

# Citrus Juice Characterization

## Identification and Estimation of the Major Phospholipids

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The major phospholipids in orange, lemon, and grapefruit juices were identified as phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidylserine (PS), and phosphatidylinositol (PI). The individual phospholipids were separated by thin-layer chromatography and estimated by their phosphorus content.

The average values (mg per 100 ml) for orange, lemon, and grapefruit juices were: PE, 13, 11, 6; PC, 14, 12, 8; PA, 2, 0.7, 0.2; PS, 1, 1, 0.2; and PI, 3, 5, 3, respectively. An unidentified phospholipid was observed in commercial orange juice and several lemon and grapefruit juices, but not in any of the fresh hand-reamed juices.

The total lipid phosphorus content of citrus juices was reported as part of an earlier paper from this laboratory (Vandercook and Guerrero, 1969). This lipid fraction of a citrus juice makes a significant contribution to the color, flavor, and cloudy appearance (Curl and Veldhuis, 1947). All three of these properties are important factors in consumer acceptance of citrus juices. Much of the color and flavor are associated with the cloud, and loss of cloud results in an unattractive clear serum and a residue on the bottom of the container. Swift and Veldhuis (1951) and Huskins *et al.* (1952) have made some studies on the lipid composition of orange juice. More recently, Nagy and Nordby (1970) studied the effects of storage on the composition of the lipid fraction.

Commercial phospholipids, such as soy bean lecithin, might be potential adulterants as clouding agents or emulsifiers in citrus juices. To forestall this possibility, and to expand the general knowledge of citrus chemistry, it would be desirable to study the composition of citrus phospholipids. The purpose of this investigation was to identify and quantitatively measure the major phospholipids of representative orange, lemon, and grapefruit juice samples.

### EXPERIMENTAL

Extraction of the phospholipids followed the procedure reported earlier (Vandercook and Guerrero, 1969) with only minor modifications. Since the phospholipids are labile compounds, all manipulations were carried out as quickly as possible and with evaporation temperatures not exceeding 30° C. All extracts were stored under nitrogen in a freezer when they could not be used immediately.

A 20 ml aliquot of juice was mixed with Celite and filtered. The filter cake was washed twice with 5 ml portions of water. The filtrate and washings were discarded. The lipids were washed through the filter cake with small portions of chloroform-methanol (C-M) (67:33) (50 to 60 ml) until it was colorless. The residue was then removed and extracted with 20 ml of the same solvent and filtered; the residue was again washed with 5 ml of solvent. The entire extract was passed through a 1.5 × 20 cm column of Sephadex G-25 (coarse) equilibrated with water-saturated C-M (95:5) to separate nonlipids from lipids (Siakotos and Rouser, 1965). An additional 125 ml of the C-M (95:5) water-saturated solvent was passed through

the column and the total eluate made up to 250 ml with chloroform. A 50 ml aliquot was digested for the determination of total lipid phosphorus. A 100 ml aliquot was concentrated to 10 ml. Three milliliters of this solution were further concentrated under a stream of nitrogen and spotted under nitrogen on a 0.25 mm thick, 20 × 20 cm pre-coated silica gel (E. Merck) thin-layer chromatographic plate used directly from the package. The plates were developed two-dimensionally using chloroform, methanol, conc. NH<sub>4</sub>OH (65:35:5) in one direction, and chloroform, acetone, methanol, acetic acid, and water (56:20:10:10:4) in the other direction.

The plates were sprayed with a ninhydrin solution (Dittmer and Lester, 1964) and heated to 100° C for 2-3 min to detect phosphatidylethanolamine and phosphatidylserine. This was followed by a molybdenum spray (Dittmer and Lester, 1964) which reacted with the phospholipids to give blue spots. The plates were again heated to 150° C to char the other compounds. The silicic acid layers containing the phospholipid spots were scraped from the plate and digested with 5 ml of HNO<sub>3</sub> and 1.0 ml of 50.0% (v/v) H<sub>2</sub>SO<sub>4</sub>. The mixture was heated to fuming, cooled, 2 ml of water added, and again heated to fuming. The remaining 0.5 ml of digested phospholipid solution was diluted in the Kjeldahl flask with 24.5 ml of water. The phosphorus was determined on this fraction, after centrifuging, by the phosphomolybdovanadate complex as modified by Gherke *et al.* (1967) for the Technicon Autoanalyzer.

A 100-ml batch of orange juice was worked up as above through the Sephadex column stage. The extract was then concentrated and chromatographed on a 2.5 × 20 cm diethylaminoethyl (DEAE) cellulose column for the separation and tentative identification of the phospholipids. The column was eluted stepwise according to Rouser *et al.* (1964) with chloroform (C); chloroform-methanol (C-M) (90:10); C-M (70:30); acetic acid (HAc); methanol; and C-M (80:20) with 20 ml conc. NH<sub>4</sub>OH per l. made 0.01M in NH<sub>4</sub>Ac. The fractions were concentrated and compared with standards (Applied Science Laboratories, Inc.) by thin-layer chromatography in the above solvent pairs.

### RESULTS AND DISCUSSION

The elution sequence reported by Rouser *et al.* (1964) was used as a guide for the tentative identification of the major orange juice phospholipids. Carotenoids,  $\beta$ -sitosterol,  $\beta$ -sitosterol glucoside, and neutral glycerides were found in the first fraction. The second and third fractions contained phosphatidylcholine (PC) and phosphatidylethanolamine (PE), respec-

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**Table I. Phospholipid Composition of Citrus Juices**

Sample	Lipid P <sup>a</sup>	PE <sup>b</sup>	PC <sup>b</sup>	PA <sup>b</sup>	PS <sup>b</sup>	PI <sup>b</sup>	X <sup>b</sup>
Orange Juice							
Com'l Cal.	1.18	32	42	3	5	3	14
Com'l Cal.	1.53	29	34	15	4	10	9
Com'l Fla.	1.40	41	38	4	trace	16	1
Com'l Fla.	1.50	38	47	5	2	1	2
Com'l Fla.	1.70	37	39	6	1	15	3
Com'l Fla.	1.60	40	38	5	trace	15	1
Fresh Cal.	1.30	44	42	12	2	trace	0
Fresh Cal.	0.70	47	34	13	1	6	0
Fresh Cal.	1.10	35	43	10	5	7	0
Fresh Cal.	1.10	33	50	1	12	5	0
Fresh Cal.	1.30	37	47	trace	3	12	0
Mean	1.31	38	41	7	3	8	5 <sup>c</sup>
Std. Dev.	0.284	5.3	5.3	5.0	3.4	5.8	5.3
Lemon Juice							
Com'l Ariz.	0.84	40	42	4	5	9	0
Com'l Ariz.	1.20	43	44	0	4	9	0
Com'l Ariz.	1.60	39	38	1	7	15	0
Com'l Ariz.	1.60	40	39	2	3	16	0
Com'l Cal.	0.90	39	40	0	4	17	0
Com'l Cal.	1.00	39	42	1	3	15	trace
Com'l Cal.	1.10	34	44	0	2	20	0
Com'l Cal.	1.10	30	31	0	6	33	0
Fresh Cal.	1.10	34	44	2	8	12	0
Fresh Cal.	1.10	40	46	3	9	15	0
Fresh Cal.	1.20	41	41	1	5	12	0
Fresh Cal.	1.10	32	41	7	8	12	0
Mean	1.15	38	41	2	5	15	...
Std. Dev.	0.234	4.0	4.1	2.1	2.3	6.4	...
Grapefruit Juice							
Com'l	1.00	33	35	trace	trace	22	9
Com'l	0.83	36	49	trace	trace	13	trace
Com'l	0.70	38	44	5	3	11	0
Fresh	0.43	40	51	trace	trace	9	0
Fresh	0.40	34	58	trace	trace	8	0
Mean	0.67	36	47	1	1	12	...
Std. Dev.	0.258	2.9	8.6	...	...	5.6	...

<sup>a</sup> Mg lipid P per 100 ml juice. <sup>b</sup> Percentage of lipid P. <sup>c</sup> Average of commercial samples only.

**Table II. Average Concentration of Phospholipid Compounds in Citrus Juices (mg per 100 ml juices)**

	PE	PC	PA	PS	PI	X
Orange	13	14	2	1	3	2 <sup>a</sup>
Lemon	11	12	0.7	1	5	0
Grapefruit	6	8	0.2	0.2	3	0

<sup>a</sup> Unknown phospholipid found in commercial samples only.

tively. The acetic acid fraction contained phosphatidylserine (PS). Methanol removed the acetic acid, and the last fraction contained phosphatidylinositol (PI) and phosphatidic acid (PA). All of these compounds were identified by comparison with standards on tlc. PE and PS both gave the same color with the ninhydrin spray as their standards.

When the fractions from the column were concentrated and chromatographed by tlc, some minor compounds were seen as a result of the concentrating effect of the column. These minor constituents have not been identified. They are present in low enough concentrations that they are not seen in the juice extracts at the normal level of spotting for tlc.

A typical chromatogram of phospholipids from fresh orange juice is shown in Figure 1. The dotted spot represents the position of an unknown phospholipid observed in some of the orange concentrates but not in freshly squeezed orange juice.

It was not observed in fresh lemon or grapefruit juice; however, several commercial samples of these juices contained the compound. The unknown spot reacted with ninhydrin to give a blue color. With the exception of the higher levels of carotenoids in the orange, the lemon and grapefruit phospholipid chromatograms are similar to the one in Figure 1.

The quantitative estimation of phosphorus values for the major phospholipids of orange, lemon, and grapefruit juices are listed in Table I. These values are averages from at least two plates, and they represent the phosphorus from a given spot as a percentage of the total recovered phosphorus from the plate. The averages of the individual phospholipids in orange, lemon, and grapefruit juices were essentially the same for all three juices. About 80% of the lipid phosphorus can be accounted for by PE and PC, another 8 to 15% by PI, the remainder by PA, PS, and in a few samples by the unknown.

The concentration of the individual phospholipids may be calculated from the amount of lipid phosphorus in the juice times the percentage of the constituent times a factor related to the phosphorus content of the constituent. Table II gives the average amounts of the phospholipids in the citrus juices from Table I using the factors reported by Rouser *et al.* (1966) (PE, PC, 25.4; PA, 25.0; PS, 26.2; PI, 31.4). The lower limit of detectability for phosphorus in a chromatographic spot was about 1 µg. When the level of an individual compound was represented as 0, no spot could be detected on the plate.

2. C/A/M/HAc/W  
(56/20/10/10/4)

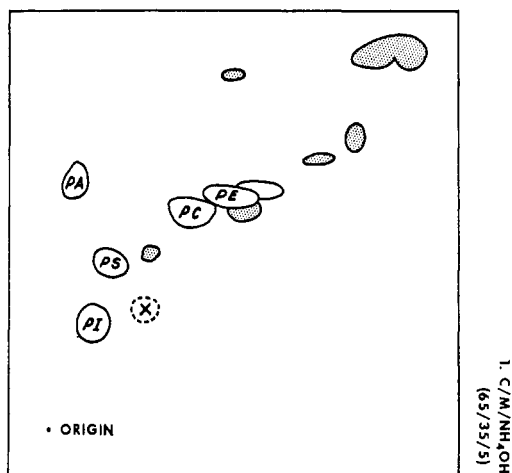


Figure 1. Chromatogram of phospholipids from orange juice PE, phosphatidylethanolamine; PA, phosphatidic acid; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; X, unknown phospholipid. Cross-hatched spots are nonphospholipids that appear on charring

When it was indicated as a trace, a spot could barely be seen, but the measured phosphorus was too low to distinguish it from the blank.

The precision of the determinations was measured in several samples by taking three aliquots of juice and working them up separately, followed by triplicate tlc and phosphorus analyses for each extract. Analysis of variance showed no significant difference between the three extracts. The standard deviations for the PE and PC for the replicates were around 7 to 9% of the means. The standard deviation was about 16% of the mean for PI, and approximately equal to the means of the minor constituents, PA, PS, and the unknown.

A study was made of the effects of lemon maturity and fruit storage at 58° F on the phospholipid content of the finished juice. The results are shown in Table III. It can be seen that there is essentially no change in the composition with either maturity or storage up to 75 days.

The amount of PC and PE found in this study was quite close to that found in orange juice by Swift and Veldhuis (1951). After converting the data to similar units, their value

Table III. Effect of Lemon Maturity and Fruit Storage on the Phospholipid Composition of the Finished Juice

Sample	Phospholipids <sup>a</sup>				
	PE	PC	PA	PS	PI
Maturity					
Green	34	44	2	7	13
Green-yellow	37	43	1	7	12
Yellow	32	41	7	8	12
Storage at 58° F					
0 days	34	44	2	7	13
26	37	43	2	7	14
48	39	40	1	6	13
75	42	39	1	5	13

<sup>a</sup> Phosphorus from phospholipid spot as a percentage of phosphorus recovered from the plate. Average of four determinations.

was 14 mg PC per 100 ml, and this study indicated an average of 15 mg PC per 100 ml. They suspected ethanolamine to be present and calculated the balance of their phosphorus and nitrogen as PE or cephalin (an older general term which includes PS and PI). They reported 16 mg "cephalin" per 100 ml in orange juice. The value reported here is 14 mg PE per 100 ml. The relative percentages of the orange juice phospholipids were also quite close to those reported by Nagy and Nordby (1970).

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