# Characterization of Volatiles in Bullock's Heart (*Annona reticulata* L.) Fruit Cultivars from Cuba

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Volatile compounds were isolated from four cultivars of bullock's heart fruit (Cenizo, Rojo, Verde, and De Ojo) by simultaneous steam distillation–solvent extraction. Compounds were identified by HRGC and capillary GC-MS. One hundred and eighty compounds were identified in the aroma extracts, of which α-pinene, β-pinene, myrcene, limonene, terpinen-4-ol, and germacrene D were found to be the major constituents. Fruit from cv. De Ojo containing the highest concentration of total volatile and the highest major terpenoid content had the highest custard-like and overall fruity aroma intensity. The presence of many terpenic compounds is thought to contribute to the unique flavor of the bullock's heart fruit.

KEYWORDS: Volatiles; bullock's heart; GC-MS; terpenic compounds

## INTRODUCTION

The Annonaceae family contains a considerable number of fruits of economic significance (*1*). Of these, bullock's heart (*Annona reticulata* L.) is very popular in Cuba, where it is commonly known as *mamón*. It has a smooth skin, which becomes dull red when the fruit is ripe. Its custard-like pulp is rather sugary and less flavorful than that of other species. It is usually eaten as a dessert fruit but can also be made into drinks and ice creams.

Despite the characteristic desirable flavor of this fruit, there is little information on its volatile components. The volatile compound composition of bullock's heart fruits from Malaysia was investigated by capillary GC and GC-MS following isolation by simultaneous steam distillation–extraction. A total of 52 components were identified in the aroma concentrate, of which terpenoid constituents were found to be dominant (*2*). Volatile components of bullock's heart growing in Cuba were isolated by simultaneous steam distillation–solvent extraction and analyzed by GC-MS. The major volatiles were α-pinene, β-pinene, and germacrene D from the 49 identified constituents (*3*).

The present investigation was conducted to identify and estimate the levels of the volatile constituents of the four most important cultivars of bullock's heart fruit grown in Cuba.

## MATERIALS AND METHODS

**Materials.** Fruits from four cultivars, Cenizo, Rojo, Verde, and De Ojo (six samples for each one), were collected at maturity from the National Botanical Garden near Havana and immediately processed.

Fruits were hand peeled, the stem and blossom portions were cut off, and seeds were separated. Tissue was rapidly pulped into a slurry with a commercial blender. Pure reference standards were purchased from Aldrich (Steinheim, Germany). Diethyl ether was purchased from Merck (Darmstadt, Germany).

**Sample Preparation.** After addition of an internal standard (methyl undecanoate, 2 mg), pulp (200 g) was blended with distilled water (800 mL) and simultaneously distilled and extracted for 60 min in a Likens–Nickerson microapparatus with 25 mL of diethyl ether (previously redistilled and checked as to purity). The volatile concentrate was dried over anhydrous sulfate and concentrated to 0.6 mL in a Kuderna–Danish evaporator with a 12 cm Vigreux column and then to 0.2 mL with a gentle nitrogen stream.

**HRGC and GC-MS Analyses.** A Konik 2000 GC equipped with a 30 m × 0.25 mm (0.25 μm film thickness) DB-5 J&W Scientific fused-silica capillary column and a flame ionization detector was used. Injector and detector temperatures were both 250 °C. Oven temperature was held at 60 °C for 10 min and then raised to 280 °C at 4 °C/min and held for 40 min. Carrier gas (hydrogen) flow rate was 1 mL/min. The retention times from a series of straight-chain alkanes ($C_6$–$C_{24}$) were used to calculate retention indices for all identified compounds and for reference standards. These conditions were used for quantitative analysis, by the internal standard method. The recovery of the method was determined by the standard addition technique applied to a sample. The analytes [α-pinene, limonene, ethyl hexanoate, 1-hexanol, (*Z*)-3-hexenol, ethyl octanoate, (*E*)-β-caryophyllene, and α-terpineol] were added at two different concentrations. The average recoveries were ∼89–102%, and their relative standard deviations were <10%.

GC-MS analyses were performed on a Hewlett-Packard model 6890 coupled to an HP 5973 mass spectrometer. It was fitted with a CP-SIL-5CB Chrompack fused-silica column (25 m × 0.25 mm, 0.25 μm film thickness). Temperature programming was as follows: from 60 °C (10 min) to 280 °C at 4 °C/min and held for 40 min. Injector temperature was 250 °C, transfer line temperature was 250 °C, and carrier gas (helium) flow rate was 1 mL/min. The spectrometer was operated in electron impact (EI) mode at 70 eV with the scan range

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Volatiles in Bullock's Heart Fruit

*J. Agric. Food Chem.,* Vol. 51, No. 13, 2003   **3837**

**Table 1.** Volatile Compounds Identified in Bullock's Heart Fruits from Cuba

| compound | RI[a] | concentration (mg/kg) in cv. Cenizo | Rojo | Verde | De Ojo |
|---|---|---|---|---|---|
| acetaldehyde[b,c] | 435 | <0.01 | <0.01 | <0.01 | <0.01 |
| ethanol[b,c] | 500 | 0.68 | 0.23 | 0.47 | 0.71 |
| 1-propanol[b,c] | 568 | <0.01 | <0.01 | 0.02 | 0.07 |
| acetic acid[b,c] | 600 | <0.01 | 0.12 | 0.01 | >0.01 |
| 2,3-butanedione[b,c] | 612 | <0.01 | <0.01 | 0.03 | 0.04 |
| ethyl acetate[b,c] | 615 | 0.01 | 0.04 | 0.01 | 0.13 |
| isobutanol[b,c] | 622 | <0.01 | 0.03 | <0.01 | 0.76 |
| 3-methylbutanal[c] | 650 | <0.01 | <0.01 | 0.01 | 0.05 |
| 2-methylbutanal[c] | 653 | <0.01 | <0.01 | <0.01 | 0.03 |
| pentanal[b,c] | 698 | nd[f] | nd | <0.01 | nd |
| 2,3-pentanedione[b,c] | 700 | nd | nd | <0.01 | 0.02 |
| 1-penten-3-ol[b,e] | 716 | nd | nd | <0.01 | nd |
| 2-hydroxy-3-oxobutane[e] | 720 | 0.08 | 0.09 | 0.01 | 0.05 |
| methyl butanoate[b,c] | 724 | <0.01 | <0.01 | <0.01 | nd |
| 3-methyl-1-butanol[b,c] | 735 | 0.16 | 0.14 | 0.05 | 0.99 |
| 2-methyl-1-butanol[b,c] | 738 | <0.01 | 0.03 | 0.01 | 0.14 |
| 1-methyl-1$H$-pyrrole[b,c] | 743 | <0.01 | <0.01 | 0.03 | 0.04 |
| pyridine[b,c] | 752 | <0.01 | 0.09 | 0.04 | 0.04 |
| ethyl isobutanoate[b,e] | 760 | nd | <0.01 | <0.01 | nd |
| 1-pentanol[b,c] | 768 | nd | <0.01 | <0.01 | <0.01 |
| toluene[c] | 773 | <0.01 | <0.01 | 0.01 | <0.01 |
| 3-methyl-2-buten-1-ol[b,e] | 778 | <0.01 | 0.02 | 0.01 | <0.01 |
| 3-methyl-2-butenal[b,e] | 781 | nd | <0.01 | <0.01 | nd |
| 2,4-pentanedione[b,e] | 786 | nd | nd | nd | <0.01 |
| ethyl butanoate[b,c] | 798 | 0.01 | 0.02 | 0.03 | 0.04 |
| hexanal[b,c] | 801 | nd | <0.01 | 0.01 | nd |
| 2-methyltetrahydrofuran-3-one[b,e] | 804 | nd | <0.01 | nd | 0.03 |
| 2-furfural[c] | 829 | <0.01 | 0.08 | 0.04 | 1.99 |
| isopentanoic acid[b,c] | 830 | nd | <0.01 | nd | <0.01 |
| (E)-2-hexenal[b,d] | 854 | <0.01 | <0.01 | <0.01 | nd |
| (Z)-3-hexenol[b,c] | 858 | <0.01 | <0.01 | <0.01 | nd |
| (Z)-2-hexenal[b,e] | 860 | <0.01 | <0.01 | <0.01 | nd |
| 1-hexanol[b,c] | 866 | 0.20 | 0.10 | <0.01 | <0.01 |
| ethylbenzene[b,c] | 868 | <0.01 | 0.01 | nd | <0.01 |
| p-xylene[b,c] | 884 | 0.10 | 0.01 | nd | 0.04 |
| 2-heptanone[b,c] | 890 | nd | nd | 0.01 | nd |
| styrene[b,c] | 891 | <0.01 | 0.02 | 0.01 | 0.16 |
| heptanal[b,c] | 899 | nd | <0.01 | <0.01 | nd |
| o-xylene[b,c] | 901 | <0.01 | <0.01 | nd | <0.01 |
| methional[b,c] | 904 | <0.01 | <0.01 | <0.01 | <0.01 |
| 2-acetylfuran[d] | 907 | <0.01 | <0.01 | <0.01 | 0.11 |
| γ-butyrolactone[e] | 908 | <0.01 | <0.01 | <0.01 | nd |
| tricyclene[b,d] | 926 | <0.01 | nd | <0.01 | <0.01 |
| α-thujene[d] | 931 | 3.94 | 2.36 | 3.10 | 2.93 |
| α-pinene[c] | 939 | 50.10 | 60.30 | 62.10 | 100.49 |
| camphene[b,c] | 953 | 0.48 | 0.56 | 0.53 | 1.04 |
| (E)-2-heptenal[b,e] | 954 | nd | nd | <0.01 | nd |
| benzaldehyde[b,c] | 961 | <0.01 | <0.01 | <0.01 | <0.01 |
| 5-methyl-2-furfural[b,c] | 962 | nd | <0.01 | <0.01 | 0.47 |
| β-pinene[c] | 977 | 71.49 | 86.44 | 77.52 | 97.06 |
| myrcene[c] | 991 | 12.62 | 17.40 | 14.86 | 20.06 |
| α-phellandrene[c] | 1005 | 6.57 | 5.70 | 7.93 | 9.37 |
| δ−3-carene[c] | 1010 | 0.12 | 0.70 | 0.13 | 0.37 |
| α-terpinene[c] | 1018 | 4.34 | 8.06 | 3.13 | 0.70 |
| p-cymene[c] | 1026 | 0.57 | 0.55 | 0.33 | 0.50 |
| limonene[c] | 1030 | 14.89 | 17.71 | 14.19 | 20.49 |
| β-phellandrene[d] | 1031 | <0.01 | <0.01 | <0.01 | <0.01 |
| 1,8-cineole[c] | 1032 | 0.01 | <0.01 | <0.01 | <0.01 |
| benzyl alcohol[b,c] | 1033 | 0.02 | nd | <0.01 | <0.01 |
| (Z)-β-ocimene[d] | 1040 | 0.09 | 0.14 | 0.12 | 0.14 |
| cyclohexyl acetate[b,e] | 1043 | 0.09 | 0.10 | 0.08 | 0.14 |
| 2-phenylacetaldehyde[b,c] | 1045 | <0.01 | 0.04 | 0.01 | <0.01 |
| 2-heptyl acetate[b,c] | 1047 | <0.01 | <0.01 | <0.01 | <0.01 |
| (E)-β-ocimene[d] | 1051 | 0.84 | 0.77 | 0.79 | 2.34 |
| isopentyl butanoate[b,c] | 1060 | nd | nd | <0.01 | nd |
| γ-terpinene[c] | 1062 | 6.54 | 11.40 | 4.68 | 2.32 |
| 2-acetylpyrrole[b,d] | 1064 | nd | nd | nd | <0.01 |
| acetophenone[b,c] | 1066 | nd | nd | nd | <0.01 |
| cis-sabinene hydrate[b,d] | 1068 | 0.48 | 0.49 | 0.29 | <0.01 |
| 1-octanol[b,c] | 1070 | <0.01 | <0.01 | <0.01 | nd |
| cis-linalool hydrate[b,d] | 1074 | nd | <0.01 | <0.01 | nd |
| p-methylbenzaldehyde[b,c] | 1080 | <0.01 | <0.01 | 0.02 | <0.01 |
| terpinolene[c] | 1088 | 2.92 | 5.07 | 2.46 | 1.71 |
| trans-linalool oxide[b,c] | 1088 | nd | nd | nd | <0.01 |
| p-cymenene[b,d] | 1090 | nd | <0.01 | <0.01 | <0.01 |
| 6-camphenone[b,d] | 1093 | nd | nd | nd | <0.01 |
| trans-sabinene hydrate[d] | 1096 | 0.42 | 0.41 | 0.22 | <0.01 |
| linalool[c] | 1098 | <0.01 | 0.04 | 0.02 | 0.04 |
| perillene[b,d] | 1099 | <0.01 | <0.01 | <0.01 | <0.01 |
| nonanal[b,c] | 1102 | <0.01 | <0.01 | <0.01 | <0.01 |
| 2-phenylethanol[c] | 1110 | <0.01 | nd | nd | 0.19 |
| α-fenchol[d] | 1112 | 0.06 | 0.12 | 0.07 | 0.19 |
| trans-thujone[b,d] | 1114 | <0.01 | <0.01 | <0.01 | nd |
| cis-p-2-menthen-1-ol[b,d] | 1120 | 0.87 | 1.31 | 0.62 | 0.19 |
| α-campholenal[b,d] | 1125 | 0.12 | 0.09 | 0.15 | 0.08 |
| nopinone[b,d] | 1135 | <0.01 | <0.01 | 0.04 | 0.02 |
| trans-pinocarveol[b,d] | 1137 | nd | <0.01 | 0.04 | 0.02 |
| 2-methylacetophenone[b,c] | 1139 | <0.01 | <0.01 | 0.04 | 0.04 |
| benzyl cyanide[b,c] | 1140 | nd | nd | <0.01 | 0.02 |
| trans-sabinol[b,d] | 1140 | 0.18 | 0.11 | 0.06 | 0.10 |
| trans-p–2-menthen-1-ol[b,d] | 1142 | 0.60 | 1.05 | 0.38 | 0.19 |
| trans-verbenol[b,d] | 1144 | 0.29 | 0.17 | 0.30 | 0.10 |
| isopulegol[b,d] | 1146 | <0.01 | nd | 0.01 | <0.01 |
| menthone[b,c] | 1154 | <0.01 | <0.01 | nd | nd |
| isoborneol[b,c] | 1156 | <0.01 | nd | nd | <0.01 |
| sabina ketone[b,d] | 1158 | <0.01 | nd | <0.01 | nd |
| pinocarvone[d] | 1161 | 0.10 | 0.13 | 0.14 | <0.01 |
| borneol[c] | 1165 | 0.18 | 0.20 | 0.10 | 0.06 |
| p-mentha-1,5-dien-8-ol[b,d] | 1167 | <0.01 | nd | nd | 0.01 |
| terpinen-4-ol[b,c] | 1177 | 11.81 | 22.02 | 7.83 | 1.11 |
| p-cymen-8-ol[b,d] | 1183 | 0.08 | 0.13 | 0.09 | 0.05 |
| α-terpineol[c] | 1189 | 2.07 | 3.13 | 1.34 | 5.28 |
| myrtenal[c] | 1191 | 0.20 | 0.20 | 0.19 | nd |
| cis-piperitol[b,d] | 1193 | 0.20 | 0.21 | 0.20 | 0.11 |
| myrtenol[b,c] | 1194 | 0.10 | 0.11 | 0.04 | 0.10 |
| ethyl octanoate[b,c] | 1195 | <0.01 | 0.01 | <0.01 | <0.01 |
| verbenone[b,d] | 1203 | 0.24 | 0.31 | 0.21 | 0.14 |
| trans-piperitol[b,d] | 1205 | 0.24 | 0.32 | 0.21 | 0.13 |
| cis-carveol[b,c] | 1229 | <0.01 | 0.04 | 0.05 | <0.01 |
| myrtenyl acetate[b,d] | 1235 | 0.01 | 0.24 | 0.09 | <0.01 |
| 3-phenylpropanol[b,c] | 1237 | nd | nd | nd | 0.07 |
| cuminaldehyde[b,d] | 1239 | <0.01 | 0.04 | <0.01 | <0.01 |
| carvone[b,c] | 1243 | <0.01 | <0.01 | <0.01 | <0.01 |
| piperitone[d] | 1252 | nd | 0.06 | nd | <0.01 |
| trans-myrtanol[b,d] | 1258 | <0.01 | 0.01 | <0.01 | nd |
| (E)-2-decenal[b,e] | 1260 | nd | <0.01 | <0.01 | nd |
| cis-chrysanthenyl acetate[b,d] | 1262 | <0.01 | 0.01 | <0.01 | <0.01 |
| phellandral[b,d] | 1265 | <0.01 | 0.02 | <0.01 | <0.01 |
| 1-decanol[b,c] | 1272 | <0.01 | <0.01 | <0.01 | nd |
| 4-ethyl-2-methoxyphenol[e] | 1281 | <0.01 | nd | nd | 0.24 |
| (E)-anethole[b,c] | 1283 | nd | 0.02 | 0.03 | nd |
| bornyl acetate[b,c] | 1285 | <0.01 | 0.02 | 0.03 | 2.26 |
| cumin alcohol[b,c] | 1287 | <0.01 | <0.01 | <0.01 | nd |
| perilla alcohol[d] | 1295 | <0.01 | nd | <0.01 | nd |
| carvacrol[c] | 1298 | <0.01 | nd | <0.01 | <0.01 |
| undecanal[b,c] | 1306 | <0.01 | <0.01 | <0.01 | nd |
| 4-vinyl-2-methoxyphenol[c] | 1308 | <0.01 | 0.11 | 0.06 | 0.22 |
| (E,E)-2,4-decadienal[b,c] | 1314 | <0.01 | nd | 0.02 | <0.01 |
| methyl decanoate[b,c] | 1326 | <0.01 | nd | nd | <0.01 |
| δ-elemene[d] | 1339 | <0.01 | 0.13 | 0.12 | 0.22 |
| α-terpinyl acetate[b,d] | 1351 | nd | 0.03 | 0.06 | nd |
| α-cubebene[d] | 1352 | 0.09 | 0.02 | 0.17 | 0.10 |
| cyclosativene[b,d] | 1368 | 0.05 | 0.16 | 0.12 | 0.10 |
| isoledene[b,d] | 1373 | 0.07 | 0.11 | 0.15 | 0.13 |
| α-copaene[d] | 1376 | 0.59 | 1.25 | 1.94 | 1.82 |
| isobornyl propanoate[d] | 1382 | <0.01 | <0.01 | <0.01 | 0.02 |
| β-cubebene[b,d] | 1390 | 0.29 | 0.54 | 0.99 | 0.09 |
| β-elemene[d] | 1392 | 0.53 | 1.08 | 0.97 | 1.94 |
| α-gurjunene[b,d] | 1409 | 0.08 | 0.24 | 0.17 | 0.18 |
| β-caryophyllene[c] | 1418 | 1.07 | 2.16 | 1.41 | 1.84 |
| β-gurjunene[d] | 1432 | 0.11 | 0.23 | 0.23 | 0.29 |
| α-humulene[b,c] | 1454 | 0.35 | 0.68 | 0.44 | 0.63 |
| allo-aromadendrene[b,d] | 1461 | 0.19 | 3.32 | 2.05 | 2.69 |
| germacrene D[d] | 1480 | 9.99 | 18.33 | 27.16 | 24.92 |

Table 1 (Continued)

| compound | RI[a] | concentration (mg/kg) in cv. | | | | compound | RI[a] | concentration (mg/kg) in cv. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cenizo | Rojo | Verde | De Ojo | | | Cenizo | Rojo | Verde | De Ojo |
| epi-bicyclosesquiphellandrene[d] | 1490 | 0.19 | 0.33 | 0.24 | 0.54 | α-cadinol[d] | 1653 | 1.89 | 2.45 | 2.24 | 3.12 |
| bicyclogermacrene[d] | 1494 | 2.98 | 5.76 | 4.53 | 7.76 | 7-epi-α-eudesmol[b,d] | 1658 | 0.15 | 0.17 | 0.15 | 0.16 |
| α-muurolene[d] | 1499 | 1.25 | 2.25 | 2.16 | 2.67 | khusinol[b] | 1674 | 0.01 | 0.35 | 0.39 | 0.39 |
| γ-cadinene[d] | 1512 | 0.90 | 0.98 | 1.40 | 0.17 | (2Z,6E)-farnesol[b,d] | 1697 | <0.01 | 0.01 | <0.01 | 0.26 |
| cubebol[b,d] | 1515 | 0.04 | 0.06 | 0.08 | 0.04 | benzyl benzoate[b,c] | 1760 | 0.05 | 0.04 | 0.01 | <0.01 |
| δ-cadinene[d] | 1525 | 1.87 | 2.89 | 3.09 | 4.77 | tetradecanoic acid[c] | 1773 | 0.88 | 0.81 | 0.47 | 0.64 |
| cadina-1,4-diene[d] | 1532 | 0.11 | 0.20 | 0.18 | 0.44 | 14-hydroxy-δ-cadinene[b,d] | 1799 | <0.01 | 0.01 | <0.01 | <0.01 |
| α-calacorene[b,d] | 1542 | <0.01 | 0.03 | 0.05 | <0.01 | benzyl salicylate[b,c] | 1864 | 0.01 | <0.01 | <0.01 | <0.01 |
| elemol[d] | 1549 | 0.20 | 0.26 | 0.28 | 0.25 | pentadecanoic acid[b,c] | 1870 | 0.08 | 0.08 | 0.20 | 0.04 |
| (E)-nerolidol[c] | 1564 | 0.66 | 0.78 | 0.72 | 0.89 | 2-heptadecanone[b,d] | 1892 | <0.01 | <0.01 | <0.01 | <0.01 |
| spathulenol[d] | 1576 | 2.51 | 2.26 | 1.37 | 0.95 | methyl hexadecanoate[c] | 1926 | 0.08 | 0.06 | 0.12 | 0.09 |
| globulol[b,d] | 1583 | 0.76 | 1.02 | 0.88 | 1.10 | hexadecanoic acid[c] | 1973 | 6.52 | 6.04 | 6.84 | 4.61 |
| viridiflorol[d] | 1590 | 0.17 | 0.70 | 0.56 | 1.07 | kaurene[d] | 2034 | <0.01 | 0.01 | 0.01 | 0.01 |
| dodecanoic acid[b,c] | 1592 | 0.17 | nd | 0.50 | nd | heptadecanoic acid[b,c] | 2071 | <0.01 | <0.01 | <0.01 | <0.01 |
| humulene epoxide II[b,d] | 1606 | <0.01 | 0.05 | <0.01 | <0.01 | octadecanoic acid[b,c] | 2172 | 1.02 | 0.83 | 1.55 | 0.45 |
| 1,10-di-epi-cubenol[d] | 1614 | 1.44 | 1.51 | 1.32 | 2.02 | kauren-16-ol[e] | 2182 | 1.17 | 0.01 | 1.23 | 6.10 |
| 1-epi-cubenol[d] | 1627 | 1.62 | 2.09 | 2.12 | 2.79 | methyl (4β)-kaur-16-en-18-oate[b,e] | 2269 | <0.01 | <0.01 | <0.01 | 0.03 |
| δ-cadinol[b,d] | 1645 | 3.70 | 4.29 | 3.58 | 5.34 | (4β)-kaur-16-en-18-oic acid[b,e] | 2358 | 1.01 | 1.81 | 0.99 | 0.48 |

[a] RI, retention index on DB-5. [b] Not previously reported. [c] Compound identified by comparing mass spectrum and retention index with standard. [d] Compound identified by mass spectrum and retention index from literature data. [e] Compound identified by mass spectral database. [f] nd, not detected.

between 35 and 400 amu and scan speed of 1000 amu/s. Compounds were preliminarily identified by use of NIST, Wiley, NBS, and our own mass spectral libraries, as well as literature MS data (*4*, *5*), and then the identities of most were confirmed by linear retention indices of reference standards or with published data (*5*).

**Odor Panel Procedures.** Samples (10 g) of fresh pulp were placed in three-digit randomly coded glass screw cap vials (8 × 4 cm) and equilibrated to room temperature until presentation to the panelists. Sensory analysis testing was conducted in the sensory analysis testing laboratory. Six panelists were recruited for each panel session from the laboratory staff. Training procedures for panel members generally followed those described elsewhere (*6*). Prior to sample testing, panelists were familiarized with attribute descriptions and anchor points ranging from imperceptible to very pronounced. Pretests were undertaken with selected fruit samples to familiarize panelists with test samples as well as the measurement tool. Panelists were presented with a tray of coded fruit samples and a descriptive odor analysis ballot consisting of unmarked five-point linear scales for each attribute (custard-like aroma and overall fruity aroma). These attributes were selected as the most important by a group familiar with bullock's heart aroma.

## RESULTS AND DISCUSSION

The volatile constituents of four bullock's heart fruit cultivars were isolated by simultaneous steam distillation–solvent extraction (SDE) and analyzed by HRGC and GC-MS. The SDE under atmospheric pressure is an elegant and rapid extraction method resulting in an aroma extract that is ready to be injected into a GC system after concentration. However, heat-induced artifact formation and decomposition of labile compounds are serious drawbacks. The fruit pulp and the corresponding concentrated aroma extracts were evaluated by fragrance chemists for their characteristic aroma. The intensity of the odor of the concentrated aroma extract was higher than that of the fruit pulp, and no differences were found in the quality of the aroma impression between the fresh pulp and the extracts. Therefore, the concentrated aroma extracts were judged to have the same composition of the fruit aroma.

**Table 1** summarizes the qualitative and quantitative (concentrations are given only as a general indication of relative amounts, as response factors for each compound were not included) analyses of the fruit volatiles according to order of elution on the DB-5 column. Identification of these compounds was based on mass spectra and Kovats retention indices. The

yields of total volatiles, estimated by the addition of a measured amount of internal standard to the pulp, were 240, 316, 279, and 332 mg/kg of fruit pulp from cv. Cenizo, Rojo, Verde, and De Ojo, respectively. These values are higher than those for Malaysian fruit (9 mg/kg) (*2*) and an unknown Cuban cultivar (109 mg/kg) (*3*).

In total, 180 volatile constituents were identified in the four cultivars. One hundred and twenty compounds were reported for the first time as volatiles of bullock's heart fruit and partly in *A. reticulata* leaves and roots (*7*). In general terms, though not in detailed composition, bullock's heart volatiles are similar to those found previously (*2*, *3*) and in other *Annona* species (*8*–*13*) in which mono- and sesquiterpenes comprise the majority of the total volatiles. The compounds α-pinene, β-pinene, myrcene, limonene, terpinen-4-ol, and germacrene D together constitute some 70–80% of the total extracts. Oxygenated terpenoids were the second most abundant group, with the monoterpene alcohols terpinen-4-ol and α-terpineol being the most notable. Oxygenated sesquiterpenoids were found in great variety. These may arise via the biogenetic transformations of germacrene D and bicyclogermacrene as postulated for hops (*14*).

Some compounds present, for example, 2-furfural and 5-methyl-2-furfural, could be degradation products of ascorbic acid and sugars (*15*, *16*), but their concentrations are very low so heat-induced artifact formation could be considered minimal.

The aroma extracts from all bullock's heart cultivars contained terpenoids in highest concentrations. However, it appeared that large variations exist among cultivars (**Table 1**). Higher concentrations of mono- and sesquiterpene hydrocarbons were found in fruits from cv. De Ojo, whereas fruits from cv. Rojo had substantially higher concentrations of acetic acid, γ-terpinene, terpinolene, *cis-* and *trans-p*-2-menthen-1-ol, terpinen-4-ol, and α-terpineol. Fruits from cv. Cenizo and Verde had lower concentrations of β-pinene, myrcene, and limonene in comparison with the two other cultivars.

Data from odor panels show that fruits from cv. De Ojo exhibited the highest intensity of custard-like aroma and overall fruity aroma of all cultivars tested (**Table 2**). Fruits from this cultivar contained the highest concentration of total volatiles as well as the highest concentrations of the major terpenoids

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**Table 2.** Mean Scores for the Descriptive Sensory Analysis of Bullock's Heart Fruits

| sensory attribute | cv. Cenizo | cv. Rojo | cv. Verde | cv. De Ojo |
|---|---|---|---|---|
| custard-like aroma | 3.4 (0.3)a[a] | 4.1 (0.2)b | 3.5 (0.2)a | 4.7 (0.2)c |
| overall fruity aroma | 3.3 (0.2)a | 4.0 (0.3)b | 3.4 (0.2)a | 4.8 (0.2)c |

[a] Standard deviations reported in parentheses. Mean scores in the same row with different letters are significantly different ($p < 0.05$)

(80% of total extract). Sensory data indicate that fruits from cv. Cenizo and Verde exhibited lower scores for custard-like aroma and overall fruity aroma, and these cultivars showed the lower quantities of total volatiles. Although moderate concentrations of terpenoids were observed in both cultivars, the distribution of volatiles from these cultivars reflected lower quantities of $\beta$-pinene, myrcene, and limonene.

Further studies are needed to determine which volatiles contribute to the aroma of these bullock's heart cultivars by measuring threshold values. It is our opinion that this fruit does not have easily identifiable flavor impact compounds; instead, its aroma would be the result of a delicate balance of several components present.

## LITERATURE CITED

(1) Herrmann, K. Über die Inhaltsstoffe und die Verwendung wichtiger exotischer Obstarten. V. Annona-Früchte wie Cherimoya, Stachel-, Schuppen- und Netzannone. *Ind. Obst.-Gemueseverwert.* **1994**, *79*, 136−138.

(2) Wong, K. C.; Khoo K. H. Volatile components of Malaysian Annona fruits. *Flavour Fragrance J.* **1993**, *8*, 5−10.

(3) Pino, J. Volatile components of cuban Annona fruits. *J. Essent. Oil Res.* **2000**, *12*, 613−616.

(4) MacLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*; Wiley: New York, 1989.

(5) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 1995.

(6) Meilgaard, M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*; CRC Press: Boca Raton, FL, 1991; Vol. II.

(7) Jirovetz, L.; Buchbauer, G.; Shafi, M. P.; Saidutty, A. Analysis of the essential oils of leaves and roots of *Annona reticulata* (Annonanaceae) from South-India. *Ernaehrung* **1998**, *22*, 9−10.

(8) Wyllie, S. G.; Cook, D.; Brophy, J. J.; Richter, K. M. Volatile flavor components of *Annona atemoya* (custard apple). *J. Agric. Food Chem.* **1987**, *35*, 768−770.

(9) Santos, A. S.; Andrade, E. H.; Zoghbi, M. G.; Maia, J. G. Volatile constituents of fruits of *Annona glabra* L. from Brazil. *Flavour Fragrance J.* **1998**, *13*, 148−150.

(10) Jirovet, Z. L.; Buchbauer, G.; Ngassoum, M. B. Essential oil compounds of the *Annona muricata* fresh fruit pulp from Cameroon. *J. Agric. Food Chem.* **1998**, *46*, 3719−3720.

(11) Jirovetz, L.; Buchbauer, G.; Ngassoum, M. B.; Mapongmetsem, P.-M. Analysis of the aroma compounds of fruit and leaf essential oils of *Annona senegalensis* from Cameroon using GC-FID, GC-MS, and olfactometry. *Ernaehrung* **2002**, *26*, 25−29.

(12) Pino, J.; Rosado, A. Volatile constituents of custard apple (*Annona atemoya*). *J. Essent. Oil Res.* **1999**, *11*, 303−305.

(13) Pino, J.; Agüero, J.; Marbot, R. Volatile components of soursop (*Annona muricata* L.). *J. Essent. Oil Res.* **2001**, *13*, 140−141.

(14) Tressl, R.; Engel, K. H.; Kossa, M.; Köpler, H. Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus* var. *Hersbrucker Spät*). *J. Agric. Food Chem.* **1983**, *31*, 892−897.

(15) Shaw, P. E.; Tatum, J. H.; Berry, R. E.. Base-catalyzed fructose degradation and its relation to nonenzymic browning. *J. Agric. Food Chem.* **1968**, *16*, 979−982.

(16) Tatum, J. H.; Shaw, P. E.; Berry, R. E. Degradation products from ascorbic acid. *J. Agric. Food Chem.* **1969**, *17*, 38−40.