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Original

Volatile compounds in noni (*Morinda citrifolia* L.) at two ripening stages

Compostos voláteis em noni (Morinda citrifolia L.) em dois estágios de maturação

Jorge Antonio PINO[1]*, Eliosbel MÁRQUEZ[1], Clara Elizabeth QUIJANO[2], Déborah CASTRO[3]

Abstract

The volatile components of noni at two ripening stages were isolated by headspace solid-phase microextraction using 65 µm Polydimethylsiloxane-Divinylbenzene (PDMS/DVB) fibers and analyzed using GC/MS. Both maturation stages had several compounds in common. Ninety-six compounds were identified, from which octanoic acid (≅ 70% of total extract) and hexanoic acid (≅ 8% of total extract) were found to be the major constituents. Due to noni maturation, octanoic acid, decanoic acid and 2*E*-nonenal decreased their concentrations, while some esters (methyl hexanoate, methyl octanoate, ethyl octanoate and methyl 4*E*-decenoate), which their fruity odor notes, increased their contents. Two unsaturated esters, reported for the first time in this fruit, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased their concentration in the ripe to over-ripe fruits.

Keywords: *noni; Morinda citrifolia; Rubiaceae; volatile compounds; HS-SPME; GC-MS.*

Resumen

Los compuestos volátiles del noni en los dos estados de madurez se aislaron mediante microextracción en fase sólida por headspace con fibras de 65 µm Polidimetilsiloxano-Divinilbenceno (PDMS/DVB) y se analizaron por cromatografía de gases/espectrometría de masas. Ambos estados de madurez poseen numerosos constituyentes en común. Se identificaron 96 compuestos, de los cuales el ácido octanoico (≅ 70% del extracto total) y ácido hexanoico (≅ 8% del extracto total) fueron los componentes mayoritarios. Debido a la maduración del noni, el ácido octanoico, ácido decanoico y 2*E*-nonenal disminuyeron su contenido, mientras que algunos ésteres (hexanoato de metilo, octanoato de metilo, octanoato de etilo y 4*E*-decenoato de metilo), con sus olororosas notas frutales, incrementaron la concentración. Dos ésteres insaturados, reportados por primera vez en esta fruta, hexanoato de 3-metil-3-buten-1-ilo y octanoato de 3-metil-3-buten-1-ilo, disminuyeron significativamente su concentración al pasar la fruta del estado maduro al sobremaduro.

Palabras clave: *noni; Morinda citrifolia; Rubiaceae; compuestos volátiles; HS-SPME; GC-MS.*

1 Introduction

Morinda citrifolia L. (Rubiaceae), commonly known as noni, is a plant typically found in the Hawaiian and Tahitian islands. It is believed to be one of the most important plants brought to Hawaii by the first Polynesians (ROSS, 2001). Different parts of the tree, including the fruit, have been traditionally used as folk remedy for many diseases such as diabetes, hypertension, and cancer (SANG et al., 2002; CHAN-BLANCO et al., 2006; POTTERAT; HAMBURGER, 2007). The consumption of the juice for health benefits has seen a tremendous growth worldwide. The plant is a small evergreen tree with large bright green elliptical leaves. The fruit results from coalescence of the inferior ovaries of many closely packed flowers, it has a smooth surface and many polygonal sections. The immature fruit is green. As it matures the fruit becomes whiter in colour, and unless it is harvested at this stage, it simply falls to the ground. As it ripens, the fruit has a very pungent smell, similar to the odor of blue vein cheese and its taste is sour.

There are two main methods used to produce noni juice. In the traditional method, the fruit is collected when it is beginning to ripen and placed in a container for several weeks. At the end of this time a large percentage of the over-ripe fruit simply disappears into the juice; the residual fruits are mashed into a puree, and the juice is filtered to remove any remaining sediment. The dark brown juice is then ready for use. In another processing method, the mature fruit (light amber colour) is pressed and the resulting juice becomes the finished product (RUSSELL, 2000). Although the noni fruits have been used as a food, very few reports on their chemical composition are available (WANG, 1999; ROSS, 2001; KAMIYA, 2004; CHAN-BLANCO et al., 2006; POTTERAT; HAMBURGER, 2007), all of them related with the non-volatile components. In this study, 51 volatile compounds of the ripe fruit were isolated and quantified by solvent extraction (FARINE et al., 1996), and 24 volatile constituents were identified by HS-SPME-GC-MS using a PDMS fiber from commercial juices (LACHENMEIER et al., 2006).

Solid-phase microextraction (SPME), developed in 1989 by Pawliszyn, is a rapid, direct, inexpensive, and efficient technique for sampling different matrixes (PAWLISZYN, 1997, 1999). SPME is a multi-analyte extraction technique that requires no

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solvents; it minimizes artefact formation by heat and provides linear results over a wide range of analyte concentrations in a large variety of matrixes. In the field of food aroma analysis, the headspace SPME (HS-SPME) has proved to be an advanced and efficient tool for studies on food aromas, including fruits and fruit juices (HARMON, 2002; MARSILI, 2002). In fact, SPME/GC is a convenient technique for providing the aromatic fingerprint descriptions of each analyzed fruit.

The present work is aimed at studying, using HS-SPME, the change of volatile compounds of noni fruits during ripening.

2 Materials and methods

2.1 Plant material and chemicals

Freshly harvested noni fruits, grown in Havana, were purchased from a local producer. Voucher specimens were deposited in the herbarium of the National Botanic Garden in Havana (JBN-2344-08). The fruits were selected at two maturity stages: ripe (light amber colour) and over-ripe (dark brown colour), by a commercial producer. Three groups, of ten fruits each one, were manually mashed and strained using a 200-mesh sieve. The homogenized pulp was analyzed immediately after sample preparation.

Authentic reference chemical compounds with purity figures above 98% were provided by Aldrich (Milwaukee, USA).

2.2 HS-SPME procedure

A manual holder (Supelco, Inc., Bellefonte, USA) and 65 µm Polydimethylsiloxane-Divinylbenzene (PDMS/DVB) (Supelco, Inc., Bellefonte, USA) were used for all the experiments. This fiber was selected according to the best results for the extraction of fruit volatiles (PAWLISZYN, 1999; HARMON, 2002). For each extraction, 10 g of pulp (without seeds) and 20 mL of 20% NaCl solution (to inhibit enzymatic reactions and to favour the transfer of the analytes from the aqueous solution to the headspace) were blended in a Braun MR 400 juicer for 2 minutes and then centrifuged at 3000 rev/min for 10 minutes, a procedure essentially identical to those previously used for other fruits (FALLIK et al., 2001; URRUTY et al., 2002; SERVILI et al., 2006). An 8 mL aliquot of the supernatant was transferred into a 15 mL Teflon-lined septum cap vial equipped with a teflon-coated magnetic bar. To favour the transfer of the analytes from the aqueous solution to the headspace, the solution was stirred (200 rev/min) at 40 °C. After a 10 minutes wait to reach equilibrium between the solution and the headspace, the fiber was exposed for 20 minutes in the headspace. The fiber was then removed and introduced into the injector port of the GC for desorption at 250 °C for 2 minutes, in the splitless mode. Then the split valve was opened (ratio of 1:50), but the fiber was kept in the injector for 5 minutes for cleaning.

2.3 Gas chromatography-mass spectrometry analyses

A Hewlett-Packard 6890 GC coupled to a HP-5973 mass selective detector was employed. An inlet of 0.75 mm I.D., which improves the GC resolution, was used. The carrier gas was helium (1 $mL.min^{-1}$) and the injector temperature was 250 °C. The analytes were separated on a HP-5MS 30 m × 0.32 mm × 0.25 µm column (Supelco, Inc., Bellefonte, USA), kept at 50 °C for 2 minutes and then ramped to 240 °C at 4 °C/min and held at the final temperature for 10 minutes. The transfer line was kept at 250 °C and the ion source was held at 230 °C. Mass spectra were measured at 70 eV and collected at the rate of 1 scan/second over an m/z range of 35 to 400. Chromatographic retention indices of separated compounds were calculated relative to a C_8-C_{22} n-alkanes mixture.

Constituents were identified by comparing their mass spectra to those in NIST/EPA/NIH, our FLAVORLIB data base and with mass spectra of authentic standards. In many compounds, the identities were confirmed by their relative retention indices with authentic standards. Mass spectra from the literature (MACLAFFERTY; STAFFER, 1989; ADAMS, 2001) were also compared.

For each compound, quantitation was performed by measuring the corresponding peak area of the total ion chromatogram and expressed as relative (percent) areas by normalization.

The original data from triplicate analyses were transformed and processed for statistical analysis by the t-Student' test.

3 Results and discussion

The volatile constituents of noni fruit were obtained by HS-SPME and analyzed by GC–MS using a fused silica capillary column. The peak areas of the compounds analyzed in triplicate on the same sample showed a coefficient of variation ≤ 5%.

In total, ninety-six volatile compounds were identified (Table 1), sixty-seven of them reported for the first time in noni fruit, although they are often found in other fruits (NIJSSEN et al., 1996). The use of a more polar fiber in this study enables us to isolate many polar compounds not previously found in a previous study using the non-polar PDMS fiber (LACHENMEIER et al., 2006). The presence of so many aliphatic esters in fruits of both maturity stages, mainly in the over-ripe ones, is interesting. This abundance of aliphatic esters has not been reported in previous studies (FARINE et al., 1996; LACHENMEIER et al., 2006). Alkyl esters of hexanoic and octanoic acids were the major ones in this family. All these esters have powerful fruity odor notes (ARCTANDER, 1969).

In general, although terpenes are present in small quantities in both maturity stages, their contribution to the fruit's flavor could be considerable, as in the case of limonene and linalool, which were found to possess intense citrus and flower-like odors (ARCTANDER, 1969). Interestingly, three sulphur compounds were found for the first time in noni fruit, *e.g.* dimethyl disulfide, dimethyl trisulfide and 3-(methylthio)-1-propanol. No nitrogen-containing volatile compounds were found.

The major volatile compounds, at both maturity stages, were octanoic acid (≅ 70% of total extract) and hexanoic acid (≅ 8% of total extract), basically the same components as those previously reported (FARINE et al., 1996), although in different

Table 1. Volatile compounds in noni at two maturity stages (mean area %).

Compound	RI_{exp}	RI_{st}	Identification	Ripe	Over-ripe
ethanol*	535	537	A	0.02[a]	0.02[a]
acetic acid	602	600	A	0.01[a]	0.02[a]
1-butanol	650	653	A	0.04[a]	0.04[a]
pentanal*	701	706	A	0.01[a]	0.01[a]
methyl butanoate*	728	729	A	0.01[a]	0.02[a]
3-methyl-3-buten-1-ol	730	731	B	0.02[a]	0.03[a]
3-methylbutan-1-ol*	741	741	A	0.02[a]	0.01[a]
2-methylbutan-1-ol*	744	743	A	t	0.02
dimethyl disulfide*	747	746	C	0.01	t
ethyl isobutanoate*	750	751	A	0.01[a]	0.02[a]
2-methylpropanoic acid	758	758	C	0.02[a]	0.01[a]
3-methyl-2-buten-1-ol	772	774	C	0.03[a]	0.03[a]
butanoic acid	793	790	A	0.03[a]	0.04[a]
ethyl butanoate*	801	804	A	t	0.03
butyl acetate*	809	811	A	t	t
3-methylbutanoic acid*	837	836	A	0.20	–
ethyl 2-methylbutanoate*	846	846	A	t	0.02
2-methylbutanoic acid	862	860	A	t	0.16
1-hexanol	870	871	A	0.29[a]	0.34[a]
3-methyl-3-buten-1-yl acetate*	888	885	C	0.01	t
2-heptanone	895	892	A	0.04[a]	0.04[a]
propyl butanoate*	898	899	A	t	t
3-methyl-2-hexanol*	906	909	C	0.01	0.01
methyl hexanoate	930	927	A	0.37[a]	0.44[b]
α-pinene*	938	939	A	0.01	t
butyl isobutanoate*	950	949	A	0.01[a]	0.01[a]
benzaldehyde	858	960	A	0.01[a]	0.01[a]
dimethyl trisulfide*	969	970	C	0.01[a]	0.01[a]
sabinene*	971	975	A	t	t
3-(methylthio)-1-propanol*	980	980	C	0.01[a]	0.01[a]
hexanoic acid	982	981	A	8.19[a]	8.16[a]
6-methyl-5-hepten-2-one*	988	986	A	–	t
myrcene*	993	991	A	0.03[a]	0.02[a]
butyl butanoate*	996	995	A	0.03[a]	0.02[a]
ethyl hexanoate	998	998	A	1.02[a]	1.07[a]
octanal*	1000	999	A	t	t
hexyl acetate*	1011	1009	A	t	0.11
3-methyl-3-buten-1-yl isobutanoate*	1014	1013	C	0.03[a]	0.03[a]
limonene	1030	1029	A	1.44[a]	1.89[a]
benzyl alcohol	1033	1032	A	t	t
Z-β-ocimene*	1034	1032	B	t	t
γ-terpinene*	1058	1060	A	–	t
acetophenone*	1067	1065	A	–	t
1-octanol	1069	1070	A	–a	0.01[a]
terpinolene*	1088	1088	B	t	t
2-nonanone*	1089	1090	A	0.04[a]	0.02[a]
propyl hexanoate*	1094	1094	A	0.01[a]	0.01[a]
ethyl heptanoate*	1097	1098	A	0.01	t
linalool*	1099	1099	A	0.06[a]	0.09[a]
nonanal*	1102	1101	A	0.03[a]	0.07[a]
heptanoic acid	1109	1108	A	0.07[a]	0.04[a]
3-methyl-3-buten-1-yl isopentanoate	1112	1113	C	0.03[a]	0.03[a]

t = lower than 0.01%, - not detected, RI_{exp} = experimental retention index, RI_{st} = standard or literature retention index; *reported for the first time in noni; Values followed by the same letter are not significantly different at $p \leq 0.05$. The reliability of the identification proposal is indicated by the following: A) mass spectrum and retention index agreed with standards; B) mass spectrum and retention index agreed with the literature data; and C) mass spectrum agreed with mass spectral database.

Table 1. Continued...

Compound	RI_{exp}	RI_{st}	Identification	Ripe	Over-ripe
2-phenylethanol*	1117	1118	A	0.04[a]	0.05[a]
methyl octanoate	1120	1120	A	4.47[a]	6.13[b]
2-ethylhexanoic acid*	1122	1122	C	0.01	t
hexyl isobutanoate*	1150	1152	B	0.03[a]	0.02[a]
isobutyl hexanoate*	1153	1156	B	0.05[a]	0.04[a]
2*E*-nonenal*	1160	1161	A	0.28[a]	–b
benzyl acetate*	1162	1162	A	0.01	t
methyl 2-phenylacetate*	1177	1176	A	0.03[a]	0.02[a]
octanoic acid	1179	1181	A	72.29[a]	70.47[b]
butyl hexanoate*	1187	1188	A	0.75[a]	0.48[a]
α-terpineol*	1190	1189	A	t	t
methyl salicylate*	1193	1192	A	t	t
ethyl octanoate	1199	1197	A	3.48[a]	4.58[b]
decanal*	1201	1202	A	t	t
methyl nonanoate*	1224	1227	A	t	t
3-methyl-3-buten-1-yl hexanoate*	1241	1244	C	0.52[a]	0.33[b]
ethyl 2-phenylacetate*	1246	1247	A	t	t
3-methylbutyl hexanoate*	1255	1254	B	t	t
γ-octalactone*	1260	1261	A	t	t
methyl 4*E*-decenoate*	1262	1263	C	–a	0.15[b]
2*E*-octenoic acid*	1269	1266	C	0.07	–
2-phenylacetic acid*	1270	1268	A	t	–
nonanoic acid	1271	1271	A	0.04[a]	0.03[a]
methyl decanoate	1321	1326	A	0.71[a]	0.77[a]
benzyl butanoate	1349	1347	A	t	t
eugenol	1356	1359	A	0.08[a]	0.05[a]
decanoic acid	1373	1371	A	0.24[a]	0.13[b]
ethyl 4*E*-decenoate*	1380	1382	C	t	0.03
propyl disulfide*	1388	1390	C	t	t
hexyl hexanoate*	1393	1394	A	0.47[a]	0.45[a]
butyl octanoate	1395	1396	A	0.76[a]	0.54[b]
ethyl decanoate	1397	1397	A	0.09[a]	0.11[a]
methyl 10-undecenoate*	1427	1429	C	0.06[a]	0.07[a]
3-methylbutyl octanoate*	1453	1450	A	0.05[a]	0.05[a]
pentyl octanoate*	1490	1491	A	t	t
3-methyl-3-buten-1-yl octanoate*	1513	1518	C	3.20[a]	1.24[b]
methyl dodecanoate*	1523	1526	A	t	t
benzyl hexanoate*	1549	1547	A	0.02[a]	0.02[a]
butyl decanoate*	1599	1590	A	t	t
hexyl 2-phenylacetate*	1630	1631	B	t	t
2-phenylethyl hexanoate*	1639	1642	A	0.01	t
isopropyl tetradecanoate*	1835	1830	B	0.01[a]	0.03[a]
2-phenyletyl octanoate*	1838	1842	B	t	t
methyl hexadecanoate	1920	1922	A	t	t

t = lower than 0.01%, - not detected, RI_{exp} = experimental retention index, RI_{st} = standard or literature retention index; *reported for the first time in noni; Values followed by the same letter are not significantly different at $p \leq 0.05$. The reliability of the identification proposal is indicated by the following: A) mass spectrum and retention index agreed with standards; B) mass spectrum and retention index agreed with the literature data; and C) mass spectrum agreed with mass spectral database.

proportions (58 and 19%, respectively). According to their odor notes (ARCTANDER, 1969), both acids are responsible for the pronounced "rancid cheese" odor of noni fruit.

Although most of the compounds remain without changes in both maturation stages, the composition of some particular volatile compounds clearly differs in both maturation stages (Table 1). Some esters, with fruity odor notes, increased, while some compounds, mainly acids, decreased or even disappeared during ripening. The over-ripe noni fruit showed significantly higher amounts of methyl hexanoate, methyl octanoate, ethyl

octanoate and methyl 4*E*-decenoate, while octanoic acid and decanoic acid concentrations significantly decreased. These changes probably indicate that esterification occurs during maturation, in a similar way as in other fruits (WILLS et al., 1989). Only two unsaturated esters, reported for the first time in this fruit, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased their concentration in the ripe to over-ripe fruits.

An unsaturated aldehyde related with lipid-degraded product, 2*E*-nonenal, decreased during fruit maturation. As reported for tomatoes (GAILLARD et al., 1977), the activities of several enzymes seem to change during the noni fruit ripening, especially those involved in the formation of this lipid-degraded product.

4 Conclusions

A total of 96 volatile components of noni at two ripening stages, 67 of them for the first time, were isolated by headspace solid-phase microextraction and analyzed using GC-MS. Both maturation stages had several compounds in common. Octanoic acid (≅ 70% of total extract) and hexanoic acid (≅ 8% of total extract) were found to be the major constituents. Due to noni maturation, octanoic acid, decanoic acid and 2*E*-nonenal decreased their concentrations, while some esters (methyl hexanoate, methyl octanoate, ethyl octanoate and methyl 4*E*-decenoate), with fruity odor notes, increased their contents. Two unsaturated esters, reported for the first time in this fruit, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased their concentration in the ripe to over-ripe fruits.

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