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Volatile compounds and descriptive odor attributes in umbu (*Spondias tuberosa*) fruits during maturation

Mércia de Sousa Galvão *, Narendra Narain, Maria do Socorro Porto dos Santos, Maria Lúcia Nunes

Laboratory of Chromatographic Analysis and of Flavor, NUCTA, Federal University of Sergipe, 49100-000 São Cristóvão SE, Brazil

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ABSTRACT

The umbu fruit is appreciated in north and northeast region of Brazil mainly because of its refreshing and acidic flavor. The objective of this work was to determine the volatile compounds in half-ripe and ripe stages of umbu fruit and to identify important characteristic compounds which could contribute for its aroma by performing sniffing evaluation of volatile extracts. The principal volatile compounds identified in the ripe umbu fruit pulp were 4-methyl-3-penten-2-one, ethyl benzene, 1-penten-3-one, 2-acetyl thiazone, *p*-xylene, limonene, 2,2-dimethyl 4-octenal, 3-hexanol, 2-nonanol, 1-nonanol, 2-pentanol, 2-octanol, 3-methylethyl 2-butanoate, butyl benzoate, 3-allyl cyclohexene, 2-acetyl furan, 2-hexyl furan, β-caryophyllene and methyl pyrazine. A total of 37 volatile compounds were diagnosed with characteristic aroma attributes. The principal volatile compounds which could be responsible for the characteristic aroma of ripe umbu fruit pulp were β-(*Z*)-ocimene, methyl pyrazine, 2-butyl-thiophene, methyl octanoate, 2-hexyl furan, 2-octanol, (*E*)-2-cyclohexen-1-one, 3-bromo-cyclohexene, 1-heptanol, 2-nonanol and 1-octanol.

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1. Introduction

Fruit flavor results from the combined effect of its constituents on the taste and olfactory organs. Although taste sensations are very important, it is the presence of trace amounts of volatile compounds which determine the flavor quality of the fruit. Aroma is generated during fruit development and is due to the presence of a large number of organic volatile compounds which are present in extremely small concentrations. When dealing with fruits of same genus, a compound which is considered to be an impact component for the flavor of one species may well be only contributory component in another or when the same fruit is encountered at different stages of maturity (Alves & Franco, 2003; Williams, 1979).

The flavor of exotic tropical fruits is of increasing interest to consumers worldwide. Fruits which possess regional importance and are only little-known outside the tropics are gaining interest of flavorists and flavor researchers for their unusual and strong sensory attributes (Fisher, Hammerschimidt, & Brunke, 1995; Moretti, Mattos, Calbo, & Sargent, 2010, Pino & Marbot, 2001). Among the tropical and subtropical fruits cultivated in the Northeast region of Brazil, the fruits of genus *Spondias* such as umbu (*Spondias tuberosa* Arruda Camara), yellow mombim (*Spondias mombim* L.), cajá-umbu (*Spondias sp.)*, purple mombin (*Spondias purpurea* L.) and ambarella (*Spondias dulcis* L. syn. *Spondias cytherea* Sonn.) are usually consumed in their natural form as juices or in products mainly ice-cream.

The umbu fruit is appreciated in the north and northeast region of Brazil mainly because of its refreshing and an acidic flavor; however, it is underutilized (Crane & Campbell, 1990; Epstein, 1998; Martin, Campbell, & Ruberte, 1987). The umbu fruits are round to ovoidal in shape, being 3–4 cm long and 2–3 cm in diameter and these possess greenish yellow color when ripe (Narain, Bora, Holschuh, & Vasconcelos, 1992). When the fruit is ripe, it's pulp is sweet (9.5 ^{0}Brix), pleasant and of mild acidic flavor (pH about 3). Narain et al. (1992) determined the physical, physico-chemical and chemical characteristics during various stages of fruit maturation.

Several fruits belonging to *Spondias* genus have been an object of study for their volatile compounds such as yellow mombin (*Spondias mombin* L.) (Adedeji, Hartman, Rosen, & HO, 1991; Allegrone & Barbeni, 1992; Augusto, Valente, Tade, & Rivellino, 2000; Ceva-Antunes, Bizzo, Alves, & Antunes, 2003; Narain, Almeida, Galvão, Madruga, & Brito, 2004; Sagrero-Nieves & De Pooter, 1992), purple mombin (*Spondias purpurea*, L.) (Augusto et al., 2000; Ceva-Antunes, Bizzo, Silva, Carvalho, Antunes, 2006), caja-umbu (*Spondias* sp.) (Franco & Shibamoto, 2000; Narain, Galvão, & Madruga, 2007). However, there is only one publication (Franco & Janzantti, 2005) available on identification of volatile compounds in umbu fruit pulp. In an analysis of headspace of ripe umbu fruit pulp, they reported the presence of only 16 volatile compounds and most of these were terpenes.

Furthermore, the fruits are usually classified in 3 stages (green, half-ripe and ripe) of maturity and hence it is important to know how the volatile compounds change during maturation of a fruit (Jiménez

* Corresponding author. Tel.: +55 79 2105 6535.
E-mail address: merciagalvao@gmail.com (M. de Sousa Galvão).

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et al., 2010, Ubeda et al., 2010). The stages of maturation when umbu fruit are usually utilized or used for processing is either half-ripe (skin of yellowish-green color) or ripe (skin of greenish yellow color) when its flavor is quite developed and when it possesses appreciable organoleptic attributes. However, no report has yet been published on characterizing the odor compounds responsible for aroma of umbu fruit. The objective of this work was therefore to identify the volatiles compounds present in pulp of umbu fruit at half-ripe and ripe stages of maturation and to characterize volatile compounds which could contribute for aroma of umbu fruit.

2. Materials and methods

2.1. Fruits and other materials

Fresh half-ripe and ripe umbu fruits were obtained from an Experimental Station Farm, administered by IPA (*Empresa Pernambucana de Pesquisa Agropecuária*), situated in the city of Itambé, in the Pernambuco state of Brazil. The fruits were transported to the laboratory in the city of João Pessoa in small cardboard boxes at ambient conditions (27 ± 2 °C; 60–75% relative humidity). At the laboratory, the fruits were washed in chlorinated water (10 ppm of available chlorine), selected and classified into two stages of its physiological maturity (half-ripe and ripe) according to the criteria of skin color, fruit texture and ease of pulp extraction from the fruit (Table 1). The classification of fruits for the green maturity stage according to these criteria led to the selection of fruits with hard texture wherein pulp extraction from the fruits to obtain a homogenous liquid mass was very difficult in domestic mixer. Therefore, in this work only half-ripe and ripe fruits were considered for the study.

The authentic standard flavor compounds used in volatiles identification were of pure grade (purity >99.9%) obtained from Sigma/Aldrich while solvents utilized in this study were purchased from Merck.

2.2. Extraction of volatiles compounds

The fruit, after being washed with distilled water, was cooled to 2 °C. The skin and kernel were separated manually by using a stainless steel knife and the pulp homogenized in a domestic mixer. The volatile compounds from the pulp of umbu fruit were extracted by using Likens and Nickerson's apparatus (Likens & Nickerson, 1964), which applies the simultaneous distillation and extraction technique.

The extraction conditions (Table 2) were initially optimized by varying the parameters such as use of solvent (*n*-hexane or a mixture of *n*-pentane and ethyl ether in proportion 2:1) and extraction period (40, 60 or 80 min), with an objective to obtain a large number of volatile compounds on chromatographic analysis of extracts. The temperatures for extraction were those of the boiling points of the solvents utilized, e.g. extraction with hexane was maintained at 69 °C, while that for the mixture of pentane and ethyl ether was 36 °C. The pulp weight (100 g), volume (100 ml) of the water added to pulp and the volume (20 ml) of the solvent utilized for extraction were fixed. The condenser was maintained cool by circulation of cold water (± 2 °C). The extraction temperature of the flask containing the pulp was 100 °C. The volatile extracts were obtained at optimized conditions by usage of 100 g of pulp diluted with 100 ml of distilled water and extraction performed with 20 ml of *n*-hexane for 60 min. The extracts were concentrated to a final volume of 0.3 ml under flow of nitrogen gas (Narain & Galvão, 2004). A blank test simulated with optimum extraction conditions using only solvent (without the presence of pulp) was also performed.

Table 1
Subjective criteria for classification of various maturation stages in umbu fruit.

Characteristic	Stage of maturity		
	Green	Half-ripe	Ripe
Prominent skin color	Green	Yellowish-green	Greenish yellow
Texture	Hard	Semi-hard	Soft
Pulp extraction	Difficult	Normal	Easy

Table 2
Description of extraction conditions for obtaining volatile extracts from ripe umbu fruit pulp.*

Experiment	Fruit maturity	Solvent	Extraction time (min)	Number of peaks
1	Half-ripe	n-hexane	40	102
2	Half-ripe	Pentane-ethyl ether	40	27
3	Half-ripe	n-hexane	60	245
4	Half-ripe	Pentane-ethyl ether	60	54
5	Half-ripe	n-hexane	80	241
6	Half-ripe	Pentane-ethyl ether	80	34
7	Ripe	n-hexane	40	109
8	Ripe	Pentane-ethyl ether	40	32
9	Ripe	n-hexane	60	246
10	Ripe	Pentane-ethyl ether	60	46
11	Ripe	n-hexane	80	240
12	Ripe	Pentane-ethyl ether	80	58

* Pulp weight of 100 g used for all experiments.

2.3. Selection and training of aroma analysts

In a preliminary test to evaluate the capacity and perception of aroma, 10 members participated initially. They were trained to perceive and develop sensitivity to relate different descriptive aroma attributes such as burnt, buttery, citric, cocoa, coconut, ethereal, fatty (or oily), garlic, grassy, minty, naphthalene, onion-like, paint-like, rancid, soapy, solvent, spicy, sweet, vanilla and woody. Finally only 3 aroma analysts were selected based on this training and posterior evaluation of their performance on perception of odor attributes.

2.4. Sniffing evaluation

A divider (1:1) was used at the end of high resolution capillary column. One part of column flow was directed to flame ionization detector in gas chromatograph system (Varian, model 3380), while the other part was sent to a sniffing port according to the methodology described by Silva, Sampaio, and Bertolini (2004). Three aroma analysts, who had better aptitude for descriptive aroma attributes as described earlier, sniffed at the port and registered their time-intensity response for aroma through the use of mouse on computer loaded with a program SCDTI (*Sistema de Coleta de Dados Tempo-Intensidade*), developed by State University of Campinas, Brazil. This program facilitated registering the data in the form of aromagrams which include initial and final time of any odor perception, point of maximum odor intensity and also duration of time corresponding to maximum intensity.

2.5. Volatiles separation in gas chromatography/flame ionization detector

Inittialy gas chromatographic (GC) experiments were performed by analyzing all volatile extracts obtained by varying extraction conditions (Table 2) in a gas chromatograph (Varian, model CP3800) coupled with flame ionization detector in order to establish optimum separation conditions. All consequent analysis were performed at standards analytical conditions, as described in item 2.2.

One microliter of the concentrated volatile extract was injected in the column in a split (1:20) mode. Capillary GC investigations were carried out on a 30 m (length) × 0.25 mm (internal diameter) innophase bondable polyethylene glycol polar capillary column (HP-INNOWax; 0.25 μm film thickness; Hewlett Packard, Inc., Palo Alto, USA). The carrier gas used was helium (99.999% pure) and column

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