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Volatiles of Mango var. Ataulfo Characterized by SPME and Capillary GC/MS Spectroscopy

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Abstract. Head space volatiles from flowers, as well as green and ripe mango fruit of cv. Ataulfo from Soconusco, Chiapas, were collected using Solid Phase Microextraction (SPME). Identification of chemical species was carried out by Gas Chromatography-Mass Spectrometry (GC-MS). A complex mixture of monoterpenes and sesquiterpenes was found. 3-carene, α -pinene, myrcene, limonene, terpinolene, β -selinene and the sesquiterpene tentatively identified as germacrene D were the major constituents. This information could be valuable to characterize certain aspects of genetic traits, which in turn will be useful for breeding programs, and to provide assessment of cross pollination techniques.

Key words: Mango, Ataulfo, volatiles, SPME, GC-MS, terpenes.

Resumen. Los compuestos volátiles emitidos por flores y frutas verdes y maduras de mangos de la variedad Ataulfo del Soconusco, Chiapas fueron colectados por medio de la técnica de Microextracción en Fase Sólida (MEFS). El análisis químico de los volátiles se llevó a cabo por Cromatografía de Gases y Espectrometría de Masas (CG-EM). El análisis mostró que los volátiles del mango están formados por una mezcla principalmente de monoterpenos y sesquiterpenos. 3-careno, α -pineno, mirceneno, limoneno, terpinoleno, β -selineno y el sesquiterpeno tentativamente identificado como germacreno D fueron los principales constituyentes. Esta información será de mucha utilidad en los programas de mejoramiento genético de variedades de mango, ofreciendo así confirmación del éxito de las técnicas de polinización cruzada.

Palabras clave: Mango, Ataulfo, volátiles, MEFS, CG-EM, terpenos.

Introduction

Mango (*Mangifera indica*) is one of the most popular and best-known tropical fruits. It is widely grown in India, but is also produced in almost every tropical country, and even some subtropical regions such as Florida, Egypt, and southern Latin America [1, 2, 3]. Chemical analysis of the flavor of several mango cultivars around the world have been reported [4, 5, 6]. A wide range of compounds has been identified, including esters, lactones, mono- and sesquiterpenes. Monoterpene hydrocarbons such as *cis*-ocimene, α and β -pinene, myrcene and limonene seem to be particularly important contributors to the flavor of the fresh fruit, depending upon the variety [7, 8]. In the region of Soconusco, Chiapas, a particular variety named Ataulfo was developed years ago [9]. Ataulfo is a medium sized fruit, with an average between 200 and 400 g, green skin which turns yellow-orange when ripe. Fruits are fibreless and fairly sweet, and mainly eaten fresh but they are also made into preserves and puree. Due to the increasing popularity of this variety of mango by the export markets, we decided to identify the chemical nature of the volatiles produced by the flowers, and by green and ripe fruit. Solid Phase Microextraction (SPME) was employed to isolate volatiles. SPME has been successfully employed for the analysis of flavor and fragrances [10] as well as for other applications, such as extraction of pesticides and drug metabolites [11, 12].

Results and discussion

Table 1 lists the volatiles, obtained by SPME, of flowers, and green and ripe fruit of mango cv Ataulfo identified by GC-MS. Monoterpene and sesquiterpene hydrocarbons were found to be the most abundant volatiles. This sort of compounds have also been found to be volatile components of many mango cultivars [4, 5, 6]. In the mango Ataulfo cultivar we found that 3-carene was the major volatile constituent for both flowers and fruits. However, flowers and green fruit contained a larger amount of 3-carene than ripe fruits. The second compound in importance in flowers and green mango is α -pinene, while in ripe fruit 3-carene is followed by the tentatively identified germacrene D. Limonene, terpinolene and myrcene were also three minor common monoterpenes present in the volatiles mixture. Recently Pino [8] reported the volatiles of 20 mango cultivars from Cuba. In that study, 3-carene which has a sweet and limonene-reminiscent odor, was found to be the major component in 10 mango cultivars: Haden, Manga Amarilla, Macho, Manga Blanca, San Diego, Manzano, Smith, Florida, Keith and Kent [8]. 3-carene was also found to be an important component in the cultivars Corazon, Bizcochuelo and Super Haden from Cuba, Venezuela and Brazil [6,15]. In an African mango, 3-carene was shown to be in a minor amounts [14,15] and the mango Parrot variety from Sri Lanka was characterized by the presence of 3-carene [14,15]. α -pinene one of

Table 1. Mean relative content (area %) of volatiles from flowers and fruit of mango cv. Ataulfo (n = 6).

	Compound	KI	Flowers	Green mango	Ripe mango
1	α -pinene *	939	22.0	16.6	16.2
2	Camphene*	956	< 1.0	< 1.0	< 1.0
3	β -pinene*	981	1.3	1.7	1.0
4	Myrcene*	989	2.7	2.1	2.1
5	3-carene*	1008	55.9	59.7	37.4
6	Limonene*	1030	1.8	2.9	2.4
7	<i>cis</i> -ocimene *	1042	< 1.0	< 1.0	< 1.0
8	Terpinolene*	1087	1.1	3.6	5.6
9	α -copaene	1375	< 1.0	< 1.0	< 1.0
10	β -bourbonene	1383	1.0	1.0	< 1.0
11	α -gurjunene**	1410	< 1.0	1.0	< 1.0
12	β -caryophyllene*	1430	1.4	2.2	2.1
13	α -humelene*	1456	< 1.0	< 1.0	1.0
14	γ -gurjunene**	1474	< 1.0	2.7	1.0
15	Germacrene D	1482	2.2	1.0	22.9
16	β -selinene**	1486	2.3	4.7	7.7

Chemical identification: * Synthetic standards ** NIST 2002 Computer library

¹ Kovat's indices calculated from retention time data on a DB-5 capillary column.

the main volatile compound in both flowers and fruits of Ataulfo, is also a common constituent in the 20 mango cultivars from Cuba [8]. The cultivars Langra, Bombay and Desi from India have already been characterized by the presence of α -pinene [15]. Limonene a volatile constituent present in similar amount either in flowers or fruits of the mango Ataulfo, has been found to be the major component in five mango cultivars from Cuba: Delicioso, Super Hadden, Ordoñez, Filipino and La Paz [8]. Cultivars from Egypt (Alphonso and Baladi) had limonene as main constituent [16]. Terpinolene a minor volatile component of mango Ataulfo is contained in larger amount in fruits than flowers. This compound is also a component of the mango Delicia, Obispo, Corazón and Huevo de Toro from Cuba [8]. In other mango cultivars terpinolene was present in high amounts: Williard from Sri Lanka and Kensington from Florida [14,4]. Mango Parrot from Sri Lanka was also characterized by the presence of terpinolene [14,5]. *Cis*-ocimene another minor component of Ataulfo is a component in mango cultivars Alphonso from India and Jaffna from Sri Lanka [14,17].

In all samples of Ataulfo cultivar, sesquiterpene hydrocarbons were constantly present but variation was found between samples. Sesquiterpenes have been found in many mango cultivars, for example, β -caryophyllene and α -humelene are common components in the volatiles mixture in the 20 mango varieties from Cuba [8]. Parrot from Sri Lanka contain β -selinene [14,5]. Langra, Bombay and Desi from India have already been found to contain, caryophyllene oxide and humulene oxide [15].

There is little information in the literature concerning the volatiles produced by the flowers of mango cultivars. The volatiles of the flowers of cv Ataulfo were first analyzed by Cruz-López [18] who reported that the mango flower volatiles were composed by a mixture of 14 terpenoid compounds,

being 3-carene the most abundant followed by α -pinene and limonene. This information was confirmed in the present study. De Jesús [19] investigated the volatiles of mango flowers cv. Carabao from the Philippines. About 138 compounds constitute the aroma of Carabao mango panicles at full bloom stage. The major components were the hydrocarbons, alcohols and esters. The minor components consisted of carboxylic acids, ketones, aldehydes, ether and amide, however. Major variations in the concentrations of volatile components produced by the different cultivars occur; the analysis of fruit volatiles is, therefore, desirable as it facilitates the classification of the various cultivars on the basis of the volatile components produced by the fruit or even leaves [20]. This information may also help to characterize certain aspects of genetic inheritance, which will be of considerable assistance for breeding programs, by confirmation of cross pollination techniques. Furthermore, the characteristic fingerprint of volatiles from each cultivar may be of use for protection of patented propagation rights to new material. On the other hand, the flowers and mango fruit volatiles could be used as insect pest attractants [10, 11, 21]. In conclusion, volatiles of flowers, and green and ripe fruits of mango var. Ataulfo are composed mainly of monoterpene and sesquiterpene hydrocarbons. Flowers and green mango are characterized by a large amount of α -pinene and 3-carene, while in ripe mango the amount of sesquiterpenes increased.

Experimental

Biological Material. Flowers and mango fruits of the variety Ataulfo were obtained from orchards near Tapachula, Chiapas, Mexico. The mango Ataulfo has a growing season from early March through the end of May.

Solid Phase Microextraction (SPME). Individual mango fruit or a flower panicle were placed into a single glass container (20 × 10 cm) and sealed with an 11 mm Teflon-lined septum. Headspace volatiles were sampled by SPME. The solid phase fiber coating was 100 µm poly (dimethylsiloxane). SPME equipment was obtained from SUPELCO (Toluca, Mexico). To obtain samples, the sheath of the SPME was inserted through the septum, the fiber was extended and exposed to the volatiles for 30-40 min. After that, the fiber was withdrawn into the needle and immediately transferred to GC-MS for analysis.

Chemical analysis. The analysis of the volatiles from mango flowers and fruit adsorbed by the SPME fibers was conducted using a Varian model Star 3400 CX GC, coupled to MS and integrated data system (Varian Saturn 4D). Ionization was done by electron impact at 70 eV, 230 °C. A DB-5 column (30 m × 0.25 mm ID) was temperature-programmed from 50 °C (held for 2 min) to 250 °C at 15 °C/min. The injection port temperature was 200 °C. The SPME fiber was placed to the hot injection port of the GC, where the analytes were thermally desorbed from the fiber for 5 min. The relative percentage of the components was calculated by adding up the recorded peaks. Chemical identification was confirmed by comparison of mass spectra pattern with the NIST 2002 computer library and retention time of synthetics. Kovat's retention indices (KI) were calculated using the formula $I_x = 100 \frac{t_x - t_n}{t_{n+1} - t_n}$, where I_x is the retention index of the compound of interest, t_x is the retention time of the compound of interest, t_n and t_{n+1} are the retention times of the n -alkanes eluting immediately before and after the compound of interest, and n is the number of carbon atoms in the n -alkane eluting immediately before the compound of interest [22]. KI observed was compared with those reported by Adams [23].

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