VOLATILE COMPOUNDS FROM *THEOBROMA BICOLOR* L.

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ABSTRACT - *Theobroma bicolor is a type of cocoa where mainly the pulp is eaten. In contrast to the other species, the pulp is thick around the seed. Yellow in color, the taste is soft and sweet. Bicolor pulp was freeze dried and analyzed with head space (SPME) GCMS. Six (6) compounds were identified with the quality of matching of 80% and above with regards to the available mass library. There were d-limonene, linalyl anthranilate, dodecamethyl-cyclohexasiloxane, tetrademethyl-cycloheptasiloxane, 5-hydroxyl-2,4-di-t-butylphenyl ester, and hexadecamethyl-cyclooctasiloxane. However, only d-limonene and linalyl anthranilate are believed to be contributing compounds to the bicolor aroma.*

Keywords: Cocoa, bicolor, volatile compounds, head space GCMS

INTRODUCTION

Theobroma bicolor is a member of the Sterculiacea family originating from the Amazon Region in South America. Its popular names are “macambo” in Peru, “cacau” from Brazil, “macambo” and “bacau” in Colombia, and “pataste” in the other Latin-American countries. The fruit of Theobroma bicolor is voluminous, ellipsoidal, measuring up to 35 cm in length and 15 cm in width and weighing 3 kg. The pod is yellowish-brown in color when ripe. It has numerous seeds, an average of 40 per fruit which are arranged in series and surrounded by a yellowish pulp (Figure 1). The pulp is fibrous and has an uncommon taste and aroma. The fruit pulp is edible, and consumed in the natural state, but it can also be used in the preparation of juices and ice creams. The seeds are consumed boiled or roasted, and are also used in cooking just as nuts and for the preparation of chocolate (Torres et al., 2002). The fruit contains a high amount of lipids (33%), 42% being unsaturated fatty acids that can be easily oxidized (Flores Paytan, 1997).

Figure 1 Theobroma bicolor

The Theobroma bicolor possesses strong “sweet and fruity” smell from its yellow pulp. This aroma can be detected even when the pods are not opened. Not much study has been done on Theobroma bicolor. The objective of this paper is to report the volatile compounds of the bicolor pulp.

MATERIALS AND METHODS

Theobroma bicolor pulp sample was freeze dried with Buchi freeze dryer (model L-200, Germany) to remove moisture and concentrating the sample for GC analysis. Its volatile compounds were analyzed with headspace GCMS (Agilent, USA) with SPME fiber assembly Polydimethylsiloxane/ Divinylbenzene (PDMS/DVB), 65µm. Freeze dried pulp sample of 100mg was placed in a 20ml headspace vials. The headspace GCMS parameters were as below:

Sampler conditions (SPME):
Incubation temperature: 100 ^{o}C
Incubation time: 15 min
Sample extraction time: 10 min
Sample desorption time: 2 min

GC settings:
Column: HP-5MS (5% Phenyl Methyl Siloxane) 30m x 0.25mm x 0.25µm (Agilent, USA)
Oven temperature: 600C to 2000C at 100C/min and hold for 1 minute at 2000C. It was then increased to 2800C at 150C/min and hold for 2 min at 2800C.
Inlet mode: splitless
MSD transfer line: 2800C
Ion source: EI (70eV)
MS1 scan: Start mass: 50 Da.; End mass: 550 Da.; Scan time: 250ms.

RESULTS AND DISCUSSIONS

Six (6) compounds were identified with the quality of matching of 80% and above with regards to the available NIST mass library. There are D-limonene, Linalyl anthranilate, dodecamethyl-cyclohexasiloxane, tetrademethyl-cycloheptasiloxane, 5-hydroxyl-2,4-di-t-butylphenyl ester, and hexadecamethyl-cyclooctasiloxane (Table 1).

Table 1 Compounds identified by NIST mass library

Retention time (min)	Compound name	Formula	Score (%)
7.21	D-Limonene	$C_{10}H_{16}$	81.6
8.33	Linalyl anthranilate	$C_{17}H_{23}NO_2$	87.5
11.58	Dodecamethyl-Cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	89.6
13.83	Tetradecamethyl-Cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	84.1
14.11	5-hydroxy-2,4-di-t-butylphenyl ester	$C_{19}H_{30}O_3$	81.6
15.84	Hexadecamethyl-cyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	83.2

Three out of six identified compounds are siloxane derivative that may contributed from column and SPME materials. Only two terpenes are identified in contributing bicolor's unique notes, namely D-limonene and linalyl anthranilate.

Compound of 5-hydroxyl-2,4-di-t-butylphenyl ester with its IUPAC name of (2,4-ditert-butylphenyl) 5-hydroxypentanoate is an unsaturated fatty acid and has anticancer property (Nurhaslina *et al*., 2020).

Linalyl anthranilate (Figure 2) is a yellow-orange liquid (NTP, 1992) and is an arene and a terpene with an IUPAC name 3,7-dimethylocta-1,6-dien-3-yl 2-aminobenzoate. It has a blossom, fresh, and gardenia taste.

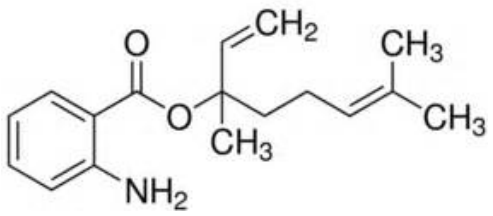

Figure 2 Molecule structure of Linalyl anthranilate

Linalyl anthranilate is insoluble in water but soluble in ethanol. It is used as flavoring agents and classified as food additives with JECFA-no. 1540 (EFSA, 2008). It is believed that the yellowish color of *bicolor* pulp is contributed by the presence of linalyl anthranilate.

D-limonene is a cyclic monoterpene and has a lemon or citrus-like odor (NTP, 1992; Lewis, 2012). Its IUPAC name is (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene with the chemical structure as shown in Figure 3 (National Center for Biotechnology Information, 2022).

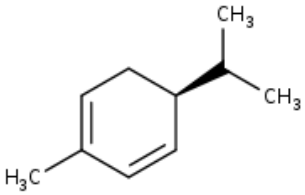

Figure 3 D-limonene

D-limonene is a colorless liquid or oil with a boiling point at 177.6°C. It is miscible with ethanol and ether (Haynes, 2015). Solubility in water is less than 13.8 mg/L at 25°C, slightly soluble in glycerin and insoluble in propylene glycol (Lewis, 2012).

CONCLUSIONS

Freeze dried *bicolor* pulp consists mainly of unsaturated fatty acid, linalyl anthranilate and d-limonene. The yellow color of *bicolor* pulp may be contributed by the existence of linalyl anthranilate and both d-limonene and linalyl anthranilate are the main contributors for the unique aroma of the *bicolor* pulp.

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