JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Characterization of Monkey Orange (*Strychnos spinosa* Lam.), a Potential New Crop for Arid Regions

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The green monkey orange (*Strychnos spinosa* Lam., Loganiaceae), a tree indigenous to tropical and subtropical Africa, produces juicy, sweet-sour, yellow fruits containing numerous hard brown seeds. The species has recently been introduced into Israel as a potential new commercial crop. However, little is known about its agronomical performance, fruit development and ripening, or postharvest physiology. The current study shows that during ripening in storage, the peel color changes from green to yellow, accompanied by a climacteric burst of ethylene and carbon dioxide emission. Total soluble solids slightly increased during storage, whereas total titratable acidity and pH did not change significantly. The major sugars that accumulated during ripening in storage were sucrose, glucose, and fructose, and the main acids, citric and malic acids. The main volatiles present in the peel of ripe fruits were phenylpropanoids, *trans*-isoeugenol being the major compound.

**KEYWORDS:** ***Strychnos spinosa*; Loganiaceae; sugars; acids; aroma; green monkey orange; ripening**

## INTRODUCTION

The green monkey orange (*Strychnos spinosa* Lam., Loganiaceae) tree is indigenous to tropical and subtropical Africa (*1*). The tree is small, 1−7 m in height, and bears edible, balled-shaped fruits, 6−12 cm in diameter. Unripe fruits have a bright green woody peel (3−4 mm thick), which turns yellow-brown upon ripening (*2*). The fruit has an edible, juicy, sweet-sour pulp, which is pale brown in color and contains numerous hard brown (1−3 cm) seeds. The seeds might be poisonous, but this has not been proven unequivocally (*3*, *4*). The fruit emits a delicate complex of aroma volatiles, which are perceived as a mixture of pineapple, apricot, melon, clove, and citrus.

Monkey orange fruit is not common in the Western world, but the local people of the Kalahari Desert harvest the fruits at the mature green stage and store them in the ground until they are ready to eat. In other parts of Africa, the fruits are usually eaten fresh (*5*), because it is commonly believed that ripe fruits cannot be stored (*1*).

Monkey orange fruits are also used in traditional medicine for the treatment of sexually transmitted diseases (*6*), and the Zulus use the green fruits as an antidote to snakebite (*7*, *8*). A few pharmacoactive compounds have been isolated from the pericarp (*9*, *10*). The pulp is nutritious, containing particularly high levels of Cu [0.46 mg/100 g of fresh weight (fw)], thiamin (0.23 mg/100 g of fw), and nicotinic acid (1.39 mg/100 g of fw), all of which are 20% higher than the average daily requirement (*11*).

There is increasing interest in monkey orange as a potential crop in the southern parts of Africa for marketing in countries already familiar with the fruit. In addition, in light of its adaptability to arid conditions, *S. spinosa* is regarded as a promising potential crop species for other arid regions (*11*). However, little is known about the agronomical performance of this species, about fruit development and ripening, or about the postharvest physiology of the fruit. As a first step toward developing monkey orange as a commercial crop, we have investigated some chemical and physiological changes occurring during the development of the fruits and during postharvest storage.

## MATERIALS AND METHODS

**Plant Material.** Fruits were harvested from 10-year-old *S. spinosa* trees grown from seed at the Besor Experimental Station in the western Negev Desert, Israel. The seeds had originally been collected in various locations in Botswana. The seedlings were planted in sandy loam soil, pH ∼7.5, and irrigated with water containing fertilizer (23N−3P−20K) having a nitrogen concentration of 30−40 mg/L. Fruits were analyzed when fresh (mature green stage) and after storage at 25 °C and 60−70% relative humidity when the color changed to yellow (∼60 days later).

**Fruit Growth and Abscission Rates.** Flowering branches were tagged on the day of anthesis. After the period of massive flower

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10.1021/jf030289e CCC: $25.00 
Published on Web 09/10/2003

abscission, which normally occurs within 2 weeks after anthesis, individual fruitlets were also tagged. For each tree, 50 fruitlets (0.5 mm in length) were selected for examination. Fruit diameter was measured, and abscission rates were recorded at weekly intervals.

**Water Content.** Immediately after harvest, the fruit tissues were separated and samples of each tissue were oven-dried at 70 °C for 48 h for determination of water content.

**Ethylene and Carbon Dioxide Determination.** Individual fruits were enclosed in 1.6-L jars for 1 h at room temperature. Then, a 2-mL sample of air was withdrawn by means of a hypodermic syringe and analyzed for ethylene and carbon dioxide, as previously described (*12*).

**Total Soluble Solids (TSS).** A refractometer (PR-100, Atago) was used for the determination of TSS in diluted sap that had been expressed from the pulp, as previously described (*12*).

**Sample Preparation for Carbohydrate and Acid Determinations.** Pulp tissue, 5 g, was homogenized with 5 mL of doubly distilled water by means of a Polytron homogenizer (PCU, Lucerne, Switzerland). The shaft of the homogenizer was washed with an additional 10 mL of doubly distilled water, which was then combined with the homogenate. The slurry was centrifuged at 10000*g* for 10 min, and the supernatant was collected and kept at −20 °C for further analyses.

**Soluble Sugars.** Total soluble sugars were determined according to the phenol−sulfuric acid method, with glucose as the standard (*13*). The soluble sugars composition was determined as described (*14*). In brief, 1 mL of the above-described extract was diluted with doubly distilled water and filtered through a 0.45-$\mu$m nylon filter (Whatman Inc., Springfield Mill, U.K.). A 10-$\mu$L sample of the filtrate was injected into a Waters Delta 4000 HPLC (Milford, MA), equipped with a Supelco-c-611 ion-exchange column (30 cm × 7.8 mm) and RI detector (Waters 410). Column temperature was 85 °C, and the mobile phase was 0.1 $\mu$M NaOH at a flow rate of 0.9 mL/min. Sugars were identified according to their retention times as compared with those of authentic standards (*14*).

**pH and Organic Acid Determinations.** The pH of freshly extracted fruit juice was measured, and total acidity was determined by titration with 0.1 N NaOH to pH 8.2. Organic acids were determined as previously described (*14*).

**Volatiles.** Peel samples were frozen in liquid nitrogen and then ground to a fine powder with a pestle and mortar. A 1-g sample of the powder was extracted with 15 mL of methyl *tert*-butyl ether (MTBE), containing 10 $\mu$g of isobutylbenzene as the internal standard. The samples were vigorously shaken for 2 h at room temperature. The extracts were dried by passage through a small glass column plugged with glass wool and containing sodium sulfate and then concentrated under a nitrogen stream. Samples of 1 $\mu$L of the concentrated MTBE extracts were analyzed by GC-MS as described before (*14*).

**Tissue Ion Analysis.** Fruit pulp was dried at 70 °C and ground to a fine powder in a coffee grinder. Samples of 0.1 g were digested overnight in 5 mL of a solution containing 70% $HClO_4$, concentrated $HNO_3$ (1:2, v/v), and a few drops of kerosene (*15*). The samples were boiled for 10 min and cooled, and the volume was then made up to 10 mL with doubly distilled water. The samples were then filtered through filter paper, and the concentrations of ions in the filtrate were determined by atomic absorption spectroscopy with an ICP-OES Optima 3000 (Perkin-Elmer, Shelton, CT) (*15*).

## RESULTS AND DISCUSSION

**Fruit Set and Development.** A single monkey orange tree can produce thousands of small flowers, but only a small fraction of them develop into mature fruits. Most of the flowers abscise as early as 2 weeks postanthesis. In addition, fruitlets drop off at a high rate, leaving only a relatively low number of fruits to mature on the tree. To study this phenomenon in more detail, individual fruitlets on four trees were tagged 2 weeks postanthesis, and fruitlet drop was recorded throughout the season. For most of the trees, the major fruitlet drop occurred within 25 days after fruit set, at which time only ∼10% of the initial fruitlets had survived (**Figure 1**). By the end of the following 2 weeks, only 4% of the initial fruitlets were left on three of the trees, but almost all of them developed to maturity. On tree 18 (see **Figure 1**), >25% of the initial fruitlets developed to maturity, in accordance with the above-average yields recorded for this tree (data not shown). It is thus evident that the excessive fruit drop is the main cause of the low yields obtained from trees of this species. The causes of the massive fruit drop are not clear, but they may possibly depend on poor pollination, unfavorable climatic conditions, or physiological stress. It is likely that ameliorating fruitlet drop, either by selection of cultivars or by developing suitable agrotechnical practices, will contribute to higher yields.

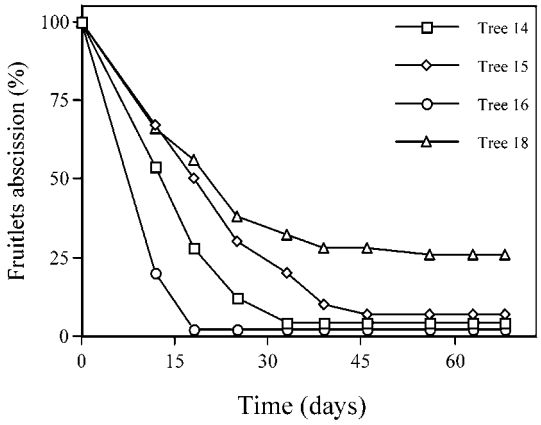


**Figure 1.** Abscission rates of monkey orange fruitlets. Fifty fruitlets were tagged on each tree, and survival was determined at weekly intervals.

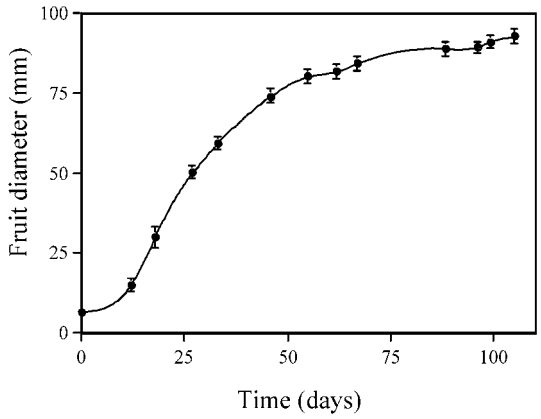


**Figure 2.** Monkey orange fruit growth. Diameters of individual fruits on one tree were determined at weekly intervals. Values are means ± SE ($n$ = 10).

The effects of cultivation conditions in Israel's Negev Desert on fruit growth rates and the patterns of fruit development are not known. This study shows that the growth of the monkey orange fruit, as measured in terms of fruit diameter, follows the sigmoid growth curve typical of many fruits. In the first 2 weeks after anthesis, during which cell division usually occurs, fruit diameter did not change significantly (**Figure 2**). Thereafter, a sharp increase in fruit diameter took place over a period of ∼7 weeks. The fruits reached their full size within ∼100 days, without a further significant increase in fruit diameter, and fruit ripening (up to color break) was completed within 12 months (**Figure 2**). Under the conditions prevailing in southern Africa monkey orange fruits require 12−14 months to develop to full maturity (*1*). Fruit weight varied from 250 to 500 g, with the average weight being ∼330 g (**Table 1**). The average dry weights of the hard fruit tissues, peel, and seeds were ∼56% of the tissues fresh weight. The dry weight of the juicy pulp was ∼18% of the fresh weight. The pulp of each fruit contained

**Table 1.** Performance and Yield Components of Monkey Orange Fruits from Individual Trees[a]

| tree | fruit wt (g) | yield (kg/tree) | pulp (%) | | peel (%) | | seeds (%) | | no. of seeds/fruit |
|---|---|---|---|---|---|---|---|---|---|
| | | | fresh | dry | fresh | dry | fresh | dry | |
| 1 | 348 ± 12 | 19.7 ± 7.5 | 40.0 ± 1.9 | 15.2 ± 0.5 | 43.6 ± 1.5 | 54.2 ± 1.1 | 16.4 ± 0.4 | 51.9 ± 0.5 | 58 ± 6 |
| 20 | 410 ± 139 | 11.2 ± 6.8 | 43.1 ± 1.2 | 18.5 ± 0.9 | 37.1 ± 0.8 | 62.3 ± 4.0 | 19.9 ± 0.5 | 50.1 ± 1.4 | 69 ± 5 |
| 22 | 279 ± 25 | 24.5 ± 9.0 | 36.9 ± 0.8 | 21.0 ± 0.4 | 48.6 ± 2.0 | 65.9 ± 1.6 | 14.6 ± 1.4 | 53.9 ± 1.1 | 53 ± 4 |

[a] Values are means ± SE ($n = 3$).

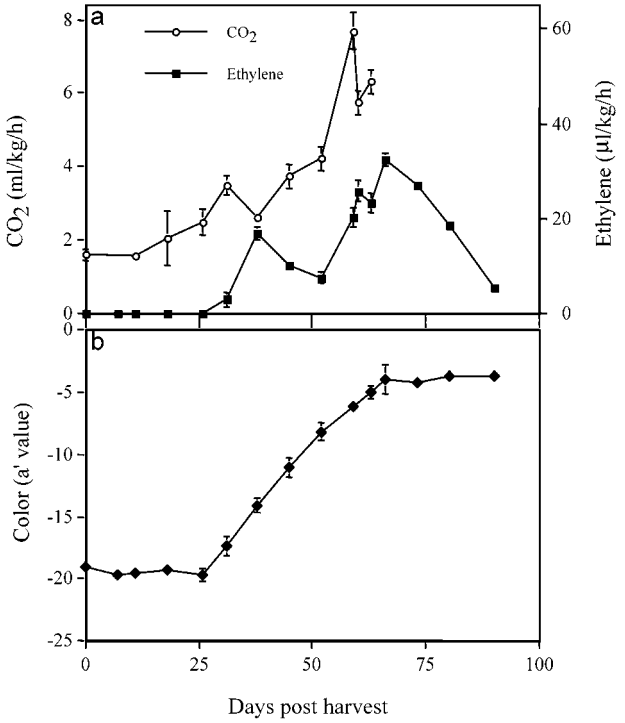


**Figure 3.** Ripening of monkey orange fruits during storage: (a) ethylene production and respiration; (b) color change. The results are shown only for fruits of one tree. Similar results were obtained for fruits from the three other trees. Values are means ± SE ($n = 3$).

~60 hard ovular nonedible seeds, comprising ~17% of the total fruit fresh weight (**Table 1**). The ratio of pulp fresh weight to peel was about 1:1 (~40% each), indicating that the edible portion of the fruit is relatively small. Selection for trees producing fruits with higher proportions of pulp would thus be one of the future goals for commercialization of this crop.

Fruit yields recorded for three successive years gave an average yield per tree of ~18 kg (**Table 1**). This figure is equivalent to a harvest of 9 tons/ha under our growth conditions (assuming 500 trees are planted per hectare). The wide range of yields obtained for the different trees, from 10 to 30 kg, serves to illustrate the potential for enhancing yields by the selection of superior germplasm.

**Carbon Dioxide and Ethylene Production and Color Development during Fruit Ripening.** In climacteric fruits, an increase in respiration and ethylene production marks the beginning of the ripening process. To ascertain whether ripening in monkey orange fruits is climacteric or nonclimacteric, we harvested fruits at the mature green stage and determined carbon dioxide and ethylene emission during ripening of stored fruits. After 20 days in storage, a gradual but slow increase in respiration started, and after 50 days respiration rates reached a peak of 8 mL of $CO_2$/kg/h (**Figure 3a**), a pattern characteristic of climacteric fruits. Ethylene production started to increase 25 days after harvest and reached a peak of 32 $\mu$L/kg/h after 66 days (**Figure 3a**). The levels of carbon dioxide and ethylene produced during ripening were similar to those found in other climacteric fruits, such as apples (*16*). The results clearly indicate that monkey orange fruit is a climacteric fruit and that levels of carbon dioxide and ethylene are reliable indicators of ripening in this fruit. However, the most obvious ripening indicator for monkey orange fruit is the change in peel color from green to yellow. The color break was found to coincide with the increase in ethylene production, indicating that ethylene might be involved in the regulation of chlorophyll degradation during ripening (**Figure 3b**), as is the case in many fruits (*17*).

**Fruit Shelf Life.** Water loss was determined in fruits that had been harvested at the mature green stage and stored at 20 °C for 3 months. The fruits lost up to 15% of their initial fresh weight at a steady rate over 60 days of storage, after which time the rate increased, with up to an additional 20% being lost over 40 days (data not shown). The increase in the rate of water loss coincided with the peak in respiration and ethylene production. It is thus possible that by developing proper storage methodologies, such as increasing the humidity, utilizing controlled atmosphere, or inhibiting ethylene production or perception, the overall shelf life of the fruits could be extended.

**Acid Concentrations and Composition.** The levels of total titratable acidity and the pH values found in the flesh of mature green fruits were similar to those in the flesh of ripe yellow fruits (**Table 2**). In many fruit species, there is a decrease in the contents of acids and an increase in pH during the final stages of ripening, especially during storage. Malic acid, for example, accumulates during the initial developmental stages in many fruits, such as apples, grapes, oranges, and pears, and then decreases to ~50% of its initial value during ripening, due to respiration or conversion of the acid to other metabolites (*18*−*21*). Such a decrease was, however, not found in monkey orange fruits that had been stored for 3 months. Analysis of the composition of acids in the fruit pulp revealed that the main acids at the mature green stage were citric (12.2 mg/g of fw) and malic (7.6 mg/g of fw) acids. Similar levels were detected in yellow fruits after 3 months of storage (**Table 3**). Succinic acid was found in much lower concentrations (2.3 mg/g of fw) and only in ripe yellow fruits (**Table 3**). Low concentrations of organic acids are often reflected in impaired fruit taste. Therefore, maintenance of optimal levels of organic acids is of critical importance for retaining the overall quality of the fruits during storage.

**TSS and Sugar Content and Composition.** TSS levels increased by ~1.6% (~20% of the initial value) during storage. This rise can be partially accounted for by increases in soluble sugars and malic acid (**Table 2**). Because high respiration rates were observed during storage (**Figure 3a**), it was expected that the concentrations of sugars and acids would decrease as a result of substrate loss due to respiration, as is the case in most climacteric fruits. Surprisingly, this was not the case as evidenced by increases in TSS and sugar and acid contents (**Table 2**). The source of carbon required for these increases is not known, and our data do not support the accepted view that

**Table 2.** Total Soluble Solids, Sugars, Acidity, and pH of Monkey Orange Fruits at Two Ripening Stages[a]

| | TSS (%) | | total sugar (mg/g of fw) | | total acidity ($\mu$mol of $H^+$/g of fw) | | pH | |
|---|---|---|---|---|---|---|---|---|
| tree | MG[b] | RY[c] | MG | RY | MG | RY | MG | RY |
| 1 | 17.3 ± 1.1 | 18.1 ± 1.0 | 112 ± 10 | 125 ± 6 | 279 ± 15 | 242 ± 16 | 2.91 ± 0.23 | 3.33 ± 0.04 |
| 20 | 14.8 ± 0.5 | 16.5 ± 1.0 | 72 ± 21 | 111 ± 8 | 187 ± 11 | 204 ± 30 | 2.98 ± 0.15 | 2.98 ± 0.06 |
| 22 | 20.4 ± 0.8 | 22.8 ± 0.8 | 128 ± 4 | 150 ± 20 | 214 ± 22 | 214 ± 36 | 2.58 ± 1.07 | 2.61 ± 0.02 |

[a] Values are means ± SE ($n = 3$). [b] MG, mature green. [c] RY, ripe yellow.

**Table 3.** Changes in Concentrations of Sugars and Acids of Monkey Orange Fruits at Two Ripening Stages[a]

| | | sugars (mg/g of fw) | | | acids (mg/g of fw) | | |
|---|---|---|---|---|---|---|---|
| tree | ripening stage | sucrose | glucose | fructose | citric | malic | succinic |
| 1 | MG[b] | 72 ± 5 | 16.7 ± 1.1 | 2.5 ± 0.7 | 13.5 ± 0.8 | 11.1 ± 1.0 | 0 |
| | RY[c] | 62 ± 5 | 16.7 ± 1.0 | 3.5 ± 2.5 | 12.4 ± 1.1 | 9.3 ± 0.3 | 2.3 ± 0.4 |
| 20 | MG | 44 ± 3 | 16.8 ± 1.2 | 13.0 ± 1.5 | 11.9 ± 1.5 | 6.3 ± 0.7 | 0 |
| | RY | 48 ± 9 | 17.2 ± 0.2 | 9.2 ± 1.2 | 10.2 ± 0.7 | 8.3 ± 1.2 | 2.9 ± 0.1 |
| 22 | MG | 86 ± 1 | 15.3 ± 2.5 | 7.8 ± 2.5 | 11.4 ± 0.4 | 5.4 ± 0.2 | 0 |
| | RY | 66 ± 10 | 29.7 ± 3.6 | 14.5 ± 2.5 | 9.6 ± 0.9 | 8.9 ± 0.4 | 1.8 ± 0.2 |

[a] Values are means ± SE ($n = 3$). [b] MG, mature green. [c] RY, ripe yellow.

**Table 4.** Elemental Composition of Monkey Orange Fruits[a]

| | element concn (mg/100 g of fw) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| tree | P | Zn | Fe | Mg | Cu | Ca | Na | K |
| 1 | 12.0 ± 2.7 | 0.28 ± 0.14 | 0.34 ± 0.11 | 38.3 ± 5.1 | 0.043 ± 0.008 | 20.1 ± 6.5 | 6.0 ± 1.4 | 582 ± 20 |
| 20 | 13.0 ± 2.3 | 0.18 ± 0.05 | 0.26 ± 0.11 | 24.6 ± 4.4 | 0.040 ± 0.012 | 24.1 ± 5.7 | 16.5 ± 3.0 | 421 ± 42 |
| 22 | 12.0 ± 2.4 | 0.29 ± 0.07 | 0.42 ± 0.13 | 23.6 ± 5.5 | 0.054 ± 0.010 | 25.0 ± 4.0 | 10.0 ± 0.9 | 442 ± 51 |

[a] Values are means ± SE ($n = 3$).

the respiratory burst is driven by the increase in respiratory substrates (*22*). The phenomenon of increases in sugars to maximal levels much earlier than the onset of the climacteric respiratory burst during storage has also been noted in apples (*23*).

Sucrose was the major carbohydrate found in monkey orange fruits, at both the mature green and yellow ripe stages (**Table 3**). In the fruits from one of the trees (tree 22), a decrease of 20% in sucrose levels was detected during ripening (**Table 3**). In the fruits of all three trees sampled, concentrations of glucose and fructose were ~3-fold lower than those of sucrose. Changes in glucose and fructose contents during ripening were not significant, except for those in the fruits of tree 22, in which the levels of glucose and fructose both increased by ~2-fold (**Table 3**). These increases were in accordance with the decrease in sucrose levels found in the fruits of this tree. These changes in concentrations of sugars were corroborated by the organoleptic test panelists, who graded the fruits of tree 22 as superior to those of the other two (data not presented). The data presented here indicate that the rates of conversion of sucrose to glucose and fructose at the onset of ripening differ among the various trees and exemplify the intrinsic variation that exists among the plant material grown from seeds brought from different locations in Botswana.

**Elemental Analysis.** The nutritional value of monkey orange fruits at the yellow ripe stage with regard to mineral composition was found to be comparable with that of other fruits. The levels of P, Mg, Fe, and Ca in fruits harvested in this study were about half the values reported for fruits grown in Africa, and the levels of Cu were 10-fold lower than reported values (**Table 4**; *11*). In contrast, the level of Zn was 2-fold higher in our study than that reported previously (**Table 4**; *11*). Levels of K and Na were also higher in fruits grown in Israel. On the basis of the mineral analysis of fruits from trees grown under our conditions, we conclude that the nutritional value of monkey orange fruits is similar to that of other fruits. The differences in concentrations of minerals between fruits of trees growing wild in Africa and those grown in Israel may be due to differences in chemistry and physical properties of the soils and in other climatic and agrotechnical conditions (including irrigation and fertilization), which might affect ion uptake.

**Table 5.** Composition of Volatiles in the Peel of Monkey Orange Fruits at the Ripe Yellow Stage

| compound identified[a] | $\mu$g/g of fw |
|---|---|
| *trans*-$\beta$-ocimene (6.7) | 161.5 ± 117.5 |
| chavicol (13.3) | 172.0 ± 75.0 |
| indole (14.6) | 3.0 ± 3.0 |
| *p-trans*-anol (16.2) | 647.5 ± 68.0 |
| eugenol (16.4) | 307.0 ± 30.0 |
| dihydroeugenol (16.8) | 123.5 ± 31.0 |
| vanillin (17.9) | 47.5 ± 16.5 |
| *trans*-isoeugenol (19.5) | 4762.5 ± 1643.5 |
| 2,6-dimethoxyphenol (23.9) | 24.0 ± 12.0 |

[a] Retention times in minutes using an HP5 column are indicated in parentheses. Values are means ± SE ($n = 9$).

**Volatiles.** Mixtures of volatile organic compounds determine the aroma properties of food. The main volatiles present in the peels of mature ripe yellow monkey orange fruits are phenylpropanoids, the major compound, *trans*-isoeugenol, accounting for >75% of the total volatiles (**Table 5**). *trans*-Isoeugenol is normally a minor component of many commercial essential oils, such as clove and cinnamon leaf, and is usually found together with eugenol as the major component and with small amounts

of the *cis* isomer (*24*). Moreover, synthetic isoeugenol is a mixture of *trans* isoeugenol with lower levels of the *cis* isomer. In monkey orange peels we have exclusively found the *trans* isomer. Synthetic isoeugenol has a delicate faint clove odor and is used extensively in perfumery as a component of floral fragrances (*24*). In addition to *trans*-isoeugenol, lower amounts of eugenol, which has a pungent clove aroma, and of phenylpropenes, such as dihydroeugenol, *p-trans*-anol [4-(1-propenyl)-phenol], and chavicol, were also found (**Table 5**). Except for eugenol, the other components do not normally accumulate in plants: *p-trans*-anol and chavicol serve as precursors for the biosynthesis for the more common 4-methoxylated phenylpropanoids, such as *trans*-anethole and estragole (*25*, *26*). In addition, small amounts of the monoterpene hydrocarbon *trans*-$\beta$-ocimene were found in the peels. Lower levels of other phenolic materials, such as vanillin and 2,6-dimethoxyphenol, as well as the nitrogen-containing compound indole were also detected. Despite the low levels of these compounds, they still might contribute significantly to the overall appealing aroma of the peel of the monkey orange fruits. None of the aforementioned compounds were found in the peels of immature fruits, which lack the typical aroma or ripe yellow fruits.

Indigenous peoples of Africa have known monkey orange for centuries, but the potential of this species for intensive cultivation has only just begun to be explored. As a crop, it has remarkable agronomical potential that has not fully been appreciated. The high variability observed in our study will facilitate selection for high-yielding and superior quality varieties. This selection, coupled with the development of adequate agrotechnical and storage practices, can make this fruit a promising profitable new crop for arid regions.

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**Received for review April 16, 2003. Revised manuscript received July 23, 2003. Accepted July 24, 2003.**

JF030289E