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Changes in Sugars, Acids, and Volatiles during Ripening of Koubo [*Cereus peruvianus* (L.) Miller] Fruits

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The columnar cactus *Cereus peruvianus* (L.) Miller, Cactaceae (koubo), is grown commercially in Israel. The unripe fruits are green, and the color changes to violet and then to red when the fruit is fully ripe. The content of soluble sugars was found to increase 5-fold during ripening. Glucose and fructose were the main sugars accumulated in the fruit pulp, and each increased from 0.5 to 5.5 g/100 g fresh weight during ripening. The polysaccharides content decreased during ripening from 1.4 to 0.4 g/100 g fresh weight. The titratable acidity decreased and the pH increased during ripening. The major organic acid found in the fruit was malic acid, which decreased from 0.75 g/100 g fresh weight at the mature green stage to 0.355 g/100 g fresh weight in ripe fruits. Citric, succinic, and oxalic acids were found in concentrations lower than 0.07 g/100 g fresh weight. Prominent accumulation of aroma volatiles occurred toward the end of the ripening process. The main volatile found in the ripe fruit was linalool, reaching concentrations of 1.5−3.5 μg/g fresh weight.

KEYWORDS: Koubo; *Cereus peruvianus*; Cactaceae; sugars; acids; aroma; ripening; koubo cactus; pitaya; volatiles

INTRODUCTION

Cereus peruvianus (L.) Miller (apple cactus, known also as koubo) is a large thorny columnar cactus, native to the subtropical southeastern coast of South America (*1*). *C. peruvianus* is also common as an ornamental plant (*1*, *2*). It has been recently introduced to cultivation in Israel in the framework of our program for developing new crops suitable for the Negev Desert (*3*, *4*). *C. peruvianus* is a crassulacean acid metabolism (CAM) plant; its water-use efficiency is high, and therefore water requirements are low (about 150 mm/year). *C. peruvianus* has a relatively high growth rate and can produce fruits 3−4 years after planting from seeds and 2−3 years after planting from cuttings. A 7-year-old plant can bear 60−80 kg of fruits annually. The berry-like nonclimacteric fruit has a smooth and spineless yellow to deep red peel. The pulp is white, juicy, and aromatic and contains numerous soft, black, edible seeds (*5*). *C. peruvianus* fruit development is characterized by a double-sigmoid curve with three stages: two periods of rapid growth separated by a period of moderate growth. Fruit ripening occurs during the last developmental stage (*6*, *7*). Toward the end of the ripening process, fruits tend to crack due to uncoordinated growth of fruit tissues (*8*). To avoid cracking, fruits are usually harvested before full ripening, a practice that might affect the quality and full flavor of the marketed fruits.

An important attribute to fruit quality and consumer acceptability is its overall flavor (*9*, *10*). An increase in sugar content, a decrease in organic acids, enhanced accumulation of aroma volatiles, and changes in fruit color characterize the ripening of fruits. These changes are caused by a series of concerted biochemical and physiological processes (*11*, *12*). Studying the phytochemistry of the ripening fruit will contribute to our understanding of the biochemical and physiological processes in the developing fruit and will lead to improving the quality of the fruit for consumers (*13*, *14*). Initial characterization of fruit development has been carried out on *C. peruvianus* growing in Israel (*6*, *15*). Here we present a detailed study of some of the changes that affect the taste and flavor of *C. peruvianus* fruits.

MATERIALS AND METHODS

Plant Materials. Fruits from clone G2 (*4*) were collected in 2000 from a commercial orchard at Sede-Nizan (situated in the western part of the Israeli Negev Desert, on sandy soil). The plants had been established from cuttings in 1995. Spacing of the plants was 1.5 m within the rows and 3 m between rows. Maximum temperatures were about 30−35 °C during the summer (June−September) and decreased to 20 °C in autumn (October−December). Minimum temperatures were about 20 °C during the summer and decreased to 10 °C in autumn. Plants were drip-irrigated during the dry season only, in an overall amount of 150 mm/year. Fertilizer containing N−P−K (23:3:20) at a

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concentration of 30 mg/L N was applied with the irrigation water. Since *C. peruvianus* is self-incompatible, the flowers were hand cross-pollinated with pollen of other clones and tagged (5). Flowering occurs in a few waves during the summer. Therefore, fruits were harvested during three independent periods along the ripening season: (A) fruit developing during June−July, (B) fruit developing during August−October, and (C) fruit developing during October−December. Four ripening stages were determined according to days post-anthesis (DPA) and peel color for periods A and B as follows: (1) mature green (37−39 DPA), (2) breaker (42−44 DPA), (3) violet (48−50 DPA), and (4) red ripe (50−52 days DPA, usually cracked). The period of fruit ripening in season C lasted longer than seasons A and B. Therefore, the stages were determined as follows: (1) mature green (45 DPA), (2) breaker (60 DPA), (3) violet (85 DPA), and (4) red ripe (90 DPA, usually cracked).

Chemicals. All reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Standards for the aroma compounds were from our collection.

Sample Preparation. Three to five fruits were randomly sampled at each ripening stage during the three described periods (A, B, and C). All measurements were performed on fresh pulp tissue. Fruit pulp was separated from the peel, and 10 g of fresh pulp from the fruit center was used for aroma determination. The rest of the tissue was frozen with liquid nitrogen and kept at −20 °C for subsequent analyses.

Peel Color. Peel color was determined with a Minolta Chroma Meter (CR-200, Ramsey, NJ) at two points on the fruit perimeter. Color was expressed as *a** values, describing color change from bluish-green to purplish-red (*16*).

pH and Total Acids. pH and total acids were measured on aqueous pulp extracts prepared as follows: Five grams of pulp tissue was homogenized with 5 mL of double-distilled water (ddw) by means of a Polytron (PCU, Lucerne, Switzerland). The shaft was washed with an additional 10 mL of ddw and added to the homogenates. The samples were then centrifuged at 10000*g* for 10 min. The pH values of the supernatants were determined. Total acidity was measured by titration with 0.05 N NaOH to pH 8.2.

Sugars and Organic Acids. Five grams of pulp was homogenized with 5 mL of ethanol (95% v/v) with a Polytron. The shaft was washed with another 10 mL of ethanol (95%), and both samples were combined and centrifuged at 12000*g* for 15 min at 4 °C. The pellet was resuspended in 5 mL of aqueous ethanol (80% v/v), and the samples were centrifuged at 12000*g* for 15 min at 4 °C. The two supernatants were combined and evaporated to a minimal volume (∼1 mL) in a water bath (30 °C) under a fan. The samples were diluted to a final volume of 10 mL with ddw and used for soluble sugars and organic acid determinations (*17*). The pellet was dried in a fume-hood and used for polysaccharide analyses (see below).

One milliliter of extract was used for determination of total soluble sugars using the phenol−sulfuric acid method (*18*). Ethanol-soluble sugars were analyzed by HPLC. One milliliter of extract was diluted with ddw (dilution volume was determined according to the fruit ripening stage) and filtered through a 0.45-μm nylon filter (Whatman Inc., Springfield Mill, UK), and 10 μL was injected to a Waters Delta 4000 HPLC (Milford, MA) equipped with a Supelcogel-C-611 (30 cm × 7.8 mm, Supelco, Bellefonte, PA) ion-exchange column and an RI Waters 410 detector. The column temperature was 85 °C. The mobile phase was 0.1 μM NaOH at a flow rate of 0.9 mL/min. Sugars were identified according to their retention time by comparison with authentic standards.

Organic acids were analyzed from the same extracts by first removing the neutral compounds by a strong anion-exchange minicolumn (3 mL, Strata SAX, Phenomenex, Hemelhempstead, UK). The column was preconditioned with 2 mL of methanol and washed with 2 mL of ddw at a flow rate of 0.2 mL/min. Eight milliliters of extract, previously adjusted to pH 8.0 with 0.5 N NaOH solution, was passed through the column at a flow rate of 0.2 mL/min. The column was washed with 10 mL of ddw, preheated to 70 °C. The organic acids were eluted with two 1-mL aliquots of 0.5 M sulfuric acid at a flow rate of 0.2 mL/min. The samples were filtered through 0.2-μm nylon filters, and 10 μL was injected into the HPLC equipped with an ICSep-ICE-ION-300 (30 cm × 7.8 mm, Transgenomic, Omaha, NE) ion-exchange column and a Transgenomic ICE-GC-801 guard column (*19*). The mobile phase was 0.0085 N sulfuric acid at a flow rate of 0.4 mL/min at room temperature. Organic acids were identified according to their retention times by comparison to authentic standards.

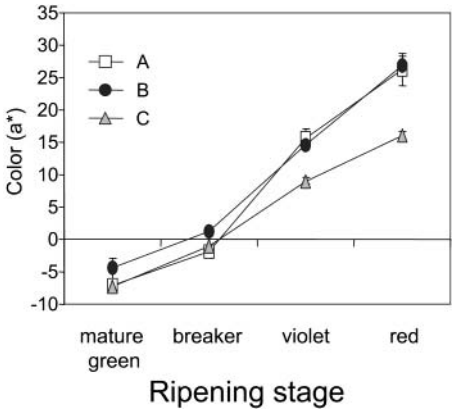

Figure 1. Change of peel color during fruit ripening of *C. peruvianus*. Peel color was determined during harvest periods A, B, and C as indicated, using a Minolta ChromaMeter at two points on the fruit perimeter. Values presented are means ± SE ($n = 3$).

Polysaccharides. The pellets of the ethanolic extracts were evaporated overnight in a fume-hood at room temperature. The polysaccharides were incubated in 10 mL of 35% (v/v) $HClO_4$ for 2 h in a water bath at 60 °C (*20*). The hydrolysate was filtered through Whatman No. 1 filter paper, and the concentration of sugars was determined by the phenol−sulfuric acid method (*18*).

Volatiles. Volatiles were extracted from the fruit pulp by solvent extraction using methyl *tert*-butyl ether (MTBE) (*21*). Seven grams of fresh pulp was frozen in liquid nitrogen and immediately ground with a pestle in a mortar to a fine powder. Five grams of the pulverized tissue was extracted with 15 mL of MTBE containing 10 μg of isobutylbenzene as an internal standard. The samples were vigorously shaken for 2 h at room temperature. The extracts were dried by passing through a small (Pasteur pipet) column containing sodium sulfate plugged with glass wool and then concentrated under a gentle nitrogen stream to a 0.5 mL volume (*22*).

Samples consisting of 1 μL of the concentrated MTBE extracts were analyzed as described before (*21*). Briefly, extracts were analyzed on a Hewlett-Packard GCD gas chromatograph equipped with a HP5 (30 m × 0.25 mm) fused-silica capillary column. Helium (1 mL/min) was used as a carrier gas with splitless injection. The injector temperature was 250 °C, and the detector temperature was 280 °C. Conditions used were as follows: initial temperature, 70 °C for 2 min, followed by a ramp of 70 to 200 °C at a rate of 4 °C/min, and 10 additional min at 200 °C. Masses between 45 and 450 *m*/*z* were recorded. Identification of the main components was done by co-injection and comparison of the EI-MS obtained with authentic standards and complemented with computerized libraries, except for 3,7-dimethyl-1,5-octadiene-3,7-diol, which was tentatively identified on the basis of EI-MS only (*21*, *38*).

RESULTS AND DISCUSSION

Color. Fruit color can serve as an index for determining ripening stage and optimal harvest time for various fruits (*23*, *24*). *C. peruvianus* (clone G2) peel color changes from green to violet at the early ripening stages and from violet to red at the end of the ripening process. The first color change appears near the perianth scar at the breaker stage; complete violet color is attained about a week later. With further ripening, the peel color changes to red, and this is usually accompanied by fruit cracking. The *a** value was found to appropriately reflect the color changes (**Figure 1**). We also found a significant correlation between fruit color (*a** value) and soluble sugars concentration [correlation coefficients (*r*) in the ripening seasons A, B, and C were 0.969, 0.973, and 0.967, respectively]. Therefore, fruit

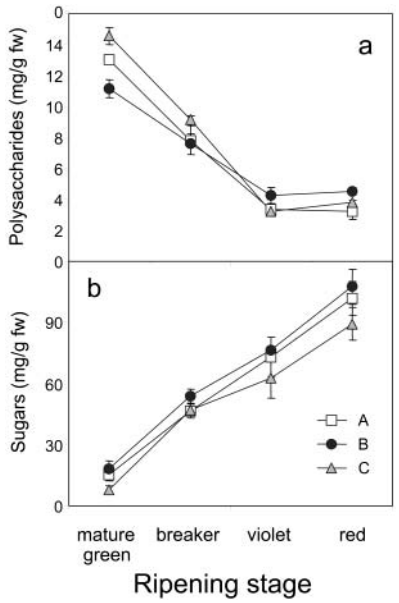

Figure 2. Changes in polysaccharides (a) and total ethanol-soluble sugars (b) during fruit ripening of *C. peruvianus*. Levels were determined during harvest periods A, B, and C as indicated. Values presented are means ± SE ($n = 3$).

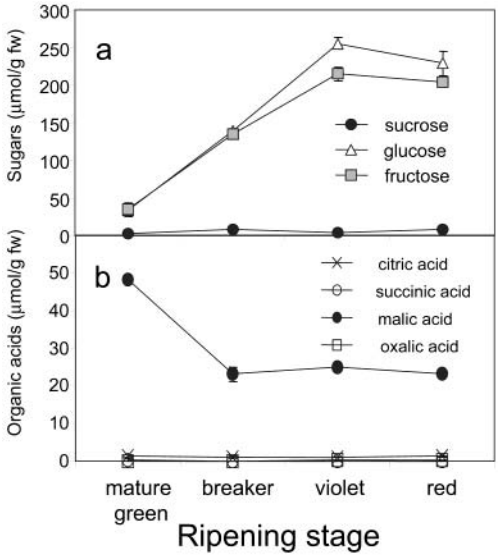

Figure 3. Sugars and organic acid composition in *C. peruvianus* pulp during fruit ripening. The data shown are for harvest period A. Values presented are means ± SE ($n = 3$).

color (a^* value) can serve as a reliable index that reflects the physiological stage of fruit ripening and can be useful for determining optimal harvest time.

Polysaccharide and Soluble Sugars Content. The ethanol-insoluble polysaccharides content decreased from 14 mg/g fw at the mature green stage to 4 mg/g fw at the violet stage and remained constant during the last ripening stage (**Figure 2a**). The levels of ethanol-soluble sugars in the fruit pulp increased 5-fold, from 20 mg/g fw at the mature green stage to 110 mg/g fw at the mature red stage (**Figure 2b**). Fructose and glucose were the main sugars accumulated in the fruit pulp, as determined by HPLC. Their ratio was 1:1, and each increased from 25 to 275 μmol/g fw during ripening. The concentrations of sucrose were low (0−10 μmol/g fw) and did not change significantly during ripening (**Figure 3**). Similar sugar compositions were found in strawberries, grapes, and *Opuntia ficus-indica* fruits (*25*−*27*). The decrease in polysaccharide content (only about 10 mg/g fw) is too low to account for the increases in soluble sugars (more than 100 mg/g fw). The starch concentration in mature green koubo fruits was about 6 mg/g fw (*8*); therefore, it is likely that the observed accumulation of fructose and glucose during ripening is dependent on assimilate transport from the mother plant. Furthermore, fruits that were harvested at the mature green stage contained lower levels of soluble sugars as compared to red ripe fruits (**Figure 2b** and ref *15*). The data presented above have major importance in determining harvest time. To obtain fruits of high quality with high sugar concentration, it is recommended to postpone fruit harvest as much as possible (before fruit cracks).

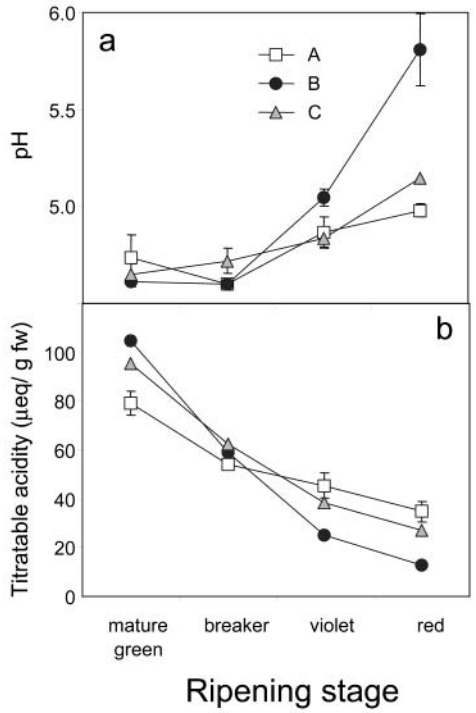

Figure 4. Changes in pH (a) and titratable acidity (b) in *C. peruvianus* pulp during fruit ripening at the seasons A, B, and C. Values are means ($n = 3$) ± SE.

pH and Titratable Acidity. The pH of the fruit pulp increased from 4.6 to about 5 during ripening, while titratable acidity decreased from 100 μequiv/g fw at mature green stage to 30 μequiv/g fw at red stage (**Figure 4**). These results are in agreement with those found in other *C. peruvianus* lines (*15*). Lower values of titratable acidity and higher values of pH were measured in ripe fruits at harvest period B as compared to other periods. Climatic and agronomical conditions such as temperature, irrigation, and fertilization regimes might influence fruit acidity (*28*). Fruits that ripened in season B were exposed to higher temperatures in their first stages of development, which can partially account for those differences. The correlation between pH and titratable acidity was not significant in all the ripening seasons. A similar phenomenon was also found for strawberries (*17*).

The major organic acid found in the fruit was malic acid, which constituted 90% of the fruit's organic acids. Upon the change from mature green stage to breaker stage, the content of malic acid decreased by half (from 50 to 25 μmol/g fw) and remained constant. Citric, succinic, and oxalic acids were found in concentrations lower than 4 μmol/g fw (**Figure 3b**). Malic acid, a key intermediate in CAM metabolism, is also a substrate in respiration and is one of the most abundant organic acids

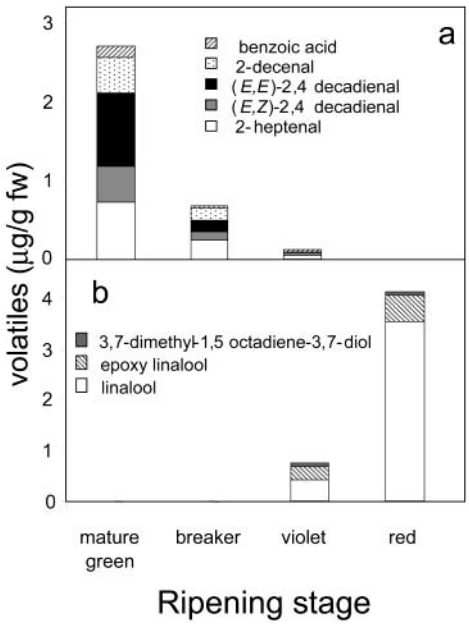

Figure 5. Major volatiles present in *C. peruvianus* pulp during fruit ripening. (a) Volatiles with decreasing concentrations during ripening. (b) Volatiles with increasing concentrations during ripening. Means ($n = 3$) are depicted.

found in fruits (*29*). In tomatoes, apples, grapes, and pears, malic acid accumulates in the first developmental stages. Malic acid content decreases by about 50% during ripening, due to respiration (*30*−*34*). It might also be that the same phenomenon occurs in *C. peruvianus* fruits during ripening. Interestingly, in *Opuntia ficus-indica*, a plant of the same family as *C. peruvianus* and a CAM plant, oxalic acid (and not malic acid) is the main acid reported in mature fruits (*35*).

Main Aroma Volatiles Accumulated in the Ripening Fruits. The major volatile compounds found in mature green fruits were benzoic acid and the following aldehydes: 2-heptenal, 2-decenal, (*E*,*Z*)-2,4-decadienal, and (*E*,*E*)-2,4-decadienal (**Figure 5a**). These compounds, which impart a "green note" to the nonripe fruit, were found in low concentrations (0.1−1 μg/g fw) and were practically absent in the ripe fruits. Decreases in volatile aldehydes during ripening have also also been found in other fruits such as peaches, apples, and melons (*36*−*38*).

A prominent accumulation of aroma volatiles, which occurred toward the end of the fruit ripening process, coincided with the change of peel color to full red. The monoterpene linalool and its derivatives (epoxy linalool and 3,7-dimethyl-1,5-octadiene-3,7-diol) constituted about 99% of total volatiles of the ripe fruits (**Figure 5b**). Linalool appeared first in the volatile profile of violet fruits, at low concentrations of about 0.1−0.5 μg/g fw. Its content rose significantly during ripening, reaching concentrations of about 1.5−3.5 μg/g fw in mature red fruits. Linalool, an acyclic monoterpene alcohol that imparts a sweet floral note, is a component of the aroma of many fruits, including peaches, guavas, strawberries, nectarines, papayas, passion fruits, tomatoes, litchi, oranges, and prickly pears (*28*, *36*, *39*−*47*). Generally, linalool is present together with other volatile chemicals that impart the aroma to the different fruits. In the case of *C. peruvianus*, linalool and its derivatives constitute more than 99% of the volatiles present in the fruits and are probably key substances in determining the unique aroma of this new crop.

Conclusions. *C. peruvianus* is a promising new crop for arid and semi-arid zones but has been cultivated under controlled agrotechnical conditions for only a relatively short time. Information on the factors that control fruit quality are essential for the future success of this crop, ensuring high revenues for farmers and acceptance by consumers. Sugar content is crucial for fruit quality and taste and needs to be easily determined before harvest. We have shown that peel color, as determined by *a** values, is a reliable parameter that is highly correlated to the sugar content of the fruits. This information is crucial for the determination of optimal fruit harvesting time. Other changes that take place during ripening and affect quality include a decrease in polysaccharide and organic acids content, increases in fructose and glucose levels, and pronounced increases in volatiles (almost exclusively linalool and its derivatives). The highest levels of the aroma compounds are attained at the red stage. However, if left on the tree, the fruits tend to crack when turning red, a problem that is usually alleviated by early harvest (at the violet stage) followed by storage under controlled conditions. Under such conditions, fruits turn red and ripen without cracking. A better understanding of the factors controlling the physiological changes involved, and the contribution of each individual factor to fruit quality, will contribute to the production of fruits with superior aroma and taste.

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