Citrus Juice Characterization

Analysis of the Phosphorus Fractions

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The organic and inorganic phosphorus compounds of orange, lemon, and grapefruit juices were fractionated into groups of total P, inorganic P, lipid P, and an ethanol-insoluble P fraction which contains nucleic acids and phosphoproteins. The averages of total P for orange, lemon, and grapefruit juices were 19.3, 11.1, and 13.6 mg. of P per 100 ml., respectively. The average percentages of total P distributed among these groups for the three juices were, respectively, 65.3, 55.0, and 69.5 for inorganic P; 7.3, 11.6, and 7.9 for lipid P; and 15.6, 17.5, and 11.0 for ethanol-insoluble P. An inverse correlation was observed between the percentages of inorganic P and ethanol-insoluble P of all juices, but particularly orange.

The problem of determining the authenticity or juice content of citrus products is becoming increasingly important because of the proliferation of synthetic drinks and adulterated fruit juices now appearing on the market. Although many chemical criteria have been proposed for this purpose, none have been completely satisfactory. Many workers (Money, 1966; Morgan, 1963; Sawyer, 1963; Yufera and Iranzo, 1965) have used mineral constituents such as Na, K, P, N, Mg, and Ca to characterize citrus juices and products. Of these, P has been one of the more popular parameters. Hulme *et al.* (1965) proposed an equation to determine juice content based on the P, K, and N values. However, any method based on these inorganic constituents could be circumvented easily and cheaply to counter a dilution of the natural juice.

Phosphorus is associated with many biochemically important classes of compounds in citrus juices such as the nucleic acids, phospholipids, and phosphoproteins. These compounds would be too difficult or too expensive to use as adulterants, so more information on their relative amounts and distribution could be helpful in characterizing these juices. The purpose of this investigation was to fractionate the P compounds into several major groups including total P, inorganic P, lipid P, and the ethanol-insoluble P which contains the nucleic acids and phosphoproteins, and to determine their usefulness in characterizing citrus juices.

EXPERIMENTAL

Sample Preparation. Single strength juice or juice concentrate diluted to single strength was filtered through a single thickness of cheese cloth to remove large particles which might clog the pipets. The juice was well mixed and stirred while removing aliquots for analysis.

Total Phosphorus. A 2-ml. aliquot of the juice was digested on a micro-Kjeldahl apparatus with 1.5 ml. of concd. H_2SO_4 and sufficient concd. HNO_3 to completely oxidize the organic matter and give a colorless solution. Usually, 2 to 5 ml. was adequate. The acid digest was heated to fuming, then cooled slightly. Water was cautiously added and the mixture again heated to fuming. To prevent the interference of nitric acid with the color formation, water and fuming steps were repeated. The digested sample was then quantitatively transferred to a 100-ml. volumetric flask. The phosphorus determination was done by a slight modification of the method of Rouser *et al.* (1966). Into a 10-ml. volumetric flask was pipetted 1 ml. of 2.5% ammonium molybdate, sufficient 10*N* H_2SO_4 to make the final acid concentration 1*N*, the digested sample containing 0.5 to 5 μg. of P, and 1 ml. of freshly prepared 10% ascorbic acid solution. The mixture was heated in a boiling water bath for 5 minutes and cooled; the absorbance was measured at 820 mμ against a reagent blank. A standard curve was prepared by using KH_2PO_4.

Inorganic Phosphorus. A 1-ml. aliquot of juice was pipetted into a 25-ml. volumetric flask and frozen until ready for analysis. The sample was diluted to volume, centrifuged until clear (10,000 G), and the P determined as above without the digestion step.

Lipid Phosphorus. A 20-ml. aliquot of juice was mixed with Celite and filtered. The filter cake was washed twice with 5 ml. of water. The filtrate and washings were discarded. The lipids were washed through the filter with small portions of chloroform-methanol (C-M) (2 to 1) until the filter cake was colorless (50 to 60 ml.). 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 lipid extract was transferred to a 100-ml. volumetric flask and made to volume with chloroform. With orange juice samples, the carotenoid content was sufficient to measure the spectrum of the extract from 500 to 380 mμ. The absorbance of the major peak, 450 to 440 mμ, was recorded. With an absorption coefficient of 250 (Goodwin, 1955), the carotenoid concentration in milligrams per 100 ml. of the original juice was equal to twice the absorbance. The lipids were separated from the nonlipids in the extract according to the method of Siakotos and Rouser (1965). A 25-ml. aliquot of the lipid extract was passed through a Sephadex G-25 (coarse) column (1.5 × 20 cm.) which had previously been equilibrated with C-M (19 to 1) saturated with water. An additional 35 ml. of the

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Table I. Compositional Data from Citrus Juices

Sample	Brix, 20° C.	Citric Acid[a]	Amino Acids[a]	Total Phenolics[b]	Total P[c]	Inorganic P[c]	Lipid P[c]	Ethanol Insol. P[c]	Carotenoids[d]
				Orange Juice					
Com'l Cal.	13.8	17.9	3.65	0.930	20.3	12.7	1.63	4.13	1.88
Com'l Cal.	13.8	11.8	2.95	0.922	21.8	14.4	1.53	3.10	1.56
Com'l Cal.	12.8	15.0	3.08	0.900	17.1	9.0	1.18	4.30	2.68
Com'l Cal.	12.4	11.8	2.84	0.980	14.8	8.8	1.53	3.07	2.14
Com'l Cal.	12.2	7.8	3.12	0.766	22.0	17.5	0.98	2.65	1.10
Com'l Cal.	14.2	12.4	3.58	0.935	21.3	14.6	1.22	3.21	1.61
Com'l Cal.	12.2	14.7	2.38	0.903	17.7	8.6	1.80	1.96	0.88
Com'l Fla.	13.2	12.6	2.10	0.657	16.3	10.0	1.76	2.75	0.32
Fresh Cal.	12.6	13.0	3.10	0.566	20.0	14.8	1.22	1.57	0.76
Fresh Cal.	11.6	14.2	2.11	0.770	21.3	14.6	1.28	3.13	0.99
Fresh Cal.	13.8	13.1	2.86	0.687	17.7	11.8	1.40	2.71	1.38
Fresh Cal.	12.6	13.0	2.07	0.861	21.6	14.1	1.37	3.64	0.98
Average	12.9	13.1	2.82	0.823	19.3	12.6	1.41	3.02	1.36
Std. Dev.	0.816	2.39	0.548	0.132	2.48	2.91	0.249	0.788	0.657
Coef. Var., %	6.3	18.2	19.5	16.0	12.8	23.1	17.7	26.1	48.4
				California Lemon Juice					
1		94.8	1.98	0.943	11.5	5.96	0.96	2.30	
2		92.0	2.16	1.114	13.4	7.50	1.62	2.52	
3		94.3	1.50	1.058	9.3	4.17	1.33	1.92	
4		93.9	1.97	0.453	9.7	5.27	1.33	1.37	
5		75.3	1.61	0.334	9.2	5.65	0.82	1.21	
6		88.1	1.81	0.522	15.2	8.49	2.18	3.16	
7		84.8	1.63	0.585	9.0	4.71	1.13	1.27	
8		81.9	1.55	0.528	10.5	5.74	1.47	1.90	
9		100.5	1.90	1.002	14.6	7.72	1.91	2.65	
10		97.2	1.33	0.533	7.3	2.87	1.22	1.75	
11		90.3	2.11	0.894	11.0	7.96	0.21	0.88	
12		84.3	2.08	0.962	12.9	7.13	1.29	2.30	
Average		89.8	1.80	0.744	11.1	6.10	1.29	1.94	
Std. Dev.		7.18	0.27	0.275	2.45	1.70	0.51	0.68	
Coef. Var., %		8.0	15.0	37.0	22.1	27.9	39.5	35.1	
				Grapefruit Juice					
Com'l Cal.	11.6	20.2	1.93	1.416	10.6	7.25	1.12	1.86	
Com'l Cal.	11.2	23.0	2.27	1.569	16.5	11.0	1.50	2.21	
Com'l Fla.	12.8	17.7	2.10	1.045	12.5	8.82	0.98	1.45	
Fresh Fla.	11.2	16.5	1.97	0.786	15.0	10.4	0.87	0.84	
Fresh Cal.	10.2	20.5	1.95	0.787	13.2	9.77	0.92	1.09	
Average	11.4	19.6	2.04	1.121	13.6	9.45	1.08	1.49	
Std. Dev.	0.939	2.55	0.143	0.359	2.28	1.47	0.254	0.557	
Coef. Var., %	8.2	13.0	7.0	32.0	16.8	15.6	23.6	37.3	

[a] Meq./100 ml. [b] Absorbance of 1 to 20 dilution with ethanol. [c] Mg. P/100 ml. [d] Mg./100 ml.

C-M (19 to 1) water-saturated solvent was passed through. The total eluate was evaporated in a Kjeldahl flask and digested. The digest was transferred to a 25-ml. volumetric flask for the P color determination.

Ethanol-Insoluble Phosphorus. A 10-ml. aliquot of juice was pipetted into a 40-ml. centrifuge tube; 0.5 ml. of 1*M* $MgCl_2$ and 20 ml. of ethanol were added. The mixture was kept in an ice bath for 30 minutes, then centrifuged. The supernatant was discarded and the residue washed three times with 10 ml. of 50% ethanol and three times or more with 10 ml. of absolute ethanol, until colorless. The entire precipitate was transferred with water to a Kjeldahl flask for digestion. The digest was diluted to 100 ml. for the P color determination.

Supplemental Analyses. The determinations of total acidity, degrees Brix, total amino acids, and total phenolics were by methods previously reported (Vandercook *et al.*, 1963; Vandercook and Rolle, 1963).

RESULTS AND DISCUSSION

The analytical data for orange, lemon, and grapefruit juices are listed in Table I. Coefficients of variation in most cases are about the same or slightly higher for the P fractions than for the other constituents. The coefficient of variation between replicates was about 2%. The sum of the phosphorus in the fractions measured by these procedures accounts for about 88% of the total. The rest of the phosphorus is probably present as organic phosphorus in the ethanol-soluble fraction, which was not routinely measured.

According to Razzell (1963), the addition of ethanol in the presence of magnesium salts would precipitate the nucleic acids and polynucleotide fragments down to 7 units. The precipitate also contains protein, as indicated by the positive biuret test.

An extract of the ethanol-insoluble precipitate by the phenol procedures of Kirby (1956) as modified

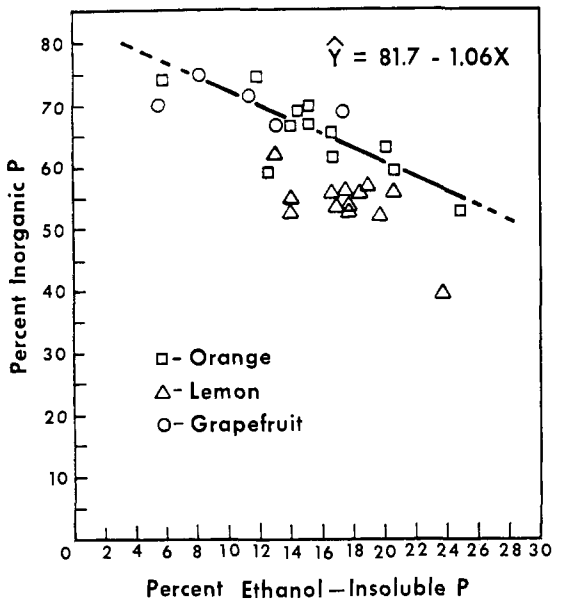

Figure 1. Relationship of inorganic P to ethanol-insoluble P in citrus juices

by Cherry and Chroboczek (1966) gave, after alcohol precipitation. a gelatinous nucleic acid fraction which contained a large amount of pectin. The precipitate was washed several times with alcohol to remove the phenol. A dilute base solution of this precipitate gave a characteristic ultraviolet spectra of the nucleic acids with the λ_{max} at 260 mμ. Hydrolysis of the precipitate in dilute KOH gave the nucleotides with essentially the same P value as the total P in the phenol-extracted, nucleic acid fraction. The nucleic acid P ranged from 2 to 4% of the total in a few selected samples. Base hydrolysis of the entire ethanol-insoluble fraction yields higher P values than when the precipitate is partially purified by the phenol procedure, as might be expected.

The physical state of the nucleic acids in citrus juice is of considerable interest. Several juice samples were clarified by filtering through Celite. There was almost no P in the alcohol precipitates. This means that the nucleic acids and phosphoproteins are largely present as part of the insoluble cloud. However, in a few samples, the alcohol precipitate from the filtrate contained considerable amounts of P. This could be from a partial degradation of nucleic acids to smaller fragments which were water-soluble, but insoluble in the 67% ethanol. The phospholipids, by the very nature of the extraction procedure, are also a part of the cloud.

Scott *et al.* (1965) examined the cloud of orange juice and found considerable amounts of P, but they did not identify its source. The P in their centrifuged fraction would correspond to our ethanol-insoluble P, since both are measuring mostly the insoluble constituents. By expressing their data in the units of this paper, they found 3.22 mg. of P per 100 ml. in the lipid-free cloud of orange juice. This agrees well with the average 3.02 mg. of P per 100 ml. for the ethanol-insoluble P fraction measured here.

Swift and Veldhuis (1951) examined the lipids of an orange juice sample and found 1.26 mg. of P per 100 ml. of juice (converted to the units used in this paper). This agrees nicely with the average lipid P value of 1.41 found in this study.

A relationship was observed between two of the P fractions which might be useful in characterizing citrus juices. When inorganic P and ethanol-insoluble P are calculated as a percentage of the total juice P, an inverse relationship can be seen (Figure 1). Part of the increased level of inorganic P might have come from a degradation of the nucleic acids and/or phosphoproteins. The correlation coefficient of these values in the orange juice samples was −0.826. The data for lemon and grapefruit juices are also plotted on the same graph, and there is an indication of a similar inverse relationship. However, because of the limited sampling, this relationship should be considered only as a preliminary finding.

On the basis of this limited survey, it seems that none of the individual P fractions could be used as the sole criteria for determining juice authenticity. However, since 25 to 45% of the P in citrus juices is present in a combined state, the distribution of P could be useful in characterizing certain samples. Further investigations into the characterization of citrus juices by multiple-constituent correlations are currently underway. In addition to juice characterization, these methods are also being used to study changes in the phospholipid and nucleic portion of the cloud under various commercial conditions. Since the appearance of cloud affects consumer acceptance, a better understanding of its composition could lead to an improved product.

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LITERATURE CITED

Cherry, J. H., Chroboczek, H., *Phytochemistry* **5,** 411 (1966).
Goodwin, T. W., "Modern Methods of Plant Analysis," K. Paech and M. V. Tracey, Eds., Vol. III, p. 308, Springer Verlag, Berlin, 1955.
Hulme, B., Morries, P., Stainsby, W. J., *J. Assoc. Public Anal.* **3,** 113 (1965).
Kirby, K. S., *Biochem. J.* **64,** 405 (1956).
Money, R. W., *J. Assoc. Public Anal.* **4,** 41 (1966).
Morgan, R. H., *Food Process. Packaging* **32,** 163 (1963).
Razzell, W. E., *J. Biol. Chem.* **238,** 3053 (1963).
Rouser, G., Siakotos, A. N., Fleischer, S., *Lipids* **1,** 85 (1966).
Sawyer, R., *J. Sci. Food Agr.* **14,** 302 (1963).
Scott, W. C., Kew, T. J., Veldhuis, M. K., *J. Food Sci.* **30,** 833 (1965).
Siakotos, A. N., Rouser, G., *J. Am. Oil Chemists' Soc.* **42,** 913 (1965).
Swift, L. J., Veldhuis, M. K., *Food Res.* **16,** 142 (1951).
Vandercook, C. E., Rolle, L. A., *J. Assoc. Offic. Agr. Chem.* **46,** 359 (1963).
Vandercook, C. E., Rolle, L. A., Ikeda, R. M., *J. Assoc. Offic. Agr. Chem.* **46,** 353 (1963).
Yufera, E. P., Iranzo, J. R., *Agroquimica Technol. Aliment.* **5,** 471 (1965).

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