# VOLATILE COMPOUNDS DURING THE RIPENING OF COLOMBIAN SOURSOP (*Annona muricata* L. cv. Elita)

COMPUESTOS VOLÁTILES DE LA GUANÁBANA COLOMBIANA (*Annona muricata* L. cv. Elita) DURANTE SU MADURACIÓN

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## ABSTRACT

Fruits of soursop (*Annona muricata* L. cv. Elita) were evaluated at different ripening stages to determine the changes in their physicochemical characteristics: total soluble solid content, percentage of acidity, pH, and ripeness index. The change in volatile compounds were determined through Headspace-Solid Phase Microextraction and subsequent Gas Chromatography-Mass Spectrometry analyses. The volatile compounds were identified through the comparison of their chromatographic and spectral properties against the ones exhibited by reference substances. The sensorial quality factors showed values that changed according to the ripening stage. It was established that the ester levels, particularly those from $C_6$, $C_4$, and $C_8$ saturated and unsaturated aliphatic acids, increased with ripening. $C_6$ aliphatic compounds, such as (*Z*)-3-hexenol and (*Z*)-3-hexenal, were observed to be the major volatile constituents in the green stage. In contrast, methyl hexanoate and methyl (*E*)-2-hexenoate were the main volatile in ripe, overripe and half ripe fruits. The overripe stage was characterized by an increase in the ethyl acetate, methyl butanoate and ethyl butanoate levels, and the appearance of butanoic and hexanoic aliphatic acids. These facts could be used as an indicator of the beginning of the fermentation stage and the loss of fruit sensory quality.

**Keywords:** Postharvest, tropical fruits, Headspace-Solid Phase Microextraction, gas chromatography.

## RESUMEN

Los frutos de guanábana (*Annona muricata* L. cv. Elita) en diferentes estados de madurez se evaluaron para determinar las características fisico-químicas: sólidos solubles totales, porcentaje de acidez, pH e índice de madurez. Los cambios en la composición de los compuestos volátiles se determinaron mediante la técnica de Espacio de Cabeza-Microextracción en Fase Sólida y el posterior análisis por Cromatografía de gases acoplada a espectrometría de masas. Los compuestos volátiles se identificaron por comparación de sus propiedades cromatográficas y espectrales con sustancias de referencia. Los factores de calidad sensorial mostraron valores que variaron con el estado de madurez. Además, se estableció que la cantidad de ésteres, especialmente los provenientes de los ácidos $C_6$, $C_4$ y $C_8$, saturados e insaturados, se incrementaron con la maduración. Los compuestos alifáticos $C_6$, como el (*Z*)-3-hexenol y el (*Z*)-3-hexenal, fueron los volátiles mayoritarios en la fruta verde; en tanto que el hexanoato de metilo y el (*E*)-2-hexenoato de metilo, fueron los más abundantes en las frutas maduras, sobremaduras y de madurez intermedia. En la

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sobremaduración aumentó la concentración de acetato de etilo, butanoato de metilo y butanoato de etilo; y aparecieron los ácidos alifáticos butanoico y hexanoico, los cuales pueden ser indicadores del inicio de la etapa fermentativa y de la pérdida de la calidad sensorial de la fruta.

**Palabras clave:** poscosecha, frutas tropicales, Espacio de Cabeza-Microextracción en Fase Sólida, cromatografía gaseosa.

## INTRODUCTION

Fruit consumption has been continuously increasing around the world due to the excellent biofunctional and chemo-preventive properties exhibited by these products. Thus, the World Health Organization recommends a minimum consumption of 120 kg/person/year (1). Colombia, in spite of being one of the top producers of tropical fruits in the world, only consumes 40 kg/person/year (2). Soursop (*Annona muricata* L.) is considered to have its origins in the low equatorial zones of Central and South America. Colombia produces an average of about 10,010 tons/year (the yield is around 7.7 ton/ha/year). The main production zones are located in the departments of Valle, Tolima, Cauca, and Cundinamarca. Some modern orchards can be found in the Cauca and Tolima valleys (3). In the ripeness stage, the soursop fruit is has a sweet, white, creamy, juicy, soft and slightly acid pulp with excellent sensory characteristics (4).

The volatile constituents of the aroma of this fruit have been studied through different extraction methods, showing outstanding differences according to the assessed species. Thus, MacLeod and Pieris, 1981 (5) characterized the volatile compounds of soursop fruits from Sri Lanka through steam distillation and a simultaneous extraction with organic solvent (DES). They found that approximately 80% of the extract was constituted by esters, among which methyl hexanoate and methyl 2-hexenoate were the most abundant ones. The volatile compounds present in Malaysian soursop fruits were also extracted by DES, revealing esters to be the major constituents (57.2%) (6, 7). Later on, soursop essential oil was extracted from Cameroon fruits through steam distillation (8). In this case, aliphatic esters were found to be predominant (51%), from which methyl 2-hexenoate, ethyl 2-hexenoate, methyl 2-octenoate, and methyl 2-butenoate the most abundant volatile compounds. Mono- and sesquiterpenes such as, β-cariophyllene, 1,8-cineole, linalol, α-terpineol, linalyl propionate, and calarene have also been characterized as constituents of soursoup. The differences found in volatile compound composition are attributable to variations in cultivars and geographic origins (9).

Most of the volatile components of fruits are saturated and unsaturated aliphatic compounds with oxygenated functional groups such as ester, alcohol, acid, aldehyde, ketone or lactone. These substances are originated during the ripening process through different metabolic paths. Thus, the fatty acid path produces esters and $C_6$ compounds, via the lipoxygenase (10). Through the isoprenoid pathway, the group of terpenes is biogenerated either via mevalonate or no-mevalonate. And finally, the shikimic acid path is involved in the biosynthesis of phenylpropanoids, which are the precursors of many aromatic compounds (11).

The Headspace-Solid Phase Microextraction (HS-SPME) technique is remarkably agile and trustable for the determination of volatile compounds in complex matrixes. It is based on the extraction of analytes from the sample matrix by means of a melted silica fiber recovered with a sorbent material, which in most of the cases is of polymeric nature. Subsequently, in order to separate and identify the products, the desorption of analytes is done by temperature once the fiber has been placed in the injection port of the chromatograph for its instrumental analysis (12, 13).

The aim of this research is to determine the changes in physicochemical parameters and volatile composition of soursop fruits during the following four ripening stages: unripe, turning, ripe and overripe fruits.

## MATERIALS AND METHODS

### Plant material

Fruits of soursop cv. 'Elita' were obtained from orchards located in 'La Española' farm (1,070 m AMSL, with an average temperature of 23°C, 1,225 mm of mean annual precipitation, average solar radiation of 4.8 W m$^{-2}$ day$^{-1}$, and a relative humidity of 83%), which belongs to the 'Agrícola Varahonda' company, established in the agricultural industry complex of the Cauca Valley, in the rural

area of the municipality of Pradera. The samples were randomly picked in an unripe state; then, they were ripened at 20°C and 65% RH until reaching the maturity stages needed for the experiment on days 1, 4, 7 and 10 after being harvested. Thus, four maturity stages were obtained: unripe, green mature, ripe and overripe.

## Physicochemical characterization of the fruits

The physicochemical characterization of the fruits was achieved by determining the total soluble solids (TSS) in °Brix by using a Leica auto ABBE refractometer. Total acidity (expressed as the percentage of citric acid) was established through titration with a Schott CG840B pH meter until reaching a final pH of 8.2. The ripeness index was obtained by dividing the obtained °Brix record by the percentage of titratable acidity. Six fruits from each maturity stage were individually used for each of the corresponding measurements (14).

## Analysis of volatile compounds

For the analysis of volatile compounds in the studied soursop fruits, 30 g of seedless pulp were obtained and homogenized during 5 min; then, they were equilibrated during 20 minutes in a hermetically sealed 110 mL vial at 20°C. The headspace was collected on a DVB/CAR/PDMS fiber (with a thickness of 30 $\mu$m, Supelco®) during 30 minutes, and then it was directly injected (desorption time was set at 5 min) into a Shimadzu® GC17A coupled to a QP5050 selective mass detector operated in splitless mode. An RTX-5 fused silica column (Restek®, 30 m x 0.32 mm i.d., with a film thickness of 0.25 mm) was used. The column oven was programmed from 50 to 300 at 4°C /min, and the final temperature was held for 5 min. The injector temperature was maintained at 250°C; the carrier gas was 1.5 mL of He/min; and the make up gas was nitrogen at a flow rate of 30 mL/min. Each of the experiments was repeated using an FFAP column (30 m x 0.32 x 0.25 $\mu$m i.d; J&W Scientific®). The conditions used were the same that were mentioned above, except for the final oven temperature, which was 250°C. Mass spectrometry data were recorded within a range of 30-250 u for a 70 eV, and later processed with a Class 5K software.

Linear retention indices were calculated according to the Kovats method using a mixture of normal $C_6$-$C_{28}$ paraffin as external reference. Mass spectral identification was completed by comparing the spectra with commercial mass spectral databases from WILEY® and EPA/NIH, and by comparison with authentic reference standards (15, 16).

## RESULTS AND DISCUSSION

The fruits were characterized at each of the ripening stages by their physicochemical properties, as it is shown in table 1.

**Table 1.** Physicochemical characterization of soursop (*Annona muricata* L. cv. Elita) fruits in different ripening stages.

| Ripening stage | N° of samples | Total soluble solids (°Brix)[a] | Acidity (%)[a,b] | pH[a] | Ripeness index |
|---|---|---|---|---|---|
| I | 6 | 5.6 ± 0.22 | 0.15 ± 0.013 | 4.90 ± 0.40 | 37.3 |
| II | 6 | 7.9 ± 0.59 | 0.59 ± 0.092 | 3.78 ± 0.21 | 13.4 |
| III | 6 | 13.4 ± 0.63 | 0.46 ± 0.022 | 3.52 ± 0.11 | 29.1 |
| IV | 6 | 12.6 ± 0.77 | 0.42 ± 0.033 | 4.00 ± 0.15 | 30.0 |

[a] All data are the mean values of triplicate measurements ± standard deviation, $p < 0.0001$.

[b] Expressed as percentage of citric acid.

These results showed that the concentration of TSS increases along with the ripening process until the consumption or ideal ripeness phase, probably due to the hydrolysis of complex polysaccharides into mono and disaccharides, organic acids and soluble pectins. The slight decrease observed during overripening might be due to the utilization of these low weight carbohydrates as substrates for the initiation of the fermentative stage (17, 18).

The initial ripening stage exhibited a low total acidity value that was concomitant with the changes in pH. Then, during the ripening process, this value decreased slowly until the overripe stage. A similar behavior has been observed in other climacteric annonaceae (19). This tendency is attributed to the use of acid substances as substrates for the respiration process; besides, they are also used as precursors for the production of other secondary metabolites in the fruit during this period. This situation is inversely related to the pH values found during the ripening stage (20-22).

Fruit characteristics at stage III (mainly TSS) were closer to the ones specified by the Colombian Technical Regulation (Norma Técnica Colombiana – NTC-5208, 2003 (23)) for soursop fruits, which has been standardized as the consumption

ripeness consisting in the following average values: 13.4 ºBrix for TSS, 0.46% for acidity, and 29.1 for ripeness index.

## Analysis of volatile compounds

The volatile compounds produced at each ripening stage were extracted through HS-MEFS and immediately analyzed through Gas Chromatography-Mass Spectrometry (G-CMS) by separately using two columns with different polarity. In general, the volatile compound profile changes during the ripening process, as it is shown in the figure 1.
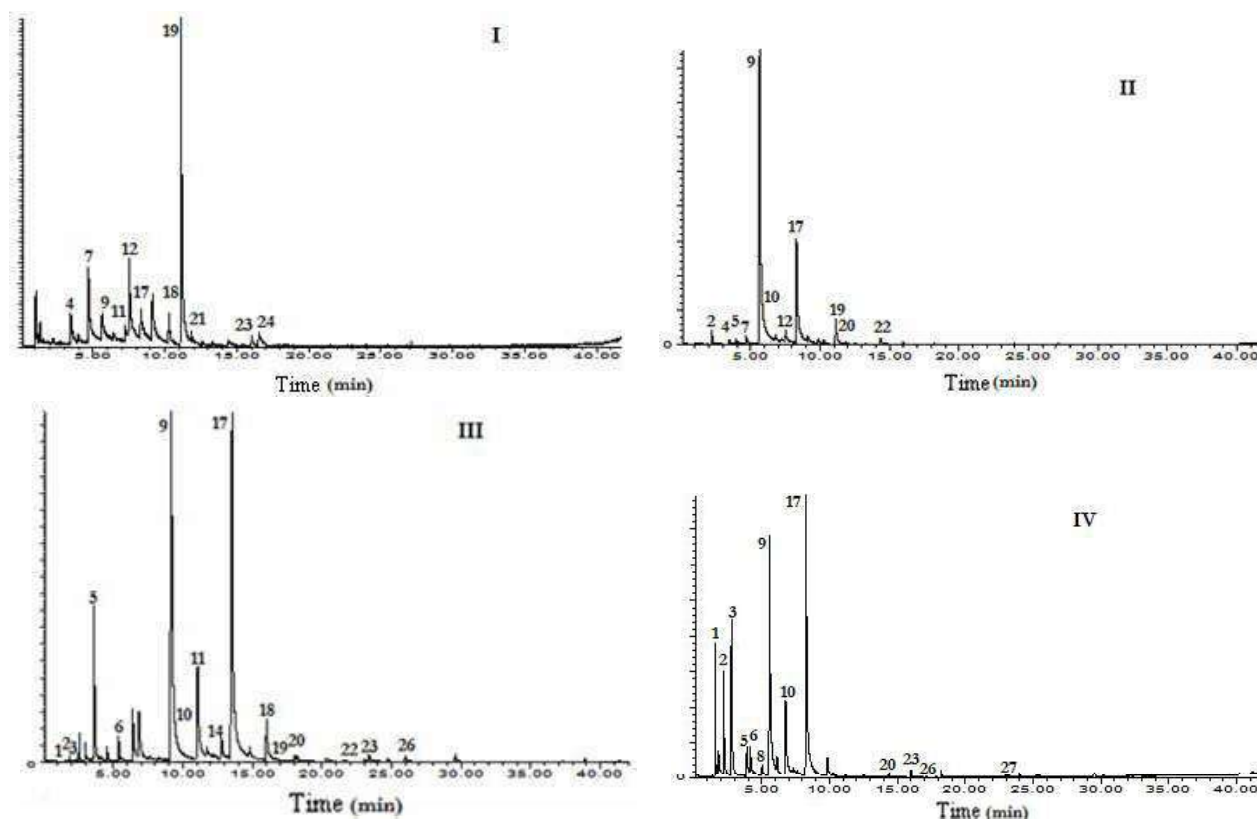


**Figure 1.** Chromatographic analysis (FFAP column) of the volatile compounds obtained through HS-MEFS from soursop fruits at the four ripening stages. Unripe (I), green mature (II), ripe (III), and overripe (IV) (peak numbers correspond to those in table 2).

The chemical compounds that were found in the four ripening stages are esters, aldehydes, alcohols, terpenes and lactones, as it is shown in table 2. Among them, esters were quantitatively dominant. Unripe fruits were mainly characterized by the presence of (Z)-3-hexenol, methyl (Z)-3-hexenoate, and (Z)-3-hexenal as their major constituents. Methyl (E)-2-hexenoate and (Z)-3-hexenol were major constituents in green mature fruits, as well as methyl (E)-2-hexenoate, methyl 2-butenoate, ethyl hexanoate, and β-ocimene in ripe fruits. Finally, in overripe fruits methyl hexanoate, methyl (E)-2-hexenoate, ethyl butanoate, methyl butanoate, and ethyl acetate were predominant.

These results indicate that the biogenetic pathway of fatty acids is activated during the soursop fruit ripening process. Saturated and unsaturated fatty acids are formed through β-oxidation, and then transformed into their corresponding esters, which subsequently produce different $C_6$ compounds by lipoxygenase oxidation, such as: hexanal, (Z)-3-hexenal, hexanol, and (Z)-3-hexenol, among others (11).

**Table 2.** Volatile compounds identified in soursop (*Annona muricata* L. cv. Elita) fruits at the four ripening stages.

| No.[a] | Compound | IRL[b] | | Relative amount[c]/Ripening stage | | | |
|---|---|---|---|---|---|---|---|
| | | FFAP | RTX-5 | I | II | III | IV |
| 1 | Ethyl acetate | 885 | <800 | ++ | + | + | +++ |
| 2 | Methyl butanoate | 999 | <800 | - | + | + | +++ |
| 3 | Ethyl butanoate | 1030 | <800 | - | - | + | +++ |
| 4 | Hexanal | 1093 | 900 | + | + | - | - |
| 5 | Methyl 2-butanoate[d] | 1118 | - | - | + | +++ | + |
| 6 | Isoamyl acetate | 1132 | 820 | - | - | + | + |
| 7 | (*Z*)-3-Hexenal | 1154 | - | ++ | + | - | - |
| 8 | Ethyl 2-butenoate[d] | 1170 | - | - | + | + | + |
| 9 | Methyl hexanoate | 1196 | 926 | + | ++++ | ++++ | ++++ |
| 10 | Ethyl hexanoate | 1242 | 1003 | - | + | ++ | ++ |
| 11 | *trans*-b-Ocimene[d] | 1257 | - | + | + | ++ | - |
| 12 | Methyl (*Z*)-3-hexenoate[d] | 1268 | 934 | ++ | + | - | - |
| 13 | α-Terpinolene | 1278 | 1103 | + | + | - | - |
| 14 | Hexyl acetate | 1281 | 1039 | - | - | + | - |
| 15 | (*E*)-2-Hexenal | 1300 | 844 | + | - | - | - |
| 16 | *trans*-2-Hexenyl acetate | 1336 | 1009 | + | - | - | - |
| 17 | Methyl (*E*)-2-hexenoate[d] | 1350 | 970 | + | +++ | ++++ | ++++ |
| 18 | Hexanol | 1365 | 931 | + | - | + | - |
| 19 | (*Z*)-3-Hexenol | 1384 | 854 | +++ | ++ | + | - |
| 20 | Methyl octanoate | 1397 | 1128 | - | + | + | + |
| 21 | (*E*)-2-Hexenol[d] | 1419 | - | + | - | - | - |
| 22 | Methyl 2-octenoate[d] | 1505 | 1172 | - | + | + | + |
| 23 | Linalol | 1562 | 1050 | + | - | + | + |
| 24 | b-Caryophyllene | 1581 | 1446 | + | - | - | - |
| 25 | g-Butyrolactone | 1610 | 915 | - | - | - | + |
| 26 | Butanoic acid | 1642 | - | - | - | + | + |
| 27 | Hexanoic acid | 1881 | 1142 | - | - | - | + |

[a] The numbering corresponds to the one of figure 1, and to the elution order in the FFAP column.

[b] Linear retention index.

[c] Relative abundance calculated on the basis of the GC area + <5%; ++ 5 - 10%; +++ 10 - 40%; ++++ 40 - 70%.

[d] Identified only based on mass spectrometry and retention index.

Unripe fruits were characterized by the presence of $C_6$-type compounds such as hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol, which are responsible for the green odor note. Additionally, the following terpenic compounds were also found in this stage: β-ocimene, α-terpinolene, and β-caryophyllene, which are produced from Acetil-CoA and piruvate through a *de novo* synthesis (24). The concentration of these substances decreased during the ripening, except for *trans*- β-Ocimeno, which contribution to the floral aroma of the ripe fruit could be important.

The amount of these $C_6$ compounds diminished or disappeared during the green mature and ripe stages, while methyl esters of saturated and unsaturated $C_6$, $C_8$ and $C_4$ acids increased, exhibiting fruity odor notes. The predominance of esters, mainly those from $C_4$, $C_6$ and $C_8$ acids, continued during the overripe stage. In this stage, the presence of butanoic acid, hexanoic acid, and γ-butyrolactone could be related to the fermented odor note, which is characteristic of this stage. The production of these volatile compounds could indicate the beginning of the fermentative stages and the loss of sensory fruit quality, process in which some esters could act as precursors (25). Thus, new volatile compounds appeared during soursoup ripening process and others, which were already present in the unripe fruits, were transformed. This situation could be interpreted as a physiological indicator of non-destructive ripening (26).

The volatile chemical composition reported here is in agreement with the data reported by other

authors. In fact, MacLeod and Pieris, 1981 (5) found 80% of esters, mainly methyl hexanoate (31%) and methyl 2-hexenoate (27%) in fruits collected in Sri Lanka; and Iwaoka *et al.*, 1993 (27) found that unripe fruits cultivated in Hawaii contained high amount of (*Z*)-3-hexenol, while the mature ones showed high ester levels: methyl (*E*)-2-hexenoate, methyl (*E*)-2-butenoate, methyl butanoate and methyl hexanoate (27).

## CONCLUSIONS

The ripening process of soursop fruits was characterized by a higher value of TSS in the consumption ripeness stage, preceded by high acid levels at the other mature stage. Methyl hexanoate and methyl (*E*)-2-hexenoate were found to be the most abundant compounds during the different post-harvesting stages, corresponding to green mature, ripe and overripe soursop fruits. In contrast, unripe fruits were characterized by the predominance of the (*Z*)-3-hexenol alcohol. The presence of esters (mainly $C_4$, $C_6$, and $C_8$) was a remarkable characteristic of soursoup fruits; the abundance of these compounds progressively increased until reaching consumption ripeness, which happened on the 7th day after the harvest. The specific monitoring of aliphatic acids could be used as an indicative of the ripening degree.

## ACKNOWLEDGEMENTS

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