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Analysis of Mineral Content and Amount of Chelated Minerals in Citrus Juice by Inductively Coupled Plasma Emission Spectroscopy

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The elemental composition of the juice and the amount of elements bound by the pectin component of orange and grapefruit juices were determined at pH 3.2 and 7.2. The minerals are bound in varying amounts from 0 to 50% with copper and zinc being the most firmly bound. No adverse nutritional effects should be caused by the bonding. Pectin has both positive and negative influences on citrus juice quality. The positive effects are the contribution to viscosity and the cloud stability. The increase of gelation is the negative effect. The soluble elements of the citrus juice and the inter- and/or intramolecular elements bound by the pectin component of citrus juice were determined at pH 3.2 and 7.2.

INTRODUCTION

Pectin is a normal component of citrus juices. It is known to bind many ions and may affect the human nutritional availability of some components of citrus juice. The literature on citrus pectin through 1980 has been extensively reviewed by Baker (1980) and was earlier reviewed by Kertesz (1951).

Pectin is a linear polysaccharide that acts as a cellular binder in the peel of many fruits and vegetables. Its basic structure consists of a chain of galacturonic acid units linked by α (1–4) glycosidic bonds. Many of the carboxyl groups on the chain have been esterified with methanol.

Pectins are classified by solubility and degree of esterification. Norman and Kimball (1990) have given the following pectin distribution in navel oranges and pink grapefruit:

	navel orange	pink grapefruit
water soluble, mg/L	270	370
ammonium oxalate soluble, mg/L	133	167
sodium hydroxide soluble, mg/L	257	230

The viscosity of a pectin solution is increased dramatically by the addition of a metal ion such as those of copper, calcium, and lead. At higher elemental concentrations, pectin precipitates may be formed. The reaction of some metal ions with pectin is so strong that they may be removed from solutions as pectin precipitates (Kertesz, 1951).

Schweiger et al. (1964) studied the bonding between pectin and metal ions which can consist of both ionic and coordination bonds. Ionic interactions involve the negative groups on the pectin that are not protected by methoxylation. Schweiger et al. (1964) acetylated pectin to various degrees and showed that hydroxyl as well as carbonyl groups are needed for effective chelation. Each divalent cation may react with two carboxyl groups and two hydroxyl groups to give coordination bonding and may result in configurations such as the egg-box structure proposed for pectin complexes (Baker, 1980). The presence of carboxyl groups on the pectin chain enhances coordination.

There is a competition between various types of cations for sites on the pectin chain. The chelates formed may involve several sites in one pectin chain or may share groups from adjacent chains. Pectin and its metal complexes act as soluble or partly soluble fiber in human nutrition. They have the beneficial property of adding bulk to the intestinal contents. They may also decrease the absorption of metals in the digestive system as reported by Kertesz (1951) and Monnier et al. (1980). This would be beneficial in the case of toxic metals but may also reduce the absorption of necessary nutrients as shown by Monnier et al. (1980).

Binding of metals by pectin is pH dependent so there are possible changes in the amount bound in various sections of the digestive system. This will vary from the usually acidic stomach to the neutral pH of the intestine. Also, there may be changes due to partial degradation of the pectin by bacteria in the colon as reported by Kertesz (1951).

It is the purpose of this study to investigate to what extent insoluble pectin binds the metals present in orange and grapefruit juices and what percent of the metals is present as soluble ions or chelates at two pHs, natural and 7.2.

EXPERIMENTAL PROCEDURES

All juice samples were single-strength juice reconstituted from concentrate and stored in either a tin can or a glass bottle. Tin cans were 1.36-L (46-oz) capacity, unlacquered tin-plated steel, hot-filled with reconstituted concentrate, heat-stabilized in a commercial operation, sealed, cooled, and stored at ambient temperature (75 °F) for 6 months. The glass bottles were also 1.36-L (46-oz) capacity, hot-filled with reconstituted concentrate, commercially heat stabilized, sealed with a plastisol-lined metal screw cap, cooled, and stored at 75 °F for 6 months, and then 25-g samples were taken for analysis.

All chemicals for spectrochemical analysis were certified high-purity materials from Spex Industries (Metuchen, NJ). Standard solutions were made by dissolving the high-purity salts of the metals in a matrix to simulate citrus juice mineral constituents. Standardization of the inductively coupled plasma atomic emission spectrometer was carried out by using a low and a high standard for the multielement matrix because because of the large, linear dynamic range of the plasma (5–6 orders of magnitude). The slope and intercept of the calibration line for each element was calculated by an automatic computerized system, and these parameters were used in sample analysis. Both the accuracy and precision of this system for standard solutions were less than 1%. The precision (coefficient of variation) of unknown sample analysis was less than 5%. The matrix contained 640 μg/mL K (K_2CO_3), 50 μg/mL P ($NH_4H_2PO_4$), 25 μg/mL each Ca ($CaCO_3$) and Mg ($MgCO_3$), and 20 μg/mL Na

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Table I. Elemental Concentration and Percent Bound in Orange Juice Stored in a Glass Bottle

element	mequiv/L	total[a]	soluble[a]	% bound of element	% RSD
		pH 3.6			
B	0.080	0.64	0.59	7.8	1.4
Mn	0.009	0.32	0.30	6.2	1.0
Zn	0.013	0.32	0.16	50.0	2.4
Cu	0.009	0.27	0.17	37.0	1.5
P	4.180	158.00	126.50	20.0	1.0
Fe	0.017	0.76	0.56	26.0	1.8
Sn					
Mg	11.10	116.00	120.00	0.0	0.7
Ca	7.57	75.00	73.00	3.9	0.9
Sr	0.03	0.56	0.40	29.8	1.2
		pH 7.2			
B	0.080	0.59	0.63	0.0	1.5
Mn	0.009	0.32	0.31	3.1	1.3
Zn	0.013	0.32	0.25	16.0	3.5
Cu	0.009	0.30	0.19	32.0	11.3
P	4.180	152.00	139.00	17.0	1.2
Fe	0.017	0.72	0.60	18.0	2.3
Sn					
Mg	11.10	116.00	120.00	0.0	0.53
Ca	7.57	75.20	74.60	7.6	1.1
Sr	0.03	0.53	0.40	25.0	1.0

[a] Elemental concentration in ppm at 11.8° Brix.

Table II. Elemental Concentration and Percent Bound in Orange Juice Stored in a Tin Can at pH 3.6

element	total[a]	soluble[a]	% bound of element
B	0.96	0.79	17.7
Mn	0.22	0.20	9.1
Zn	0.51	0.37	27.5
Cu	0.30	0.17	43.3
P	126.70	120.10	5.2
Fe	2.60	2.15	17.3
Sn	33.65	26.32	21.8
Mg	137.20	129.30	5.8
Ca	162.80	122.50	24.7
Sr	1.70	1.30	23.5

[a] Elemental concentration in ppm at 11.8° Brix.

Table III. Elemental Concentration and Percent Bound in Grapefruit Juice Stored in a Glass Bottle

element	total[a]	soluble[a]	% bound of element	% RSD
		pH 3.2		
B	0.47	0.50	0.0	2.0
Mn	0.14	0.13	7.0	2.0
Zn	0.39	0.35	10.0	2.5
Cu	0.33	0.21	36.0	9.5
P	134.20	111.30	17.0	1.0
Fe	0.63	0.53	16.0	2.6
Sn				
Mg	99.30	97.10	2.0	0.7
Ca	104.60	95.40	8.7	0.8
Sr	1.40	1.13	19.0	1.2
		pH 7.2		
B	0.45	0.46	0.0	1.7
Mn	0.13	0.13	0.0	2.1
Zn	0.37	0.37	0.0	3.2
Cu	0.32	0.23	2.8	10.5
P	131.00	118.00	9.9	0.75
Fe	0.65	0.53	26.0	2.3
Sn				
Mg	98.70	98.70	0.0	0.4
Ca	104.30	98.70	6.0	0.6
Sr	1.41	1.15	18.5	1.5

[a] Elemental concentration in ppm that 11.8° Brix.

Table IV. Elemental Concentration and Percent Bound in Grapefruit Juice Stored in a Tin Can at pH 3.2

element	total[a]	soluble[a]	% bound of element
B	0.30	0.26	13.3
Mn	0.14	0.14	0.0
Zn	0.51	0.38	25.5
Cu	0.21	0.15	28.6
P	111.80	101.50	9.2
Fe	1.90	1.72	9.5
Sn	26.79	23.94	10.6
Mg	121.30	120.90	0.3
Ca	80.80	79.20	2.0
Sr	1.11	1.00	10.0

[a] Elemental concentration in ppm at 11.8° Brix.

(Na_2CO_3). Trace elements at levels of parts per billion (ng/mL) were dissolved and added to the reference solution.

Reagent grade ammonia diluted with deionized, distilled water was used for pH adjustment. About 4 mL of 7 N NH_4OH was required to raise the juice pH to 7.2.

To determine the amount of soluble element, 25 g of grapefruit or orange juice was centrifuged at 19745*g* for 20 min at 4 °C and then left to reach room temperature (20 °C). The supernatant fluids were then removed, and 10 mL of aqua regia (2.5 mL of concentrated HNO_3 and 7.5 mL of concentrated HCl) was added to the solutions at 20 °C. The volumes of the final solutions were promptly brought to 100 mL by adding deionized distilled water. The subsequent analysis of these solutions by inductively coupled plasma atomic emission spectrometry (ICP-AES) gave the amount of soluble elements in the juice.

Total element concentrations in juice were obtained by weighing accurately the 25 g of grapefruit juice or orange juice into a Teflon digestion vessel (MDS-81D; CEM Co., Indian Trail, NC) and adding 10 mL of aqua regia. The Teflon vessel was closed with a 100-psi pressure relief valve and threaded cap using a CEM capper. The vessel was placed in the rotating turntable located inside the CEM programmable microwave oven, heated for 8 min at 100% power, cooled for 5 min at 0% power, and, finally, heated again for 8 min at 100% power. The digestion vessel was brought to room temperature and opened using the capping station, and the contents were filtered through ashless filter paper (Whatman No. 42).

The amount of bound element was then calculated from the difference of the above values according to the following equation:

$$\% \text{ bound element} = 100(\text{total concn of element} - \text{soluble concn of element})/(\text{total concn of element})$$

RESULTS AND DISCUSSION

Tables I–IV show total concentrations of elements, concentration of free elements, and percent of bound elements. The concentrations determined by ICP-AES are independent of the chemical/physical form present, although we can assume that the metals are present as cations or chelated atoms. The phosphorus and boron are present as anions such as $H_2PO_4^-$ and $H_2BO_3^-$. A column has been included in Table I data showing the milliequivalents per liter of each element. Using the pectin amount of 660 mg/L (Norman and Kimball, 1990) and a unit molecular weight of 176 (assuming 50% esterification with methanol), there are about 2 mequiv of pectin present. This is not enough to bind all of the cations; therefore, the cations must compete for sites on the pectin chain. The sum of the bound cations in Table I is 1.7 mequiv, which is close to the 2 mequiv assumed to be present.

The data in Tables I and II show that there were no large differences between metal binding to pectin orange juice stored in a tin can or in a glass bottle. Tin was not detected in the orange juice packed in glass, and 33 ppm was observed in the sample stored in a tin can. Comparison of data in Tables I and II vs Tables III and IV shows that the amount of binding of metal to pectin is less in grapefruit juice at pH 3.2 than in orange juice at pH 3.6. This may be due to the differences in pH or in the various anions present. The results are similar at pH 7.2.

Data in Tables III and IV indicate differences in the amounts of various metals bound to pectin in grapefruit juice with and without tin. The presence of tin at the grapefruit juice pH appears to change the amount of several elements bound, notably iron and calcium. In all cases, copper is the most strongly chelated element.

The data in Tables I and III show some differences in binding at the two pHs tested. Notable differences are the values for boron, zinc, phosphorus, iron, and calcium. Zinc, at 50%, is the most completely bound, followed by copper, iron, and strontium.

CONCLUSION

In conclusion, we have shown that the pectin contained in citrus juices can bind a portion of the elements present in the juice. This portion is not large, 0–20%, except for zinc and copper at 35–50%. We can conclude that the pectin content has negligible effect on the nutritional properties of the minerals contained in citrus juice.

Other studies using higher concentrations of pectin (Kertesz, 1951; Monnier, 1980) show decreased iron absorption, especially in subjects affected with hemochromatosis.

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