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## LITERATURE CITED

- Bestmann, H. J.; Roth, K.; Ettliger, M. Eine Stereoselektive Synthesemethode für (Z)- $\alpha,\beta$ -Ungesättigte Aldehyde. *Chem. Ber.* 1982, 115, 161-171.
- Boar, R. B.; Damps, K. Resolution of 2,3-Dihydrosqualene-2,3-diol, 10,11-Dihydro-10,11-dihydroxyfarnesyl Benzoate, and 6,7-Dihydro-6,7-dihydroxygeranyl Benzoate. Synthesis of (3R)- and (3S)-2,3-Epoxy-2,3-dihydrosqualene. *J. Chem. Soc., Perkin Trans. 1* 1977, 709-712.
- Canonica, L.; Rindone, B.; Santaniello, E.; Scolastico, C. A Total Synthesis of Mycophenolic Acid, Some Analogues and Some Biogenetic Intermediates. *Tetrahedron* 1972, 28, 4395.
- Chang, C. E. Lauraceae. In *Flora of Taiwan*; Li, H. L., et al., Eds.; Epoch: Taipei, Taiwan, 1976; Vol. II, p 417.
- Chen, Y.; Li, Z.; Xue, D.; Han, H.; Yang, Y. Analysis of Volatile Constituents of Chinese Medicinal Herbs by Flash Distillation-Capillary Gas Chromatography-Mass Spectrometry. *Gaodeng Xuexiao Huaxue Xuebao* 1985, 6, 1075-1079; *Chem. Abstr.* 1986, 104, 136143x.
- Chen, Y.; Li, Z.; Wang, R.; Xue, D. Identification of Chinese Medical Herbs by Pyrolysis-Capillary Gas Chromatography. *Fenxi Ceshi Tongbao* 1987, 6, 12-16; *Chem. Abstr.* 1987, 107, 121172u.
- Furia, T. E.; Bellanca, N. *Fenaroli's Handbook of Flavor Ingredients*, 2nd ed.; CRC Press: Cleveland, OH, 1975; Vol. I, pp 323-325.
- Hu, T. W.; Lin, Y. T.; Ho, C. K. Natural Variation of Chemical Components of Leaf Oil of *Cinnamomum osmophloeum* Kaneh. in Taiwan. In Proceedings of the Annual Meeting of Agricultural Associations of the Republic of China, Taichung, Taiwan, 1985; pp 45-62.
- Hussain, R. A.; Kim, J.; Hu, T. W.; Pezzuto, J. M.; Soejarto, D. D.; Kinghorn, A. D. Isolation of a Highly Sweet Constituent from *Cinnamomum osmophloeum* Leaves. *Planta Med.* 1986, 403-404.
- Juell, S. M. K.; Hansen, R.; Jork, H. New Substances isolated from the Essential Oils of Various *Artemisia* Species, Part 1. Spathulenol, an Azulenic Sesquiterpene Alcohol. *Arch. Pharm.* 1976, 309, 458-466.
- Klibanov, A. M.; Giannousis, P. P. Geometric Specificity of Alcohol Dehydrogenases and Its Potential for Separation of Trans and Cis Isomers of Unsaturated Aldehydes. *Proc. Natl. Acad. Sci. U.S.A.* 1982, 79, 3462-3465.
- Lawrence, B. M. Progress in Essential Oils. *Perfumer Flavorist* 1978, 3, 54-55.
- Majlat, P.; Erdos, Z.; Takacs, J. Calculation and Application of the Retention Indices in Programmed-Temperature Gas Chromatography. *J. Chromatogr.* 1974, 91, 89-103.
- McMurry, J. E. Total Synthesis of Copacamphene. *Tetrahedron Lett.* 1970, 3731-3734.
- Nohara, T.; Kashiwada, Y.; Nishioka, I. Cinnassiol E, a Diterpene from the Bark of *Cinnamomum cassia*. *Phytochemistry* 1985, 24, 1849-1850.
- ter Heide, R. Qualitative Analysis of the Essential Oil of Cassia (*Cinnamomum cassia* Blume). *J. Agric. Food Chem.* 1972, 20, 747-751.
- Thomas, A. F. Two New Sesquiterpenoid Ketones from Cassia Oil. *Helv. Chim. Acta* 1980, 63, 1615-1618.
- von Rudloff, E. Scope and Limitation of Gas Chromatography of Terpenes in Chemosystematic Studies. In *Recent Advances in Phytochemistry*; Seikel, M. K.; Runeckles, V. C. Eds.; Appleton-Century-Crofts: New York, 1969; Vol. II, pp 127-162.
- Yuan, A.; Tan, L.; Wei, S.; Kang, S.; Jiang, D. Chemical Constituents of Gui Zhi (*Cinnamomum cassia*), a Traditional Chinese Medicine. *Zhongyao Tongbao* 1984, 9, 127-128; *Chem. Abstr.* 1984, 101, 60032a.

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## Volatiles from Naranjilla Fruit (*Solanum quitoense* Lam.). GC/MS Analysis and Sensory Evaluation Using Sniffing GC

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Aroma volatiles from naranjilla fruit (*Solanum quitoense* Lam.) were concentrated by either a closed-loop stripping or a solvent extraction method. Compounds were identified by capillary GC/MS and sensorially characterized by sniffing GC. The main aroma constituents were esters of butanoic acid and ethyl acetate. Aroma impact compounds could not be found.

Naranjilla or lulo, which looks like a small orange, is the fruit of the tropical nightshade *Solanum quitoense* Lam. It is consumed in Ecuador and northern parts of Brazil either in fresh form or as a drink (Duke, 1970). At present, it is commercially important only in the region of its production. The flavor of naranjilla has been described by several people not accustomed to the fruit as sweet and resembling a mixture of banana, pineapple, and strawberry. Nothing has been published yet about the substances responsible for this interesting aroma. We now communicate

the results of our work on the volatile components of naranjilla.

The current goal of aroma analysis is not only to identify components but also to determine their importance to the flavor of the product under investigation. For this reason, we used a gas chromatographic sniffing detector to assess the sensory properties of every separated peak.

### MATERIALS AND METHODS

All solvents used were distilled through a 40-cm Vigreux column. Deionized water was purified through a 30-cm column of activated carbon.

Ripe fruits were obtained by air freight from Ecuador, stored at 4 °C, and used within 7 days of arrival.

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**Table I. Components Identified in Headspace of Naranjilla Fruit Juice**

substance	odor at sniffing port <sup>a</sup>
ethyl acetate	estery, fruity
methyl propanoate	
methyl 2-methylpropanoate	herbal, sweet
ethyl propanoate	} sweet, naranjilla
ethyl 2-methylpropanoate	
methyl butanoate	fruity, naranjilla
2-methylpropyl acetate	
ethyl butanoate	synthetic, fruity
ethyl 3-methylbutanoate	
butyl acetate	
methyl pentanoate	green, herbal
methyl ( <i>E</i> )-2-butenolate	
3-methylbutyl acetate	banana
methyl hexanoate	} sweet, spicy, herbal
methyl ( <i>E</i> )-2-methyl-2-butenolate	
ethyl hexanoate	} fruity, very sweet, herbal
ethyl ( <i>E</i> )-2-methyl-2-butenolate	
hexyl acetate	
( <i>Z</i> )-3-hexenyl acetate	green banana
methyl octanoate	fruity, pungent
ethyl octanoate	green, sour-fruity
methyl benzoate	sweet, fruity
ethyl benzoate	fruity, heavy
( <i>E</i> )-2-hexenal	sweet, fruity, green

<sup>a</sup> Identified GLC peak need not be responsible for the observed odor impression.

**Sample Preparation. Closed-Loop Stripping.** Pulp (275 g, from eight fruits) was mixed in a blender (Braun) with 100 mL of 50% aqueous MeOH. A 100-mL aliquot of this mixture was purged at 25 °C for 2.5 h in an apparatus similar to the one presented by Grob (1973). However, we used a cold trap (0 °C) instead of a heater to prevent water from entering the carbon traps. The activated carbon traps (22 mg; Klimes, Dübendorf, Switzerland) were desorbed with 50  $\mu$ L of CS<sub>2</sub> (Merck, Darmstadt, West Germany). Extracts not used immediately for GC were stored at -20 °C.

**Solvent Extraction.** The edible parts of 15 fruits were cut into the blender and mixed with 250 mL of ice-cold MeOH to inhibit enzyme activities. Insoluble material was separated with a hydraulic press (Hafico, Neuss, West Germany) and discarded. The liquid containing 500 g of fruit juice was made up to 2.2 L with water and extracted with 100 mL of an 8:2 mixture of ether/pentane in a continuous liquid-liquid extractor for 8 h (Drawert et al., 1969). The resulting solution was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to 4 mL in a Kuderna-Danish evaporator with a 12-cm Vigreux column.

**Prefractionation.** The concentrate from solvent extraction was subjected to flash chromatography over silica gel (LiChroprep Si 40; Merck) in a 15 cm  $\times$  1.9 cm (i.d.) column. A stepwise gradient of ether in pentane (100 mL of 10, 90, and 100% ether, respectively) was used. Fractions of 10 mL were collected and pooled according to their odor. The pooled solutions were concentrated to approximately 0.1 mL and stored at -20 °C.

**Capillary GLC Analysis.** A 60-m-long J&W DB-Wax 60 W column (ICT, Frankfurt, West Germany) with 0.32-mm i.d. and a film thickness of 0.25  $\mu$ m was used in a HP 5890 with FID detector and a Spectra Physics SP4290 integrator. For GC/MS a HP 5986 system with an identical column was employed. Helium was used in both cases as carrier gas with a flow of 19.9 cm<sup>3</sup>/s at 150 °C. Samples were injected in split mode and chromatographed with a temperature program from 60 to 220 °C at 4 °C/min and a final hold of 30 min. If not otherwise stated, identification is based on comparison of mass spectra of unknowns with the spectra of authentic reference materials and on retention time.

**GLC Sniffing Analysis.** A 25-m-long CP-WAX-52CB column (Chrompack, Müllheim, West Germany) (0.53-mm i.d., 2.19- $\mu$ m film thickness) was used in a Packard 438A gas chromatograph equipped with a packed-column injector, an effluent splitter (Chrompack-Packard, Maintal, West Germany), and a Shimadzu C-R3A integrator. Approximately 80% of the effluent was diverted through a heated glass-lined capillary to a sniffing mask,

**Table II. Components Identified in Solvent Extract of Naranjilla Juice**

substance	area, %	odor at sniffing port <sup>a</sup>
(a) Esters and Lactones		
ethyl acetate	5.9	estery, fruity
ethyl propanoate	0.2	} sweet, naranjilla
ethyl 2-methylpropanoate	0.2	
methyl butanoate	12.1	cheesy <sup>b</sup>
2-methylpropyl acetate	0.6	
ethyl butanoate	26.7	sweet, naranjilla
ethyl 3-methylbutanoate	<0.1	
butyl acetate	0.1	
methyl ( <i>E</i> )-2-butenolate	0.9	sharp, aldehydic
ethyl ( <i>Z</i> )-2-butenolate	0.1	
3-methylbutyl acetate	1.0	banana
ethyl ( <i>E</i> )-2-butenolate	0.9	fruity, synthetic
methyl hexanoate	0.4	} musty, wet paper
methyl ( <i>E</i> )-2-methyl-2-butenolate	0.2	
ethyl hexanoate	0.7	} sweet, fruity
ethyl ( <i>E</i> )-2-methyl-2-butenolate	0.5	
hexyl acetate	0.2	
( <i>Z</i> )-3-hexenyl acetate	0.4	green banana
2-hexenyl acetate	0.1	
methyl octanoate	<0.1	chemical
ethyl octanoate	0.1	fruity, metallic, slightly sweet
1,2-propanediol diacetate	<0.1	cocoa sweet
ethyl 3-hydroxybutanoate	11.4	tarry
ethanediol diacetate	0.1	
methyl 3-(methylthio)propanoate	<0.1	sweet, synthetic, fruity
methyl benzoate	0.3	pungent
methyl 3-hydroxyhexanoate	<0.1	
ethyl benzoate	0.2	chemical
3-oxo-2-butyl butanoate	<0.1	
ethyl 3-hydroxyhexanoate	17.1	
$\gamma$ -hexalactone	8.2	slightly coconut
ethyl 3-acetoxyhexanoate	2.9	spicy
benzyl acetate	0.1	
phenylethyl acetate	0.1	fresh, floral
methyl 3-hydroxyoctanoate <sup>c</sup>	<0.1	lemon
methyl palmitate	<0.1	
$\gamma$ -dodecalactone	0.1	floral, heavy
ethyl linolate	<0.1	
ethyl linolenate	<0.1	
(b) Alcohols		
2-methylpropanol	0.3	sweet
3-pentanol	<0.1	
2-pentanol	0.1	
butanol	0.1	
3-methylbutanol	0.4	
pentanol	<0.1	
hexanol	0.6	
( <i>Z</i> )-3-hexenol	0.8	
linalool	1.6	floral, sweet
2,4-hexadienol <sup>d</sup>	0.4	
2,4-hexadienol <sup>d</sup>	0.1	
$\alpha$ -terpineol	0.6	
benzyl alcohol	0.2	
(c) Carbonyls		
hexenal	0.1	green, grassy
( <i>Z</i> )-3-hexenal	0.5	green, fruity
( <i>E</i> )-2-hexenal	<0.1	sweet, fruity-green
phenylacetaldehyde	<0.1	sweet, floral, slightly herbal
(d) Acids		
acetic acid	1.5	acidic
butanoic acid	0.4	sweaty
phenylacetic acid	0.2	

<sup>a</sup> Identified GLC peak need not be responsible for the observed odor impression. <sup>b</sup> Probably partial decomposition in sniffing port. <sup>c</sup> Tentatively identified from MS data. <sup>d</sup> Configuration not assigned.

where it was mixed with humidified air. Each sample was sniffed twice by two people trained in that technique. In general, sniffing sessions were finished after 40 min, since preliminary tests showed that no sensorially interesting compounds eluted with higher retention times. Only odor impressions, which have been recorded at least twice in all four runs, albeit with occasionally differing odor descriptions, were recorded as real properties of the eluting

substances. A FID was used as monitor detector. The flow of the carrier gas (He) was adjusted to 31 cm/s with a constant-flow controller. The following temperature program was used: 50 °C for 2.5 min, 4 °C/min to 160 °C, 6 °C/min to 250 °C, hold for 30 min.

## RESULTS AND DISCUSSION

The headspace above a particular sample is considered satisfactory for analysis of flavor substances because it contains the different components in the same concentrations as present to the human nose (Dirinck et al., 1984). However, it is necessary to concentrate headspace volatiles, e.g. by purge and trap methods, in order to obtain sufficient amounts of minor components for identification. We used small charcoal traps in a closed-loop assembly (Grob, 1973) because of the high sampling capacity and the ease of desorption with a few microliters of solvent.

Since purge and trap methods discriminate against less volatile constituents (Parliment, 1986), we also analyzed conventional solvent extracts (Drawert et al., 1969) of naranjilla juice.

Table I shows the purgeable constituents of naranjilla juice and the sensory description of the corresponding peaks at the sniffing port of a gas chromatograph. The results obtained from a juice not mixed with methanol were almost identical and confirmed that the many methyl esters detected are not artifacts.

The coincidence of an identified peak with some odor impression does not require that this specific compound is solely responsible for the odor. The possibility of an underlying minor component strongly influencing the sensory results can never be ruled out.

The components that could be identified in the different fractions of a solvent extract after silica gel flash chromatography are given in Table II with their sensory description. The reported concentrations (area percent in gas chromatograms) are values from single GC runs and therefore only show approximative relationships.

Additionally, to the above-mentioned compounds, we found three regions with interesting sensory properties (sweet, resembling naranjilla) that we could not attribute to any identified substance.

It can be concluded from the results shown in Tables I and II that the methyl and ethyl esters of lower carboxylic acids contribute much to the typical naranjilla flavor. However, it was not possible to find one or more impact compounds. A combination of the identified flavor components did not simulate the naranjilla flavor either. Therefore, we suspect that the yet unidentified substances, which possess strong odors, play an important role in the flavor complex of naranjilla. Further research is required to elucidate these compounds.

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**Registry No.** Methyl propanoate, 554-12-1; methyl 2-methylpropanoate, 547-63-7; ethyl 2-methylpropanoate, 97-62-1; ethyl 3-methylbutanoate, 108-64-5; methyl pentanoate, 624-24-8; ethyl acetate, 141-78-6; ethyl propanoate, 105-37-3; methyl butanoate, 623-42-7; 2-methylpropyl acetate, 110-19-0; ethyl butanoate, 105-54-4; ethyl 2-methylbutanoate, 7452-79-1; butyl acetate, 123-86-4; methyl (*E*)-2-butenate, 623-43-8; ethyl (*Z*)-2-butenate, 6776-19-8; 3-methylbutyl acetate, 123-92-2; ethyl (*E*)-2-butenate, 623-70-1; methyl hexanoate, 106-70-7; methyl (*E*)-2-methyl-2-butenate, 6622-76-0; ethyl hexanoate, 123-66-0; ethyl (*E*)-2-methyl-2-butenate, 5837-78-5; hexyl acetate, 142-92-7; (*Z*)-3-hexenyl acetate, 3681-71-8; 2-hexenyl acetate, 10094-40-3; methyl octanoate, 111-11-5; ethyl octanoate, 106-32-1; 1,2-propanediol diacetate, 623-84-7; ethyl 3-hydroxybutanoate, 5405-41-4; ethanediol diacetate, 111-55-7; methyl 3-(methylthio)propanoate, 13532-18-8; methyl benzoate, 93-58-3; methyl 3-hydroxyhexanoate, 21188-58-9; ethyl benzoate, 93-89-0; ethyl 3-hydroxyhexanoate, 2305-25-1;  $\gamma$ -hexalactone, 695-06-7; ethyl 3-acetoxyhexanoate, 21188-61-4; benzyl acetate, 140-11-4; phenylethyl acetate, 103-45-7; methyl 3-hydroxyoctanoate, 7367-87-5; methyl palmitate, 112-39-0;  $\gamma$ -docecalactone, 2305-05-7; ethyl linolate, 544-35-4; ethyl linolenate, 1191-41-9; 2-methylpropanol, 78-83-1; 3-pentanol, 584-02-1; 2-pentanol, 6032-29-7; butanol, 35296-72-1; 3-methylbutanol, 123-51-3; pentanol, 30899-19-5; hexanol, 25917-35-5; (*Z*)-3-hexenol, 928-96-1; linalool, 78-70-6; 2,4-hexadienol, 120204-34-4;  $\alpha$ -terpineol, 98-55-5; benzyl alcohol, 100-51-6; hexanal, 66-25-1; (*Z*)-3-hexenal, 6789-80-6; (*E*)-2-hexenal, 6728-26-3; phenylacetaldehyde, 122-78-1; acetic acid, 64-19-7; butanoic acid, 107-92-6; phenylacetic acid, 103-82-2.

## LITERATURE CITED

- Dirinck, P.; DePooter, H.; Willaert, G.; Schamp, N. Application of a dynamic headspace procedure in fruit flavour analysis. In *Analysis of Volatiles*; Schreier, P., Ed.; Walter de Gruyter: Berlin, 1984.
- Drawert, F.; Heimann, W.; Emberger, R.; Tressl, R. Gas-Chromatographische Untersuchung pflanzlicher Aromen II. *Chromatographia* 1969, 2, 57-66.
- Duke, J. A. Ethnobotanical observations on the Choco indians. *Econ. Bot.* 1970, 24, 344-366.
- Grob, K. J. Organic substances in potable water and in its precursor Part 1. *J. Chromatogr.* 1973, 84, 255-273.
- Parliment, T. H. Sample preparation techniques for GLC analysis of biologically derived aromas. In *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986.

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