Rev. CENIC Cienc. Quím.; Vol. 52. (2): 092-100. Año. 2021. e-ISSN: 2221-2450.

ARTICULO DE INVESTIGACION

Volatile compounds of *Morinda royoc* L. fruit at two ripening stages

Compuestos volátiles de la fruta de *Morinda royoc* L. en dos estados de maduración

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Recibido: 1 de abril de 2021; **Aceptado:** 14 de julio de 2021;

ABSTRACT

Morinda royoc L. (Rubiaceae), commonly known in Cuba as *garañón*, *piñipiñi* or *piña ratón arbusto*, is a plant typically found in coastal hammocks with medicinal properties. Application of headspace-solid phase microextraction combined with gas chromatography-mass spectrometry was used to analyze, for the first time, the volatile compounds of *Morinda royoc* L. fruits at to ripening stages. A fiber of polydimethylsiloxane-divinylbenzene (PDMS/DVB) was used for the extraction. The analyses led to the identification of 137 compounds, including 42 esters, 22 acids, 17 alcohols, 14 ketones, 14 terpenes, 9 aldehydes, 9 sulfur-compounds, 4 lactones, 3 phenols and derivatives, 3 paraffins and a nitrogen-compound. Qualitative and quantitatively, acids were a major chemical class of compounds in both maturity stages, mainly in the ripe stage. Although some compounds remain without changes in both ripening stages, the composition of certain volatile compounds clearly differs in both stages. The over-ripe fruit showed significantly higher amounts of 3-methyl-3-butenyl butanoate, 3-methyl-3-butenyl hexanoate and 3-methyl-3-butenyl octanoate, while 3-methyl-3-butenyl acetate, benzyl acetate and benzyl butanoate significantly decreased. The content of butanoic acid and 2-methylbutanoic acid increased from mature to over-ripe stage, while the major part of the other acids decreased during maturation.

Keywords: *Morinda royoc*; fruit; volatile compounds; headspace-solid phase microextraction; gas chromatography-mass spectrometry.

RESUMEN

Morinda royoc L. (Rubiaceae), comúnmente conocida en Cuba como garañón, piñipiñi o piña ratón arbusto, es una planta que se encuentra típicamente en las maniguas costeras con propiedades medicinales. La aplicación de la microextraction de fase sólida del espacio de cabeza combinada con la cromatografía de gases-espectrometría de masas de gas fue usada para analizar, por primera vez, a los compuestos volátiles de la fruta de *Morinda royoc* L. en dos estados de madurez. Se usó una fibra de polidimetilsiloxano-divinilbenceno (PDMS/DVB) para la extracción. Los análisis permitieron la identificación de 137 compuestos, que incluyen 42 ésteres, 22 ácidos, 17 alcoholes, 14 cetonas, 14 terpenos, 9 aldehídos, 9 compuestos azufrados, 4 lactonas, 3 fenoles y derivados, 3 parafinas y un compuesto nitrogenado. Los ácidos fueron la clase química más abundante cuali- y cuantitativamente en ambos estados de madurez, particularmente en el estado maduro. Aunque algunos compuestos permanecieron sin cambios en ambas etapas de maduración, la composición de ciertos compuestos volátiles se diferenció claramente en ambas etapas. La fruta sobremadura mostró cantidades significativamente más altas de butanoato de 3-metil-3-butenilo, hexanoato de 3-metil-3-butenilo y octanoato de 3-metilo-3-butenilo, mientras que el acetato de 3-metil-3-butenilo, acetato de bencilo y butanoato de bencilo disminuyeron significativamente. El ácido butanoico y ácido 2-metilbutanoico aumentaron su contenido de la fruta madura a sobremadura, mientras la mayor parte de los otros ácidos disminuyeron durante la maduración.

Palabras clave: *Morinda royoc*; fruta; compuestos volátiles; microextracción en fase sólida, cromatografía de gases-espectrometría de masas.

Este articulo no presenta conflicto de intereses.

INTRODUCTION

Morinda royoc L. (Rubiaceae), commonly known in Cuba as *garañón*, *piñipiñi* or *piña ratón arbusto*, is a plant typically found in coastal hammocks, and rarely inland (Roig y Mesa, 1988). The plant is a vine sprawling shrub with large bright green elliptical leaves and small white flowers throughout the year. 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 yellower in color, and unless it is harvested at this stage, it simply falls to the ground. As it ripens, the fruit change to a dark brown color with a very pungent smell, like the odor of rancid cheese and its taste is sour. In Cuba, the roots are used to make a product with stimulating, revitalizing and anti-stress activity that also increases the libido (Scull et al., 2000).

Although the fruit of noni (*Morinda citrifolia* L.), a related species used as folk remedy, has been broadly studied (Chan-Blanco et al., 2006; Potterat & Hamburger, 2007; Pino et al., 2009, 2010a, 2010b), very few reports on the chemical composition of *M. royoc* are available, particularly related to the non-volatile components in other parts of the plant (Borroto et al., 2008, 2010). This plant has some medicinal properties reported by several authors (Hernández & Volpato, 2004; Borroto et al., 2008, 2010).

Solid-phase microextraction (SPME) is a rapid, direct, inexpensive, solvent-free and efficient extraction technique for sampling different matrixes (Souza-Silva et al., 2015). 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 (Xu et al., 2016). In fact, HS-SPME combined with gas chromatography-mass spectrometry (GC-MS) is a convenient technique for providing the aroma profile of each analyzed fruit.

The aim of this study was to evaluate the changes of volatile compounds of the fruits of *Morinda royoc* L. at two ripening stages, using HS-SPME combined with GC-MS.

MATERIALS AND METHODS

Sample and chemicals

Fruits of *M. royoc* were collected directly from plants grown in the coastal area of the city of Havana. They were selected at two maturity stages: ripe (peel with light amber color) and over-ripe (peel with dark brown color). Two groups, six fruits each one, were cut and seeds removed. The pulp was manually mashed analyzed immediately after sample preparation.

Authentic reference compounds were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstad, Germany), and some others were generously given by Robertet (Grasse, France). A normal paraffin solution (C_5-C_{24}) was supplied by Sigma-Aldrich (Steinheim, Germany). Sodium chloride was provided by Merck (Darmstadt, Germany).

Headspace-solid phase microextraction

A similar procedure reported earlier was used for the HS-SPME of the volatile compounds (Pino et al., 2010). A manual holder (Supelco, Inc., Bellefonte, PA, USA) and a 1-cm long, 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) (Supelco, Inc., Bellefonte, PA, USA) were used. For each extraction, 5 g of the

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homogenized pulp and 10 mL of 20 % NaCl solution (to inhibit enzymatic reactions and to favor the transfer of the analytes from the aqueous solution to the headspace) were centrifuged at 3000 min^{-1} for 10 min and the supernatant was transferred to a 15-mL Teflon-lined septum cap vial equipped with a Teflon-coated magnetic bar. The extraction was carried out under magnetic stirring at 600 min^{-1} at 40 °C for 20 min, after equilibration of the samples for 10 min at the same temperature. The fiber was then removed and introduced into the injector port of the GC for desorption at 250 °C for 2 min, in the splitless mode.

Gas chromatography-Mass spectrometry

GC-MS analysis was carried out using a Shimadzu QP-2010 Ultra (Kyoto, Japan), equipped with a 30 m x 0.25 mm i.d., 0.25 µm film thickness DB-Wax capillary column (J&W Scientific, Folsom, CA, USA). Thermal desorption was carried out at 250 °C in splitless mode for 2 min, using and inlet liner of 0.75 µm i.d. Carrier gas was helium at 1 mL/min. Oven temperature was held at 50 °C for 2 min, increased at 4 °C/min up to 250 °C and held for 3 min. The MS worked in electron impact mode at 70 eV ionization energy and in scanned mode from m/z 35 to 350, at 1.3 scan/s. Ion source and connecting parts temperature, 250 °C.

Identification of compounds was achieved by comparison of their linear retention index and mass spectra with those shown by reference standards when they were available. In other cases, comparison was made with those in commercial databases (NIST 05, NBS 75 k, Wiley 6 and Adams 2001).

Linear retention indexes were calculated using a mixture of normal paraffins. Semiquantitative determinations were based on the internal normalization method assuming similar calibration factors for all the compounds.

The original data from triplicate analyses were transformed as arc sen $p^{½}$, in which p is the proportion of each compound and processed for statistical analysis by the t-Student' test.

RESULTS AND DISCUSSION

The volatile constituents of *M. royoc* fruits were obtained by HS-SPME and analyzed by GC-MS using a polar 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, 137 volatile compounds were identified, all of them for the first time, in *M. royoc* fruits (Table 1). These compounds comprise 42 esters, 22 acids, 17 alcohols, 14 ketones, 14 terpenes, 9 aldehydes, 9 sulfur-compounds, 4 lactones, 3 phenols and derivatives, 3 paraffins and a sulfur-nitrogen compound (benzothiazole).

Qualitative and quantitatively, acids were a major chemical class of compounds in both maturity stages, mainly in the ripe stage. They are represented by linear carboxylic acids generated from fatty acid biosynthesis, branched acids such as 2-methylpropanoic, 2-methylbutanoic, 3-methyl-3-butenoic, and 2-ethylhexanoic acid related to the synthesis of aliphatic amino acids, unsaturated aliphatic acids produced by the peroxidation of lipids and (*E*)-cinnamic acid as the only aromatic acid (Sanz & Pérez, 2010). Of them, octanoic acid and hexanoic acid were the major acids at both maturity stages. Both acids were also found as the most abundant in *M. citrifolia* fruits (Pino et al., 2010a). According to their odor notes (Burdock, 2010), both acids contribute to the noticeable rancid cheese odor of *M. royoc* fruit.

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Esters were the second most abundant chemical class of compounds in both maturity stages, mainly in the over-ripe stage. Volatile esters are formed by esterification of alcohol and carboxylic acid through the enzymatic routes previously described (Sanz & Pérez, 2010). Alkenyl esters were the major ones in this class, particularly those related to 3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol. Among these esters, 3-methyl-3-butenyl acetate, 3-methyl-3-butenyl butanoate, and 3-methyl-3-butenyl hexanoate were the major ones. These esters have powerful fruity odor notes (Burdock, 2010). In the previous study about the volatile composition of *M. citrifolia* fruits, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate were found the major esters (Pino et al., 2010a).

Some lactones, as intramolecular esters of 4-hydroxy acids, were also detected. They comprise γ-butyrolactone, γ-hexalactone, γ-octalactone and γ-decalactone. The biosynthesis of lactones is associated with the β- oxidation pathway and they are considered important contributors to the aroma of many fruits (Sanz & Pérez, 2010), but their contribution to the aroma of *M. royoc* fruits seem to be improbable due to the low contents.

Several aliphatic (13) and aromatic alcohols (4) were also detected. Like carbonyls, two main biogenetic pathways can be considered for alcohols: amino acid metabolism and lipid peroxidation pathways leading to unsaturated alcohols (Sanz & Pérez, 2010). The branched unsaturated alcohol, 3-methyl-3-buten-1-ol, was the most abundant in both maturity stages.

Amongst the identified carbonyl compounds, 9 aldehydes and 14 ketones were found. All of them are produced via lipid or aromatic amino acid metabolism (Sanz & Pérez, 2010). Of them, only heptan-2-one was present in notable amounts in the fruit at ripe stage.

Terpenes, although comprised 14 compounds, were present in small quantities at both maturity stages, excepting linalool in the ripe stage. Its contribution to the fruit's aroma could be considerable due the low odor threshold of this monoterpene alcohol, which possess an intense floral odor (Burdock, 2010).

Interestingly, nine sulphur compounds were found in *M. royoc* fruit, including thiomethanol, dimethyl disulfide, dimethyl trisulfide, three S-methyl thioesters, 3-(methylthio)propanoic acid and two esters of this acid. In *M. citrifolia* fruit only dimethyl sulfide, dimethyl trisulfide and 3-(methylthio) propanol were found (Pino et al., 2010a). Benzothiazole was the only nitrogen-containing compounds in this fruit.

Three phenols and derivatives and three *n*-paraffins were identified, all of them at very low amounts.

Many significant differences were found based on the quantitative distributions of the compounds in both ripening stages. According to the chemical class, major changes noticed were the increase in esters contents and a decrease in acids, aldehydes, ketones and terpenes (Table 1).

Table 1. Volatile compounds (%) of *Morinda royoc* fruits at two ripening stages

Compound	LRI	Ripe fruit	Over-ripe fruit
Thiomethanol	643	0.8 a	0.2 b
Ethyl acetate	893	tr	tr
n-Nonane	900	tr	-
Butan-2-one	905	-	tr

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2-Methylbutanal	916	tr	-
Butan-2,3-dione	974	-	tr

Table1. (continued)

Compound	LRI	Ripe fruit	Over-ripe fruit
3-Buten-2-one	977	-	tr
Pentan-2-one	978	0.3 b	0.8 a
n-Decane	1000	0.2	-
Methyl 2-methylbutanoate	1007	0.5	-
1-Penten-3-one	1019	tr	-
Ethyl butanoate	1041	-	tr
S-Methyl thioacetate	1044	tr	-
Hexan-3-one	1046	-	tr
Dimethyl disulfide	1065	0.5	tr
Hexanal	1077	0.1	-
Hexan-2-one	1088	-	tr
n-Undecane	1100	tr	-
(*E*)-2,6-Dimethyl-2-6-octadiene	1107	tr	-
3-Methylbutyl acetate	1112	tr	-
Pentan-2-ol	1138	-	tr
α-Phellandrene	1150	tr	-
Myrcene	1158	0.2	-
2-Methylpropyl butanoate	1161	tr	-
Butan-1-ol	1164	tr	-
3-Methyl-3-buten-1-yl formate	1168	-	tr
α-Terpinene	1176	-	tr
Pentyl acetate	1179	0.1	-
Limonene	1183	tr	-
Heptan-2-one	1186	5.2 a	1.7 b
3-Methyl-3-butenyl acetate	1190	15.1 a	2.9 b
1,8-Cineole	1204	tr	-
3-Methylbutan-1-ol	1211	tr	tr
(*E*)-2-Hexenal	1230	tr	-
Butyl butanoate	1234	-	tr
(*Z*)-2-Pentenyl acetate	1236	0.3	-
(*Z*)-β-Ocimene	1238	tr	-
Ethyl hexanoate	1241	tr	tr
γ-Terpinene	1245	tr	-
(*E*)-β-Ocimene	1247	0.1	-
Octan-3-one	1250	tr	-
3-Methyl-3-buten-1-ol	1252	1.5 b	2.5 a
p-Cymene	1254	tr	-
3-Methylbutyl butanoate	1256	0.3	tr
3-Methyl-3-butenyl propanoate	1260	tr	tr
Terpinolene	1271	tr	-
Octan-2-one	1297	tr	tr
Octanal	1301	0.1	-
Methyl (*E*)-2-hexenoate	1305	tr	tr
(*E*)-3-Hexenyl acetate	1309	tr	-
1-Octen-3-one	1314	tr	-
Pentyl butanoate	1320	tr	-

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Table1. (continued)

Compound	LRI	Ripe fruit	Over-ripe fruit
Heptan-2-ol	1327	0.8	-
Ethyl heptanoate	1341	tr	-
(*E*)-2-Hexenyl acetate	1346	tr	-
3-Methyl-3-butenyl butanoate	1353	4.7 b	24.9 a
(*Z*)-4-Hepten-2-ol	1361	0.1	-
3-Methyl-3-butenyl 3-methylbutanoate	1371	0.1	0.1
Hexan-1-ol	1373	0.1	-
(*Z*)-2-Pentenyl butanoate	1376	tr	-
Dimethyl trisulfide	1383	tr	tr
Nonan-2-one	1398	-	1.2
Nonanal	1408	0.3 a	0.4 a
3-Methyl-2-butenyl butanoate	1410	tr	0.3
S-Methyl thiohexanoate	1412	-	tr
Butyl hexanoate	1414	-	tr
Hexyl butanoate	1420	-	tr
(*E*)-2-Octenal	1425	tr	-
Ethyl octanoate	1433	tr	-
1-Octen-3-ol	1451	0.2	tr
3-Methylbutyl hexanoate	1459	tr	-
Acetic acid	1463	1.2	tr
3-Methyl-3-butenyl 3-methyl-3-butenoate	1466	tr	0.1
6-Methyl-5-hepten-2-ol	1469	tr	-
Decanal	1485	0.1	-
Camphor	1490	tr	-
Octyl acetate	1493	tr	-
Benzaldehyde	1520	0.3	tr
Methyl 3-(methylthio)propanoate	1525	tr	0.1
2-Furfuryl acetate	1534	tr	-
Linalool	1547	6.0	tr
Octan-1-ol	1561	0.1	tr
2-Methylpropanoic acid	1576	tr	0.1
3-Methyl-3-butenyl hexanoate	1585	tr	15.9
Undecan-2-one	1595	0.4 b	0.7 a
S-Methyl thiooctanoate	1598	-	tr
Methyl benzoate	1605	tr	tr
(*Z*)-3-Octenyl acetate	1616	tr	-
γ-Butyrolactone	1641	tr	-
Butanoic acid	1644	1.9 b	5.5 a
3-Methyl-2-butenyl heptanoate	1649	-	0.1
Acetophenone	1655	0.3 a	0.5 a
Nonan-1-ol	1664	tr	-
2-Methylbutanoic acid	1668	0.1 b	0.6 a
Borneol	1700	tr	-
γ-Hexalactone	1708	-	tr
Benzyl acetate	1738	1.1 a	0.1 b

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Table 1. (Continued)

Compound	LRI	Ripe fruit	Over-ripe fruit
3-Methyl-3-butenyl octanoate	1744	0.3 b	3.2 a
Methyl phenylacetate	1754	tr	tr
2-Phenylethyl acetate	1804	tr	-
3-Methyl-3-butenoic acid	1852	tr	-
(Z)-Cinnamaldehyde	1870	tr	tr
Hexanoic acid	1873	11.5 a	9.8 b
Benzyl butanoate	1876	tr	0.4
Benzyl alcohol	1884	0.2	tr
Pentyl 3-(methylthio)propanoate	1898	-	1.5
2-Phenylethanol	1923	tr	tr
γ-Octalactone	1934	tr	-
2-Ethylhexanoic acid	1962	tr	-
Benzothiazole	1968	tr	tr
Heptanoic acid	1971	0.3 a	0.3 a
(E)-3-Hexenoic acid	1976	tr	0.2
Dodecan-1-ol	1981	tr	tr
3-Methyl-3-buten-2-yl benzoate	1994	-	0.1
(Z)-Methyl isoeugenol	2059	tr	-
Octanoic acid	2072	32.3 a	20.2 b
γ-Decalactone	2126	tr	tr
Eugenol	2167	-	tr
Nonanoic acid	2174	0.7 a	0.2 b
(E)-2-Octenoic acid	2197	tr	0.5
Decanoic acid	2246	1.3 a	0.8 b
(E)-Cinnamyl alcohol	2257	-	tr
3-(methylthio)Propanoic acid	2320	-	0.2
Chavicol	2346	-	tr
Undecanoic acid	2365	tr	1.7
Benzoic acid	2436	0.1 a	0.3 a
Dodecanoic acid	2529	1.8 a	0.1 b
Tridecanoic acid	2603	0.1 a	0.1 a
Tetradecanoic acid	2714	4.5 a	0.2 b
Pentadecanoic acid	2819	0.2	tr
(E)-Cinnamic acid	2835	tr	-
Hexadecanoic acid	2928	2.4 a	1.1 b
Esters		2.0	48.1
Acids		tr	41.7
Lactones		2.0	tr
Alcohols		tr	2.6
Aldehydes		tr	0.4
Ketones		2.0	4.9
Phenols and derivatives		2.0	tr
Terpenes		tr	tr
Paraffins		2.0	-
S-compounds		tr	2.0
Others		2.0	tr

LRI: Lineal retention index in DB-Wax column. tr: <0.1%. Different letters in the same row indicate significant difference ($p \leq 0.05$).

Este articulo no presenta conflicto de intereses.

Although some compounds remain without changes in both ripening stages, the composition of certain volatile compounds clearly differs in both stages. Some esters, with fruity odor notes, increased, while some compounds, mainly acids, decreased or even disappeared during maturation. The over-ripe noni fruit showed significantly higher amounts of 3-methyl-3-butenyl butanoate, 3-methyl-3-butenyl hexanoate and 3-methyl-3-butenyl octanoate, while 3-methyl-3-butenyl acetate, benzyl acetate and benzyl butanoate significantly decreased. These changes probably indicate that esterification occurs during maturation, in a similar way as in other fruits (Sanz & Pérez, 2010). On the other hand, butanoic acid and 2-methylbutanoic acid increase their content, while the major part of the other acids decreased during maturation.

CONCLUSIONS

A total of 137 volatile constituents of *Morinda royoc* fruits at two ripening stages, all of them for the first time, were isolated by headspace solid-phase microextraction and analyzed by gas chromatography-mass spectrometry. Both ripening stages had several compounds in common. The compounds comprised 42 esters, 22 acids, 17 alcohols, 14 ketones, 14 terpenes, 9 aldehydes, 9 sulfur-compounds, 4 lactones, 3 phenols and derivatives, 3 paraffins and a nitrogen compound. Higher amounts of 3-methyl-3-butenyl butanoate, 3-methyl-3-butenyl hexanoate and 3-methyl-3-butenyl octanoate were present in the over-ripe fruits, while 3-methyl-3-butenyl acetate, benzyl acetate and benzyl butanoate significantly decreased. The content of butanoic acid and 2-methylbutanoic acid increased from mature to over-ripe stage, while the major part of the other acids decreased during maturation.

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