The Effects of Storage Conditions on the Lipid Composition of Commercially Prepared Orange Juice

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An examination of the neutral lipid and polar lipid fractions of chilled orange juice was conducted upon storage over a 16-month period at 40 and 85° F. The neutral lipid content of both 40 and 85° F juice increased over 16 months and was due to fatty acids being hydrolyzed primarily from phospholipids. Free fatty acids increased three-fold in 40° F juice and eight-fold in 85° F juice. Phospholipid phosphorus decreased 69.6% while phosphatidyl choline, -ethanolamine, -serine, and -inositol showed decreases ranging from 46 to 91% at 85° F.

Commercially processed citrus juice is highly susceptible to off-flavor and off-odor development when stored at adverse temperatures and prolonged storage periods. Early studies by Nolte and von Loesecke (1940) on fresh and canned Florida Valencia orange juice showed that temperature aged juice differed from fresh juice by increased acidity, increased saponification and peroxide values, and by the presence of carbonyl compounds. Curl and Veldhuis (1947), and Swift (1951) showed that the suspended matter of citrus juice, which includes the lipid fraction, was the principal contributor to off-flavor in aged orange juice. Huskins *et al.* (1952) investigated the change in lipid composition of Valencia orange juice stored at 72° F for 2 years. These investigators found that the phospholipid phosphorus content had decreased to one-tenth its original value, lipid nitrogen had decreased to one-fifth, and lipid choline had completely disappeared. Changes were also noted in several fatty acids.

The indicated importance of lipids in off-flavor development stimulated further investigations of citrus lipids. The following study is concerned with the effects of storage temperatures and length of storage on the neutral lipid and polar lipid composition of chilled orange juice.

MATERIALS AND METHODS

Chemicals and Orange Juice Samples. Fatty acid methyl esters and neutral lipid standards, *viz.* cholesterol, cholesterol oleate, triolein, oleic acid, monoolein, and diolein, were obtained from the Lipids Preparation Laboratory of The Hormel Institute, Austin, Minn. Phospholipid standards, *viz.* phosphatidyl choline (PhC), -inositol (PhI), -serine (PhS) and -ethanolamine (PhE), were purchased from Applied Science Laboratories, State College, Pa. Chilled orange juice samples in 1 qt glass jars were purchased from Adams Packing Co., Auburndale, Fla. The juice had been pasteurized at 220° F for 3 sec, then brought to 40° F in 3 sec and, finally, aseptically filled into glass containers (industrially termed "cold-fill"). These samples were taken directly from the assembly line and stored at 40° F for 2 weeks. During this 2 week period, samples were visually inspected for any signs of fermentation.

Sample Preparation. After the 2-week inspection period, chilled orange juice samples were divided equally and stored at 40 and 85° F. At varying time periods between 0 and 16 months, samples were removed from both the 40 and 85° F storage chambers and freeze-dried to a powder possessing a moisture content no greater than 4%.

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Total lipids were extracted from 15 g orange powder samples in the following manner. The sample was placed in a Waring Blendor and 25 ml water added for rehydration. Three hundred ml of $CHCl_3$-MeOH (2 to 1, v/v) was next added and the total mixture blended at room temperature for 10 min at low speed. The mixture was filtered on a coarse sintered glass funnel and the residue subjected to two more successive 150 ml extractions ($CHCl_3$-MeOH, 2 to 1, v/v). The mixtures were filtered and the combined filtrate concentrated (*in vacuo*, under nitrogen at 86° F) until a thick syrup resulted. The syrup was transferred to a separatory funnel with alternate aliquots of 50 ml $CHCl_3$ and 50 ml water. Ammonium sulfate (*ca.* 1 g) was added to the funnel to enhance separation of the biphasic system, and the contents shaken vigorously. Upon removal of the $CHCl_3$ layer, the aqueous layer was reextracted with four 50 ml $CHCl_3$ aliquots until free of chloroform-soluble pigments. The combined $CHCl_3$ phases were reduced to dryness (*in vacuo*, under nitrogen at 86° F) and the crude lipid purified by the Sephadex method of Wuthier (1966). Dry weights were taken on the Sephadex-purified lipid by vacuum drying under desiccation at room temperature.

Column Chromatography. The Sephadex-purified lipid was fractionated into neutral lipids and polar lipids by silica gel chromatography. Columns used in separation were 30 cm in length × 0.9 cm i.d. and contained 11 g of silica gel (60 to 200 mesh, J. T. Baker Chemical Co., Phillipsburg, N.J.). Fractionation of the purified lipid was conducted by percolating 20 to 30 mg lipid in absolute $CHCl_3$ (no methanol stabilizer added) onto a gel column prepared in absolute $CHCl_3$. Neutral lipids were eluted with 300 ml absolute $CHCl_3$ and finally with 50 ml of 1% methanol in $CHCl_3$. Twenty-five ml aliquots were monitored by tlc to insure elution completeness. This solvent sequence was also tested on a standard mixture of 10 mg of triolein and 10 mg of oleic acid, and found to elute completely the highly retentive free fatty acid. Polar lipids were not eluted from the column. Regardless of the amount of methanol used to elute this lipid group, elution completeness, as measured by phospholipid phosphorus, was never greater than 93%. Polar lipids were determined by difference between total lipid percolated onto the gel column and the weight of the neutral lipids eluted.

Thin-Layer Chromatography. Quantitative determination of neutral lipid constituents was accomplished by tlc densitometry on 20 × 20 cm precoated silica gel G plates (250 μ, Analtech, Inc., Wilmington, Del.). The precoated plates were prewashed with ethyl ether and activated at 230° F for 1 hour prior to sample application. Plates were ruled in 9

mm width lanes. The standard neutral lipid and unknown neutral lipid mixtures were spotted on the same plate with disposable capillary pipets at a concentration that would yield *ca.* 1 to 10 μg for each individual lipid in the mixture. Development of the plate was by a sequential two-step elution procedure similar to that of Biezenski *et al.* (1968). The plate was first developed in ethyl ether-benzene-ethanol-acetic acid (40:50:2:0.2 v/v) to a distance 10 cm above the point of sample application. After this first development, the plate was dried in a vacuum desiccator for *ca.* 20 min. The plate was then placed in a solvent of hexane-benzene-acetic acid (80:25:1 v/v) and developed to a point 16 cm above the origin. The plate was vacuum dried, sprayed lightly and evenly with 50% H_2SO_4 and finally charred in a muffle furnace at 410° F for 20 to 30 min. Densitometry was carried out with a Photovolt Densitometer Model 530 (Photovolt Corp., New York, N.Y.). The light source unit was fitted with a specially designed 1 × 6 mm substage slit to limit the incident light beam. A commercially available 0.2 × 6 mm collimating slit was also placed over the photocell. The technique of normalizing all density values to the observed density area of triolein (Blank *et al.*, 1964) was adopted to account for the differences in chemical structure and R_f values for each of the neutral lipid constituents.

Quantitative determination of PhC, PhE, PhI, and PhS was accomplished through use of two-dimensional tlc according to the method of Nagy (1965). Glass plates, 20 × 20 cm, were coated to a thickness of 400μ with silica gel H (Brinkmann Instruments, Inc., Westbury, N.Y.). The H plates were prewashed with MeOH-$CHCl_3$-conc. HCl (80:10:10 v/v) to remove organic impurities, air dried for 1 hr and activated at 230° F for 1 hr prior to sample application. The Sephadex-purified lipid mixture (both neutral lipids and polar lipids) was placed in a 1 cm streak on the lower right hand corner of the plate. The plate was developed to a height of 18 cm with $CHCl_3$-MeOH-7*N* NH_4OH (70:30:1 v/v). The plate was removed from the chamber, vacuum dried for *ca.* 20 min to remove this first solvent, turned 90° and placed in $CHCl_3$-acetone-MeOH-acetic acid-water (125:50:12:10:5 v/v) for development in the second direction. Neutral lipids migrated with one and/or both of the solvent fronts while the polar lipids showed excellent resolution. PhC, PhE, PhI, and PhS were cleanly resolved from one another and from other constituents as determined by charring and the specific phospholipid spray of Dittmer and Lester (1964).

Gas-Liquid Chromatography. Fatty acid methyl esters were determined with an F and M Model 5750 gas chromatograph equipped with hydrogen flame detectors. Dual aluminum columns (3.05 m in length and 3 mm, i.d.) coated with 10% stabilized DEGS (Analabs, Inc., Hamden, Conn.) on 100/120 mesh, DMCS treated, acid washed Chromosorb W were used for all quantitative analyses. Methyl ester samples were determined isothermally at 392° F oven temperature, 482° F detector temperature, 455° F injection temperature, and a helium flow-rate of 80 ml per min. Quantitative results were obtained by measuring peak areas with the aid of a disc integrator.

Fatty Acid Methyl Ester Preparation. Methyl esters were prepared from the following lipid compounds, *viz.* free fatty acids, total polar lipids, phosphatidyl choline, and phosphatidyl ethanolamine. Free fatty acids and total polar lipid were resolved by streaking a total lipid mixture (both neutral lipids and polar lipids) dissolved in benzene-absolute ethanol (4 to 1, v/v) on precoated G plates. Lipid mixtures containing less than 20% free fatty acids were streaked on 250μ plates while lipids containing greater than 20% were streaked on 500μ plates. Plates were sprayed with Rhodamine 6G and the free fatty acid band, and the area from the origin to the free sterol band (polar lipids) scraped from the plate. The silica gel plus lipid mixture was subjected to transmethylation by the BF_3-methanol method (Metcalfe *et al.*, 1966) with slight modifications. The mixture was refluxed 5 min with 1*N* NaOH in methanol (alkaline reflux being eliminated for free fatty acid mixture). After reflux, 5 ml of 14% BF_3-methanol reagent (Applied Science Labs., State College, Pa.) was added and refluxing continued for an additional 5 min. The esters were extracted with heptane (Van Wijngaarden, 1967), the heptane layer washed with water saturated with NaCl, and finally, the heptane layer was dried over Na_2SO_4. All esters were stored in 0.1 ml heptane under helium at 41° F.

Phosphatidyl ethanolamine and phosphatidyl choline fractions were resolved from a 12 to 17 mg total lipid sample. The sample, dissolved in benzene-absolute ethanol (4 to 1, v/v) was streaked on a 400 μ, 20 × 20 cm silica gel H plate. The plate was activated 1 hr at 230° F prior to sample application. The plate contained, in addition to the unknown sample, phospholipid standards spotted on two sides of the plate. The plate was developed in $CHCl_3$-MeOH-7*N* NH_4OH (70:30:4 v/v) and after development was dried 15 min in a vacuum desiccator. Two cm strips on both sides of the plate were sprayed with the phosphate spray (Dittmer and Lester, 1964). The areas in the central unsprayed portion of the plate corresponding to the two phospholipids were scraped from the plate and eluted with 40 ml methanol. Upon removal of the methanol by rotary evaporation, the samples were dissolved in benzene-ethanol and individually restreaked on separate 400 μ *H* plates. Plates were developed in chloroform-acetone-methanol-acetic acid-water (10:4:2:2:1 v/v) and after development dried in a vacuum desiccator for 15 min. The phospholipids were detected by spraying the edges of the plate with the phosphate spray and the lipid scraped from the plate. Methyl esters were obtained from these two phospholipids by transmethylation of the silica gel-phospholipid mixture in a manner similar to total polar lipids. Comparative analyses of the esterification procedures between samples eluted from silica gel and those esterified without prior elution revealed no differences in quantitation of methyl esters.

Other Chemical Methods. Sephadex-purified lipid was analyzed for phospholipid phosphorus by the method of Bartlett (1959).

RESULTS AND DISCUSSION

Neutral Lipid Composition of Stored Juice. The weight of the neutral lipid fraction, as a function of storage time and temperature, was recorded over a 16-month period. At zero storage time, 40.8 mg of neutral lipids is extracted from 15 g of orange juice powder. After 16 months, the amount extracted from 40° F juice is 46.3 mg, an increase of 11%. The amount of neutral lipid obtained from 85° F juice for the same period is 66.4 mg, an increase of 63%. To determine the reason for this increase, neutral lipid fractions from both 40 and 85° F juice were taken at 0, 3, 5, 11, 15, and 16-month storage periods and subjected to quantitative tlc densitometry. Table I shows the storage time *vs.* wt% distribution of neutral lipid constituents for 85° F juice. The weight distribution of 40° F juice was similarly tabulated but is not reported here. Each of the lipid constituents is the average of from 3 to 5 separate determinations, and each is accompanied by a standard deviation. The most important feature of Table I is the

Table I. Neutral Lipid Constituents of 85° F Chilled Orange Juice
Wt %

Storage (mo.)	MG[a]	FFA	FS	UNK	1,2DG	1,3DG	TG	SE	HC
0	1.7 ± 0.2	11.1 ± 2.6	28.1 ± 1.3	0.6 ± 0.1	4.4 ± 0.4	1.7 ± 0.9	24.3 ± 3.8	20.8 ± 1.2	8.8 ± 3.3
3	0.7 ± 0.1	36.3 ± 0.5	19.7 ± 1.0	0.2 ± 0.1	1.4 ± 0.3	1.3 ± 0.2	20.8 ± 0.7	14.7 ± 0.3	5.0 ± 1.4
5	1.4 ± 0.1	45.6 ± 1.9	17.1 ± 0.4	0.3 ± 0.1	1.5 ± 0.1	1.0 ± 0.2	18.9 ± 0.0	14.0 ± 0.8	4.1 ± 2.4
11	1.1 ± 0.2	50.9 ± 3.2	15.7 ± 0.9	0.4 ± 0.1	0.8 ± 0.4	1.5 ± 0.5	14.1 ± 1.1	11.2 ± 0.2	4.0 ± 2.4
15	0.4 ± 0.1	53.4 ± 2.9	15.6 ± 0.7	0.3 ± 0.2	0.9 ± 0.3	1.2 ± 0.3	12.5 ± 0.6	10.9 ± 1.3	4.2 ± 1.2
16	0.4 ± 0.2	54.6 ± 0.5	15.2 ± 0.5	0.2 ± 0.1	0.5 ± 0.2	1.5 ± 0.5	12.4 ± 0.8	11.3 ± 0.2	4.0 ± 1.4

[a] Monoglyceride—MG; free fatty acid—FFA; free steroid—FS; unknown—UNK; 1,2 diglyceride—1,2DG; 1,3-diglyceride—1,3DG; triglyceride—TG; steryl ester—SE; hydrocarbon—HC.

percentage increase in free fatty acid, *i.e.*, 11.1 to 54.6%. The free acid increase determined for 40° F juice was to 26.9%. All other neutral lipid constituents from both storage temperatures show a relative wt% decrease over the 16-month period. This relative wt% decrease is, however, misleading. A component possessing a constant concentration throughout the storage period would show a wt% decrease if another component, *e.g.*, free fatty acid, increases. To obtain an accurate representation of a component's concentration, the component's wt% is multiplied by the weight of the total neutral lipid fraction at that specific storage period. Table II shows the weight of three major neutral lipids, *viz.* free fatty acid, triglyceride, and steryl ester, at 40 and 85° F, and varying storage periods. Triglyceride and steryl ester were selected because they would be the major contributors of free fatty acid if hydrolyzed. At 40° F, triglyceride shows no apparent breakdown, while at 85° F, the loss is only 1.7 mg. For steryl ester, the breakdown rate is higher at 85° F, but the total amount lost is approximately similar for both temperatures, *i.e.*, 1.3 mg. The free fatty acid concentration range presents a markedly different profile. At 40° F, fatty acids show a three-fold increase, while 85° F juice shows an eight-fold increase.

Table III shows the four major fatty acids comprising this free fatty acid fraction. Collectively these acids, *viz.* palmitic ($C_{16:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$), comprise greater than 88% of all fatty acids in orange juice (Nordby and Nagy, 1969). For 40° F juice, oleic acid increases *ca.* 2.6-fold while palmitic, linoleic, and linolenic acids increase three-fold. The free acids of 85° F juice presents a different concentration range, *i.e.*, palmitic and oleic acids show a seven-fold increase, linoleic 9.3-fold increase, and linolenic 9.7-fold increase.

Table II reveals that the small breakdown in triglyceride and steryl ester could not possibly account for the large concentration gain in the free fatty acid fraction. It appears, *a priori*, that the increase in free acids is due to breakdown of constituents in the polar lipid fraction.

Polar Lipid Composition of Stored Juice. The polar lipid group of orange is composed of a number of different lipids, *viz.* galactose-containing glycerides, cerebrosides, phospholipids, steryl glucosides, acylated steryl glucosides, and a sulfolipid (unpublished observations). In this investigation we have focused our attention on the phospholipid group because this group has been implicated by a number of investigators in several food product areas to be highly susceptible to enzymic breakdown (Olley and Lovern, 1960; Bligh and Scott, 1966; Bosund and Ganrot, 1969).

Figure 1 shows the percentage decrease of phospholipid phosphorus in 85° F stored juice *vs.* storage time. After 16 months storage, the phospholipid concentration decreased to 30.4% of its original value (77.4 μM of lipid phosphorus from 15 g of orange juice powder is regarded as 100% at zero storage time).

From the phospholipid group, the concentrations of four major phospholipids, *viz.* PhC, PhE, PhI, and PhS, were monitored by two-dimensional tlc during storage at 85° F over this 16-month period. Figure 2 shows a concentration *vs.* storage time profile for these four phospholipids. As measured by phospholipid phosphorus, PhC decreases 91%, PhE 88%, PhI 88%, and PhS 46%. PhC, PhE, and PhI appear similar in breakdown magnitude, while PhS shows a slower rate of enzymic destruction. Examination of the tlc profiles of these four phospholipids did not show formation of any lyso derivatives. It is concluded, therefore, that phospholipid breakdown is complete, and suggests further that both fatty acids associated with the phospholipid molecule are split off.

Positional heterogenity exists in all phospholipid classes because of the number of fatty acid combinations possible. It would be of interest to determine whether hydrolysis of a particular phospholipid proceeds in a random or selective

Table II. Weight of Triglyceride, Steryl Ester, and Free Fatty Acid as a Function of Storage Time and Temperature

Time (mo.)	Weight (mg/15 g powder) TG[a] 40° F	TG 85° F	SE 40° F	SE 85° F	FFA 40° F	FFA 85° F
0	9.9	9.9	8.5	8.5	4.5	4.5
3	9.7	9.4	8.4	6.6	6.8	16.4
5	10.1	8.7	7.8	6.8	7.8	22.0
11	10.1	8.6	7.0	6.8	10.8	31.9
15	10.2	8.1	7.4	7.0	11.7	34.7
16	10.2	8.2	7.2	7.2	12.3	36.2

[a] Triglyceride—TG; steryl ester—SE; free fatty acid—FFA.

Table III. Weight of Free Fatty Acids as a Function of Storage Time and Temperature

Time (mo.)	Weight (mg/15 g powder) 40° F 16:0[a]	40° F 18:1	40° F 18:2	40° F 18:3	85° F 16:0	85° F 18:1	85° F 18:2	85° F 18:3
0	1.3	1.3	1.1	0.6	1.3	1.3	1.1	0.6
3	2.0	1.9	1.7	0.9	4.5	4.1	4.3	2.4
5	2.3	2.0	2.0	1.1	5.7	5.3	5.7	3.3
11	3.3	2.7	2.7	1.4	8.7	7.6	9.1	4.5
15	3.5	3.4	3.0	1.6	9.1	8.8	10.1	5.6
16	4.0	3.3	3.4	1.8	9.1	8.8	10.2	5.8

[a] Number of carbons in chain: number of double bonds.

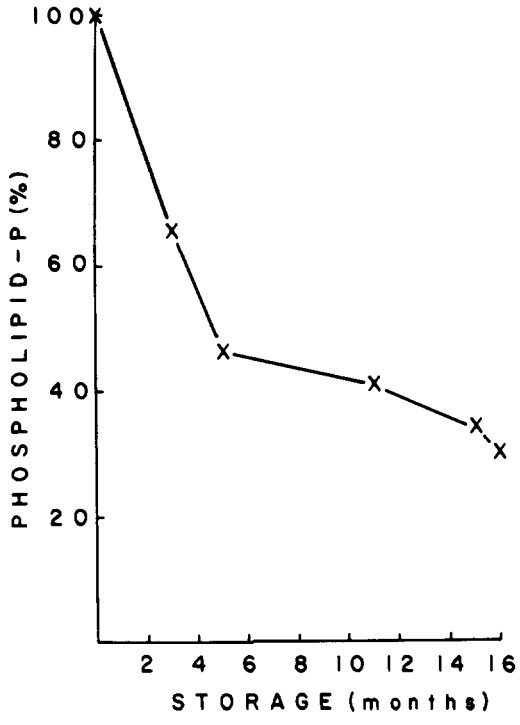

Figure 1. Percent change in phospholipid phosphorus over a 16-month period for orange juice stored at 85° F

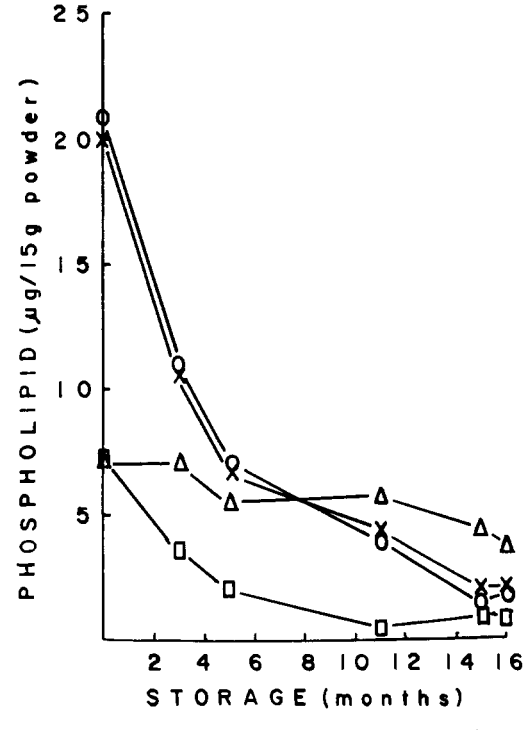

Figure 2. Effect of storage at 85° F on the concentrations of PhC (○), PhE (×), PhI (□), and PhS (△) over a 16-month period; phospholipid concentrations expressed as phospholipid phosphorus

manner. To this end, fatty acid profiles were determined for two phospholipids, *viz.* PhC and PhE, at 3 and 16 months for the two storage temperatures, *i.e.*, 40 and 85° F. Table IV shows these comparative fatty acid profiles. For juice stored at 40° F there appear only slight differences in the fatty acid pattern for both PhC and PhE at the 3- and 16-month periods. The fatty acid profile of PhC at 85° F shows a marked change between these storage periods. The most

Table IV. Comparative Fatty Acid Composition of PhC and PhE

Fatty acid	PhC				PhE			
	40° F		85° F		40° F		85° F	
	3 mo	16 mo	3 mo	16 mo	3 mo	16 mo	3 mo	16 mo
14:0	0.2	0.2	0.2	0.4	0.1	0.9	0.3	0.6
16:0	21.9	22.2	23.5	17.6	28.6	27.5	26.4	21.5
16:1	4.2	3.1	4.1	3.2	3.8	2.3	2.9	3.2
18:0I[a]	0.6	0.6	0.8	0.5	0.5	0.6	0.5	0.6
18:0	1.4	1.2	1.6	2.3	1.0	1.0	1.3	1.7
18:1	30.3	31.6	29.6	37.9	20.9	21.5	21.6	29.7
18:2	27.9	28.2	27.7	26.8	34.2	35.1	34.1	31.4
18:3	13.5	13.1	12.5	11.3	10.9	11.1	12.9	11.3

[a] Iso derivative of stearic acid.

Table V. Contribution of Phospholipid Fatty Acid to Total Free Fatty Acid Increase

Fatty acid	Polar Lipid Fatty Acid (%)	M.W.	M.W. × % FA
14:0	0.2	228.4	0.4
16:0	24.5	256.4	62.9
16:1	3.2	254.4	8.2
18:0[a]	0.7	284.5	2.0
18:0	1.3	284.5	3.7
18:1	25.3	282.5	71.5
18:2	29.7	280.5	83.4
18:3	15.3	278.5	42.6
		mean M.W. =	274.7

(53.8 μM P) (2 μM FFA) (274.7 μg/μM) = 29.6 mg

$\frac{29.6}{31.3}$ = 94.6% = relative percent phospholipid fatty acid contribution

[a] Iso derivative of stearic acid.

noticeable change is in the concentration of palmitate (25% decrease) and oleate (22% increase). PhE shows a similar profile change at 85° F storage. The most affected acids are again palmitate (19% decrease) and oleate (27% increase). It would appear from examination of the data in Table IV that hydrolysis of PhE and PhC does not proceed in a random manner, but rather proceeds through selective hydrolysis. Another possibility is that all PhE and PhC molecules are not equally exposed to the hydrolytic action of the phospholipases.

From the breakdown of phospholipids, it was possible to account for a considerable percentage of the free fatty acid increase manifest in the neutral lipid fraction. Table V shows the calculations which were employed to approximate the amount of fatty acids liberated from the phospholipid group. The first calculation was the determination of the mean molecular weight of the fatty acid associated with the phospholipid group. The phospholipid group, however, could not be completely separated from the polar lipid fraction *in toto;* therefore, fatty acid percentages of the total polar lipid fraction were employed to determine the mean molecular weight. As shown in Table V, the calculated mean molecular weight is 274.7 μg per μM. This weight, although relative, closely approximates the mean fatty acid molecular weight of two major phospholipids, *viz.* PhC (274.6) and PhE (272.9). During the 16-month storage period at 85° F, the loss in phospholipid phosphorus amounted to 53.8 μM. For every μM of phospholipid phosphorus lost, approximately 2 μM of fatty acid is generated. Multiplying the μM of lost phospholipid phosphorus by 2 μM of fatty acid times the mean fatty acid molecular weight yields a value of 29.6 mg fatty acid. From Table II, the gain in free fatty acid over the 16-

month storage period at 85° F is 31.3 mg. The relative percentage contribution of phospholipid fatty acid to the free fatty acid gain is approximated at 94.6%. This value, while relative, can be regarded as closely approximating the true value of fatty acids contributed by the phospholipid group.

The relative importance of lipase, steryl esterase, and phospholipase activity in the formation of free fatty acids during storage has received little attention. Lipases are enzymes which hydrolyze glyceryl esters of fatty acids (neutral fats), steryl esterases hydrolyze steryl esters, and phospholipases hydrolyze phospholipids (Kates, 1960). From these studies (Table II and Figure 2) it appears, *a priori*, that neutral lipase and steryl esterase activities are relatively weak, while phospholipase activity is quite pronounced.

The production at elevated storage temperatures of large quantities of unsaturated fatty acids (Table III) predisposes commercial orange juice to off-odor and off-flavor development. High carbon number fatty acids, as found in orange juice, contribute very little to flavor; however, their importance is as precursors to many volatile off-flavor compounds. Fatty acid oxidative products, formed by autocatalytic and enzymic (lipoxidase) mechanisms, have been implicated in off-flavor development in aged orange juice (Huskins and Swift, 1953; Huskins *et al.*, 1952). Many of these fatty acid oxidative products, *e.g.*, alk-2,4-dienals, 2-octenal, and *n*-hexanal from linoleic acid, possess extremely malodorous and malflavorous properties.

Storage of citrus juice at low temperatures, *i.e.*, −5° F for concentrate and 40° F for single-strength, is commercially employed to maintain flavor stability. It would appear from these studies that control of phospholipase activity would serve as an important adjunct to low temperature storage.

LITERATURE CITED

Bartlett, G. R., *J. Biol. Chem.* **234,** 467 (1959).
Biezenski, J. J., Pomerance, W., Goodman, J., *J. Chromatogr.* **38,** 148 (1968).
Blank, M. L., Schmit, J. A., Privett, O. S., *J.A.O.C.S.* **41,** 371 (1964).
Bligh, E. G., Scott, M. A., *J. Fish. Res. Bd. Can.* (Canada) **23,** 1025 (1966).
Bosund, I., Ganrot, B., *J. Food Sci.* **34,** 13 (1969).
Curl, A. L., Veldhuis, M. K., *Fruit Prod. J. Amer. Food Mfr.* **26,** 329 (1947).
Dittmer, J. C., Lester, R. L., *J. Lipid Res.* **5,** 126 (1964).
Huskins, C. W., Swift, L. J., *Food Res.* **18,** 360 (1953).
Huskins, C. W., Swift, L. J., Veldhuis, M. K., *Food Res.* **17,** 109 (1952).
Kates, M., in "Lipid Metabolism," K. Bloch, Ed., p. 165, Wiley, New York, 1960.
Metcalfe, L. D., Schmitz, A. A., Pelka, J. R., *Anal. Chem.* **38,** 514 (1966).
Nagy, S., PhD Thesis, Rutgers, The State University, New Brunswick, N.J. (1965).
Nolte, A. J., von Loesecke, H. W., *Food Res.* **5,** 457 (1940).
Nordby, H. E., Nagy, S., *Phytochemistry* **8,** 2027 (1969).
Olley, J., Lovern, J. A., *J. Sci. Food Agr.* **11,** 644 (1960).
Swift, L. J., *Proc. Fla. State Hort. Soc.* **64,** 181 (1951).
Van Wijngaarden, D., *Anal. Chem.* **39,** 848 (1967).
Wuthier, R. E., *J. Lipid Res.* **7,** 558 (1966).

Received for review January 19, 1970. Accepted April 6, 1970. References to brand names are for identification and do not imply endorsement. A laboratory of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.