

Differences of volatile constituents between unripe, partially ripe and ripe guayabita del pinar (*Psidium salutare* H.B.K.) fruit macerates

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Received 13 October 2007; received in revised form 13 November 2007; accepted 16 January 2008

Abstract

The effect of the maturation stages on the volatile constituents of the guayabita del pinar (*Psidium salutare* H.B.K.) fruit macerates was investigated during three different stages (unripe, partially ripe and ripe). Volatile compounds were isolated by continuous liquid–liquid extraction with pentane and analyzed by means of GC–FID and GC–MS. In unripe fruit macerate the fruit volatiles were predominantly the mono- and sesquiterpenes. During maturation, levels of the mono- and sesquiterpenes decreased drastically in macerates, whereas levels of some esters (ethyl nicotinate, ethyl malate, ethyl 3-phenylpropanoate, pentyl benzoate, benzyl benzoate and ethyl cinnamates) and cinnamic acid increased significantly.

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Keywords: Guayabita del pinar; *Psidium salutare*; Maturation; Macerate; Volatile compounds; GC–MS

1. Introduction

The species *Psidium salutare* H.B.K., commonly called guayabita del pinar very much resembles the guava in size of tree and in general growth. The small tree, which is endemic of the western part of Cuba, produces a thin-skinned, green fruit about the size of a little olive, with a short sharp point at the flower end. The fruit has scanty flesh and a pleasant sweet flavour. It is very aromatic and is commonly used at its partially ripe stage to prepare traditional liquor. A possible way of extending the time of production of the liquor is to explore the possibility of the use of green or ripe fruits. The changes which occur in fruits during development may affect the quality of the product obtained but information in this field is limited and widely scattered in the literature. To date the volatile compounds of *P. salutare* ripe fruits has been the subject of only one previous study (Pino, Marbot, & Bello, 2002). It is known in the case of the common guava (*Psidium guajava* L.) that considerable changes occur in the composition and quality of fruit

during maturation, particularly C₆ aldehydes decreased and some esters increased (Chyau, Chen, & Wu, 1992; Soares, Pereira, Marques, & Monteiro, 2007).

The objective of this study was to further evaluate the volatile constituents between the unripe, partially ripe and ripe guayabita del pinar macerates.

2. Materials and methods

2.1. Fruits and macerates

Fresh unripe, partially ripe and ripe fruits (1 kg each one) were picked from the same bushes grown in western Cuba in Pinar del Río. Fruits were harvested at three ripening stages, based on their manually measured firmness: unripe fruits were very firm, partially ripe fruits were slightly softer than immature fruits, and ripe fruits were very soft. The alcoholic macerates (1 l) were prepared at room temperature in the laboratory simulating the technological process in the industry with the use of 95% ethanol from white cane spirit (1:3 fruit:alcohol ratio) during 90 days. Those macerates presented a final alcoholic grade of 42–45%.

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2.2. Isolation of volatile compounds

One liter of each macerate of the three maturation stage fruits previously diluted at 20% was subjected to the isolation of volatile compounds by continuous liquid–liquid extraction with 150 ml of pentane for 6 h (Pino, Villareal, & Roncal, 1994). Internal standard (0.2 mg of methyl nonanoate) was added before extraction. The extracts were dried over anhydrous sodium sulphate and concentrated with a Kuderna–Danish apparatus with a vigreux column to 0.2 ml for analysis. Extractions were made in triplicate.

2.3. Gas chromatography (GC–FID)

An HP 6890 GC with FID equipped with a fused silica HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The temperature program was 2 min isothermal at 50 °C and then 40–280 °C at a rate of 4 °C/min. The carrier flow rate (hydrogen) was 1 ml/min. Injector and detector temperatures were kept at 220 °C. Volumes of 1 μl were injected with a split ratio of 1:10. Retention indices were calculated of separated compounds relative to an *n*-alkanes mixture.

2.4. Gas chromatography–mass spectrometry (GC–MS)

An HP 6890 Series II coupled to a HP-5973N mass detector was used. A similar column and temperature program as GC–FID were used. The carrier flow rate (helium) was 1 ml/min. Mass spectra were recorded in the electron-impact mode at 70 eV by 1.8 scans/s. Detection was performed in the scan mode between 30 and 400 Da.

2.5. Qualitative and quantitative analysis of volatile compounds

Constituents were identified by comparison of their mass spectra with those in NBS, NIST or our FLAVOR-LIB data base created with authentic standards, and confirmed in many compounds by relative retention indices of authentic standards. Mass spectra from the literature (Adams, 2001; MacLafferty & Staffer, 1989) were also compared.

Quantitative determinations were carried out by internal standard method from GC–FID peak areas, without the use of calibration curves. Concentrations were uncorrected and were based on the ratio of the area of a compound compared to the internal standard. Results of the analyses for replicate samples were averaged.

2.6. Sensory evaluation

A panel of 10 people (five women and five men, staff from the laboratory) between the ages of 24 and 55, all with previous experience serving on sensory panels, was used for all sensory testing. Panelists evaluated the odour of the macerates, previously diluted to 30% ethanol, between

10:00 a.m and 11:30 a.m. A triangle test was used to determine if the panel could detect differences between the samples. The samples, 30 ml per glass, were served at ambient temperature in clear glasses covered by watch glasses in individual testing booths. The panelist was asked to smell the samples and indicate the odd sample by its three-digit coded. No special training preceded the test.

2.7. Statistical analysis

The concentrations were assessed as average and analyzed by one way-ANOVA and average were compared using Duncan's test, with a probability $p \leq 0.05$.

3. Results and discussion

The volatile compounds of guayabita del pinar fruit macerates were obtained by liquid–liquid extraction and analyzed by GC and GC–MS. Valid aroma concentrates were prepared by using established procedures with an acceptable extraction efficiency and relatively low danger of artefact formation (Parada, Duque, & Fujimoto, 2000). The concentrated extracts showed aroma notes resembling the flavour of fresh fruit, described as sweet–grassy–fruity.

Table 1 lists the identified compounds and quantitative distribution. In total, 109 volatile constituents were identified. Of them, 45 are reported for the first time in this fruit. In quantitative distribution, total amounts of 188.9, 97.3 and 85.4 mg/l of unripe, partially ripe and ripe fruit macerates, respectively, were determined.

Many of the new volatile compounds have their origin in the ethanol used for the maceration, e.g. ethyl esters, acetals, fatty acids and their ethyl esters (Pino, 2007), and therefore they were not found in the previous study of the fruit (Pino et al., 2002). For these compounds, similar amounts in the three macerates were found, independently of the fruit maturity stage. Other constituents should have their origin in the fruit, but they were not probably detected previously (Pino et al., 2002), probably because a different isolation method was used.

In general, those fruit macerates were rich in mono- and sesquiterpenes, as it was reported previously for the fruit (Pino et al., 2002). As it was expected, there are some differences in the proportions of the volatile compounds in the macerate of the mature fruit that is the only one that could be comparable with the composition reported in the previous study. However, these differences could be considered with caution because, as it was mentioned previously, the isolation method used in both studies was very different. Table 1 showed clearly that mono- and sesquiterpenes decreased from unripe to ripe fruit macerates. In fact, this reduction in terpene amount ruled the decrease in total concentration of all the fruit macerates with the advance in the maturity stage of the fruit. This result was similar to the previous studies in common guava fruit at different maturity stages (Chyau et al., 1992; Soares et al., 2007).

Table 1
Composition of guayabita del pinar macerates at different fruit maturity stage (mg/l)

Compound	KI	Identification ^a	Unripe	Partially ripe	Ripe
Ethyl acetate	615	A	<0.01	<0.01	<0.01
2-Pentanone	692	A	–	<0.01	<0.01
Ethyl isobutanoate ^b	751	A	<0.01	<0.01	<0.01
1-Pentanol	771	A	–	<0.01	–
Ethyl butanoate	804	A	0.15a	0.19a	0.17a
Ethyl 2-methylbutanoate ^b	846	A	<0.01	<0.01	<0.01
Ethyl isopentanoate ^a	858	A	<0.01	<0.01	<0.01
Ethylbenzene	868	A	<0.01	–	–
1,1-Diethoxyisobutane ^b	872	C	0.09a	0.08a	0.10a
Isopentyl acetate ^b	876	A	0.07a	0.08a	0.10a
2-Heptanone	892	A	0.05a	0.04a	0.05a
Ethyl pentanoate ^b	898	A	0.21a	0.20a	0.21a
Santolina triene	909	B	0.11	<0.01	–
α -Pinene	939	A	2.65a	0.51b	0.40c
Camphene	954	A	0.54	<0.01	<0.01
1,1-Diethoxy-3-methylbutane ^b	957	C	0.27a	0.25a	0.27a
Benzaldehyde	960	A	<0.01	<0.01	<0.01
β -Pinene	979	A	3.54a	2.08b	1.57c
Hexanoic acid ^b	981	A	<0.01	<0.01	<0.01
Myrcene	991	A	0.90a	0.31b	0.24b
Ethyl hexanoate ^b	998	A	0.62a	0.56a	0.66a
<i>p</i> -Cymene	1025	A	0.80a	0.22b	0.13b
Limonene	1029	A	4.63a	2.28b	1.76b
1,8-Cineole	1031	A	4.63a	1.52b	0.19c
(<i>Z</i>)- β -Ocimene	1037	A	1.94a	1.34b	0.63c
(<i>E</i>)- β -Ocimene	1050	A	0.76a	0.31b	0.21b
γ -Terpinene	1060	A	0.35a	0.08b	0.05b
Heptanoic acid	1071	A	0.05	–	–
<i>trans</i> -Linalool oxide (furanoid)	1073	A	1.02a	0.31b	0.42b
Fenchone	1087	A	<0.01	<0.01	<0.01
Terpinolene	1089	A	<0.01	<0.01	<0.01
<i>cis</i> -Linalool oxide (furanoid)	1087	A	1.66a	0.72b	0.56c
<i>p</i> -Cymenene	1091	A	<0.01	<0.01	<0.01
2-Nonanone	1090	A	<0.01	<0.01	<0.01
Linalool	1097	A	12.88a	7.46b	6.08c
<i>cis-p</i> -Menth-2-en-1-ol	1122	B	0.22	<0.01	<0.01
Myrcenol	1123	B	–	0.01	<0.01
Allo-ocimene	1132	B	0.79a	0.19b	0.12b
<i>trans-p</i> -Menth-2-en-1-ol	1141	B	0.13	–	–
Camphene hydrate ^b	1150	B	0.22a	0.10b	0.10b
Menthone ^b	1154	A	<0.01	–	–
<i>iso</i> -Menthone ^b	1163	A	0.17	–	–
Pinocarvone ^b	1165	B	<0.01	–	–
Ethyl benzoate ^b	1173	A	0.15a	0.12a	0.13a
<i>cis</i> -Pinocamphone ^b	1175	B	0.20a	0.11b	0.03c
Terpinen-4-ol	1177	A	1.28a	0.84b	0.47c
Diethyl succinate ^b	1179	A	0.25a	0.29a	0.24a
Octanoic acid ^b	1180	A	–	–	<0.01
Cryptone ^b	1186	B	<0.01	<0.01	<0.01
α -Terpineol	1189	A	4.73a	4.53a	3.04b
Methyl salicylate ^b	1192	A	<0.01	–	–
Ethyl octanoate ^b	1197	A	<0.01	<0.01	<0.01
Verbenone	1205	A	<0.01	<0.01	<0.01
Ethyl nicotinate ^b	1233	A	0.59a	0.33b	0.09c
Carvone	1243	A	0.64a	0.21b	0.08c
Ethyl phenylacetate ^b	1244	A	<0.01	<0.01	<0.01
Piperitone	1253	B	0.02	<0.01	0.01
Geraniol	1253	A	0.08	–	<0.01
Ethyl salicylate	1270	A	0.01	–	–
Ethyl malate ^b	1282	A	0.10a	0.12a	0.11a
Ethyl nonanoate ^b	1298	A	0.01	<0.01	<0.01
Carvacrol	1299	A	<0.01	0.07	0.07
(2,2-Diethoxyethyl)-benzene ^b	1312	C	0.12a	0.11a	0.12a

(continued on next page)

Table 1 (continued)

Compound	KI	Identification ^a	Unripe	Partially ripe	Ripe
Ethyl 3-phenylpropanoate ^b	1349	B	1.12a	0.06b	0.07b
Eugenol ^b	1359	A	0.30	–	–
Geranic acid ^b	1370	B	–	–	0.11
Isoledene	1376	B	0.93	–	–
Ethyl (Z)-cinnamate ^b	1378	A	0.01a	0.08b	0.17c
Decanoic acid ^b	1380	A	<0.01	0.03	0.03
Ethyl decanoate ^b	1396	A	0.01	0.01	0.01
1-Methylhexyl hexanoate	1405	C	–	0.01	0.01
β-Caryophyllene	1419	A	0.92a	0.08b	0.10b
Aromadendrene	1441	B	1.12a	0.10b	0.10b
(E)-Cinnamic acid	1454	A	–	0.01	0.09
α-Humulene	1455	B	1.00a	0.05b	0.06b
allo-Aromadendrene	1460	B	1.02a	0.20b	0.19b
Ethyl (E)-cinnamate ^b	1467	A	0.69a	2.82b	3.24c
Pentyl benzoate ^b	1478	A	0.08a	0.12a	0.24b
β-Selinene	1490	B	1.00a	0.26b	0.30b
Viridiflorene	1497	B	1.82a	0.27b	0.32b
α-Muurolene	1500	B	0.72a	0.09b	0.01c
γ-Cadinene	1514	A	0.30	0.05	<0.01
δ-Cadinene	1523	A	2.43a	0.11b	0.16b
α-Calacorene	1546	B	1.57a	0.11b	0.11b
(E)-Nerolidol	1563	A	–	1.25	1.02
Caryophyllene alcohol ^b	1572	B	–	0.31	0.29
Dodecanoic acid ^b	1580	A	0.68a	0.70a	0.70a
Caryophyllene oxide	1583	A	<0.01	–	–
Globulol	1585	B	–	5.32	5.19
Ethyl dodecanoate ^b	1595	A	<0.01	<0.01	<0.01
Viridiflorol	1593	A	37.28a	8.72b	9.11b
Guaiol	1601	B	4.14a	3.25b	1.62c
1-epi-Cubenol	1629	B	–	3.07	3.04
γ-Eudesmol	1632	B	8.08a	3.85b	3.42b
epi-α-Cadinol	1640	B	28.79	18.02	17.75
α-Eudesmol	1646	B	0.81a	1.91b	1.82b
α-Cadinol	1654	B	12.34a	3.82b	3.64b
Benzyl benzoate	1760	A	0.25a	0.52b	1.00c
Tetradecanoic acid	1780	A	<0.01	<0.01	<0.01
Ethyl tetradecanoate ^b	1793	A	0.03a	0.03a	0.04a
Cryptomeridiol ^b	1814	C	8.22a	3.83b	2.73c
Ethyl (Z)-9-hexadecenoate ^b	1989	A	–	0.27	0.27
Hexadecanoic acid ^b	1991	A	1.08a	0.98a	1.09a
Ethyl hexadecanoate ^b	1993	A	0.30a	0.29a	0.32a
Octadecanoic acid ^b	2171	A	0.01	<0.01	<0.01
Ethyl octadecanoate ^b	2197	A	0.01a	0.02a	0.02a

– Not detected.

Different letters indicate significant differences at $p \leq 0.05$.

^a The reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index agreed with standards; B, mass spectrum and Kovats index agreed with literature data; C, mass spectrum agreed with mass spectral database.

^b No previously reported in this fruit.

Another important class of compounds was esters. In the individual order, some of them significantly increased their concentration from the unripe to ripe fruit macerates, e.g. ethyl nicotinate, ethyl malate, ethyl 3-phenylpropanoate, pentyl benzoate and benzyl benzoate. These esters are reported for the first time in this fruit and they were not reported in white cane spirit (Pino, 2007).

A further important group of guava aroma constituents was represented by the cinnamyl derivatives, including cinnamic acid and ethyl cinnamates. All of them increased their concentrations from unripe to ripe fruit macerates. Cinnamic acid and ethyl cinnamates contribute to balsamic

and honey-like odour (Arctander, 1969). These increments in concentration could influence the fruity note of the ripe fruit.

These results confirm that the formation of aroma compounds in fruit is a dynamic process, during which concentrations of volatile constituents change both qualitatively and quantitatively, which can already cause changes in the quality of a commercially established product.

All 10 judges could distinguish the unripe macerate from partially ripe and ripe macerates, but they could not find differences between partially ripe and ripe macerate in the triangle test for a $p \leq 0.05$ significance level. These results

clearly indicate that ripe fruits could be used for preparing the traditional liquor which is prepared only with mature fruits. Because guayabita del pinar fruits are often, for commercial reasons, harvested for producing the liquor, the possible sensory changes in the final product will be the subject of further investigations.

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