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Volatile compounds from pitanga fruit (*Eugenia uniflora* L.)

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Abstract

Extracts from pitanga leaves are considered to be effective against many diseases, and are therefore used in popular Brazilian medicines. In this study, the volatile constituents of pitanga fruits (*Eugenia uniflora* L.) were trapped on to Porapak-Q and eluted with ethyl acetate, and the chemical composition of the extract was analyzed by gas chromatography and gas chromatography/mass spectrometry. Fifty-four compounds were detected, and twenty-nine of those were identified by close matches with standard MS spectra. Monoterpenes (75.3% in mass) were found to comprise the largest class of the pitanga fruit volatiles, including *trans*-β-ocimene (36.2%), *cis*-ocimene (13.4%), the isomeric β-ocimene (15.4%) and β-pinene (10.3%). Several known therapeutic constituents of pitanga leaf extract, such as selina-1,3,7(11)-trien-8-one (the major constituent) were also found to be present in the fruit volatile extract, suggesting that the fruit may display therapeutic properties similar to those of the leaf extract.

Keywords: *Eugenia uniflora* L.; Aroma; Monoterpenes; GC/MS

1. Introduction

Secondary metabolites in foods and their potential effects on human health are attracting increasing interest. Consumers, who are also increasingly aware of diet-related health problems are demanding natural foods that are expected to be safe and health promoting.

Exotic fruits, whose use was once restricted to people living in limited geographic areas, are gaining popularity worldwide owing to their nutritional value and exotic flavours that appeal to the consumer. Furthermore, such fruits may contain compounds with important therapeutic properties against human diseases. The pitanga (*Eugenia uniflora* L.) is a tree widely distributed in South American countries, mainly in Brazil, Argentina, Uruguay and Paraguay (Consolini & Sarubbio, 2002). Pitanga fruits are round, about 3 cm in diameter, with eight furrows on the surface, and their colour ranges from orange to purple (Bezerra, Junior, & Lederman, 2000). Pitanga has an exotic and pleasant flavour that has not yet been chemically characterized. The identification of the volatile constituents of pitanga is therefore important for the delineation of processing procedures aimed at retaining the pleasant and unique pitanga flavour in industrialized products. In the Brazilian food industry, pitanga has mostly been used to produce juice, which has high economic potential, owing to its consumer appeal, arising from its high concentrations of antioxidant compounds, such as anthocyanins, flavonols and carotenoids (Lima, Melo, & Lima, 2002).

Studies have also shown that the pitanga fruit may be useful for preventing human diseases. In Brazilian folk medicine, pitanga fruit is used as an anti-diarrheic, diuretic, anti-rheumatic, anti-febrile and anti-diabetic

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agent. Recently, extracts from pitanga leaves have been found to show pronounced anti-inflammatory action (Schapoval, Silveira, Miranda, Alice, & Henriques, 1994), considerable contractile activity, with a resulting effect on intestinal transit (Gbolade, Ilesanmi, & Aladesnmi, 1996), endothelium-dependent vasorelaxant effects (Wazlawik et al., 1997) and hypotensive effects (Consolini, Baldini, & Amat, 1999; Consolini & Sarubbio, 2002), and to inhibit the increase of plasma glucose and triglyceride levels (Arai et al., 1999; Matsumura et al., 2000). Some compounds present in pitanga leaf extracts have also been shown to inhibit the Epstein–Barr virus, known to be closely associated with nasopharyngeal carcinoma (Lee, Chiou, Yen, & Yang, 2000), and to have antimicrobial activity (Adebajo, Oloke, & Aladesanmi, 1989; Holetz et al., 2002) and antifungal activity (Lima, Gompertz, Giesbrecht, & Paulo, 1993; Souza et al., 2002). Owing to these promising and diverse pharmacological effects, there is an increasing interest in the isolation and synthesis of pitanga constituents (Kanazawa, Patin, & Greene, 2000; Lee et al., 1997).

It is widely known, from epidemiological studies, that consumption of fruits and vegetables imparts many health benefits, owing mainly to their organic micronutrients, such as carotenoids, polyphenolics, tocopherols, and vitamin C. Pitanga fruit is one such source of functional compounds; its juice is rich in vitamins and antioxidant compounds. The volatile constituents of the pitanga fruit have not however been, fully characterized. Therefore, this study was conducted to identify the major volatile compounds from pitanga fruits, so that its pleasant and characteristic flavour can be understood. This knowledge can aid attempts to stimulate consumption of industrialized pitanga fruit products with their potential therapeutic effects and health benefits.

2. Materials and methods

2.1. Raw material

Pitanga fruits (*Eugenia uniflora* L.), harvested from a plantation in the State of Pernambuco, Brazil, were acquired (ripe) from a fruit and vegetable market centre (CEASA – São Paulo, Brazil). The fruits that were immature or over ripe were discarded, providing uniform samples, with fruits in the same stage of ripeness. The ripe fruits were processed in the Laboratory of Natural Products in the Faculty of Animal Science and Food Engineering at the Sao Paulo State University – Pirassununga, SP. The pulp was separated from the seeds and 300 g portions stored in sealed bags under vacuum at −18 °C. To obtain the juice, 300 g portions of pitanga fruits were homogenized with 450 ml of distilled water and 30% w/w NaCl to inactivate fruit enzymes during the isolation (Franco & Shibamoto, 2000). A rotating blade working under at 1000 rpm for 2 min at room temperature was used.

2.2. Trapping procedure

Aliquots (450 ml) of four homogenized juice samples were transferred to a volumetric flask and magnetically stirred at low speed. The flask was connected to a Porapak-Q trap, which was connected in turn to a vacuum pump. The volatiles from the pitanga juice were swept to the trap by suction (Franco & Rodrigues-Amaya, 1983). A gauge monitored the vacuum to ensure constant pressure (15 psi) throughout the trapping period. The trap was prepared by packing Porapak-Q (80–100 mesh, Waters Corporation, USA) between silinized glass wool plugs in the glass tube. Trapped volatiles were desorbed by solvent extraction using pure ethyl acetate (2000 μl) applied at one end of the column, and the solvent containing the eluted volatiles was forced to the other end of the column, where a sample (2 μl) was immediately injected into the GC–MS. Thus, four injections were done, one for each sample (four samples). The volume for solvent that allowed easy manipulation and efficient elution without over-diluting the sample was found to be 200 μl, for a trap dimension of 0.5 cm id × 50 mm. The best trapping period allowing detection of the greatest amount of volatile constituents was found to be 4 h at room temperature.

2.3. Gas chromatography–mass spectrometry (GC–MS) analyses

The GC–MS apparatus was a gas chromatograph (HP 5890 series III) and a mass spectrometer (HP 5988A) equipped with a split/splitless injector (maintained at 250 °C) and an HP Ultra 2 capillary column (25 m, 0.2 mm, 0.33 μm). Helium was used as the carrier gas with an inlet pressure of 15 psi. The split ratio was 1:50 and the volume of each injected sample was 2.0 μl. The GC oven was programmed to operate from 50 to 180 °C, maintaining the temperature at 50 °C for 2 min and then increasing it from 50 to 180 °C at 4 °C/min, the detector temperature being 280 °C. Mass spectrum acquisition was performed in the mass range from 40 to 500 m/z. Ionization using 70 eV electrons (EI) was employed. The compounds were identified by comparing the experimental mass spectra with those found in the Wiley 275 l mass spectra library and by comparing their Kovats indices (Table 1).

2.4. Retention indices

Co-injection of a sample with a standard mixture of a homologous series of *n*-alkanes (C_{10}–C_{25}) prepared

Table 1
Volatile compounds of pitanga fruit trapped on Porapak-Q and identified by GC–MS analysis

Compound	Peak	Retention time (min)	Retention index[c]	Area (%)
Propyl acetate	1	3.22	–	1.8
Ethyl propionate	2	4.00	226	0.4
Isobutyl acetate	3	4.08	240	0.8
n-Butyl acetate	4	4.58	321	0.3
N.I.	5	4.89	367	0.1
N.I.	6	5.00	383	0.1
N.I.	7	5.63	466	0.1
N.I.	8	5.99	509	0.2
N.I.	9	6.30	545	0.2
3-Methyl butyl acetate	10	6.49	565	2.0
α-Thujene[a]	11	8.00	712	0.2
α-Pinene[a]	12	8.21	730	0.3
N.I.	13	8.99	793	0.2
β-Pinene[a]	14	10.26	886	9.3
N.I.	15	10.54	903	0.3
1,5,8-*p*-Menthatriene	16	10.70	915	1.8
β-Myrcene[a,b]	17	10.88	927	0.4
α-Terpinene[b]	18	11.12	942	0.3
p-Cymene[b]	19	11.44	962	1.2
trans-Ocimene[b]	20	11.57	970	1.2
cis-Ocimene[b]	21	11.98	995	13.4
trans-β-Ocimene	22	12.46	1024	36.2
N.I.	23	12.68	1035	0.2
λ-Terpinene[a,b]	24	12.75	1041	0.6
N.I.	25	13.31	1069	0.4
p-Mentha-1,5,8-triene	26	13.50	1084	0.3
N.I.	27	13.71	1090	0.4
Terpinolene[a,b]	28	13.79	1100	0.9
Rosefuran	29	14.13	1116	0.4
Linalool[b]	30	14.27	1122	0.4
N.I.	31	14.82	1144	0.2
N.I.	32	15.06	1155	0.2
β-Ocimene	33	15.38	1172	15.4
Allo-ocimene[a]	34	15.79	1189	1.5
N.I.	35	16.81	1232	0.2
N.I.	36	17.43	1258	0.1
N.I.	37	17.97	1279	0.1
N.I.	38	18.35	1294	0.2
Acetophenone	39	20.38	1288	0.1
N.I.	40	24.53	1497	0.1
β-Elemene[a,b]	41	24.78	1409	1.3
β-Caryophillene[a,b]	42	25.75	1440	0.1
λ-Elemene[a,b]	43	26.14	1452	1.1
N.I.	44	26.86	1561	0.1
N.I.	45	27.11	1567	0.1
Germacrene-D	46	27.76	1502	0.2
N.I.	47	27.94	1588	0.1
Curzerene[b]	48	28.25	1576	0.7
N.I.	49	30.51	1650	0.1
N.I.	50	30.86	1658	0.5
Caryophyllene oxide	51	31.02	1729	0.2
β-Elemenone[a]	52	31.60	1744	0.2
Selina-1,3,7(11)-trien-8-one[b]	53	32.45	1767	0.8
N.I.	54	35.85	1823	0.1

[a] These volatile components were also identified in Cuban pitanga fruit using steam distillation and solvent extraction (Pino et al., 2003).
[b] Volatile constituents also found in pitanga leaf extracts (Weyerstahl et al., 1988).
[c] On HP Ultra II column.

in pure hexane as the solvent provided the Kovats indices under the same chromatographic conditions as those used for the separation of the volatile compounds.

3. Results and discussion

Fig. 1 shows the total ion chromatogram for the volatiles of only one sample from the pitanga fruit trapped

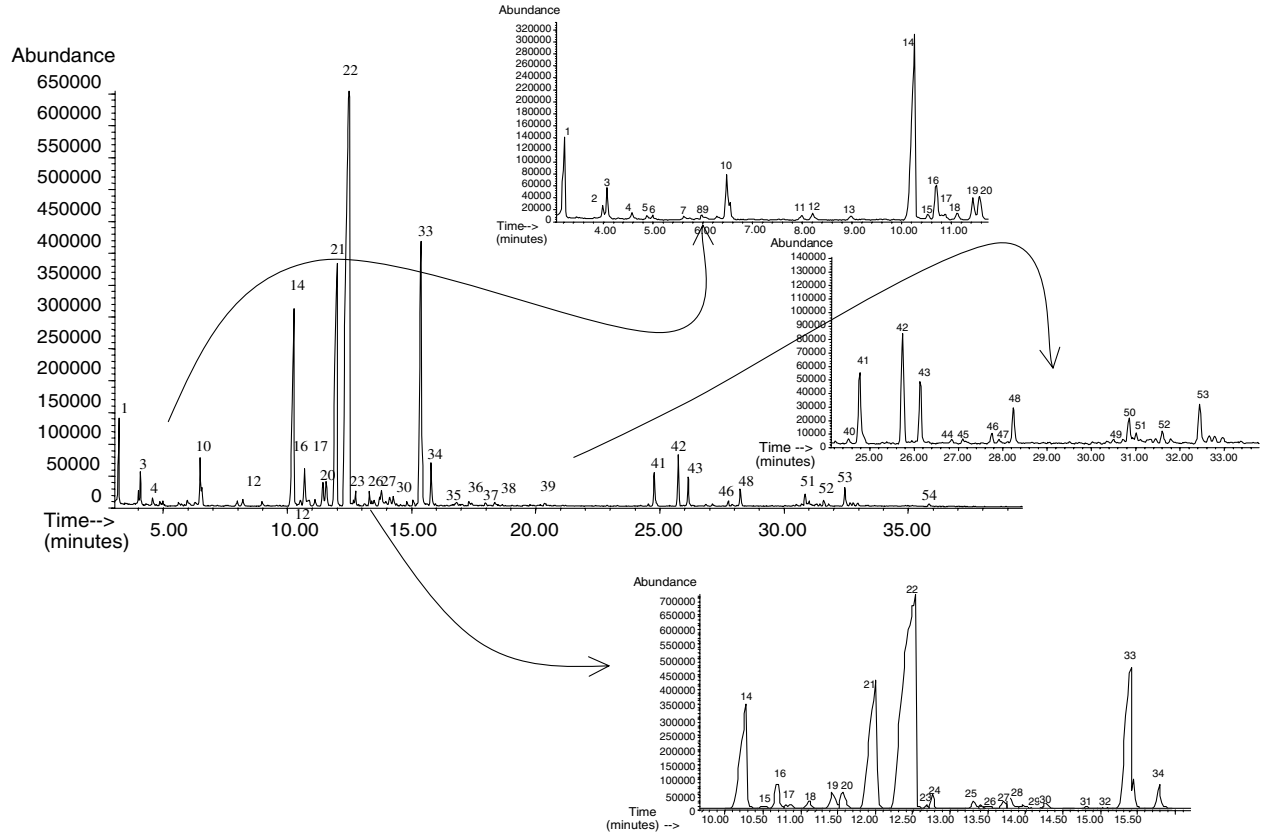

Fig. 1. Total ion chromatogram of the pitanga fruit extract trapped on Porapak-Q. The inserts show expansions of the chromatogram in which less abundant compounds are detected. Well-defined chromatographic peaks are sequentially numbered as a function of increasing retention time.

on to Porapak-Q during 4 h. Fifty-four chromatographic peaks can be clearly detected, and Table 1 shows the 29 volatile compounds that could be identified by mass spectra comparison. The retention indices were calculated for all compounds using a homologous series of *n*-alkanes under the same operational conditions. The majority of them are monoterpenes, among which *trans*-β-ocimene predominates (36.2%), followed by *cis*-ocimene (13.4%), the isomeric β-ocimene (15.4%) and β-pinene (10.3%). Fourteen of the identified constituents have also recently been identified by Pino, Bello, Urquiola, Aguero, and Marbot (2003), using GC–MS analysis

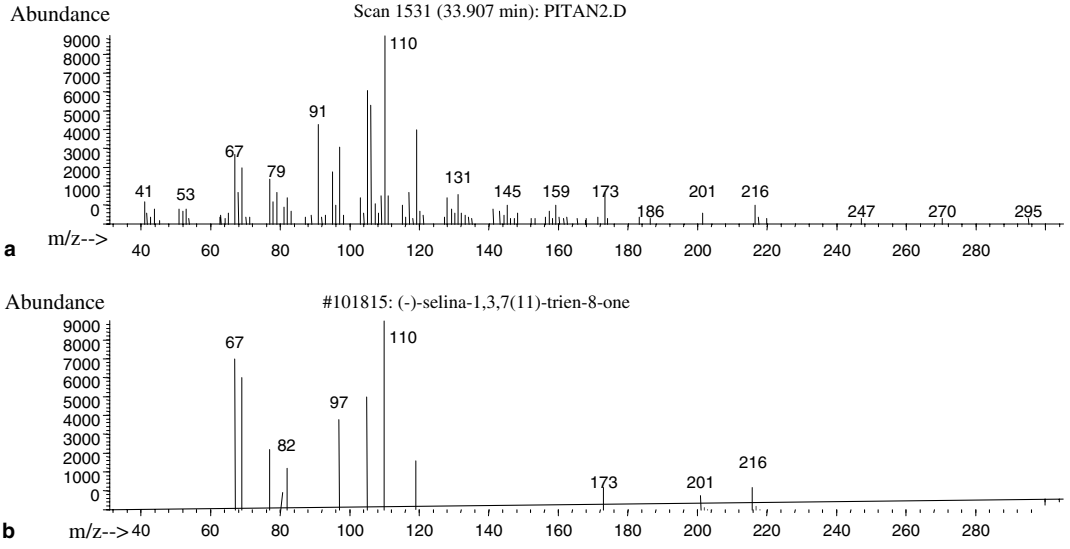

Fig. 2. (a) 70 eV EI mass spectrum of the pitanga volatile extract constituent numbered as peak 49 in the GC–MS chromatogram of Fig. 1. (b) 70 eV EI reference mass spectrum of (−)selina-1,3,7(11)-trien-8-one, as provided by the Wiley 275 l mass spectra library.

on the volatile extract of Cuban pitanga fruit obtained by simultaneous steam distillation and solvent extraction. Surprisingly, when using Porapak-Q trapping and solvent extraction, followed by GC–MS analysis, we were unable to identify the two major volatile constituents of pitanga fruit found by Pino et al. (2003), namely, curzenene and bergaptene.

The major volatile component here identified for the pitanga fruit, *trans*-β-ocimene (Table 1), is also an important volatile constituent of other tropical Brazilian fruits, such as umbu-caja (*Spondias citherea*). The second one, β-pinene, is also an important constituent of araça-boi (*Eugenia stipitata*), umbu-caja (*Spondias citherea*) and camu-camu (*Myrciaria dubia*) (Franco & Shibamoto, 2000).

Twelve of the volatile compounds found in the pitanga fruit extract have also been found in the essential oil of pitanga leaves (Weyerstahl, Marschall-Weyerstahl, Christiansen, Oguntimein, & Adeoye, 1988). For instance, the major constituent of the pitanga leaves, that is, selina-1,3,7(11)-trien-8-one, has also been identified in the volatile extract from the pitanga fruits as peak 53 (Fig. 1), as confirmed by mass spectra comparison (Fig. 2). This natural compound has recently been synthesized (Kanazawa et al., 2000). Aqueous alcoholic extracts or infusions made from pitanga leaves have been used as traditional remedies in folk medicine for a number of diseases. The present finding that the leaves and fruits from pitanga share several constituents is important, since it can stimulate the consumption of the fruit for similar health benefits.

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