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# Abstract

The entire range of fatty acid composition is found for seeds of self-pollinated fruit from 22 individual plants representing 17 named species of *Cucurbita*. It appears that some varieties merit interest as a source of drying oil and edible oils. Xerophytes that are genetically related have similar types of unsaturation and molecularweight distribution.

# Introduction

A ULT ET AL. (1), Chisholm and Hopkins (2) reported that C. palmata and C. digitata had conjugated unsaturation in the range of 10-17%. Results of the present authors are consistent, and these two species had conjugated unsaturation. The bulk of *Cucurbita* however contained less than 1% conjugated unsaturation. *Cucurbita ficifolia* had an unusually high concentration of oleic acid.

The xerophytic plants of this genus could be of interest in semi-arid areas. The seeds yield approximately 30% oil and make up approximately 60% of the air-dry weight of the fruit. As the need for additional fats and oils increases, the possibility of converting arid land to useful production by growing these plants and harvesting the seed would conceivably provide an additional source of food.

# Experimental Procedure and Data

All of the seeds analyzed were harvested from selfpollinated fruits from plants grown during the 1966 season at the Arizona Agricultural Experiment Station, Tucson, with the exception of C. palmata, Fort Irwin, Calif.

Two methods of analysis were employed to define the oil composition: vapor-phase chromatography and ultraviolet spectroscopy.

Oil was extracted from the Cucurbita seeds in the following manner. Five grams of seeds were weighed to 0.02 g and placed in a Waring Blendor. To these were added 100 ml of petroleum ether (bp 30-60C). The Blendor was placed in a hood and run for 10 min. The resultant mixture was filtered through a No. 12 fluted Whatman filter paper. Residue in the Blendor was carefully washed out with more petroleum ether and filtered. This mixture was placed in a tared round-bottom flask and evaporated to dryness in a rotary vacuum evaporator. The percentage yield of oil was calculated by using an oven-dry seed weight. The moisture content was determined by drying another portion of seeds (1 g) at 105C for 4 hr (Table I). The oil samples were flushed with nitrogen, capped, and stored in a refrigerator.

Transesterification was carried out by using a potassium methylate methyl alcohol system (3). In a 25-ml Erlenmeyer flask were placed two drops of oil. To the oil were added 10 ml of 0.2 N potassium methylate; the mixture was agitated by a magnetic stirrer for 5 min in a hot-water bath ( $60C \pm 5C$ ). Upon removal from the bath, distilled water was added until the mixture turned cloudy. One ml of carbon tetrachloride was added to the cloudy mixture,

which was then shaken gently. The carbon tetrachloride was separated from the aqueous solution and evaporated to dryness by a nitrogen stream. Two drops of carbon tetrachloride were added when the material was ready to be injected into the chromatography apparatus.

Vapor-phase chromatography, utilizing a thermal conductivity cell and a  $\frac{1}{4}$ -in.  $\times$  5-ft -15% ethylene glycol succinate column operating at 175C and 20 lb of helium pressure, was used. The data are presented in Table I.

The ultraviolet spectra analysis was carried out in the following manner. Samples (0.02-0.05 g) of the fatty acid triglycerides were weighed accurately to 0.0002 g in tared 10.0 ml volumetric flasks and diluted to volume with cyclohexane (Spectral Grade). The solutions were analyzed in 1-cm quartz cells on a Perkin-Elmer Model 202 Spectrophotometer with slit setting of 25, and data were recorded as linear absorbance vs. wavelength  $(m\mu)$ . Two or more solutions at variable concentrations were prepared for an individual sample; the optical density was measured at the strongest absorption maximum (270-278 m $\mu$ ) observed in the conjugated triene region and, in the conjugated diene region, about 230 mµ. In concentrated solutions [optical density (at 275  $m\mu$ ) >0.7], the diene region could not be measured (optical density >1.5).

To test the limits of analysis, six solutions of one oil were analyzed. The maximum absorbance was constant over a range of 1–3 m $\mu$  for a given solution, and the midpoint was reported as  $\lambda$ maximum. The latter varied from 272 to 277 m $\mu$  in the six solutions and averaged at 275 m $\mu$ . A plot of absorbance at a given wavelength vs. concentration conformed with Beer's law in the region of 0.3 to 0.9 optical density; however calculation of extinction coefficients indicated that acceptable accuracies could be achieved over a range of 0.2–1.4 optical density.

The percentage of absorbing triene was calculated as the methyl ester of punicic acid  $[E_{lom}^{g/1} (pure) =$ 161.3]<sup>2</sup> by using the respective average specific extinction coefficients at average  $\lambda$ maximum reported herein.

Species with high resulting conjugated trien (C.palmata, C. digitata, C. cordata, and C. digitatapalmata hybrid) displayed three distinct absorption maxima in the regions at 263.5-267, 275-276.5, and  $287-288.5 \text{ m}\mu$ . These positions are consistent with the ultraviolet data for C. digitata and C. palmata reported by Chisholm and Hopkins (2), but they are inconsistent with curves (4) reported for a- and  $\beta$ eleostearic acids [λmax (cyclohexane) 261, 271.5, 283 mu and 259, 269.0, 280 mu respectively] and also inconsistent with the curve (5) reported for isomerized methyl linolenate  $[\lambda \max (\text{methanol}) 234 \ \text{m}\mu (\text{diene});$ 258, 268, 278 m $\mu$  (triene)]. Absorption maxima for conjugated diene were not observed for these samples, but inflection at 235 ma was present in both samples of C. palmata, and a plot of  $E_{lem}^{g/l}$  vs.  $\lambda(m\mu)$  supports  $\lambda$ shoulder at 235 mµ. The data are shown in Table I.

<sup>&</sup>lt;sup>1</sup>Agricultural Experiment Station No. 1204.

<sup>&</sup>lt;sup>2</sup> This value was obtained from the molar extinction coefficient ( $\epsilon = 47,168$ ), which was calculated from the data of punicic acid;  $\lambda \max$  (cyclohexane) 275 m $\mu$ , k<sub>275</sub> = E  $\frac{g/1}{1 \text{ cm}} = 169.4$  (2).

|  | _   | Fatty Acid Composition of Cucurbita Seed Oils<br>Fatty Acid Distribution, wt. % |                        |   |                            | s<br>Conjugated triene region           |   |  | Conjugated diene region  |                                |  |
|--|---|---|------------------------|---|----------------------------|---|---|--|--|--------------------------------|--|
| Species  | Oil in<br>seed<br>% d.b.                  | Palmitic<br>wt %  | Stearic<br>wt %        | Oleic<br>wt %   | Linoleic<br>wt %           | Linoleic<br>wt %                        | λmax <sup>b</sup><br>in mµª                     | E lem  | Wt %<br>in oil   | λmax<br>in mµª                 | E <sup>g/1</sup> lmc   |
| Maxima, Cv, Pink Banana<br>Pepo, Cv, Yellow Crookneck                        | $\begin{array}{c} 31.9\\ 24.6\end{array}$ | $\begin{array}{c} 16 \\ 12 \end{array}$   | 6<br>4                 | 47<br>33  | 31<br>45                   | 5                                       | $\begin{array}{c} 274\\ 276\end{array}$         | $\begin{array}{c} 0.11\\ 0.55\end{array}$  | 0.07<br>0.33   | 229                            | 1.06   |
| Pepo, Var., Ovifera P.I. 173,681   | 31.3                                      | 12  | 3                      | 43  | 42                         |   | 275   | 0.27   | 0.16   | 234°<br>230°<br>235°           | 1.01ª<br>0.77ª<br>0.68ª  |
| Pepo, Chapingo Exp. Sta.,<br>Mexico City                                     | 31.9                                      | 12  | 6                      | 47  | 26                         | 9                                       | 274   | 0.37   | 0.22   | $227^{\circ}$<br>$230^{\circ}$ | $0.64^{d}$<br>$0.60^{d}$   |
| Moschata, Cv, Butternut<br>Moschata, Cv, Seminole Pumpkin<br>Mixta, Tucson 2 | $33.5 \\ 31.2 \\ 37.4$                    | $19 \\ 17 \\ 12$  | 7<br>7<br>8            | $   \begin{array}{r}     40 \\     50 \\     46   \end{array} $                         | 34<br>26<br>34             | TRα                                     | $274 \\ 277 \\ 273$                             | $\begin{array}{c} 0.35 \\ 0.12 \\ 0.43 \end{array}$  | $0.21 \\ 0.07 \\ 0.26$   | 230                            | 0.85   |
| Ficifolia<br>Andreana<br>Texana<br>Sororia<br>Gracilor                       | $27.6 \\ 39.4 \\ 23.6 \\ 34.1 \\ 27.7$    | $14 \\ 19 \\ 8 \\ 14 \\ 12$   | ${}^{5}_{\mathrm{TR}}$ | $57 \\ 34 \\ 52 \\ 48 \\ 40$  | 24<br>42<br>37<br>26<br>30 | 15<br>11                                | $272 \\ 274 \\ 274 \\ 274 \\ 274 \\ 274 \\ 275$ | $0.09 \\ 0.35 \\ 0.15 \\ 0.36 \\ 0.20$   | $\begin{array}{c} 0.05 \\ 0.21 \\ 0.09 \\ 0.21 \\ 0.12 \end{array}$  | 234°<br>230°<br>225°<br>227°   | 0.85 <sup>d</sup><br>0.23 <sup>d</sup><br>0.72 <sup>d</sup><br>0.47 <sup>d</sup> |
| Palmeri<br>Lundelliana<br>Martinezii<br>Okeechobeensis                       | 34.5<br>13.7<br>32.0<br>10.9              | $16 \\ 19 \\ 16 \\ 26$  | 8<br>8<br>7<br>5       | $33 \\ 22 \\ 34 \\ 19$  | 43<br>51<br>42<br>50       | TRª<br>TRª                              | 274<br>274<br>274<br>274<br>273                 | 0.35<br>0.26<br>0.17<br>0.29   | 0.21<br>0.15<br>0.10<br>0.17   | 229°<br>236°                   | 0.72a<br>0.62a   |
| Foetidissima<br>Palmata, Fort Irwin, Calif.                                  | 30.4<br>31.6                              | 11<br>8   | 1<br>5                 | 50<br>30  | 38<br>35                   | 6                                       | $273 \\ 274 \\ 276$                             | 0.29<br>0.60<br>26.3   | 0.36   | 230<br>235<br>235              | 1.60<br>1.60<br>9.9 d  |
| Palmata, Yuma, Ariz.<br>Digitata<br>(Dig. × Pal.) Hybrid<br>Cordata          | $27.9 \\ 20.2 \\ 27.0 \\ 16.1$            | 10 $8$ $10$   | 3<br>5<br>3<br>7       | $     \begin{array}{r}       19 \\       24 \\       29 \\       38     \end{array}   $ | $43 \\ 43 \\ 40 \\ 36$     | TR <sup>a</sup><br>3<br>TR <sup>a</sup> | $276 \\ 275 \\ 275 \\ 275 \\ 275 \\ 275.5$      | $     \begin{array}{r}       20.5 \\       42.3 \\       29.5 \\       28.1 \\       14.5 \\     \end{array} $ | $     \begin{array}{r}       10.30 \\       26.21 \\       18.31 \\       17.42 \\       8.99 \\     \end{array} $ | 235°                           | 10.2 d   |

<sup>a</sup> TR = trace.<sup>b</sup> Cyclohexane solvent.

<sup>c</sup> Inflection. <sup>d</sup> Based on inflection.

The ultraviolet spectra of oils with low resulting conjugated triene contained a single broad triene absorption band in the general range of 272-277 m $\mu$ ; however, in concentrated solutions, the triene region approached a trimaxima curve with inflections about 268 and 280 m $\mu$  in addition to the  $\lambda$  maximum. These samples also indicated the presence of conjugated diene with absorption maxima in the region of 228-236 m $\mu$  and/or with inflections between 225 and 245 m $\mu$ . Only the specific extinction coefficients are reported for the diene region.

The variation between  $\lambda$  maxima of these species and the literature value of various standards could be attributed to the environmental effect of other components present in the oils or to another isomeric conjugated triene. Although the assumption may not be valid, the conjugated triene is reported as punicate, using the maximum specific extinction coefficients of the oils and methyl punicate.

## Discussion

Whitaker and Bemis (6) have divided Cucurbita species into groups with interspecific cross-compatibility indicating a genetic similarity. The fatty acid composition data presented in Table I show an interesting relationship to the previously determined groups of genetically similar species. The most striking group is the xerophytic complex of species composed of C. digitata, C. palmata, and C. cordata. This group of species is unique in having a relatively high percentage of conjugated unsaturation in its fatty acid composition. These three species are considered by Whitaker and Bemis to be genetic variants within a closely related group. This particular group of species is indigenous to the deserts of Baja California, Mexico, and the Arizona Sonoran desert. Cucurbita *foetidissima*, the only other species sampled which has xerophytic properties, has a conjugated unsaturation composition like the mesophytic species. However C. foetidissima is unable to grow under the extreme conditions of high temperature in which the other xerophytic species are found and is genetically separated from this group.

The molecular-weight distribution of the fatty acids in the mesophytic species is, in general, not indicative of genetically similar groups. Of the mesophytic species the exceptions are C. lundelliana and C. okeechobeensis, which are cross-compatible and do have the lowest percentage of oleic and the highest percentage of linoleic acids as well as the lowest dry percentage of oil.

It does not appear that the xerophytic species would be particularly desirable as edible oils because of the high percentage of conjugated unsaturation. The C. foetidissima, although not an extreme xerophyte, would appear to yield an acceptable edible oil.

#### REFERENCES

- 1. Ault, W. C., M. L. Swain and L. C. Curtis, JAOCS 24, 289-290 (1947).
- (1947).
  Chisholm, M. J., and C. Y. Hopkins, Ibid. 43, 390-392 (1966).
  Luddy, F. E., R. A. Barford and R. W. Riemenschneider, Ibid.
  447-451 (1960).
  Hoffman, J. S., R. T. O'Connor, D. C. Heinzelman and W. C.
  Bickford, Ibid. 34, 338-342 (1957).
  Brice, B. A., N. L. Swain, S. F. Herb, P. L. Nichols and R. W.
  Riemenschneider, Ibid. 29, 279-287 (1952).
  Whitaker, T. W., and W. P. Bemis, Evolution 18, 553-559 (1965).

(1965).

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