Relationship of Epicuticular Wax Composition of Grapefruit to Chilling Injury

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Cold storage affects amounts of alkanes, squalene, and long-chain aldehydes in the epicuticular wax of grapefruit. Levels of these components were determined in fresh grapefruit prior to cold storage and in temperature-conditioned and nonconditioned fruit after storage at various times and temperatures. Levels of C_{27}–C_{33} alkanes and C_{28}–C_{34} aldehydes declined during storage. An increase in squalene, C_{23}–C_{25} alkanes, and C_{24}–C_{26} aldehydes occurred at the previously determined optimal temperature for synthesizing squalene and temperature-conditioning grapefruit against chilling injury, e.g., 15 °C. The synthesis of these three compounds could be turned on or off by moving the fruit back and forth between 5 and 15 °C. Squalene and the C_{24} and C_{26} aldehydes were preferentially extracted from the epicuticular wax within 10 s. Fruit dewaxed prior to temperature conditioning at 15 °C failed to produce squalene and the C_{24} and C_{26} aldehydes. Fruit dewaxed partially with hexane or with chloroform showed increased amounts of chilling injury in 5 °C storage compared with control fruit; more lipids were extracted with chloroform, and these fruit showed the most injury.

Many fruits and vegetables, when stored at low but nonfreezing temperatures for various periods of time, develop chilling injury (CI). CI symptoms include surface pitting, discoloration, internal breakdown, and decay. There is little doubt that the disorder has its origin at the membrane level (Wang, 1982); however, the cuticular area of the commodity undoubtedly has some influence on the onset and extent of the injury. Grapefruit CI has been studied extensively due in part to economic factors. Grapefruit stored 3 weeks at 5 °C will show surface pitting or brown staining on over 5% of the fruits. This percentage in many instances is much higher in early- and late-season fruit (Grierson, 1974). Many studies have dealt with means of reducing or possibly eliminating this disorder in grapefruit. The postulate behind these studies is that the outer "coat" of the commodity becomes more porous at 5 °C than at room temperature, allowing oxygen to flow in toward the membrane and water to flow out; thus, the fruit becomes dehydrated. These studies have shown that reducing oxygen levels (Hatton et al., 1975), waxing (Grierson, 1971), oiling (Aljuburi and Huff, 1984), applying growth regulators (Kawada et al., 1979), and film wrapping (Grierson, 1971; Wardowski et al., 1975; Ben-Yehoshua et al., 1981; Purvis, 1985) all reduced the incidence of CI in grapefruit. Unfortunately, these reported successes are not universally repeatable or adaptable to all CI-sensitive commodities, evidence that we do not know all the optimal parameters for coating or reinforcing the coat which occurs naturally on the commodity. One method of preparing grapefruit to withstand the stress of storage at 5 °C is called temperature conditioning (Hatton and Cubbedge, 1982, 1983). Grapefruit stored 7 or more days at 15 °C prior to storage at 5 °C show a near absence of CI. In a 3-year study, we determined that squalene was produced by the fruit during this 7-day period and was deposited in the epicuticular wax (Nordby and McDonald, 1990). The optimal temperature for this synthesis was 15 °C, and the level of injury protection was directly correlated with the level of squalene on the fruit. Further studies showed other isoprenoids were also synthesized under these optimal conditioning parameters (Nordby and McDonald, unpublished data). When squalene was applied to grapefruit, a correlation was obtained between the level applied and the level of protection from CI. With a high level of application the protection was greater than that obtained under the 7-day, 15 °C conditioning treatment (Nordby and McDonald, unpublished data). A quick method for analyzing squalene in the wax was devised to reduce the time the highly unsaturated compound was exposed to possible degradation (Nordby and McDonald, 1990). Although the method was selective for squalene, during gas chromatographic analyses replication was difficult with samples having high levels of squalene. Long-chain aldehydes were determined to be the major interfering substances. These lipids have been reported to be major constituents of the epicuticular wax of sugarcane (Lamberton and Redcliffe, 1960; Lamberton, 1965), grapes (Radler and Horn, 1965), cranberry (Croteau and Fagerson, 1971), cauliflower and wheat (Barber and Netting, 1968), cabbage (Baker, 1974; Macey and Barber, 1970), and citrus (Baker et al., 1975; Freeman et al., 1979b), as well as minor constituents of other fruits and leaves (Freeman et al., 1979a; Kolattukudy, 1970; Holloway and Baker, 1970; Starratt and Harris, 1971). However, none of these studies gave any explanation as to why these unstable compounds should be present in relatively high concentrations in a medium whose purpose is to protect the commodity. In a cursory thin-layer chromatographic assay of these interfering aldehydes it was found that these aldehydes not only increased with conditioning against CI but decreased when the fruits were stored at temperatures conducive to CI. Thus, this more extensive study was devised to determine whether a possible cause of CI in grapefruit is a breakdown or formation of an improper balance of specific lipids of the natural protective coating, epicuticular wax, when the fruit is stored at 5 °C. A corollary to this purpose is to determine whether fatty aldehydes synthesized along with squalene during temperature conditioning might also protect the fruit from CI.

EXPERIMENTAL PROCEDURES

Sample Collection. Marsh grapefruits (*Citrus paradisi* Macf.) were harvested monthly from August through December during the 1989–1990 season from a grove near Merritt Island

on the east coast of Florida. Due to a severe freeze in late December, grapefruit samples for January were selectively sampled from harvesting bins at a packinghouse on the lower east coast of Florida. Fruits were washed and dried under citrus packinghouse procedures, except waxes and fungicides were not applied.

Treatments. Fruits were loosely packed (25–35/box) in nonwaxed, cardboard citrus boxes. Depending on the study being conducted, boxes were stored in 1, 5, 10, 15, and 20 °C constant-temperature storage rooms without light and at 70% relative humidity for 1–18 weeks. During this storage period, the location of the boxes of fruit was sometimes changed to study the planned effect of storage time and temperature on the changes in the grapefruit epicuticular wax. Fruits were examined for frequency and intensity of CI (pitting or brown staining) after 3 weeks of storage. The intensity was quantified on a 10-point scale (1 = very slight; 10 = very severe) to produce a CI index for each set of fruits on each of the inspection dates. Fruits were examined in a 20 °C examining room with 90% relative humidity and then returned to their respective storage rooms within 30 min.

Special Treatments. To determine whether conditions other than time and temperature are involved in the changes in epicuticular wax composition, special treatments were given to a portion of the fruit from four of the samples taken during 6 months. Individual fruits from two boxes of September grapefruit were placed stylar side up on Styrofoam fruit partitioners in a fume hood. A 20% v/v squalene in hexane solution was sprayed on each fruit for 5 s from a TLC sprayer. Fruits were turned over, and the squalene application was repeated. Fruits were boxed and stored within 10 min of spraying. Before being sorted and washed, one-third of the November fruits were degreened for 3 days under normal packinghouse procedures (Grierson, 1974). To determine the lability of the squalene and fatty aldehyde synthesizing enzymes, the December grapefruits, which were dewaxed for December fresh wax analyses, were placed in a cardboard box and stored at 15 °C for 14 days. Prior to being boxed, one-sixth of the January fruits were individually dipped for 10 s in a beaker of chloroform. Fresh chloroform was used after 20 successive dips. In a similar manner another sixth of the January fruits were dipped in hexane. All dipped fruits were boxed and stored within 10 min of treatment.

Wax Isolation and Analyses. Samples of epicuticular wax were obtained from fruit prior to storage or special treatment. Five fruits, each weighing 300–450 g, were weighed and sequentially placed in a 14-cm porcelain crucible with 100 mL of chloroform. Each fruit was swirled in the solvent for 5 min. An additional 10 mL of chloroform was added before the next fruit was bathed, and the combined wax extract was poured into a 300-mL round-bottom flask. The crucible was rinsed with 10 mL of chloroform and the extract reduced to near dryness on a rotoevaporator at 30 °C under reduced pressure. The wax residue was transferred to a 5-mL volumetric flask with chloroform containing 0.544 mg/mL cholestane internal standard. One milliliter of sample was placed in a 2-mL vial, reduced to approximately 0.1 mL with N_2, and streaked with two rinses on half of a 20 × 20, 250-μm silica gel G TLC plate. Plates developed in benzene were sprayed with Rhodamine 6G and viewed under UV light, and the aldehyde, squalene, and alkane bands were outlined. The alkane and squalene bands combined were scraped off the plate into a sintered glass funnel, eluted with chloroform (10 + 3 × 5 mL), reduced to dryness on the rotoevaporator, and made up in a vial with hexane to a concentration of 0.5 mg/100 μL of sample for GLC analyses. The aldehyde band was likewise scraped and eluted with the cholestane internal standard being added just prior to concentration. Alkanes and squalene were analyzed on a 0.75 mm × 30 m × 1 μm Supelcowax column (Supelco Inc., Bellefonte, PA), isothermally at 215 °C with injection and FID at 270 °C and a helium flow of 18 mL/min. Aldehydes were injected onto a 30 m × 0.53 mm megabore DB-1 column, chromatographed isothermally at 265 °C with injection and FID at 300 °C and a helium flow of 18 mL/min. Each analysis consisted of three pooled extracts (15 fruits) with the above two GLC assays being run on each of these triplicate pooled extracts. Alkanes, squalene, and aldehydes were quantified as micrograms per 100 g of fruit.

In the extraction time study, six November fruits (three fruits stored 21 days at 5 °C and three fruits stored 7 days at 15 °C after storage at 5 °C for 14 days) were individually dipped for 10 s in a series of three crucibles, each containing 100 mL of chloroform. Each of the 18 extracts was quantitatively transferred to TLC plates, and the plates were developed as above. Cholestane internal standard was added to both fractions prior to removal of the chloroform. An assay consisting of six GLC analyses (three for alkanes and squalene, three for aldehydes) was determined for total extraction times of 10, 20, and 30 s.

Mass spectra of the aldehydes were determined on a Hewlett-Packard 5971 GC–MS (Hewlett-Packard, Avondale, PA) with a 12 m × 0.2 mm × 0.3 μm HP-1 column. The oven temperature was held at 150 °C for 5 min, raised to 270 °C at 5 °C/min, and held there for 5 min. Transfer line and injection port were at 280 °C; mass analyzer was at 70 eV, 180 °C, 30 Torr with a helium flow of 1.0 mL/min. Three representative samples of the grapefruit wax aldehydes were reduced with $NaBH_4$ in methanol to alcohols, purified by TLC, and analyzed by GC–MS under the above parameters for the aldehydes.

RESULTS AND DISCUSSION

Alkanes (R_f 80), squalene (R_f 70), and fatty aldehydes (R_f 56) were clearly resolved by utilizing benzene as the TLC solvent. The authenticity of the aldehydes was determined by their mass spectra. Only two of the 11 gave M ions, a characteristic cracking pattern of saturated long-chain compounds. The M – 18 ion, characteristic of aliphatic aldehydes, increased in relative intensity from 3% for C_{22} to 29% for C_{32}. Major ions in all aldehydes and their ranges of relative intensities were 43 (100), 57 (68–100), 44 (46–89), 82 (31–43), and 96 (21–32). Mass spectra of the alcohol derivatives of these aldehydes and of the spectra of alcohol standards further confirmed the aldehyde structures with the alcohol spectra showing M – 18 ions 2 units greater than their parent aldehydes. Present just below and not completely resolved from the aldehyde band on TLC was a series of four or five compounds that interfered with the integration of the C_{32} aldehyde on the nonpolar DB-1 GLC column. Mass spectra indicated these compounds to be esters of α- and β-amyrins. Corrections were made for these interfering compounds by analyzing a representative sample on the polar GLC column, where the esters were well-resolved from the C_{32} aldehyde. Also present as minor components in the aldehyde fraction were compounds having equivalent chain lengths (ECL) corresponding to iso and anteiso structures. These branched structures were also observed as alkanes in the hydrocarbon fraction as they were in a previous study with citrus peel (Nordby and Nagy, 1977). In freshly harvested grapefruit linear aldehydes were present in the epicuticular wax (296–560 μg/100 g of fruit) at about 8 times the level of linear alkanes (32–61 μg/100 g of fruit) (Table I). The ranges for the two lipid classes are aldehydes C_{24}–C_{34} and alkanes C_{23}–C_{33}. Within these two ranges, even-numbered aldehydes and odd-numbered alkanes accounted for 90–92% and 70–90% of the total aldehydes and alkanes present, respectively. On a relative percentage basis, the minor even-numbered alkanes and odd-numbered aldehydes showed only slight variation over the 6-month season, whereas the major components of these two lipid classes varied greatly over the 6-month season (Figure 1). Thus, to emphasize these differences, the base levels of aldehydes and alkanes are subtracted from the levels of aldehydes and alkanes with storage or special treatment.

Figures 2 and 3 show the effect of 5 or 15 °C storage on aldehydes and alkanes in grapefruit epicuticular wax. At 5 °C there is a very slight (6.3 μg/100 g of fruit) but significant increase in the C_{24} aldehyde, tetracosanal, in all 4 months of the investigation. With C_{25}–C_{34} aldehydes,

Table I. Linear Alkanes, Long-Chain Aldehydes, and Squalene in Grapefruit Submitted to Temperature Conditioning and Storage at Various Times and Temperatures over a 6-Month Season[a]

date	storage cond, °C-days	μg/100 g of fresh fruit			
		alkanes	aldehydes	squalene	total
Aug 1989	fresh	50.5	336.8	0.4	387.7
	5–7	35.9a	190.1a	0.3	226.4a
	15–7	69.9b	360.6b	13.9a	442.4b
	15–7, 5–74	132.1c	351.1b	29.8b	544.2c
	15–14	131.1c	534.1c	62.4b	727.8d
	15–41	323.9d	720.5d	181.1c	1225.5e
	5–14, 15–67	726.1e	885.7e	194.3c	1806.1f
Sept 1989	fresh	39.6	295.9	ND[c]	335.5
	5–21	23.4a	180.6a	ND	204.0a
	5–63, CI[b]	29.9b	143.8b	ND	173.7a
	5–63, NCI[b]	25.1b	139.2b	1.1	165.4a
	20% sqe[d], 5–117	21.3b	118.9c	1657.0	140.2
	5–49, 20–13	61.7c	419.8d	1.9a	483.4b
	15–21	84.8d	520.5e	91.6b	696.9c
	15–60	414.5e	722.6f	190.6c	1327.7d
Oct 1989	fresh	60.9	404.0	2.7	467.6
	5–7	31.3a	218.7a	0.8a	253.9a
	15–7	35.1b	317.0b	26.4b	378.5b
	15–7, 5–80	29.0b	295.4b	25.8b	350.2b
Nov 1989	fresh	41.1	355.3	ND	396.4
	5–7	26.1a	257.4	1.3a	284.9a
	15–7	46.6b	447.7a	40.7b	535.0b
	degreened	56.0b	421.1a	3.1c	480.2b
	degreened, 15–7	48.5b	398.2a	5.6d	452.3b
Dec 1989	fresh	53.4	559.7	2.0	615.1
	5–7	31.8a	364.5a	3.6	399.9a
	15–7	52.6b	642.9b	23.7	719.2b
	dewaxed, 15–14	9.2c	84.5c	0.2b	93.9c
Jan 1990	fresh	31.7	331.3	2.3	365.3

[a] Within each month, values within a column with different letters from preceding values are significantly different at the 95% level, Student *t*-test. [b] Fruit were NCI (non-chill-injured) or CI (chill-injured). [c] ND, not detected at 0.1 μg/100 g level. [d] Applied squalene (20% in hexane spray) not counted in total extracts.

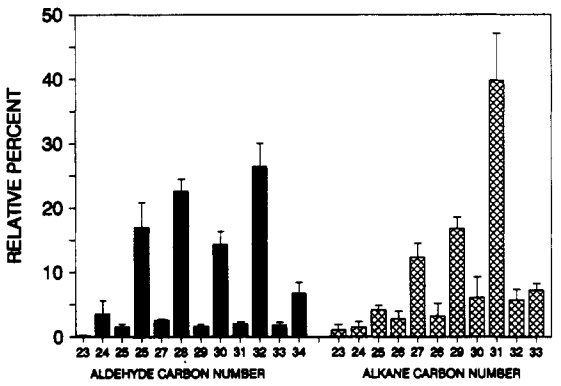

Figure 1. Relative percent and standard deviations of aldehydes and alkanes present in fresh grapefruit wax on a monthly basis from August 1989 to January 1990.

however, a drastic decrease occurred at this temperature over the 7 days. The decrease was most pronounced in the even-numbered but occurred to a lesser extent with the relatively minor odd-numbered aldehydes. The combined aldehyde decrease over this 7-day period averaged 156.4 μg/100 g of fruit, representing a 41.1% decline in base aldehydes. Conditioning the fruit for 7 days at 15 °C also tended to decrease the C_{27}–C_{34} aldehydes. Again, the major aldehydes to decline were C_{32} and C_{30}. The combined decline for these eight aldehydes was 91.8 μg/100 g of fruit or 24.1%. The final level for total aldehydes was greater than base due to substantial increases in three aldehydes: C_{24} (486%), C_{25} (92%), and C_{26} (64%).

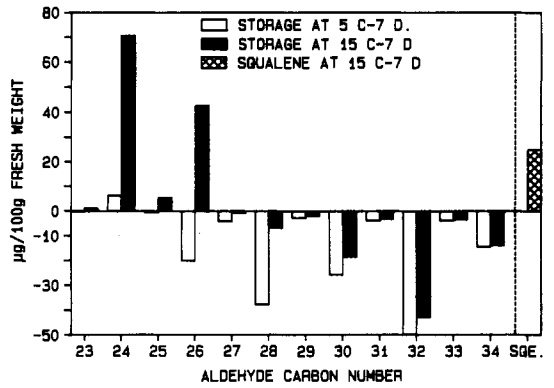

Figure 2. Changes in aldehydes and squalene present in grapefruit stored 7 days at 15 (temperature conditioned) or 5 °C (nonconditioned) compared with fresh grapefruit; mean of four monthly studies during August 1989–January 1990 season.

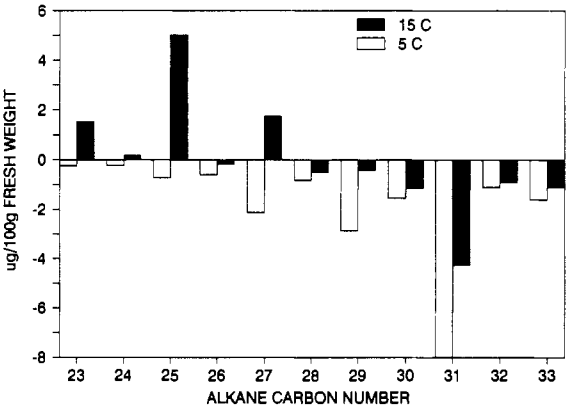

Figure 3. Changes in alkanes present in grapefruit stored 7 days at 15 (temperature conditioned) or 5 °C (nonconditioned) compared with fresh grapefruit; mean of four monthly studies during August 1989–January 1990 season.

Squalene's increased level after 7 days of conditioning was less than half that of tetracosanal (Figure 2); however, it contributed to a 14% rise in total wax isolates observed during the 7-day conditioning treatment (Table I). The general pattern for alkanes over this 7-day period was nearly identical with that of the aldehydes except for specific carbon numbers. Alkane C_{31} replaced aldehyde C_{32}, and pentacosane replaced tetracosanal (Figure 3). However, at both temperatures the final levels were the same as those present before storage.

Although 7 days was shown to be adequate for conditioning grapefruit against CI (Hatton and Cubbedge, 1983), we showed in a previous paper (Nordby and McDonald, 1990) that synthesis of squalene continues if the fruit is left in storage at 15 °C. Figure 4 and Table I clearly show that this increase with time at 15 °C also occurs for $C_{24} > C_{26} > C_{28}$ aldehydes and for $C_{27} > C_{25} > C_{29}$ alkanes, as well as for squalene. Tetracosanal continued to be synthesized in August fruit after being transferred to 5 °C for 74 days following 7 days at 15 °C. High numbered aldehydes declined under these conditions. We have found that temperature conditioning can be delayed; that is, fruit stored at a CI temperature of 5 °C for 2 weeks without showing injury can be temperature-conditioned at 15 °C for 2 weeks and show less injury compared with fruit left at 5 °C during this time (data not shown). In this study, when we examined August fruit left 14 days at 5 °C and then 67 days at 15 °C, we had maximum synthesis of tetracosanal, heptacosane, and squalene. The combined level of these lipids was substantially higher than the level of respective lipids in September fruit conditioned continuously for 60 days (Table I).

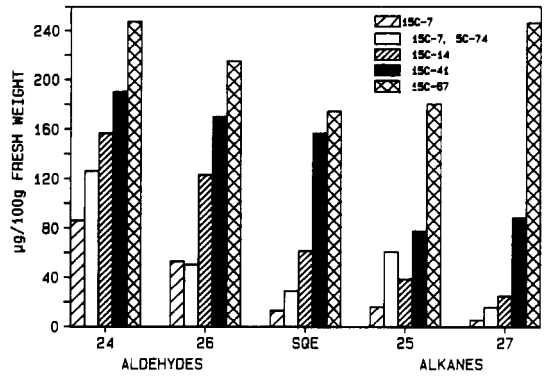

Figure 4. Levels of C_{24} and C_{26} aldehydes, squalene, and C_{25} and C_{27} alkanes in August 1989 grapefruit temperature conditioned and stored under five regimes.

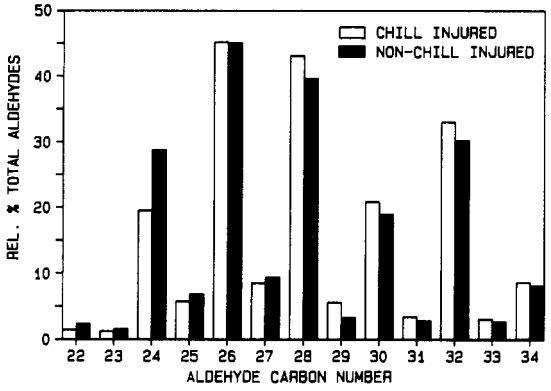

Figure 5. Relative percentage of aldehydes (individual of total) present in chill-injured and non-chill-injured, nonconditioned September 1989 grapefruit stored 115 days at 5 °C.

A study on the degree of decrease in aldehydes and alkanes with time of storage at 5 °C was conducted on September fruit (Table I). As the time of storage was extended from 7 to 21, 63, and 117 days, total aldehydes and alkanes declined. Exceptions to this were the lower C_{23}–C_{25} aldehydes. When the C_{22}–C_{25} aldehyde area of waxes from non-CI and CI fruit stored 63 days at 5 °C was examined more closely by GLC, tetracosanal and, to a lesser extent, tricosanal and pentacosanal had higher levels in the non-CI than in the CI fruit, while in the higher aldehydes the reverse was true (Figure 5). This seems to indicate that even though these grapefruits were not conditioned against CI, some fruits are hardier than others; the fruits with higher levels of tetracosanal did not show any signs of CI.

In a previous study, we found spraying grapefruit with squalene provided protection from CI (unreported data). When September fruits were sprayed with 20% squalene in hexane for 5 s, the level of CI after 12 weeks of storage was much less than for temperature-conditioned fruit (Table II). The level of squalene in the sprayed fruit, when analyzed after 117 days at 5 °C, was over 1600 µg/100 g of fruit (Table I). This was at the approximate level of alkanes, aldehydes, and squalene combined from fruit conditioned 60 days at 15 °C. During these 117 days, the presence of this high level of squalene did not protect or excite the aldehyde- or alkane-synthesizing enzymes; the levels of these two lipid classes continued to decrease.

Degreening of fruit has become a necessary evil for citrus growers wanting to sell fresh fruit that is mature on the inside but still quite green on the outside. Previous studies have shown that degreened fruits are more susceptible to CI than nondegreened fruit (Hatton and Cubbedge, 1981), and we have further confirmed this here (Table II). During the degreening process the levels of all three lipid classes increased; however, degreening reduced the ability of the fruit during temperature conditioning to produce tetracosanal, hexacosanal, and squalene by 73%, 79%, and 94%, respectively (Table I).

Figure 6 shows a number of trends in the aldehyde levels when November fruits were stored or conditioned at five temperatures. Higher numbered aldehydes tended to decrease the most at 1 °C and the least at 20 °C. These decreases were mainly during the first 7 days of storage. Aldehydes of shorter chain length were synthesized at storage temperatures of 5–20 °C. Within fruit at 5 and 10 °C for 7 days only hexadecanal and squalene were readily synthesized. At 20 °C the decreasing trend of aldehyde synthesis was $C_{28} > C_{26} > C_{24} > C_{30}$. As observed previously (Nordby and McDonald, 1990), less squalene was produced at 20 °C than at 15 °C. At 15 °C for 21 days a decreasing trend was observed, e.g., $C_{24} > C_{26} > C_{28}$, with the tetracosanal level at 123 being slightly greater than the squalene level at 111 µg/100 g of fruit. These trends show a negative correlation with CI in these fruits after 8 and 12 weeks of storage (Table II). Thus, there is further evidence for tetracosanal having a positive role in preventing CI.

Previous work showed squalene to be preferentially extracted in the first 30 s (Nordby and McDonald, 1990), which provided evidence that it is deposited in the epicuticular wax of grapefruit as a result of temperature conditioning. This study examined the extraction of alkanes, aldehydes, and squalene at 10-, 20-, and 30-s intervals. The mean values for the first 10-s extractions were 46.7% for aldehydes, 53.1% for alkanes, 72.4% for squalene. This clearly shows that squalene is deposited in the epicuticular wax. Total percentages extracted after the second 10-s extraction were 75.3% for aldehydes, 81.8% for alkanes, and 94.6% for squalene. Of the aldehydes, tetracosanal had the highest extractability at 85.6%, with tricosonal and the higher numbered aldehydes showing progressively lower values. Whether this is due to the lower carbon numbered aldehydes being more soluble in chloroform than higher numbered aldehydes still has to be determined. However, there is a distinct possibility that this high tetracosanal value is due to its specific location in the wax, since squalene has an even higher value. Due to the very low concentrations of specific alkanes in this study, it is too early to determine if the higher concentrations of lower numbered alkanes are significant.

It has been reported (Schulman and Monselise, 1970) that citrus fruit will replenish its wax if dewaxed with an organic solvent and placed back in storage. December fruits that were dewaxed for the fresh fruit analyses were stored at 15 °C for 14 days. The levels of all aldehydes, alkanes, and squalene for the dewaxed fruit "conditioned" these 14 days at 15 °C were below the levels of respective lipids in the fresh December fruits (Table I). The difference between respective aldehyde levels of dewaxed and normal temperature-conditioned fruit was greatest for tetracosanal, showing that dewaxing restricts or eliminates all further synthesis of "protective aldehydes" such as tetracosanal and of other protective compounds such as squalene. This is in contrast to the findings of Schulman and Monselise (1970).

To determine the ability of aldehydes to restrict or reduce the incidence of CI in grapefruit, levels of the three lipid classes were reduced by dipping the fruit in chloroform or hexane for 10 s. The decreases are presented in Figure 7. Hexane decreased the three lipid classes by 18.4%. However, alkanes were reduced by only 5.5%,

Table II. Chill Injury Index (CI) and Percent Chill Injury (%CI) of Stored Grapefruit

		exam date, weeks							
		4		8		12		16	
month	5 °C/15 °C, treatment	CI	%CI	CI	%CI	CI	%CI	CI	%CI
Aug	15					0.2	16.7	1.4	55.0
Sept	5	1.3	73.9	3.2	90.0				
	15	0.0	0.0	0.0	2.3	0.8	35.7		
	5, +20% sqe	0.2	15.5	0.1	10.0	0.1	8.9		
Oct	5	3.0	96.7	4.6	96.7	5.2	98.3		
	15	0.1	5.4	1.2	60.8	2.0	66.2	2.5	93.5
Nov	1			3.7	100.0	3.5	100.0		
	5			3.4	85.2	3.4	88.9		
	10			2.0	83.3	2.8	84.8		
	15			0.6	40.7	1.3	70.4		
	20			1.1	64.3	1.5	82.1		
	5, degreened			5.5	100.0				
Dec	5	1.5	55.6	1.7	88.9				
	15	0.1	7.1	0.3	26.7				
Jan	5	0.8	48.3						
	15	0.2	17.8						
	5 hex, dip	1.0	58.0						
	5 $ChCl_3$, dip	5.6	100.0						

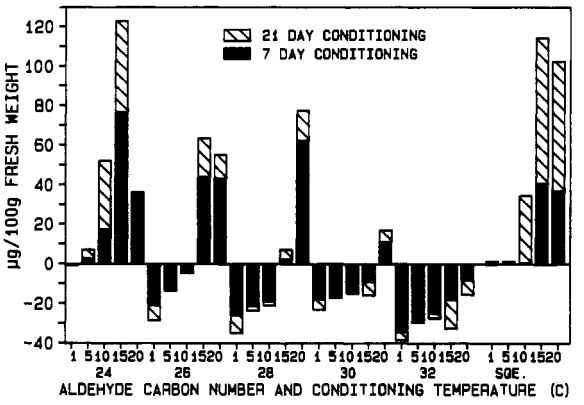

Figure 6. Changes in five aldehydes and squalene in the epicuticular wax of grapefruit after 7 and 21 days of temperature conditioning at 1, 5, 10, 15, and 20 °C.

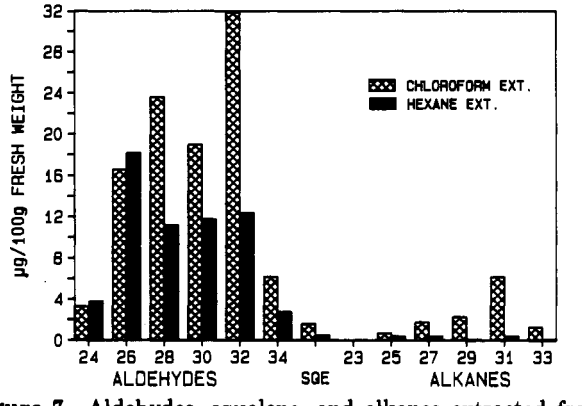

Figure 7. Aldehydes, squalene, and alkanes extracted from January 1990 grapefruit in a 10-s dip in chloroform or hexane.

while aldehydes and squalene were each reduced by 20%. Chloroform extraction reduced these three lipids by 35%. Individually these reductions were 70% for squalene, 45% for alkanes, and 34% for aldehydes. Hexane preferentially extracted the lower alkanes and aldehydes, whereas chloroform extracted the higher carbon numbered aldehydes. Fruit dipped in chloroform showed very intense CI on every fruit, whereas hexane-dipped fruits were not much worse than nondipped fruit (Table II). All three nonconditioned sets were definitely worse than the fruit conditioned 7 days at 15 °C. The above experiment does not rule out the possibility that chloroform harmed the membrane itself and that this destruction was the cause of the intense CI and not the partial extraction of the fruit's coat. Further studies are being conducted to determine an answer to this question.

Wax composition studies have been conducted for nearly a century, although only for the past 10–30 years have the isolations been carried out in such a manner that labile constituents were not destroyed in the isolation procedure. Long-chain aldehydes are a case in point. Sugarcane wax was known to contain compounds that appeared to be in dimeric or trimeric forms (Lamberton and Redcliffe, 1960) and which could not be quantified. Grape wax when dried in an oven or saponified failed to show any aldehydes. However, when not oven-dried but fractionated directly, the wax showed the presence of about 12% fatty aldehydes (Radler and Horn, 1965). Schulman and Monselise (1970) likewise failed to find aldehydes in orange wax, presumably because the wax was subjected to saponification prior to isolation. This was in contrast to a study by Baker et al. (1975), who reported aldehydes to be in the epicuticular wax of four citrus species in a range of 29–44%. However, grapefruits were not studied. Aldehydes are reported to be present in only trace amounts in blueberry wax (Freeman et al., 1979a), but in the range of 20–50% in citrus (Freeman et al., 1979b). In both studies, however, the authors extracted their wax with hot chloroform and dried the isolated wax overnight in an oven at 55 °C. Croteau and Fagerson (1971) reported cranberry wax to contain 14.3% aldehydes. The above-reported absences and variances of levels of aldehydes in various plants point out how potentially labile aldehydes really are and, also, that they may deteriorate once the plant or fruit has been harvested and stored as observed in the present study. Long-chain aldehydes, alkanes, and alcohols are reported (Grncarevic and Radler, 1967) to be excellent restrictors of the plant transpiration system in contrast to shorter chain aliphatics, terpenoids, and other cyclic compounds. Thus, our finding that 35–46% of the aldehydes in grapefruit wax are lost when the fruit is stored for 7 days seems to indicate that the fruit's natural barrier to water loss and influx of harmful gases has deteriorated. This deterioration by loss of C_{28}–C_{34} aldehydes can be

ameliorated by the "good aldehydes", namely tetra- and hexacosanal, which are synthesized along with squalene during a 7-day conditioning treatment at 15 °C, the optimal regime for temperature-conditioning grapefruit against CI. Therefore, one may postulate that C_{24}–C_{26} aldehydes, C_{25}–C_{27} alkanes, and squalene separately or in combination effectively "plug" the holes that develop in the wax coating of the grapefruit when it is placed in cold storage.

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Received for review August 16, 1990. Revised manuscript received December 6, 1990. Accepted December 14, 1990. Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Registry No. Squalene, 111-02-4; tricosanal, 72934-02-2; tetracosanal, 57866-08-7; pentacosanal, 58196-28-4; hexacosanal, 26627-85-0; heptacosanal, 72934-03-3; octacosanal, 22725-64-0; nonacosanal, 72934-04-4; triacontanal, 22725-63-9; hentriacontanal, 125507-60-0; dotriacontanal, 57878-00-9; tritriacontanal, 58196-30-8; tetratriacontanal, 132912-09-5; tricosane, 638-67-5; tetracosane, 646-31-1; pentacosane, 629-99-2; hexacosane, 630-01-3; heptacosane, 593-49-7; octacosane, 630-02-4; nonacosane, 630-03-5; triacontane, 638-68-6; hentriacontane, 630-04-6; dotriacontane, 544-85-4; tritriacontane, 630-05-7; docosanal, 57402-36-5.