# Epicuticular Wax and Its Hydrocarbons from Inter-Juice-Sac Spaces in Citrus Fruit Segments

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The epicuticular wax of juice sacs includes the common groups of constituents as in other plant waxes. The saponifiable matter, which includes the fatty acids, was found to constitute 33.8–42.3% of the total wax in the examined fruits; the unsaponifiable matter included hydrocarbons (38.4–47.8%), primary alcohols (6.6–17%), and secondary alcohols (7–8.6%). Among the hydrocarbons, the chain lengths of 23, 24, and 25 carbons were found to be the major constituents (~50% or more of the total fraction). The hydrocarbon fraction between docosane and nonacosane contained branched isomers in addition to the linear structures. Some quantitative fluctuations were found in the hydrocarbons during the development and maturation of the fruits. The present findings verify the assumption that wax is the accumulated substance in the inter-juice-sac spaces. Thus, wax is the adhesive agent between the juice sacs and contributes to the ability of the segment to withstand disintegration.

Segments of citrus fruits are built of juice sacs adhered to one another. One of the main phenomena which promote deterioration of peeled segments during industrial processing for canning is the loss of the segments' whole fine macrostructure. This occurs as a result of the separation of juice sacs from each other.

In previous works, attempts were made to find an explanation for or to evaulate the tendency of peeled segments to disintegrate (Bakal and Mannheim, 1968; Blundstone et al., 1971; Levi et al., 1969, 1971; Ludin et al., 1969; Mannheum and Bakal, 1968). These led to our studies of the mechanism by which the juice sacs adhere to each other, in order to understand the reasons for the loss of the adhesiveness between them (Fahn et al., 1974; Shomer et al., 1975).

Waxy components are known to appear in juice sacs, and the composition of lipids extracted from the bulk tissue of juice sacs originating in various citrus fruits has been investigated extensively (Nagy and Nordby, 1972a,b; Nordby and Nagy, 1975, 1977b). Fahn et al. (1974) have described the occurrence and structure of epicuticular deposits on outer surfaces of juice sacs ("inner wax"). Nordby and Nagy (1977b) have indicated that hydrocarbons are derived from the outer surfaces of juice sacs. The present work verifies a previous assumption (Shomer et al., 1975) that hydrocarbons are part of the accumulated epicuticular wax.

Plant waxes are factors which contribute to the classification possibilities of plants according to chemotaxonomical aspects (Holloway and Challen, 1966; Martin and Juniper, 1970), as are the inner lipids of juice sacs' tissue in citrus fruits (Nordby and Nagy, 1975, 1979). Hence, it is reasonable to assume that the epicuticular wax of juice sacs is well protected from environmental effects and can thus provide more accurate additional data for citrus taxonomy.

In the present work the main classes of constituents of epicuticular wax of juice sacs were identified, and the ratio between them was studied. In addition, the hydrocarbons' profiles of the wax were investigated. The hydrocarbons as an adhesive agent and the natural function of epicuticular wax in the inter-juice-sac spaces are discussed.

## EXPERIMENTAL SECTION

**Citrus Fruits.** The following species and cultivars were examined: Marsh Seedless grapefruit (during the harvest season from November to July), Shamouti orange (early season: December), Valencia orange (late season: April through May), and Lisbon lemon (December). The wax for the chemical analyses was removed from outer surfaces of juice sacs of peeled segments. Whole and undamaged segments were chosen carefully, in order to prevent any possible leakage of lipids from the inner tissue of the juice sacs into the wax solvent. Removal of the wax was accomplished by immersion of several portions of 3 kg of whole peeled segments in ~500 mL of chloroform ($CHCl_3$), at room temperature. The immersion was accompanied by gentle shaking until complete disintegration of the segments into separated juice sacs was achieved. The solvent was then transferred to a separated flask, and the separated juice sacs were rinsed 3 times more with 200 mL of analytical chloroform. The combined chloroform was evaporated under vacuum and the wax obtained. The wax was weighed and kept at −4 °C in chloroform (analytical grade) for the analyses described below. In addition, epicuticular wax of segments from grapefruit was removed with freezing of peeled segments by Freon-12 ($CCl_2F_2$). The Freon was evaporated at room temperature, and the wax remaining was kept in chloroform at −4 °C. From three to five replicates were analyzed for each fruit sample. The relative amount values are means of these replicates.

**Thin-Layer Chromatography (TLC).** The chromatography was based on the work of Holloway and Challen (1966). Kieselgel G activated plates of Desaga (20 × 20 cm) were employed for TLC. For each chromatography run, 50–150 μg of wax was loaded on plates with chloroform. The following solvent systems were used for TLC: (I) benzene–chloroform, 7:3 (v/v); (II) benzene–methanol–acetic acid, 45:8:2 (v/v/v); (III) benzene–chloroform–ethyl acetate, 1:2:1 (v/v/v). For identifications, representative constituents from the following classes were used: saturated and unsaturated hydrocarbons, primary and secondary alcohols, sterols, and fatty acids. In addition, for comparison, waxes of cabbage leaves and bees were chromatographed. The plates were developed with a spray of an aqueous solution of 0.05% Rhodamine-6G. The observations were done under UV light at 254 and 366 nm.

**Fractionation of the Wax.** Fractionations were done according to Morice and Shorland (1973) and Fernandes et al. (1964). Saponification was done by boiling of 0.5 g of wax in 2 N KOH, for 2 h under reflux. The cooled solution was combined with 40 mL of distilled water. This mixture was rinsed 4 times with dimethyl ether. The

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Table I. Amount of Epicuticular Wax of Juice Sacs (Milligram per Kilogram of Peeled Segments) and Composition of Lipid Constituents (as Percent of Total Wax) in Grapefruits and Oranges

| wax fraction | grapefruit, Marsh Seedless | oranges | |
|---|---|---|---|
| | | Shamouti | Valencia |
| total wax | 193 | 70 | 126 |
| saponifiable matter (acids) | 33.8 | 35.3 | 42.3 |
| unsaponifiable matter | 65.8 | 64.8 | 59.3 |
| hydrocarbons | 41.5 | 47.8 | 38.4 |
| primary alcohols | 12.9 | 7.0 | 6.6 |
| secondary alcohols | 7.6 | 7.0 | 8.6 |
| unidentified matter | 3.9 | 3.0 | 3.8 |

combined ethereal solution was evaporated under vacuum at 60 °C to obtain the unsaponifiable matter. The remaining aqueous solution with the saponified matter was acidified by concentrated HCl to pH 3–4. The acidified solution was rinsed 3 times with 50 mL of diethyl ether, and the combined ether was dried with $Na_2SO_4$. The ether was evaporated under vacuum, and the remaining fraction, which included the acids, was weighed and kept at −4 °C under $N_2$. The unsaponifiable matter was separated by a column of grade II alumina (9 × 1.2 cm). The elutions were done by hexane to obtain the secondary alcohols, by diethyl ether for primary alcohols, and by 20% methanol in diethyl ether for unidentified matter.

**Gas–Liquid Chromatography (GLC).** The hydrocarbons were analyzed by a Varian Aerograph Series 2400 gas chromatograph, with a flame ionization detector. Hydrocarbons which were obtained by column chromatography were injected into an SS column (5 ft × $^1/_8$ in.) packed with 3% SE-30 on 100/200 Var-A-Port 30 under the following conditions: the column temperature was programmed from 50 to 330 °C at the rate of 10 °C/min; injection at 175 °C; detector at 250 °C; nitrogen was used as a carrier gas at 30 mL/min. Analytical hydrocarbons were used as a standard. In addition, crude petroleum, which included linear and branched hydrocarbons, was compared for identifications.

## RESULTS

The epicuticular wax of juice sacs from grapefruit, orange, and lemon contains hydrocarbons, primary and secondary alcohols, sterols, and fatty acids (Figure 1A, chromatograms 1–3). Waxes from the outer surfaces of rinds from the same fruits were found to be composed of the same groups of constituents (chromatograms 6–8). Additonal support for these identifications was provided by comparison with waxes of cabbage leaves (chromatogram 4) and bees (chromatogram 5). Similar results were obtained in other solvent systems as well (Figure 1B,C). Analysis of waxes at different stages of fruit maturation and ripening showed that the same groups of wax constituents were present. Chromatograms of waxes which were extracted by Freon-12 were found to contain unidentified spots in $R_f$ 0.6 in Figure 1C and $R_f$ 0.88 in Figure 1D.

**Separation of the Epicuticular Wax from Juice Sacs to Classes of Constituents.** The separation of wax into classes of constituents was carried out in two stages: (1) saponification of the wax and release of the acids and (2) separation of the unsaponifiable compounds by column chromatography. The relative amounts of acids, alcohols, and hydrocarbons are summarized in Table I. In all samples the unsaponifiable matter was the major part of the wax (between 59 and 66%), and within this fraction

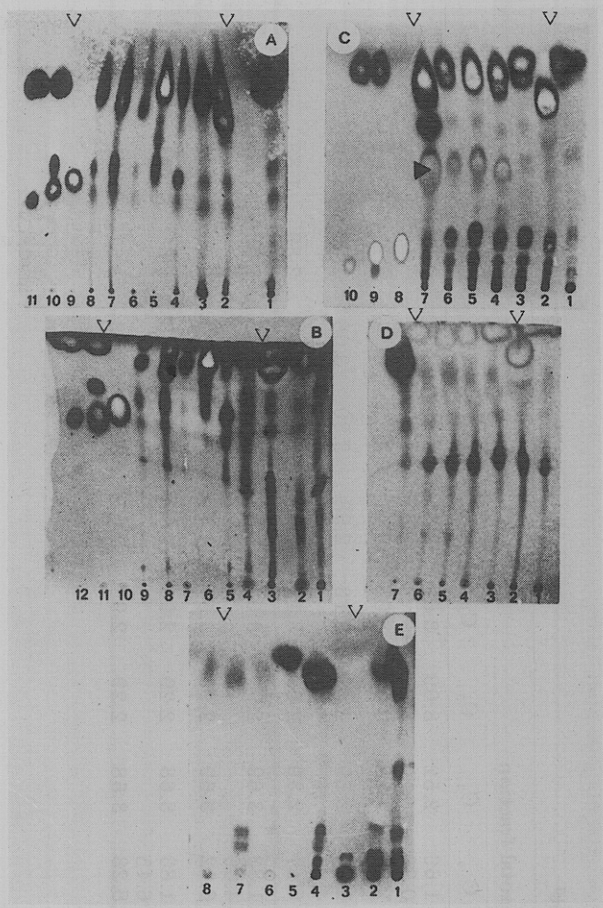


Figure 1. Thin-layer chromatograms of epicuticular wax from inter-juice-sac spaces. (A) Chromatography by solvent system III: 1, grapefruit; 2, Valencia orange; 3, Lisbon lemon; 4, cabbage leaves; 5, bees; 6, grapefruit, from outer surfaces of the rind; 7, as in 6 but of Shamouti oranges; 8, as in 6 but of Valencia orange; 9, analytical 1-tetracosanol; 10, analytical docosanoic acid, tetracosanol, and docosane, from bottom to top, respectively; 11, analytical β-sitosterol and docosene from bottom to top, respectively. (B) Solvent system II: 1, grapefruit; 2, Shamouti orange; 3, Valencia orange; 4, Lisbon lemon; 5–12, as in 4–11 of part A. (C) Solvent system I: 1–6, grapefruit at various harvest times from October to May; 7, grapefruit, but extracted by Freon-12; 8–10, as in 9–11 of part A. Black triangle indicates unidentified spots [reprinted with permission from Shomer et al. (1975); copyright 1975 Institute of Food Technologists]. (D) As in 1–7 part C, but by solvent system II. (E) Solvent system III: 1, grapefruit in December; 2–8, separated fractions of wax of the following classes: solubilized and unsolubilized acids in petroleum ether, unsaponifiable matter, hydrocarbons, primary and secondary alcohols, and unidentified matter, respectively.

the hydrocarbons constituted the major part (between 38 and 48%).

Figure 2 shows characteristic hydrocarbon profiles which were fractionated from the epicuticular wax of juice sacs of grapefruit, orange, and lemon. The profiles show a marked proportion of the $C_{23}$, $C_{24}$, and $C_{25}$ chain lengths in relation to the other constituents. The hydrocarbons of chain lengths between 23 and 29 carbons include linear and branched isomers. The relative amounts of the various hydrocarbons from juice sacs of grapefruit at several dates during the harvest season are shown in Table II. Linear and branched isomers of $C_{25}$ attained the highest relative amount of hydrocarbons at the beginning of the harvest season (14% in December). Figure 3 shows changes in proportions of the various hydrocarbons during the harvest season. The relative amounts of hydrocarbons with chain lengths of 23, 24, and 25 carbons were found to decrease and those with 27, 28, 29, and 30 carbons to increase during

Table II. Hydrocarbon Profiles of Epicuticular Wax of Juice Sacs of Marsh Seedless Grapefruit[a]

| harvest time | isomer sum | % of total fraction | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{12,21}$ | $C_{22}$ | $C_{23}$ | $C_{24}$ | $C_{25}$ | $C_{26}$ | $C_{27}$ | $C_{28}$ | $C_{29}$ | $C_{30}$ | $C_{31}$ | $C_{32}$ | $C_{33}$ | $C_{34}$ | $C_{35}$ | $C_{36}$ | $C_{37}$ | $C_{38}$ |
| mid-Dec | l | 1.93 | 1.20 | 10.15 | 5.66 | 11.26 | 1.52 | 2.17 | 1.30 | 1.66 | 2.52 | 3.09 | 3.61 | 3.41 | 3.29 | 3.37 | 2.17 | 0.89 | |
| | b | tr | | 9.12 | 8.29 | 12.64 | 5.20 | 3.72 | 0.96 | 0.90 | | | | | | | | | |
| | t | 1.93 | 1.20 | 19.27 | 13.95 | 23.90 | 6.72 | 5.89 | 2.26 | 2.56 | 2.52 | 3.09 | 3.61 | 3.41 | 3.29 | 3.37 | 2.17 | 0.89 | |
| mid-Feb | l | | 2.67 | 7.95 | 4.03 | 8.80 | 2.07 | 2.35 | 1.56 | 1.27 | 2.39 | 2.59 | 3.13 | 2.98 | 3.30 | 3.08 | 2.39 | 1.88 | |
| | b | | | 10.41 | 8.53 | 12.54 | 5.81 | 4.79 | 1.56 | 4.20 | tr | | | | | | | | |
| | t | | 2.67 | 18.35 | 12.56 | 21.34 | 7.88 | 7.14 | 3.12 | 5.47 | 2.39 | 2.59 | 3.13 | 2.98 | 3.30 | 3.08 | 2.39 | 1.88 | |
| mid-April | l | 1.53 | 1.43 | 5.04 | 5.00 | 10.45 | 3.06 | 3.36 | 2.24 | 1.83 | 3.69 | 2.96 | 3.57 | 3.46 | 3.63 | 3.36 | 2.29 | 1.63 | 1.02 |
| | b | | | 8.25 | 5.86 | 11.31 | 4.05 | 5.14 | 1.15 | 4.69 | | | | | | | | | |
| | t | 1.53 | 1.43 | 13.29 | 10.86 | 21.77 | 7.11 | 8.50 | 3.39 | 6.52 | 3.69 | 2.96 | 3.57 | 3.46 | 3.63 | 3.36 | 2.29 | 1.63 | 1.02 |
| mid-June | l | | 0.93 | 7.40 | 4.88 | 8.54 | 1.93 | 4.48 | 1.37 | 1.83 | 3.68 | 2.29 | 2.93 | 2.97 | 3.18 | 2.63 | 1.61 | 1.71 | |
| | b | | | 8.24 | 6.85 | 12.82 | 5.23 | 5.47 | 1.53 | 6.45 | | | | | | | | | |
| | t | | 0.98 | 15.64 | 11.73 | 21.36 | 7.16 | 9.95 | 2.90 | 8.28 | 3.68 | 2.29 | 2.93 | 2.97 | 3.18 | 2.63 | 1.61 | 1.71 | |

[a] tr, trace; l, b, and t, linear, branched, and total, respectively.

Table III. Hydrocarbon Profiles of Epicuticular Wax of Juice Sacs[a]

| fruit and harvest time | isomer sum | % of total fraction | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{21}$ | $C_{22}$ | $C_{23}$ | $C_{24}$ | $C_{25}$ | $C_{26}$ | $C_{27}$ | $C_{28}$ | $C_{29}$ | $C_{30}$ | $C_{31}$ | $C_{32}$ | $C_{33}$ | $C_{34}$ | $C_{35}$ | $C_{36}$ | $C_{37}$ | $C_{38}$ |
| Shamouti orange, end Dec | l | 0.33 | 2.37 | 10.98 | 6.03 | 9.31 | 1.81 | 2.40 | 1.75 | 0.97 | tr | 2.24 | 2.20 | 2.49 | 2.66 | 1.90 | 1.66 | 1.10 | |
| | b | | | 10.98 | 12.44 | 11.43 | 6.49 | 4.15 | 1.55 | 1.33 | 1.62 | | | | | | | | |
| | t | 0.83 | 2.37 | 21.86 | 18.37 | 20.74 | 8.20 | 6.45 | 3.20 | 2.20 | 1.62 | 2.24 | 2.20 | 2.49 | 2.66 | 1.90 | 1.66 | 1.10 | |
| Valencia orange, end April | l | 0.69 | 2.41 | 10.00 | 6.19 | 11.21 | 3.60 | 3.21 | 2.23 | 1.24 | 0.86 | 2.24 | 1.93 | 2.58 | 2.49 | 2.52 | 2.40 | 1.42 | 1.29 |
| | b | | | 10.89 | 9.74 | 9.66 | 4.57 | 3.61 | 1.29 | 0.45 | 1.29 | | tr | | | tr | | | |
| | t | 0.69 | 2.41 | 20.89 | 15.93 | 20.87 | 8.17 | 6.82 | 3.52 | 1.69 | 2.15 | 2.24 | 1.93 | 2.58 | 2.49 | 2.52 | 2.40 | 1.42 | 1.29 |
| Lisbon lemon, mid-Dec | l | 0.40 | 3.04 | 5.97 | 4.16 | 10.73 | 5.11 | 5.16 | 3.13 | 4.02 | 2.16 | 3.43 | 2.19 | 2.68 | 2.32 | 2.63 | 2.50 | 1.79 | 0.82 |
| | b | | | 4.79 | 5.31 | 8.25 | 6.9 | 4.95 | 2.57 | 4.25 | | | | | | | | | |
| | t | 0.40 | 3.04 | 10.76 | 9.47 | 18.98 | 12.06 | 10.11 | 5.70 | 8.27 | 2.16 | 3.43 | 2.19 | 2.68 | 2.32 | 2.63 | 2.50 | 1.79 | 0.82 |

[a] tr, trace; l, b, and t, linear, branched, and total, respectively.

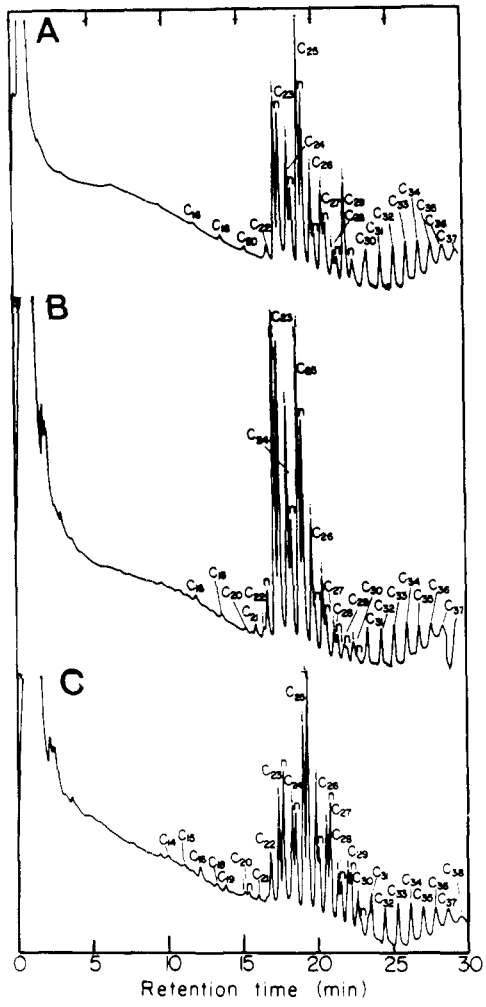


**Figure 2.** Representative chromatograms of hydrocarbons from epicuticular wax of juice sacs. (A) Grapefruit (from mid-July); (B) Shamouti orange; (C) Lisbon lemon. i and n, branched and linear isomers, respectively.

the harvest season. Table III summarizes the relative amounts of the various hydrocarbons in orange and lemon. Significant differences in relation to grapefruit were found in the relatively high percentage of the $C_{23}$ chain length (~21%) and its low percentage in lemon (10.7%). In addition, the wax of lemon was found to contain the highest percentage of the $C_{26}$ (12%) and $C_{27}$ chain lengths (10%).

## DISCUSSION

It is difficult to find an acceptable explanation for the disintegration of peeled segments into separate juice sacs, due to the fact that no tissue or cytoplasmic connections were found between the juice sacs (Shomer and Fahn, 1976). Moreover, no interruptions were found in the succession of the distinct cuticle and wax (Fahn et al., 1974; Shomer et al., 1975), through which enzyme activity (such as pectinases or cellulases) could be catalyzed separation between the juice sacs. In accordance with the above approach, it was assumed that there exists a wax which functions as an adhesive agent between the juice sacs (Shomer et al., 1975). Further investigations have indicated (unpublished experiments) that the hydrocarbons are the main constituents of the wax which contribute to the adhesion ability of the juice sacs to each other. It was found that the rate of disintegration of peeled segments is directly affected by damage to the natural deposition of the hydrocarbons in vivo. The rate of disintegration was

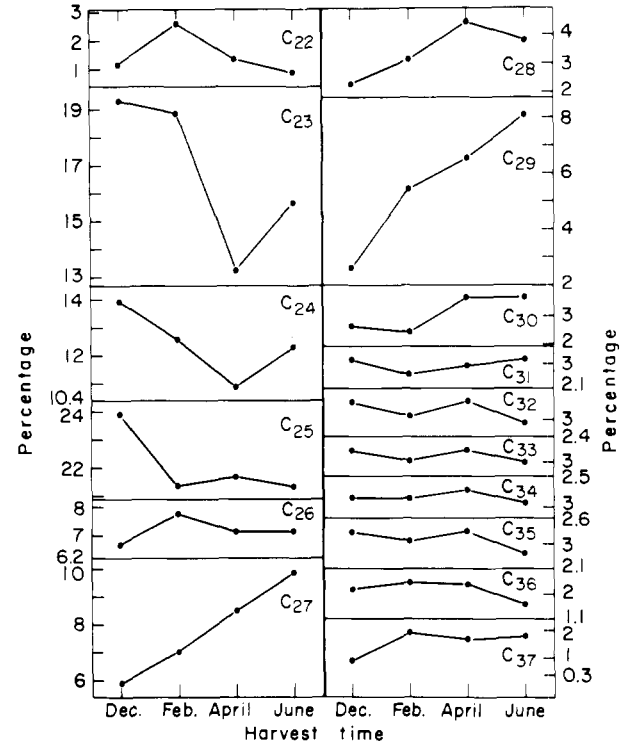


**Figure 3.** Variations in relative amounts of hydrocarbons during the harvest season.

found to decrease with the increase in the higher hydrocarbons' portion in the wax (Figure 3). One possible reason for this phenomenon may be the increase in the melting range of the wax due to an increase in relatively higher hydrocarbons in the wax as the harvest season progresses.

This led to the study of the chemical composition of the epicuticular deposits from the inter-juice-sac spaces and particularly of hydrocarbons, in order to prove their role in the adhesion of juice sacs to each other.

The TLC analyses showed that the epicuticular deposits of the juice sacs include components of plant waxes. Similar chromatograms were described by Holloway and Challen (1966) for waxes of external organs of other plants, such as sugar cane, *Papaver somniferum*, *Pisum sativum*, and others. As shown by Baker et al. (1975), in each citrus fruit organ there is a specific proportion between the classes of wax constituents. In addition, the constituents of each class are found in a specific quantitative ratio. The quantitative proportions between the wax classes in outer surfaces of juice sacs were found in the present work to be different from those of Baker et al. (1975) and of Nordby and Nagy (1977a).

In the epicuticular wax of juice sacs, the hydrocarbons are the predominant fraction. This fraction comprises 38–47% of the total wax and more than 66% of the unsaponifiable matter (Table I). These values are similar to those found by Baker et al. (1975) for waxes from leaves and fruit surfaces. However, the hydrocarbons' content in the wax of citrus fruits is higher than that in wax of other fruits, such as apples (Morice and Shorland, 1973; Fernandes et al., 1964). The second predominant class of components found in the epicuticular wax of juice sacs is the fatty acids, which are found in higher percentages than in citrus fruit peel and leaves (Baker et al., 1975) but in lower ones than that of apple fruits (Morice and Shorland, 1973). The total content of alcohols, especially the primary alcohols, is higher in the epicuticular wax of grapefruit juice sacs. In Valencia orange the secondary alcohols were predominant. The values were lower than those of wax

from the outer surface of citrus fruits and leaves (Baker et al., 1975). The amount of alcohols in the juice sacs wax was found to be in the range of the waxes of apples (Morice and Shorland, 1973). Baker et al. (1975) found that the aldehydes' content in the wax of the outer surface of citrus fruit is relatively high and that it is low in the wax of leaves. In juice sacs it reaches relatively low proportions, as can be concluded from the amounts of the last fraction of the column separation (i.e., unidentified substances). Freon-12 ($CCl_2F_2$) dissolves wax well, as seen in Figure 1C,D. Removal of wax by Freon-12 during the freezing process is one of the reasons for the disintegration of frozen peeled segments.

Previous reports have described constituents of lipids which were extracted from the intra-plant-organ zone but did not find them to be part of the mixture of wax components (Eglinton et al., 1962; Grice et al., 1968; Gülz, 1968; Kaneda, 1969; Kolattukudy, 1970; Morice et al., 1971). In this regard, if wax is a secretion mixture of lipid constituents on the outer surfaces of plant organs (Fernandes et al., 1964; Hatt and Lamberton, 1956; Gurr and James, 1971; Kolattukudy, 1970; Lange, 1967; Martin and Juniper, 1970), then it is not accurate to define as wax any lipid from the intratissue zone.

In the present work, we proved that the lipid constituents from outer surfaces of juice sacs are those of secreted wax. This point is emphasized because only secreted and accumulated substances from outer surfaces of inner organs, i.e., juice sacs, were extracted, and their structure (Fahn et al., 1974) and chemical composition were determined.

The extraction zone of the examined lipids in the present work is well-defined and differs from that described by Nagy and Nordby's works, who investigated lipids from the entire body of the juice sacs. On the other hand, it differs from the outer surfaces of external organs, which are exposed to the surrounding atmosphere.

It is possible that the quantitative ratio between the various classes of constituents of epicuticular wax differs from that of lipids from the bulk tissue. This may be explained by the various amounts of each class which were secreted as a wax. An additional possibility is that some constituents may be converted into components of another class (Kolattukudy, 1970) in the epidermal secretion region of the wax.

The hydrocarbons, which were fractionated from the epicuticular wax of juice sacs in the present work, were similar in composition to those extracted by Nagy and Nordby from the tissue bulk (Nagy and Nordby, 1971, 1972a,b, 1973; Nordby and Nagy 1972, 1974, 1975, 1977b).

Kolattukudy (1970) has described the biosynthesis pathway of wax and found that hydrocarbons are the major finished product of the secreted constituents. Previous work, which showed secreted deposits on the outer surfaces of the juice sacs (Fahn et al., 1974), and preliminary studies of Nordby and Nagy (1977b), in addition to the present findings, confirm that the hydrocarbons are a fraction of epicuticular wax. This means that in the juice sacs the hydrocarbons are biosynthesized in the secretion regions or within adjacent sites in the epidermal cells. Hence, it is concluded that the hydrocarbons of the epicuticular wax include the same linear saturated and unsaturated isomers as described by Nagy and Nordby (1972a,b, 1973) and Nordby and Nagy (1972, 1977b) and the branched hydrocarbons consisted of both iso and anteiso isomers, as was reported by Hunter and Brogden (1966) as well as by Nagy and Nordby (1971, 1972a).

The most prominent difference between hydrocarbons of the epicuticular wax from juice sacs (Tables II and III) and those of the outer surfaces of citrus fruit and leaves (Baker et al., 1975; Nordby and Nagy, 1977a) is the higher proportion in the juice sacs of hydrocarbons of relatively short chain length such as tricosane, tetracosane, and pentacosane. A distinct difference between intratissue hydrocarbons (which were not a fraction of inner epicuticular wax) and those of outer surfaces was shown by Kaneda (1969) in spinach leaves.

This distinct difference between the "inner" hydrocarbons and the "outer" ones may be due to one or both of the following factors: loss of relatively low hydrocarbons to the atmosphere from outer surfaces; chemical changes which create higher hydrocarbons or derivates as a result of external effects. Some intimation of the increased proportion of relatively high hydrocarbons during the development of the fruit was found in the juice sacs' wax (Figure 3). This may be explained by chemical changes of the secreted wax or in the proportions between the biosynthesized constituents prior to secretion. However, the epicuticular wax of the juice sacs contains the original constituents. If so, this means that the relatively low hydrocarbons which comprise the highest proportion of the total wax in the juice sacs are lost or changed in the exposed surfaces of leaves and fruits. Other factors, which may affect "outer" wax and modify its original features, are external factors such as pesticides, insecticides, UV irradiation, nutritive elements, and others (Martin and Juniper, 1970; Baker 1974).

In regard to the natural function of the epicuticular wax of the juice sacs, it is reasonable to assume that the wax contributes to the ability of the fruit to withstand cuticular transpiration. Reed [unpublished project notes on file at the University of California, Citrus Experiment Station [see Sinclair (1961)], 1931] and Curtis and Clark (1937) found that there is no ordinary movement of water and assimilates through citrus fruits (affected by temperature gradients), as in other fruits. In addition, Kaufmann (1970) has presented evidence in support of the hypothesis that osmotic potential in vesicles would be affected but little by changes in transpiration or in the carbohydrate-translocation pathway. It seems that the wax, due to its functional properties, is the main factor which prevents the usual movement of liquids in the whole fruit and particularly in the segment and contributes to the ability of the juice sacs to accumulate and store liquids. One may assume that the epicuticular wax is the main factor which contributes to the retaining ability of the juice sacs and not only to the cuticle itself, as mentioned by Sinclair (1961).

Support for this argument was obtained in experiments (as a part of an unpublished study) in which a significant loss in weight was obtained in juice sacs due to cuticular transpiration, after removal of wax from the outer surfaces by an organic solvent such as chloroform or hexane. These solvents do not remove cuticle (Baker and Procopiou, 1975; Kolattukudy and Walton, 1972). In addition, fixatives such as $OsO_4$ or glutaraldehyde did not penetrate into the juice sacs if wax was not removed from their surfaces (Shomer and Fahn, 1976). Hence, it appears that the epicuticular wax of the juice sacs is the main barrier against the movement of water or solutes in the absence of vascular tissues through which rapid passage of liquids from the juice sacs could occur. This, in addition to the special compact packing of the juice sacs in the whole segment and fruit, creates an efficient isolated construction (similar to isolation bodies such as polystyrene foam). These factors

enable the citrus fruit to withstand loss of water, especially during a relatively long hot period.

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# Nutritional Composition of Okra Seed Meal

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The nutrient content of okra seed (*Abelmoschus esculenta* Moench) was investigated. Okra seed contained 21% protein, 14% lipids, and 5% ash. Removal of the seed hulls by grinding and sifting produced a meal with 33% protein, 26% lipids, and 6% ash. The protein of okra seed has a chemical score of 55, with isoleucine the first limiting amino acid. A saturated/unsaturated fatty acid ratio of 1:1.55 was found in the oil, with principal fatty acids as follows: 42% linoleic, 34% palmitic, and 18% oleic. Minerals of whole seeds included 135 mg of Ca/100 g and 335 mg of Mg/100 g with much lesser amounts (<5 mg/100 g) of copper, iron, manganese, and zinc; sifting out hull material resulted in an increase in iron (11 mg/100 g), zinc (14 mg/100 g), and magnesium (518 mg/100 g) concentrations.

The potential of the seed of okra, *Abelmoschus esculenta* Moench, as a high-oil, high-protein crop for the temperate zone and the tropics has now been amply demonstrated. Probably Woodruff (1927), who pointed out that after oil is extracted from okra seeds a high-protein meal remains similar to that of cottonseed meal, was the first to recognize this. Nevertheless, in spite of considerable investigation of the oil and its qualities during the period from 1930 to the present, the potential of okra seed has never been realized. In 1975, Karakoltsidis and Constantinides reviewed part of the literature and studied the protein, oil, vitamin, and mineral content of the whole seed. Their studies have stimulated other investigators who see okra seed as an important crop of high potential.

The problem of removal of the okra seed hull from the kernel has not been sufficiently studied. Nevertheless, in an attempt to devise a system for small-scale use, Martin and Ruberté (1979) ground the seed by a hand mill and separated two fractions, chiefly hulls and chiefly kernels, by sieving. The okra meal so produced contained 33% protein and 32% oil. This meal was used in partial substitute for wheat flour and cooking oil to prepare a wide variety of baked products, including bread. The high acceptability of such products further stimulated interest

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