Characterization of odor-active compounds in yellow pitaya (*Hylocereus megalanthus* (Haw.) Britton *et* Rose)

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Key words: yellow pitaya, Hylocereus megalanthus, odor-active compounds, GC-MS, GC-O.

RESUMEN. La pitaya amarilla [*Hylocereus megalanthus* (Haw.) Britton *et* Rose] es una cactácea nativa de la región del Caribe de América del Sur. Es una fruta exótica de gran importancia económica en Colombia debido a que es el segundo país exportador en el mundo. A pesar de su importancia, la literatura en relación con los compuestos del aroma y sabor de la pitaya amarilla es escasa. En el presente trabajo, los compuestos volátiles de la pitaya amarilla fueron aislados simultáneamente por destilación-extracción con disolvente y analizados por cromatografía de gases con detector de llama, cromatografía de gases-olfatometría y cromatografía de gases-espectrometría de masas. Un total de 146 constituyentes volátiles fueron detectados, 121 de ellos fueron positivamente identificados. La composición de la fruta comprende 29 terpenos (26,0 % del total de la composición volátil), 20 aldehídos (14,3 %), 19 alcoholes (5,2 %), 15 parafinas (4,0 %), 14 ésteres (18,2 %), 14 ácidos (15,9 %), 13 cetonas (8,8 %) y 22 compuestos de distinta naturaleza química (7,6 %). Los componentes mayoritarios fueron hexanal (5,1 %) y δ-cadineno (4,7 %). Las áreas de olor activas en el cromatograma fueron evaluadas por la aplicación del análisis de dilución del extracto de aroma y mediante valores de actividad de olor. Este estudio reveló los odorantes potentes que son responsables del aroma global de la fruta de pitaya amarilla. Nueve odorantes fueron considerados como los más activos en el olor: (*E*)-β-damascenona, 3-metilbutanal, decanal, hexanal, octanal, fenilacetaldehído, nonanal, 1,8-cineol y limoneno.

ABSTRACT. Yellow pitaya [*Hylocereus megalanthus* (Haw.) Britton *et* Rose] is a native cactaceae of the South American Caribbean region. It is an exotic fruit of great economical importance in Colombia because this country is the second main exporter of yellow pitaya in the world. Despite its importance, literature about the flavor and arama compounds of the 1yellow pitaya is scarce. The volatile compounds of yellow pitaya fruit were isolated by simultaneous distillation-solvent extraction and analyzed by gas chromatography-flame ionization detector, gas chromatography-olfactometry and gas chromatography-mass spectrometry. A total of 146 volatile constituents were detected, 121 of them were positively identified. The composition of the fruit included 29 terpenes (26.0 % of the total volatile composition), 20 aldehydes (14.3 %), 19 alcohols (5.2 %), 15 paraffins (4.0 %), 14 esters (18.2 %), 14 acids (15.9 %), 13 ketones (8.8 %), and 22 miscellaneous compounds (7.6 %). Major compounds were hexanal (5.1 %) and -cadinene (4.7 %). The odor-active areas in the gas chromatogram were screened by the application of the aroma extract dilution analysis and by the odor activity values. This study revealed potent odorants that are responsible for the overall aroma of yellow pitaya fruit. Nine odorants were considered as the most odor-active compounds: (*E*)-β-damascenone, 3-methylbutanal, decanal, hexanal, octanal, phenylacetaldehyde, nonanal, 1,8-cineole, and limonene.

INTRODUCTION

Yellow pitaya [*Hylocereus megalanthus* (Haw.) Britton et Rose; syn. *Selenicereus megalanthus* (K. Schumann ex Vaupel) Moran] is a native cactaceae the South American Caribbean region. Yellow pitaya is an exotic fruit of great economical importance in Colombia because this country is the main exporter of the yellow pitaya after Israel in the world. The fruit is a medium-sized oblong berry and at its commercial ripe stage, the peel is yellow, and the pulp that contains many small soft digestive seeds is juicy, delicate, sweet and white.[1,2] The aroma of this fruit is delicate and it is described as fruity and herbaceous with a faint floral note. Although some experiments has been

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carried out on fruit development and storage,[1-5] there are no studies about the composition of volatile compounds and their sensory contribution to the flavor of yellow pitaya.

Fruit volatile fraction could be extremely complex, due to the great number of compounds generally present, which may have different polarities, volatilities and morver may be found in a wide range of concentrations.[6] Nevertheless, despite this extremely complex composition, only a small number of the so-called key odorants is obviously detected by the human odorant receptors.[7] An approach to separate odor-active volatiles from the bulk of odorless food volatiles is GC-Olfactometry (GC-O), odor activity values (ratio of concentration to odor threshold) or, a more comprehensive one, dilution to odor threshold techniques, such as aroma extract dilution analysis[7,8] Numerous publications have dealt with the identification of the odor-active volatiles using these techniques in fruits.[9-11]

This study was conducted to determine the composition of yellow pitaya (Hylocereus megalanthus (Haw.) Britton et Rose) fruit from Colombia and to determine which volatile components are primarily responsible for its flavor.

MATERIALS AND METHODS

Chemicals and reagents

The fresh, healthy and ripe yellow pitaya fruits used were harvested at Anolaima (Department of Cundinamarca) in Colombia. The standards used for identifications were supplied by Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland). Some standards were provided by Dallant (Barcelona, Spain). An n-alkane solution (C_8-C_{32}) from Sigma-Aldrich (St. Louis, MO) was employed to calculate the linear retention index (RI) of each analyte. Diethyl ether was purchased from Merck (Darmstadt, Germany) and it was previously redistilled and checked to purity.

Isolation of volatile compounds by simultaneous distillation-solvent extraction

Two hundred grams of the pulp fruit were blended with 500 mL of distilled water; 0.2 mg of methyl nonanoate were added as internal standard, and the volatile compounds were isolated by means of SDE apparatus using 30 mL of redistilled diethyl ether for 1 h. The extract was dried over anhydrous Na_2SO_4 and concentrated to 0.6 mL in a Kuderna-Danish evaporator with a Vigreux column and then to 0.2 mL with a gentle nitrogen stream. The concentrated extract was stored in a glass screw-top vial at –20°C until being analyzed. Two independent extractions were done and each extract was injected twice into the GC-FID and GC-MS.

GC-FID and GC-MS analysis

An HP-6890 instrument gas-chromatograph (Hewlett-Packard Co., Palo Alto, CA), equipped with a HP-5 ms column (30 m x 0.25 mm, 0.25 μm film thickness) and with a flame ionization detector were used. Oven temperature was held at 50 °C for 2 min and then raised to 280 °C at 4 °C/min and held for 10 min. Carrier gas (helium) flow rate was 1 mL/min. The injection and detector temperatures were 240 and 250 °C, respectively. The retention times of a series of straight-chain alkanes (C_8-C_{32}) was used to calculate the retention indices for all identified compounds and for reference standards. Concentration of each volatile compound is expressed as mg internal standard equivalents per kg of fruit, obtained by normalizing the compound peak area to that of the internal standard and multiplied by the concentration of the internal standard. All analyses were replicated two times. GC-MS analyses were performed on a HP-6890 instrument gas-chromatograph (Hewlett-Packard Co., Palo Alto, CA) interfaced with a HP-5973 mass-selective detector fitted with a similar fused capillary column as in GC-FID. The temperature program and carrier gas flow rate were the same as in GC-FID. EIMS, the electron energy, 70 eV; the ion source and the connecting parts temperature, 250 °C. The acquisition was performed in scanning mode (mass range m/z 35-400 u). Compounds were preliminarily identified by using NIST, Wiley, NBS, Adams 2001, and in-house Flavorlib libraries, and then the identities of most were confirmed by comparison of their linear retention indices with those of reference standards or with published data.[12]

Gas Chromatography-Olfactometry analysis (GC-O)

GC-O analyses were performed with a gas chromatograph Konik 4000A instrument (Konik, Barcelona) equipped with HP-5 ms (30 m x 0.25 mm, 0.25 μm film thickness). Analytical conditions were the same as GC-FID analyses. The end of the capillary column was connected to a deactivated Y-shaped glass splitter dividing the effluent into two equal parts, which were transferred via two deactivated fused silica capillaries (25 cm × 0.25 mm) to a sniffing port and an FID, respectively. The sniffing port, mounted on a detector base of the GC, consisted of a cylindrically shaped aluminum device (40 mm × 25 mm i.d.) with a beveled top and a central drill hole housing the capillary. Nitrogen (30 mL/min) was used as the make-up gas. The injection volume was 1 μL. During a GC-O run, the nose of the assessors was placed closely above the top of the sniffing port and the odor of the effluent was evaluated. If an odor was recognized, the retention time was marked in the chromatogram, and the odor quality was assigned. The GC-O analyses were performed by two trained assessors.

Aroma Extract Dilution Analysis (AEDA)

The yellow pitaya extract was stepwise diluted to obtain dilutions of 1 : 2, 1 : 8, 1 : 16, ..., 1 : 256 of the original solutions.[7] Each dilution was submitted to GC-O, using capillary HP-5 ms column. Analytical conditions were the same as GC-FID analyses. The odor-active compounds were located in the chromatograms, and each odorant detected was assigned an FD factor representing the highest dilution in which the odorant was detectable. Two trained assessors evaluated the sample in duplicate, thus producing four individual aromagrams. The average FD factor from the four runs was calculated for each odorant detected.

Odor detection threshold determination

A previously described multiple paired comparison test was used.[10] Samples were prepared in capped, wide-mouthed, 50-mL glass bottles. A group of 30-40 unscreened and untrained assessors was used for determining the odor thresholds. In each case, panels were replicated a sufficient number of times so that a minimum of 100 responses was obtained for each concentration used in determining a particular threshold. The test involved presenting the assessors with several samples, along with an aqueous solution for reference. Each sample was compared individually in smell with the reference to determine a possible difference. Six samples were presented to each judge during each session. The first bottle was the reference and the next five coded bottles contained four different dilutions and an aqueous solution identical to the reference. The four dilutions were placed in order to increase concentrations to prevent fatigue. The position of the aqueous solution coded sample among the different samples was arbitrarily changed from day to day. The statistical analyses for determining the odor detection threshold values involved calculating the concentration corresponding to 50 % positive responses from the total judgments. The calculation was made from the linear regression of percentage detection against log concentration. The 95 % confidence limit calculated for the threshold values was used as a measure of error. The relative standard deviations were lower than 6 %.

RESULTS AND DISCUSSION

All of the volatiles isolated from yellow pitaya fruit were evaluated by two experts smelling a drop of the extract onto a cardboard smelling strip as done by perfumers. After evaporation of the solvent, both experts agreed that the distillate evoked the characteristic odor of the fruit, thereby indicating that the isolation method used for aroma isolation was appropriate.

A total of 146 volatiles were detected (0.84 mg/kg of fruit), 121 of them were positively identified in yellow pitaya fruit (Table 1). The composition of the fruit included 29 terpenes (26.0 % of the total volatile composition), 20 aldehydes (14.3 %), 19 alcohols (5.2 %), 15 paraffins (4.0 %), 14 esters (18.2 %), 14 acids (15.9 %), 13 ketones (8.8 %), and 22 miscellaneous compounds (7.6 %). Major compounds were hexanal (5.1 %) and -cadinene (4.7 %). Although an overwhelming number of chemical compounds has been identified as volatile compounds in fruits, only a fraction of these compounds has been identified as impact compounds of fruit flavor based on their quantitative abundance and olfactory thresholds.[6]

Table 1. Volatile compounds identified in yellow pitaya.

Compound	LRI	ID	mg/kg
2-Butanone	600	a	0.02
2,3-Butanedione	605	a	t
Ethyl acetate	612	a	0.04
2-Methylpropanol	625	a	0.02
Acetic acid	645	a	0.03
2-Methyl-2-butanol	650	a	0.01
3-Methylbutanal	654	a	0.02
Cyclohexane	655	a	t
1-Penten-3-ol	681	a	0.01
1-Penten-3-one	685	a	t
n-Heptane	700	a	t
3-Pentanone	703	a	t
Propyl acetate	707	a	t
Methylcyclohexane	720	a	t
3-Methyl-3-buten-1-ol	731	a	t
4-Methyl-2-pentanone	735	b	t
3-Methylbutan-1-ol	741	a	t
(*E*)-2-Pentenal	758	b	t
1-Pentanol	771	a	t
(*Z*)-2-Pentenol	774	b	t
2-Methylpropyl acetate	780	a	t
3-Methyl-2-butenal	784	b	0.01
n-Octane	800	a	t
Hexanal	806	a	0.05
Butyl acetate	813	a	t
2-Methyl-2-pentenal	849	b	0.01
2,4-Dimethyl-1-heptene	851	b	t
Isobutylisothiocyanate	853	b	t
(*E*)-2-Hexenal	856	a	t

2,3-Dimethylheptane	858	b	t
(*Z*)-3-Hexenol	860	a	t
Ethylbenzene	866	a	t
4-Methyloctane	869	b	t
1-Hexanol	872	a	t
p-Xylene	875	a	t
3-Methylbutyl acetate	881	a	t
n-Nonane	900	a	t
Heptanal	902	a	t
2-Butoxyethanol	904	a	t
3-(Methylthio)propanal	908	a	t
Diethyl disulfide	929	a	t
α-Pinene	939	a	t
Propylbenzene	955	a	t
1,1-Diethoxy-3-methylbutane	958	b	t
Benzaldehyde	960	a	t
1-Heptanol	967	a	t
1-Octen-3-ol	979	a	t
2,3-Octanedione	982	a	t
Pentanoic acid	985	a	t
6-Methyl-5-hepten-2-one	988	a	t
1,3,5-Trimethylbenzene	990	a	t
1,2,4-Trimethylbenzene	993	a	t
(*E*,*Z*)-2,4-Heptadienal	995	a	t
Octanal	997	a	0.01
Isopropenylbenzene	999	b	t
n-Decane	1000	a	t
Hexanoic acid	1005	a	0.01
(*E*,*E*)-2,4-Heptadienal	1011	a	t
α-Terpinene	1017	a	t
p-Cymene	1023	a	t
Limonene	1026	a	0.01
1,8-Cineole	1030	a	0.02
2-Ethyl-1-hexanol	1033	a	t
(*Z*)-β-Ocimene	1037	a	t
Phenylacetaldehyde	1042	a	0.03
(*E*)-β-Ocimene	1053	a	t
(*Z*)-4-Hepten-2-yl acetate	1055	b	t
(*E*)-2-Octenal	1057	a	t
3-Methylbutyl butanoate	1059	a	t
γ-Terpinene	1062	a	0.03
Acetophenone	1066	a	0.01
1-Octanol	1068	a	t
(*E*)-2-Octenol	1072	a	t
1-Methylindan	1079	a	t
trans-Linalool oxide (furanoid form)	1085	a	t
Terpinolene	1089	a	0.01
p-Cymenene	1093	a	t
n-Undecane	1100	a	0.01
Perillene	1101	b	t
Nonanal	1103	a	0.01
3-Methylbutyl isopentanoate	1106	a	t
cis-Rose oxide	1109	b	t
2-Ethylhexanoic acid	1128	a	t
Phenylacetonitrile	1142	a	0.02

(*Z*)-3-Nonen-1-ol	1153	a	t
Nerol oxide	1157	b	t
Benzoic acid	1160	a	t
Ethyl 2-methyloctanoate	1164	b	0.03
1-Nonanol	1169	a	t
Terpinen-4-ol	1177	a	t
Octanoic acid	1183	a	0.01
α-Terpineol	1189	a	0.00
n-Dodecane	1200	a	t
Decanal	1205	a	t
(*E*,*E*)-2,4-Nonadienal	1212	a	t
Benzothiazole	1222	a	t
Nerol	1230	a	t
Thymol methyl ether	1235	a	t
Neral	1238	a	t
(*Z*)-2-Decenal	1250	a	t
Geranial	1267	a	t
1-Decanol	1270	a	t
m-Diisopropenylbenzene	1272	b	t
Nonanoic acid	1275	a	0.01
n-Tridecane	1300	a	t
p-Diisopropenylbenzene	1308	b	0.01
Methyl 2-hydroxy-5-methylbenzoate	1314	b	0.01
Citronellyl acetate	1353	a	t
(*E*)-2-Undecenal	1367	a	t
4-Isopropenyl-acetophenone	1374	b	0.04
Decanoic acid	1378	a	0.01
Biphenyl	1382	a	t
(*E*)-β-Damascenone	1385	a	0.01
n-Tetradecane	1400	a	0.01
Longifolene	1408	a	0.02
Dodecanal	1412	a	t
Aromadendrene	1441	a	t
p-Acetylacetophenone	1450	a	0.01
Geranyl acetone	1453	b	t
(*E*)-β-Farnesene	1457	a	t
1-Dodecanol	1471	a	0.01
α-Amorphene	1485	b	0.01
(*Z*,*E*)-α-Farnesene	1491	a	t
cis-Cadina-1,4-diene	1495	b	0.01
Viridiflorene	1498	a	0.02
n-Pentadecane	1500	a	t
δ-Cadinene	1523	a	0.05
trans-Calamenene	1530	a	t
trans-Cadina-1(2),4-diene	1536	b	0.01
Dibutyl maleate	1540	a	t
α-Calacorene	1546	b	0.02
Dodecanoic acid	1568	a	0.02
n-Hexadecane	1600	a	t
Benzophenone	1631	a	t
n-Heptadecane	1700	a	t
Tetradecanoic acid	1779	a	0.03
Ethyl tetradecanoate	1793	a	t
n-Octadecane	1800	a	t
Hexadecanal	1811	a	t
Pentadecanoic acid	1868	a	0.02

n-Nonadecane	1900	a	t
(Z)-11-Hexadecenoic acid	1947	a	t
Hexadecanoic acid	1960	a	0.01
Ethyl hexadecanoate	1990	a	0.03
Octadecanoic acid	2200	a	0.02
Ethyl octadecanoate	2209	a	0.02

ID: identification, [a]Compound definitely identified (comparison of RI and mass spectra with reference compound), [b]by mass spectra data and retention index data from literature. t: <0.01 mg/kg

The volatiles isolated by SDE from yellow pitaya fruit were analyzed by AEDA to find the most potent odorants. The results yielded 12 odor-active regions with important flavor dilution factors (ranging from 32 to 256), which have been arranged following their retention indices (Table 2). Six of them corresponded to aldehydes. The compounds with the highest FD factors were 3-methylbutanal, hexanal, and (E)--damascenone. Three compounds were found with FD = 128, octanal (sweet, honey-like), phenylacetaldehyde (rosy, floral), and decanal (sweet, waxy). Other compounds with significant FD factors (32-64) were nonanal (fatty-floral), ethyl acetate (fruity), propyl acetate (fruity), limonene (pungent green, citrus-like), 1,8-cineole (fresh, camphoraceous), and δ-cadinene (wood, floral).

Dilution to odor threshold techniques, such as AEDA, does not permit a study on the influence of the food matrix in odorant binding nor in the interactions of odorants when matching the overall odor impression of the food. Therefore, the OAV concept (Schieberle 1995) was applied in this study to the odorants of mango (Table 2). However, it is necessary that the threshold of the components is determined in a matrix as close as possible to the food itself. Therefore, the odor thresholds for nearly all the volatiles under investigation were determined in water or taken from papers with similar conditions.[13] Results suggested that nine odorants should contribute to the characteristic aroma of yellow pitaya fruit, because their contents clearly exceeded their odor thresholds (Table 2). Following this procedure, the compound with the highest OAV was identified as (E)-β-damascenone, with its characteristic fruity and sweet odor. Other two odorants with higher OAV were 3-methylbutanal (fruity, malty) and decanal (sweet, waxy). Moreover, other six odorants had OAVs > 1 and probably contributed to the aroma of yellow pitaya fruit (Tabla 2). Another three odorants, ethyl acetate, propyl acetate, and δ-cadinene presented OAV < 1, which probably means that its contribution is not sensory important. The potentially important odorants obtained with the odor activity approach is a refinement of that provided by the AEDA and corrects some of the defects of the AEDA technique.

To confirm the aroma contribution of these compounds, aroma recombination experiments should be done. However, the odor-active compounds identified can already be suggested as indicators to assess the odor quality of yellow pitaya fruit.

Table 2. Odor-active compounds identified in yellow pitaya.

Compound	Odor note	FD[a]	Odor threshold (μg/kg)	OAV[d]
Ethyl acetate	fruity	32	5000[b]	<1
3-Methylbutanal	fruity, malty	256	0.2[b]	77
Propyl acetate	fruity	32	54[b]	<1
Hexanal	herbaceous	256	4.5[b]	10
Octanal	sweet, honey-like	128	0.7[c]	12
Limonene	pungent green, citrus-like	32	10[bc]	1
1,8-Cineole	fresh, camphoraceous	32	12[b]	2
Phenylacetaldehyde	rosy, floral	128	4[c]	8
Nonanal	fatty-floral	64	1[c]	8
Decanal	sweet, waxy	128	0.1[b]	37
(*E*)-β-Damascenone	fruity, sweet	256	0.002[c]	975
δ-Cadinene	wood, floral	64	100[b]	<1

[a]Flavor dilution factor. [b]Experimental. [c]From Leffingwell & Assoc. (2011).
[d]Odor-activity values were calculated by dividing the concentrations by the respective odor threshold.

CONCLUSIONS

A total of 121 volatile compounds, belonging to several classes, were positively identified in yellow pitaya fruit. This study revealed potent odorants that are responsible for the overall aroma of yellow pitaya fruit by application of the odor activity value concept. Nine constituents were considered as odor-active volatiles, from which the most important were (E)-β-damascenone, 3-methylbutanal, decanal, hexanal, octanal, phenylacetaldehyde, nonanal,

1,8-cinole, and limonene. The provided information about cape gooseberry flavor can be used in the food industry as quality-freshness markers of yellow pitaya and in developing new products from this fruit.

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