Phosphorus Components of the White Potato

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The solubilities of the barium salts and preliminary chromatographic analysis of the acid-soluble phosphates of two varieties of potatoes as affected by postharvest storage temperature have been studied. In general, the barium-soluble esters go through a concentration maximum (at the expense of the barium-insoluble phosphate) during storage. Several phosphate esters have been separated chromatographically, and some tentatively identified as nucleotide, phytate, orthophosphate, glycerol phosphate, and occasionally the phosphates of fructose and glyceric acid. Five additional unknown phosphates have been detected. Orthophosphate, and not the hexose phosphates, may play a direct role in the subsequent nonezymatic browning of processed potatoes. A probable nonpolysaccharide fraction has been isolated from potatoes, which affects the barium solubility of the acid-soluble phosphorus of potatoes. The relationship of the phosphorus content of starch to the metabolism of phosphorus is discussed.

THE ENZYME PATTERN of the potato tuber as revealed by recent literature (*12*) indicates the presence in easily detectable and, in several instances, isolable quantities of specific phosphate-transforming enzymes, thus pointing to the possible special importance of phosphate in the metabolism of the tuber. Many previous investigations have been concerned with the influence of cultural practice on total phosphorus content (*8, 9, 16*). Compositional investigations have been mainly confined to the insoluble phosphate fraction including starch (*10, 11*) and phytate (*7, 17*). In one instance, a relatively high accumulation of some hexose phosphates in potatoes upon storage at both high and low temperature has been reported (*2*). These phosphates, if present, could account for at least a fraction of the "reducing" sugar involved in the nonezymatic browning of processed potato products (*14*).

As part of a study of the compositional and metabolic factors that may affect processing quality of potato (*13*), the present paper presents data on changes in the composition of the acid-extractable phosphorus as well as the starch phosphate of potatoes as affected by temperature and duration of storage. Preliminary results are presented on the composition of these fractions as revealed by paper chromatography.

Materials and Methods

The tubers were White Rose (California) and Russet Burbank (Idaho) varieties. Their preharvest history and other characteristics have been described (*13*). A 25-gram sample of potatoes (sampled from about 10 kg. of peeled and diced tubers) was blended at 0° C. (by means of a jacketed blender through which cold water was circulated) with an equal volume of 10% cold trichloroacetic acid. After centrifugation at 2000 times gravity for 10 minutes (it was frequently necessary to place the tube in an evacuated chamber to facilitate sedimentation of a fraction of the debris), the supernatant liquid was removed and the residue washed twice with 25-ml. portions of 5% trichloroacetic acid. Table I, A, shows that two washings are adequate for essentially quantitative extraction of the total acid-soluble phosphate. The subsequent standard procedure for the fractionation of the barium salts of the phosphate esters was essentially that described as Procedure A by Umbreit and coworkers (*15*). This procedure results in three classes of phosphate: barium-insoluble; barium-soluble, alcohol-insoluble; and barium-soluble, alcohol-soluble. After neutralization the fractions were lyophilized as the barium salts, weighed quantitatively, and analyzed for total phosphorus by the method of Allen (*1*). The samples were prepared for chromatographic analysis by suspension of enough in water to give approximately 1 mg. of phosphorus per ml. After mixing intimately with 100 mg. of Dowex-50 cation exchange resin to remove the barium, 3 to 15 μl. of the sample were spotted on Schleicher & Schüll paper (Blue Ribbon No. 589). Extensive experimentation showed that this choice of paper was essential for elimination of the preliminary acid wash recommended for most analytical papers by Bandurski and Axelrod (*3*). After irrigation for 65 hours at room temperature with a mixture of ethyl acetate, acetic acid, and water in the proportion 5:2.5:1 (descending), the paper was dried at room temperature, and either

Table I. Balance Data on Extraction of Acid-Soluble Phosphorus[a]

A. Adequacy of Extraction

Extraction number	1	2	3	4
Extracted, mg. P	8.4	10.2	10.5	10.7

B. Error Due to Freeze-Drying (F-D)

Mg. P			
Before F-D	8.4	9.1	11.0
After F-D	8.7	8.6	11.0

C. Comparison of Total Tuber P with Acid-Soluble (I) and Insoluble Fraction (II)

Fraction	I	II	I + II	Total P
Mg. P	10.7	4.8	15.5	15.7

D. Comparison of Total Acid-Soluble P with Fractions[b]

Fraction	I	II	III	I + II + III	Total P
Mg. P	4.9	4.8	0.6	10.3	10.7

[a] Mg. P per 25 grams of tuber.
[b] I = barium-insoluble fraction; II = barium-soluble, alcohol-insoluble fraction; III = barium-soluble, alcohol-soluble fraction.

sprayed with acid molybdate (*6*) or dipped in a mixture of acid molybdate and acetone recommended by Burrows and coworkers (*5*). After 1 minute in the oven at 85°, the paper was subjected to ultraviolet irradiation according to the technique of Bandurski and Axelrod (*3*). The blue phosphomolybdate complex of the spots was intensified and background color diminished by exposing the paper to ammonia fumes. The mobility of the spots is expressed as *Rp* (extent of movement as compared to orthophosphoric acid, which moved 180 to 210 mm. in 65 hours at room temperature).

Table II. Phosphorus Content of Potatoes and Potato Starch

Variety	Mg. P/100 G. Tuber	Mg. P/100 G. Starch	Ratio, Tuber P/ Starch P
Russet Burbank	79	100	0.79
White Rose	62	80	0.78

Solubility Distribution of Phosphorus

The total phosphorus content of the Russet variety as determined by repeated analyses of samples taken during storage was significantly higher than that of the White Rose (Table II). Balance (Table I) and distribution data (Figure 1) reveal that half to two thirds of the total phosphorus was acid-soluble. For at least one set of conditions, only about 4% of the total phosphorus was found in the barium-soluble, alcohol-soluble fraction (Table I). The results of barium fractionation are shown in Figure 1. For both varieties the control samples contained more barium-insoluble than barium-soluble phosphorus. Under almost all of the conditions investigated, the effect of storage was initially to increase the barium-soluble fraction apparently at the expense of the barium-insoluble fraction. Upon prolonged storage, the distribution characteristics again tended to revert to the original picture—that is, the barium-insoluble fraction seemed to be ascendant. Only the samples obtained from the White Rose variety after storage at 21° did not quite follow this pattern after the first week of storage.

Chromatography of Phosphate Fractions

A summary of the substances separated by paper chromatography of the acid-soluble fractions of the White Rose and Russet Burbank varieties is shown in Figure 2. Of the known phosphate esters used, spots whose *Rp* corresponded to orthophosphate, glycerol phosphate, phosphoglyceric acid, fructose phosphates, and nucleotides (or phytic acid) were found. At least one sample gave evidence of faint trace of fructose-1,6-diphosphate. In addition, five additional substances were detected whose *Rp* did not correspond to any of the control phosphate esters used. Thus, the position of spot B consistently fell in between those of fructose-6-phosphate and the glucose phosphates. Cochromatography of a sample showing this spot with the hexose phosphates revealed it to move at a significantly different rate.

The distribution of these substances with respect to temperature, variety, and barium solubility is summarized in Table III. The nucleotide fraction was found more frequently and in apparently greater amounts judged by the intensity of the spots in the barium-insoluble fraction from the Russet variety than in either fraction from White Rose. Fructose diphosphate was detected only twice at 21° in the barium-insoluble fraction, whereas fructose-6-phosphate appeared infrequently at both storage temperatures. Glycerol phosphate appeared more frequently at 21° than at 4.4°. Phosphoglycerate and spot E, in the same general area as the three-carbon phosphates, appeared only once each. Spot A appeared only in the barium-soluble fraction and most frequently at 21°. Spot B was more intense in White Rose, especially at 21° in the barium-soluble fraction, whereas spot C occurred as an intense spot in fractions from Russet Burbank stored more than 18 weeks at 4.4°. Spot D, whose mobility was greater than that of orthophosphate, was detected only in Russet Burbank at 21°.

The intensity and size of the spots showed that there was more orthophosphate in samples at the beginning and end of the storage series than in samples obtained from intermediate intervals of storage, and in general more at 4.4° than at 21°.

Effect of Precipitation on Solubility of Orthophosphate

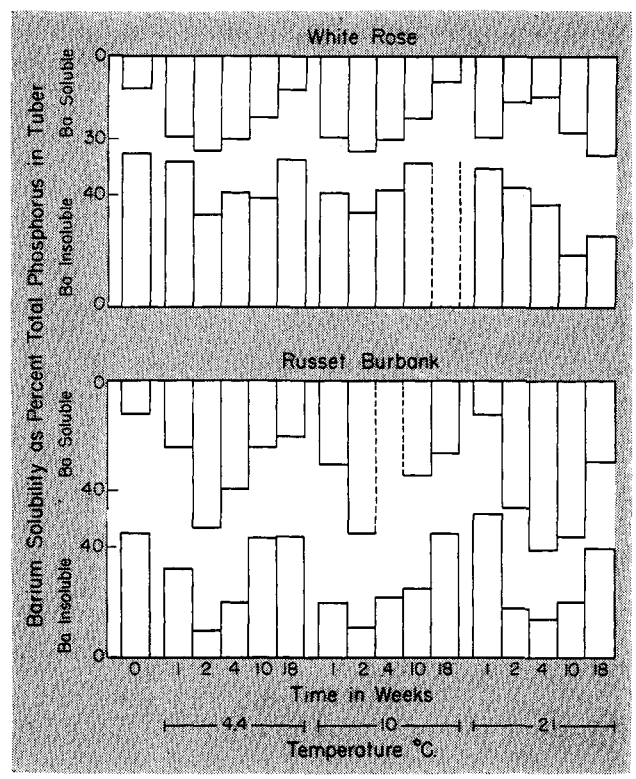
Table III shows that orthophosphate appears occasionally in the barium-soluble fraction of acid-soluble phosphorus. According to Umbreit and coworkers (*15*), this situation will occur when large amounts of glycogen (and presumably other polysaccharides) are present in the acid extract. This polysaccharide is removed in method B by precipitation with 50% ethyl alcohol prior to barium precipitation; in method A, barium precipitation is carried out prior to addition of ethyl alcohol.

To determine whether a carbohydrate did indeed interfere with the barium precipitation, a freshly prepared trichloroacetate extract of Russet Burbank potatoes stored at 4.4° for 40 weeks was divided into two portions and each portion subjected to fractionation procedures A and B (*15*), respectively. Upon addition of ethyl alcohol to the acid extract, only a very slight opalescence developed. Centrifugation sedimented a few milligrams of a flaky precipitate. However, when the pH of the centrifuged alcohol-treated extract was adjusted to 8.2 before addition of barium acetate, a heavy precipitate formed, which after centrifuging and freeze-drying, amounted to about 0.4% of the fresh potato. When the other portion of extract was subjected to procedure A, a precipitate formed when the barium-soluble, alcohol-insoluble fraction was adjusted to pH 7.0 after solubilization of this fraction by removal of the barium with dilute sulfuric acid. Previous to this experiment, all samples were lyophilized in the presence of excess barium.

Tracings of the chromatograms of the barium-insoluble and barium-soluble fractions obtained by each of the two procedures are schematically

Figure 1. Barium solubility of acid-soluble phosphorus in two varieties of potatoes as influenced by time and temperature of storage

Open dashed bars indicate absence of data for corresponding conditions



shown in Figure 3. Orthophosphate occurs in the barium-soluble and -insoluble fractions from procedure A but only in the barium insoluble fraction from procedure B. The solubility characteristics and chromatographic behavior before and after acid hydrolysis of the alcohol-precipitable material (only a trace of fructose was found) indicate that the precipitate is not of common carbohydrate nature.

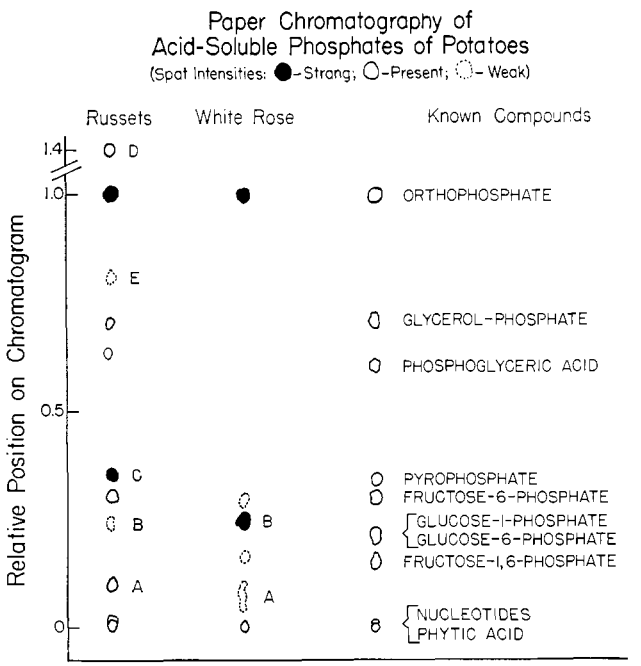


Figure 2. Diagrammatic summary of spots found in paper chromatography of phosphate ester fractions of potatoes

Discussion

It had been observed (*13*) that the phosphorus content of the starch isolated from these same lots of potatoes was constant with respect to time and temperature of storage, although the starch content varied widely. This would indicate that the phosphate of the starch is not utilized independently of the remainder of the starch molecule. For at least the two varieties used in the present study, the phosphorus content of the starch seems to bear a direct relation to the total phosphorus content of the tuber (Table II). The phosphorus content of potato starch increases with increasing amounts of this element applied as fertilizer (*16*). These results would indicate that the starch functions not only as a metabolic storage reservoir for carbon but also, along with phytic acid (*7, 17*), for phosphorus. These two insoluble components can account for as much as one half of the total phosphorus of the tuber.

The changes in the barium solubility of the phosphate with respect to time at the various temperature of storage illustrate the fact that the dormancy period of the tuber is still characterized by significant metabolic activity.

The failure to detect hexose phosphates frequently in appreciable quantities would indicate that these compounds do not enter directly into nonenzymatic browning. This does not necessarily mean, however, that the phosphates play no role in nonezymatic browning. They may be important indirectly, in that they are probably precursors of reducing sugars and may control the rate of metabolism of these sugars. Orthophosphate itself may enter into the browning directly, as it has been shown to affect profoundly the rate and extent of browning (*14*). The percentage (by dry weight) of phosphorus calculated from the phosphate ester content of Russet Burbank, as previously reported (*2*), was found to be as high as 0.6%, the upper limit of total phosphorus in potatoes (*4*), as compared to a maximum 0.15% of phosphorus found in the barium-soluble fraction in the present study.

Although most phosphate fractionation schemes, especially as applied to animal organs, stress the necessity of the preliminary removal of polysaccharide to obtain clear-cut fractionation (*15*), the results reported here would indicate that other nonpolysaccharide material may also interfere with efficient fractionation.

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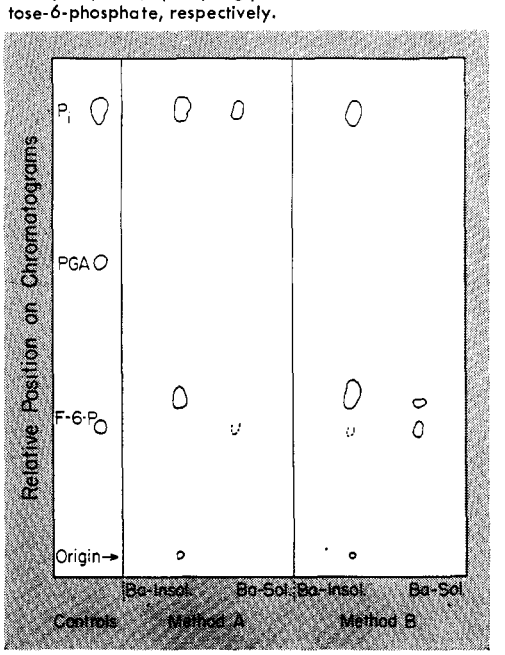
Table III. Chromatographic Analysis of Phosphate Compounds of Potatoes

	Russet Burbank				White Rose			
	Ba-insoluble		Ba-soluble		Ba-insoluble		Ba-soluble	
Storage temp., °C.	4.4	21	4.4	21	4.4	21	4.4	21
Nucleotide phytic acid	+++ +++	+++ +++ +	++	+++	+++	++	++	...
Fructose-1,6-P	...	...	...	...	...	++	...	...
Fructose-6-P	...	...	+	...	...	...	...	++
Glycerol-P	...	++	+	+++ +	...	...	...	...
P-Glycerate	+	...	...	...	...	...	...	...
Orthophosphate	+++ ++	+++ +++ +	++	++	+++ +++ +	+++	+	...
A	...	...	+	+++ +	...	...	+	+++
B	...	+	+	...	...	...	+	++
C	++	++	++	...	...	...	...	...
D	...	...	...	+++	...	...	...	...
E	...	...	...	+	...	...	...	...

Number of plus signs (+) indicates number of storage samples out of 7 showing spots with *Rp* in region of indicated phosphates.

Figure 3. Effect of fractionation procedures on barium solubility of acid-soluble phosphorus of potato

Methods A and B are described in text. P_i PGA, and F-6-P represent control compounds orthophosphate, phosphoglycerate, and fructose-6-phosphate, respectively.



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PLANT TISSUE ANALYSES

Test for Pectin Based on Reaction of Hydroxamic Acids with Ferric Ion

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The reaction of the ester groups in pectin with aqueous alkaline hydroxylamine at room temperature for 2 minutes produces hydroxamic acids. Water-insoluble, red-colored complexes are formed upon subsequent addition of ferric ion. These reactions serve satisfactorily as a histochemical test for pectin in plant tissues and as a qualitative test for pectic substances. The test seems to be specific for pectin under the conditions recommended.

CARBOXYLIC ACID DERIVATIVES, such as esters, lactones, anhydrides, amides, nitriles, and other compounds, react with hydroxylamine to form hydroxamic acids which give colored complexes with ferric ion (*8–11*). Acyl phosphates (*15*) and acetylcholine (*9*) have been determined quantitatively by this reaction. Anhydrides, lactones, and esters react with aqueous alkaline hydroxylamine at room temperature and can be detected by paper chromatography (*1, 23*). Other compounds, such as amides, nitriles, and fatty acid esters, must be heated with alkaline hydroxylamine in high-boiling organic solvents before the reaction occurs (*10, 11, 19*).

Pectin esters react with hydroxylamine in aqueous alkali at room temperature, at a more rapid rate than de-esterification, and on addition of ferric ion an insoluble colored ferric–hydroxamic acid complex is formed.

Reagents and Procedure

The reagents are those used by Hestrin (*9*) for quantitative determinations of acetylcholine.

Hydroxylamine, 13.9 grams dissolved in 100 ml. of water.

Sodium hydroxide, 14.0 grams dissolved in 100 ml. of water.

Hydrochloric acid solution, 1 volume of concentrated hydrochloric acid reagent (specific gravity 1.18) diluted with 2 volumes of water.

Ferric chloride reagent, 10 grams of ferric chloride hexahydrate dissolved in 100 ml. of 0.1*N* hydrochloric acid.

To about 0.005 gram of the test substances suspended or dissolved in 1 ml. of water is added accurately 1 ml. of the hydroxylamine reagent, then 1 ml. of the sodium hydroxide solution. The reactants are allowed to stand for 2 minutes; then 1 ml. of the diluted hydrochloric acid is added, followed by 1 ml. of the ferric chloride reagent. A red precipitate is produced in the positive tests. A control is obtained by adding the test substances to the reactants after the addition of the hydrochloric acid, because the reaction with esters to form hydroxamic acids does not proceed in strongly acid solution.

Experimental

Application to Sections of Plant Tissues. Fresh fruit and vegetable tissues were sectioned serially on a sliding microtome at thicknesses (80 to 520 microns) dependent on their structure and cell size. All freshly cut sections were placed immediately in water containing about 300 p.p.m. of dissolved sulfur dioxide. All sections were tested in dishes of 10-ml. capacity, and the amount of each reagent was doubled in order to ensure complete coverage and suspension of the section. The containers were swirled after addition of each reagent to provide thorough mixing.

Positive color production was immediate following the addition of the ferric ion and color intensity was fully developed within 1 minute. The sections were then transferred to water and excess ferric chloride and salts were washed out before further examination.

Examinations at oil immersion magnifications (ca. 1200x) were most readily made after tested sections had been vacuum infiltrated with water to remove the profuse gas bubbles evolved during the test reaction. Transverse cuts of adjacent cell walls (at the section surface) showed that localization of the color complex was confined to the so called "compound middle lamella" (*7*).

Selectivity for Pectin and Color Reflection Measurements. The selectivity of the color test for pectin was determined as follows: Fresh sections of young Meyer lemon, ripe apple, and potato were tested immediately and some were de-esterified at room temperature for 16 hours in purified orange pectinesterase (*16*) solution at pH 5.5. Other sections were soaked for 30 minutes in 10% aqueous sodium hydroxide at room temperature. Some of the enzymatically de-esterified sections were dehydrated in absolute methanol and then re-esterified for 2 hours in 0.5*N* hydrochloric acid in absolute methanol at room temperature. These conditions were suggested by results of esterification of galacturonides by Jansen and Jang