction method.

Introduction

The acerola (Malpighia emarginata DC), also known as "cherry of the Antilles" or "Barbados cherry", is native to Central America (Araújo & Minami, 1994) is grown mainly in the Northeast and Southeast regions of Brazil; this plant is member of the Malpighiaceae family, which comprises about 71 genera and 1250 species (Robertson 1972; Judd et al., 1999).

The variety "Jaburu BRS-366" has a high amount of Vitamin C, produces an average of 100 kg of fruit per plant per annum, representing a productivity of about 57 tons per hectare / year. It is a plant having seven production cycle throughout the year, and shows good adaptability for both the mechanical or manual harvesting (EMBRAPA, 2012).

However, as is a climacteric fruit after harvest readily undergoes changes in color, flavor, aroma and texture (Araujo, 1994). The fruit of acerola is characterized by its pleasant taste and it stands out by its recognized nutritional value, a source of vitamin C, vitamin A, iron, calcium and B vitamins (thiamine, riboflavin and niacin), due to its high capacity of utilization can be used industrialized or consumed "in nature" (Ferreira et al., 2009).

The taste is a decisive attribute in the selection and acceptance of food and drinks, which are perceived by the sense of taste and smell , so the demand for new and exotic flavors has attracted the attention of the flavoring industry for the characterization of volatile compounds , which are responsible for the characteristic flavor of the fruit, which explains the importance they play in the quality of fruits and their derivatives (Thomazini & Franco, 2000; Franco, 2003; Narain et al., 2004).

The volatile compounds responsible for flavor are mostly thermolabile substances subject to rearrangement, cyclization, oxidation, when undergoing any temperature increases, thus, for the analytical separation and identification of these volatile compounds are used gas chromatography-mass spectrometry (GC-MS), being preceded by an extraction operation, using solid phase microextraction method (SPME), the which it has been used by many authors for the analysis of

volatile substances in food (Hawthorne et al ., 1992; Yang & Peppard., 1994; Pelusio et al, 1995;. & Thomazini Franco, 2000).

In this context, the purpose of this study was to identify the volatile compounds of the variety BRS-366 Jaburú.

Methods

Preparation of the samples

The fruits of acerola 'BRS-366 Jaburú' were collected in December 2014, obtained from a plantation located at 19 ° 13'42 " south latitude and 44 ° 02'17 " west longitude, with 644 m altitude in Jequitibá-MG, Brazil. The climate is Cwa (warm temperate or tropical climate), with dry winter and hot summer, according to Köppen climate classification.

The fruits were harvested at random at maturity stage "once" (France and Narain, 2003), placed in polietilene bags and transported in coolers styrofoam for the Plant Production Laboratory of UFSJ / CSL. After collection, the fruits were selected, the damaged were eliminated and then were washed and stored in a freezer at -18 ° C until analysis.

For the extraction of volatile compounds, the fruits were removed from the freezer and placed in running water for its easy defrosting. Subsequently, the seeds of fruit were manually removed and after, with a mixer brand "Quick mixer", the fruits were crushed to prepare the samples.

The solid phase microextraction (SPE)

For the extraction of volatile compounds, it was used for SPE method, which were evaluated three fibers: a polar fiber polyacrylate (PA) 85 m and two semipolar fibers, Polydimethylsiloxane / Divinylbenzene (PDMS / DVB), 65 m and Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB / CAR / PDMS) 50/30 micrometers. The fibers were conditioned according to the instructions provided by the manufacturer.

For the analysis of SPME, was weighed 2.0 g of the pulp of the fruit, which was placed in headspace vials of 20 ml capacity and sealed with aluminum seal and rubber septum. Thereafter, the vial headspace of 20 ml was placed in an aluminum block and heated at 60 ° C on a magnetic stirrer with stirring from 0 to 10 rpm. After 10 minutes of heating, the fiber SPME (BP, PDMS / DVB, DVB / CAR / PDMS) mounted on a holder is exposed to the gas phase above the acerola pulp (headspace) for a time of 5 min, and then the holder containing the fiber was removed and manually inserted into the injector gas chromatograph coupled to mass spectrometer by exposing the fiber with 5 min desorption (Belo, 2009).

Gas chromatography-mass spectrometry (GC-MS).

Samples acerola BRS-366 Jaburú were separated using a gas chromatography system Thermo Finnigan Trace GC Ultra model coupled with mass spectrometry detector model Polaris Q equipped with an injector split / splitless used in the splitless mode. It was used as carrier gas, helium at a constant flow of 1 ml min-1. The chromatographic column used was a capillary column TR-1 MS (100% dimethylpolysiloxane, 60 m long x 0.25 mm internal diameter x 0.25 uM thick film). The chromatographic analysis conditions were: injector temperature 250 ºC, desorption time 5 min, temperature of ion source 200 ºC, interface temperature 275 ºC. The column heater was programmed with temperature started at 40 ° C lasting for 5 minutes and then with a heating rate of 2.5 ° C min-1 to 125 ° C and then 10 ° C min-1 to 245 ° C, at which temperature was maintained at isotherm for 3 minutes (Belo, 2009).

The volatile compounds were identified by mass-to-charge ratio (m/z) corresponding to each peak generated by the chromatogram by ion total in each sample analyzed, and compared with the mass spectra obtained by impact ionization of electrons (IE), the which uses an energy of 70 eV with a scan range (full scan, 50 to 650m / z) and compared with the data of the NIST library (National Institute of Standards and Technology) equipment.

The RSI index consisting of a numerical factor comparison between an unknown compound and the compounds NIST library is used to identify volatile compounds and is considered a value greater than 500.

Identification and correlation of the detected volatiles

The volatile compounds were identified based on mass-to-charge ratio (m/z) of ions of each sample by means of each mass spectrum. It was made a comparison of the mass spectra of each analytes detected to each peak corresponding observed in the total ion by chromatogram each sample with the mass spectra data obtained from the NIST library (National Institute of Standards and Technology), using the data reported in the literature as auxiliary tool for confirmation of volatile compounds present in the fruits of acerola.

To determine of the peaks was considered a relative abundance greater than 5%, whereas those with the greatest area. The relative area of each peak was performed by a correlation of the total area of detected peaks obtained for each of the compounds. The peak area of each of the extracted compounds was expressed as percentage (%) to indicate the relative concentration of each. The fibers were evaluated according to the highest amount of extracted compounds, considering an RSI greater than 500.

Results and discussion

The fiber of microextraction in solid phase (SPME) BP, PDMS / DVB and DVB / CAR / PDMS were evaluated for the number of extracted volatile compounds. In Figures 1, 2 and 3, are shown the

chromatograms obtained for each of the fibers, which showed different behaviors in the detection of volatile compounds of the acerola BRS-366 Juburú '.

They identified 33 volatile compounds by means of three fiber evaluated by gas chromatography-mass spectrometry. Compounds identified were classified in different chemical classes: carboxylic acids, alcohols, terpenes, phenylpropanoids, aldehydes, ketones, esters and hydrocarbons, as shown in Table 1.

According to the number of compounds extracted in each class, carboxylic acids, alcohols and terpenoids are the most abundant, however, according to the concentration relative by area, the most abundant compounds were alcohols, aldehydes, esters, phenylpropanoids and terpenoids.

Of the thirty three compounds identified, 27 were extracted by PA fiber 22 by DVB / CAR / PDMS, and 29 for the PDMS / DVB fiber so that nineteen compounds were common between fibers: myristic acid, hexadecanoic acid, nonanoic, lauric acid, benzyl alcohol, 1-tetradecanol, 1-butanol, furfural, acetofenona, miristato de isopropilo, hexadecanoato isopropilico, etilbenzeno, anetol, eugenol, vanilina, terpinoleno, p-cimeno, timol e isopreno.

The PA fibers and DVB / CAR / PDMS extracted the least amount of volatile compounds. In Figure 1, it can be seen that the polyacrylate fiber (PA) gave chromatographic peaks with the highest abundance relative area, which refers to the compounds: ethyl acetate (29.32%), terpinolene (10:50%), eugenol (10:59 %) and isopropyl hexadecanoate (4.65%). For this fiber, the most abundant classes are alcohols, esters and terpenes. The peaks observed in the chromatogram are listed in Table 1.

According to Vendramini & Trugo (2000), the main groups of compounds that contribute to the acerola aroma are esters, alcohols and ketones, especially ethyl acetate, 1-octadecanol and acetophenone, responsible for aromatic notes not fruity.

The abundance of esters fruit is quite common and reported by several authors in studies of volatile various fruits profiles as caja, soursop, cupuassu, apple, banana, grape and acerola and usually this class is engaged with the flavor notes fruity fruit (Augusto et al., 2000; Pino e Marbot, 2001; Janzantti et al., 2003; Nascimento Junior et al, 2008; Yang et al., 2009).

Figure 2 shows the chromatographic profile of the PDMS / DVB fiber, which has a semipolar coverage of dual phase, consisting of divinylbenzene Polydimethylsiloxane liquid polymer. In the chromatogram can be seen the peaks of compounds that could extract fiber, having the most volatile compounds compared with other fibers. The major peaks refer to the compounds: ethyl acetate, furfural, and 2-ethylhexanol, these compounds are shown in Table 1.

The esters were one of the main classes of compounds that were identified, having in its composition 28.62% of relative area. Alcohols are placed as the second largest class (18.7%), followed by aldehydes (18.33%), terpenes (13.73%), carboxylic acids (6.16%), phenylpropanoids (5.37%), hydrocarbon (4.65%), ketones (3.46 %) and 1% of unidentified compounds. Of all compounds identified by PDMS / DVB fiber, only 4 were detected too in the NIST library, isopropyl myristate, anethole, thymol and isoprene.

In the same manner, Figure 3 shows the chromatogram for the DVB fiber / CAR / PDMS, note that it was the fiber that has the lowest number of chromatographic peaks, however, succeeded in extracting compounds belonging to the class of phenylpropanoids more abundantly relative area. The three main peaks appear in the chromatogram are related to the compounds eugenol (20:41%), vanillin (9.95%) and furfural (7:49%).Some of the chemical classes mentioned in this study provide a taste and aroma characteristic, thereby providing the notes not fruity to the acerola, as in the case of phenylpropanoids and esters, giving the fruity flavors (Christensen et al., 2007); terpenoids, mainly monoterpenes and sesquiterpenes, which give the floral aromas, lemon, sweet, herbaceous, fruity and woody (Tamura et al., 2001) and aldehydes, which give the fruit fresh notes, green, citrus, flowers, soap and oily (rousseff & Perez-Cacho, 2007), to mention a few.

The PA fibers and PDMS / DVB, able to extract the ethyl acetate, the compound belonging to the class of esters. According Carasek & Pawliszyn (2006), the compounds responsible for the aroma and flavor of acerola fruits belong to the class of esters, however, these authors did not identify ethyl acetate.

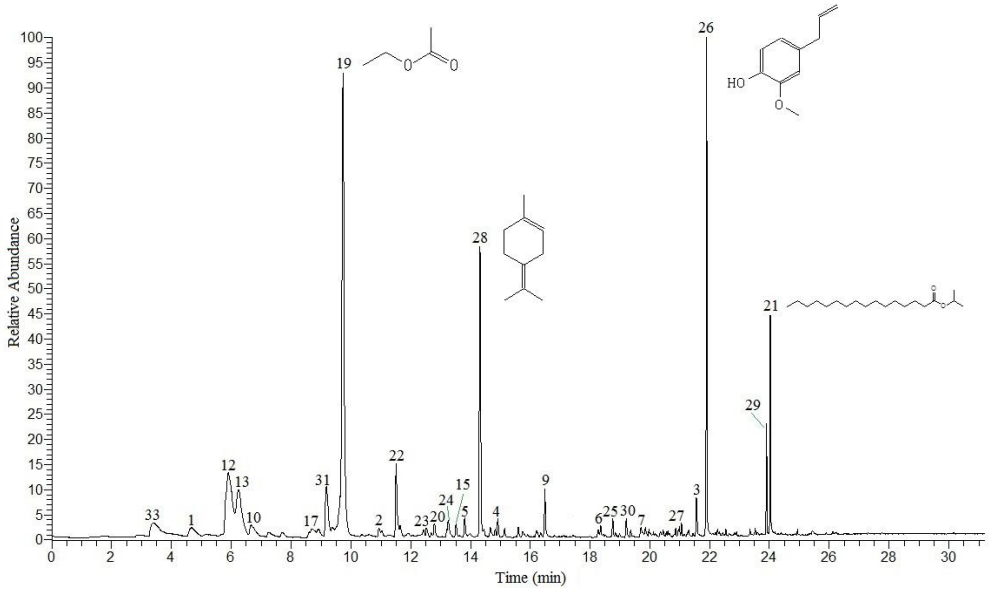

Figure 1. Profile chromatograms of volatile compounds of fruit acerola 'BRS-366 Jaburú' extracted by PA fiber.

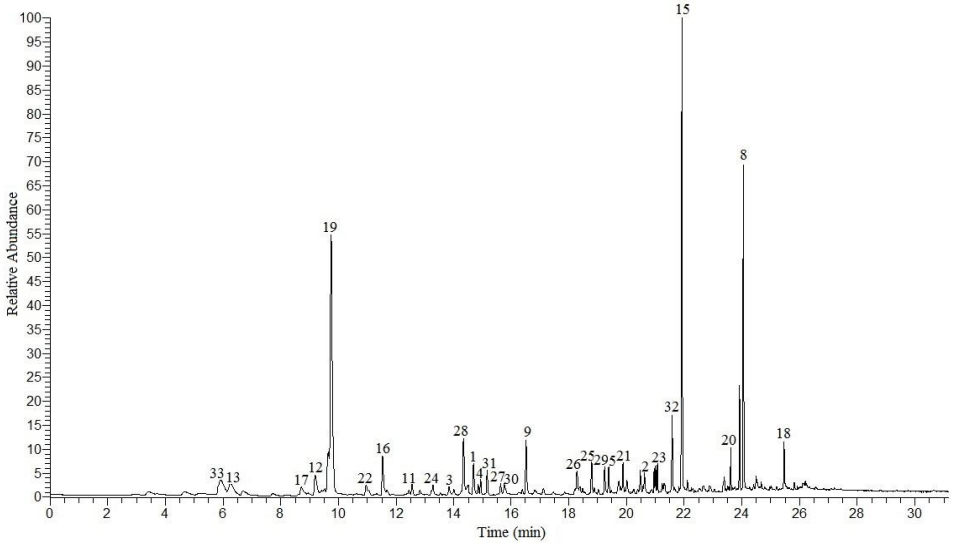

Figure 2. Gas chromatogram profile of volatile compounds of acerola 'BRS-366 Jaburú' extracted by PDMS / DVB fiber.

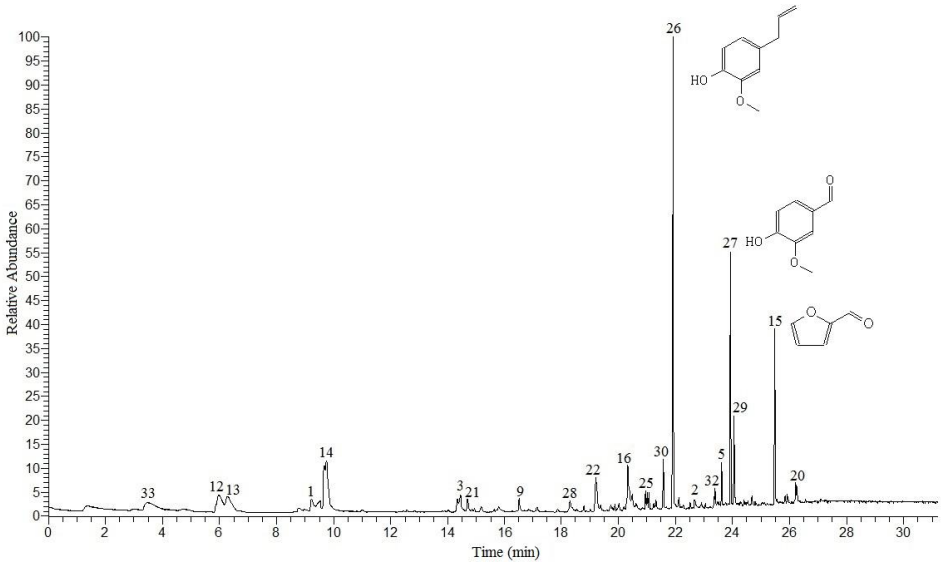

Figure 3. Gas chromatogram profile of volatile compounds of acerola 'BRS-366 quark' extracted by DVB / CAR / PDMS fiber.

Table 1. Volatile compounds identified in fruit acerola 'BRS-366 quark' through three fiber by solid phase microextraction method (SPE).

Peak	Compounds	Concentration (% de area)		
		PA	PDMS/DVB	DVB/CAR/PDMS
			Carboxylic acids	
1	Tetradecanoic acid[a,b]	1.40	1.72	1.91
2	Hexadecanoic acid[a,b,d]	0.73	1.30	0.83
3	Nonanoic acid[a]	1.19	0.59	3.43
4	Decanoic acid[a]	0.88	1.17	-
5	Dodecanoic acid[a,b]	0.78	1.38	1.72
6	Heptanoic acid[c]	0.89	-	-
7	Octadecenoic acid[b]	0.55	-	-
			Alcohols	
8	2-Ethyl-hexanol[a]	-	10.30	-
9	Benzilic alcohol	1.61	2.66	0.93
10	2-Methyl-3-butyn-2-ol[a]	1.34	-	-
11	Octadecan-1-ol[a,b]	-	1.19	-
12	1-Tetradecanol[a]	10.11	2.13	5.44
13	Butan-1-ol[a]	6.44	2.42	4.80
14	3-Methyl-1-butanol[d,f]	-	-	11.87
			Aldehydes	
15	Furfural[a,d]	0.51	15.39	7.49
16	Benzaldehyde[a]	-	2.94	6.44
			Ketones	
17	Acetophenone[a,b,d]	1.25	1.48	2.18
18	Cyclohexanone[a]	-	1.98	-
			Esters	
19	Ethyl acetate[a,b,e]	29.32	26.02	-
20	Isopropyl myristate	0.69	1.22	2.22
21	Isopropyl hexadecanoate[a]	4.65	1.38	1.29
			Hydrocarbons	
22	Ethylbenzene[a]	3.99	1.08	4.66
23	Heptadecane[b]	0.90	2.65	-
24	*m*-Xylene[a]	1.11	0.92	-
			Phenylpropanoids	
25	Anethole	0.76	2.01	0.93
26	Eugenol[b]	10.59	2.69	20.41
27	Vanillin[b]	0.79	0.67	9.95
			Terpenoids	
28	Terpinolene[a]	10.50	3.50	1.58
29	*p*-Cymene[a]	2.20	1.30	4.21
30	Thymol	0.62	1.16	2.44
31	Terpinen-4-ol[a]	3.75	1.18	-
32	*α*-Terpinene[a]	-	3.16	1.93
33	Isoprene	2.32	3.43	3.25
	Total of compounds	27	29	22

Microextraction fiber solid phase: polyacrylate (PA), of 85 micrometres Polydimethylsiloxane / Divinylbenzene (PDMS / DVB) of 65 m and Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB / CAR / PDMS) with 50/30 micrometers. The letters indicate compounds that have been identified by other authors [(a)] Pino & Marbot, 2001; [(b)] Vendramini & Trugo, 2000; [(c)] Boulanger & Crouzet, 2001; [(d)] Bicas et al., 2011; [(e)] Carasek & Pawliszyn, 2006; [(f)] Pinto, 2006. (-) Compounds not detected.

Conclusions

The microextraction method of solid phase extraction (SPME) identified thirty-three volatile compounds through the use of PA fibers, PDMS / DVB and DVB / CAR / PDMS, it is possible to determine the profile of volatiles emitted by fruits of acerola BRS -366 Jaburú.

Moreover, with SPME technique, it was possible to identify new volatiles in all the evaluated fibers, such as the isopropyl myristate, anethole, thymol and isoprene, compounds identified in the first acerola pulp.

The largest extraction of volatiles compound was obtained with the PDMS / DVB fiber, which extracted a total of 29 volatile compounds, therefore, the compounds which determines the volatile profile of acerola are alcohols, aldehydes, esters, phenylpropanoids and terpenoids has the highest area concentration on the chromatogram, as well as the higher intensity peaks in the mass spectrum.

References

ARAÚJO, P. S. R.; MINAMI, K. Acerola. Campinas, Fundação Cargill. p.81, 1994.

AUGUSTO, F., VALENTE, A. L. P., TADA, E. S., & RIVELLINO, R. S. (2000). Screening of Brazilian

fruit aromas using solid-phase microextraction–gas chromatography–mass spectrometry. Journal of Chromatography A, 873: 117−127.

BELO, RENATA FRANÇA CASSIMIRO. Caracterização de genótipos de pequizeiro (*Caryocar brasiliense* camb.) pelo perfil cromatográfico de voláteis. 125 f. (Tese de Mestrado) - Faculdade de Farmácia da UFMG, Belo Horizonte, MG, 2009.

BICAS, J. L., MOLINA, G., DIONÍSIO, A. P., BARROS, F. F. C., WAGNER, R., MARÓSTICA, M. R., & PASTORE, G. M. Volatile constituents of exotic fruits from Brazil. Food Research International 44(7): 1843-1855, 2011.

BOULANGER, R.; CROUZET, J. Identification of the aroma components of acerola (Malphigia glabra L.): Free and bound flavour compounds. Food Chemistry 74(2): 209-216, 2001

CARASEK, E.; PAWLISZYN, J. Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. Journal of Agricultural and Food Chemistry 54(23): 8688-8696, 2006.

CHRISTENSEN, L. P., EDELENBOS, M., & KREUTZMANN, S. Fruits and vegetables of moderate climate. In: *Flavours and fragrances*, Springer Berlin Heidelberg. p. 135-187), 2007.

EMBRAPA. (Embrapa Agroindústria Tropical). Cultivar Acerola BRS 366 Jaburú. 2015. http://ainfo.cnptia.embrapa.br/digital/bitstream/item/77673/1/CGL12001.pdf

FERREIRA, R. M. A.; AROUCHA, E. M. M.; SOUZA, P. A.; QUIROZ, R. F.; FILHO, F. S. T. P. Ponto de colheita da acerola visando à produção industrial de polpa. Revista Verde. Mossoró, 4(2): 13-16, 2009

FRANÇA, V. C.; NARAIN, N. Caracterização química dos frutos de três matrizes de acerola (Malpighia emarginata). Ciência e Tecnologia de Alimentos 23 (2): 157-160, 2003.

FRANCO, M. R. B. Aromas de frutas tropicais: acerola, cupuaçu, bacuri, jenipapo, umbu-cajá, aracá-boi, camu-camu, umbu e murici; Aroma e sabor de alimentos: Tema atuais. São Paulo, Editora Varela, p. 87-89, 2003.

HAWTHORNE, S.; MILLER, D.; PAWLISZYN, J.; ARTHUR, C. Solventless determination of caffeine in beverages using solid-phase microextraction with fused silica fibers. Journal of Chromatography, Amsterdam, v. 603, p. 185-191, 1992.

JANZANTTI, N. S.; FRANCO, M. R. B.; WOSIACKI, G. Efeito do processamento na composição de voláteis de suco clarificado de maçã Fuji. Cienc. Tecnol. Aliment 23: 523-528, 2003.

JUDD, W. S.; CAMPBELL, C. S.; KELLOGG, E. A. & STEVENS, P. F. Plant systematics, a phylogenetic approach. Sinauer Assoc., Inc. Publishers, Suderland, Massachusetts, USA. 1999.

NARAIN, N; ALMEIDA, J. N; GALVÃO, M, S, MADRUGA, M. S; BRITO, E.S. Compostos voláteis dos frutos maracujá (passiflora edulis forma flavicarpa) e de cajá (Spondias mombin L) obtidos pela técnica de headspace dinâmico. Ciência e Tecnologia de Alimentos, Campinas 24(2): 212-216, 2004.

NASCIMENTO, R. J. S. Composição em ácidos graxos do óleo da polpa de açaí extraído com enzimas e com hexano. Rev. Bras. Frutic., Jaboticabal – SP 30(2): 498-502, 2008.

PELUSIO, F.; NILSSON, T.; MONTANARELLA, L.; TILIO, R.; LARSEN, B.; FACCHETTI, S.; MADSEN, J. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. Journal of Agriculture and Food Chemistry, Washington, DC 43: 2138-2143, 1995.

Pino, J. A.; Marbot, R. Volatile flavor constituents of acerola (*Malpighia emarginata* DC.) fruit. Journal of Agricultural and Food Chemistry 49(12): 5880-5882, 2001.

PINTO, L. G. C. Efeito do processamento sobre o perfil sensorial e os constituintes voláteis do suco. 102 f. (Tese Mestrado) - Universidade Federal do Ceará, Fortaleza-Ceará, 2006.

ROUSEFF, R., AND PEREZ-CACHO, P.R. Citrus Flavour. In: Flavours and Fragrances. Chemistry, Bioprocessing and Sustainability; R.G Berger, Ed.; Springer-Verlag: Berlin, p. 117-134, 2007.

TAMURA, H., BOONBUMRUNG, S., YOSHIZAWA, T., AND VARANYANOND, W. The volatile constituents in the peel and pulp of a green thai mango, Khieo Sawoei cultivar (Mangifera indica L.). Food Sci. Technol. Res 7(1,): 72-77. 2001.

THOMAZINI, M.; FRANCO, M. R. B. Metodologia para análise dos constituintes voláteis do sabor. Boletim SBCTA 34 (1): 52-59, 2000.

Vendramini, A. L.; Trugo, L. C. Chemical composition of acerola fruit (Malpighia punicifolia L.) at three stages of maturity. Food Chemistry 71(2): 195-198, 2000.

YANG, C. et al. Volatiles of grape berries evaluated at the germplasm level by headspace-SPME with GC–MS. Food Chem 114: 1106–1114, 2009.

Yang, X.; Peppard, T. Solid-phase microextraction for flavor analysis. Journal of Agriculture and Food Chemistry, Washington, D.C., 42: 1925-1930, 1994.