Carbohydrate Components of the Potato Tuber

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Russet Burbank tubers were separated into soluble and insoluble fractions with 80% ethyl alcohol. Free sugars in the soluble fraction identified by paper chromatography were sucrose, glucose, and fructose. The alcohol-insoluble fraction was further fractionated. These fractions were hydrolyzed and the constituent sugars identified by paper chromatography. Fractions isolated included an araban-galactan soluble in 50% ethyl alcohol, starch, pectin, small quantities of araban-galactan extracted during pepsin hydrolysis, hemicellulose, and a cellulose fraction.

POTATOES WITH HIGH STARCH and low sugar content generally possess better cooking and processing qualities than potatoes of low starch and high sugar content. Because of this relationship many studies have been made of potato starch, factors affecting starch content, and the sugar-starch conversion in the tuber (*3, 4, 7, 12-14*). In contrast, little is known about the other constituent polysaccharides of the potato tuber. Results of scattered pectin and crude fiber determinations have been compiled (*3, 4*). Attempts to determine a possible correlation of pectin content and cooking quality have yielded negative results (*8*). No reports have been found in the literature on the occurrence of hemicellulose in potato tubers. As these nonstarch polysaccharides may also influence cooking and processing quality, it is of interest to characterize these fractions more fully. The present study was undertaken to separate out the various polysaccharide fractions and to determine qualitatively the constituent sugars of these fractions.

Materials and Methods

Whole Russet Burbank tubers stored at 40° F. were brushed and washed lightly to remove extraneous material. The raw tubers were chopped in a Waring Blendor for 5 minutes with sufficient 95% ethyl alcohol to yield a final alcohol concentration of approximately 80%. Following blending, the mixture was brought rapidly to boiling and refluxed 10 minutes. The hot solution was filtered, and the residue was washed once with 80% ethyl alcohol, further extracted with ethyl alcohol, and finally extracted with ether in a Soxhlet apparatus. The alcohol-ether–insoluble fraction was allowed to air-dry, ground to pass a 60-mesh screen in a Wiley mill, and stored for future experiments.

The various fractions were hydrolyzed with hydrochloric acid in sealed tubes (*17*) or with sulfuric acid and the sulfate was removed with barium carbonate after hydrolysis. Hydrolyzates were chromatographed (descending technique) on Whatman No. 1 paper with the organic phase of one of the following mixtures as the irrigating solvent: phenol-water (adjusted to pH 5.0 to 5.5), ethyl acetate–pyridine-water (2:1:2, v./v./v.), or 1-butanol–acetic acid–water (4:1:5, v./v./v.). The sugars were located on the chromatogram by the use of one or more of the following spray reagents: aniline hydrogen phthalate, 3,5-dinitrosalicylic acid, and resorcinol–hydrochloric acid (*18*). Unknown sugars were identified by comparison with known sugars included on the same chromatogram. Nitrogen was determined by a semimicro-Kjeldahl method, using the digestion mixture of Umbreit and Bond (*15*) and boric acid titration with Ma and Zuazaga's (*10*) mixed indicator.

Results

Free Sugars In addition to the determination of the sugars of the polysaccharide fractions, qualitative determinations of the sugars of the alcohol-soluble fraction also were made. Chromatograms of the alcoholic extracts gave spots indistinguishable from those formed by known sucrose, fructose, and glucose. In the concentration of extract used, no other sugars were detected. These results agree with those of Schwimmer and others (*14*), who also detected several other minor sugars.

Polysaccharides Microscopic examination of the residue showed that although many of the cells had ruptured and released the starch granules, some intact cells and groups of cells still remained. Most of the starch granules showed various cracks or markings on the surface, which were not seen on granules not subjected to boiling alcohol. In some cases the granules were distended or broken. When viewed in polarized light, the ruptured granules did not give the characteristic birefringent pattern of normal starch granules.

The alcohol-ether–insoluble residue (hereafter referred to as the total crude polysaccharide) contained 2.6% ash and 4.5% protein (Kjeldahl nitrogen × 6.25). When hydrolyzed with hydrochloric acid according to the AOAC method for starch (*1*), most of the total crude polysaccharide went into solution. The amount of insoluble material was determined by collecting the residue on a sintered-glass crucible and drying over sulfuric acid. The residue represented 3% of the total crude polysaccharide. Negligible precipitates formed when 3 volumes of 95% ethyl alcohol were added to the acid filtrate. Substances identified chromatographically in the acid filtrate were glucose, arabinose, xylose, and a uronic acid. Other sugars were not easily identifiable because of the excess amounts of glucose. To obtain further information concerning the total crude polysaccharide, it was separated into several fractions. A summary of the procedures and results is given in Figure 1.

The total crude polysaccharide was first extracted six times with 50 volume % ethyl alcohol. Each extraction was made using 50 ml. of aqueous ethyl alcohol per gram of sample. Such treatment removes arabans and galactans without extracting the pectin (*8*). Eight per cent of the total crude polysaccharide was removed by extraction. The soluble fraction when concentrated, hydrolyzed, and chromatographed gave spots indistinguishable from those of known arabinose and galactose. Judging from spot size and intensity, there was more arabinose than galactose in this fraction.

Subsamples of the total crude polysaccharide were also extracted with dis-

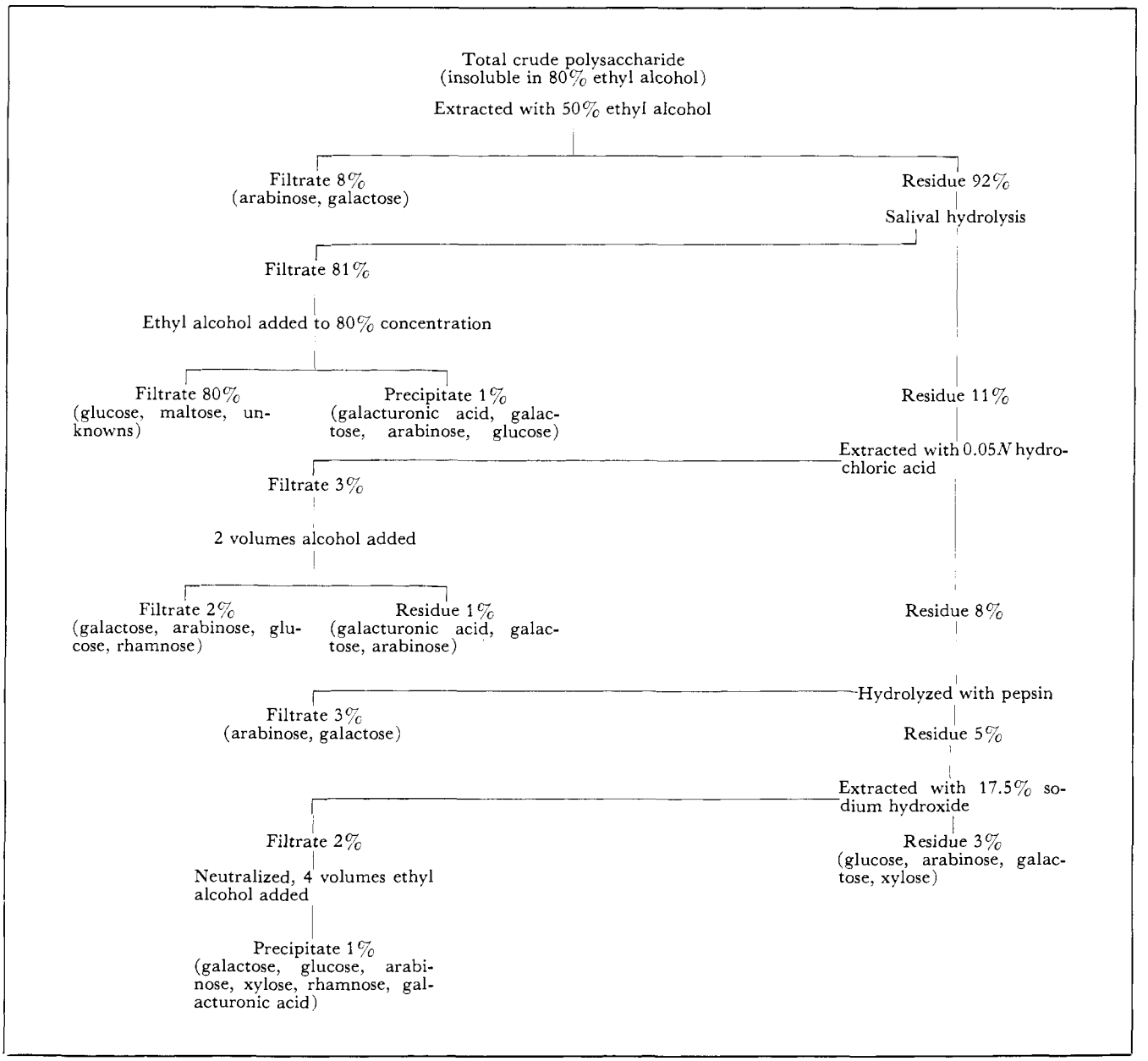

Figure 1. Flow diagram of extraction procedure and sugars found in various polysaccharide fractions of potato tuber

Percentage figures refer to per cent of original total crude polysaccharide

tilled water rather than 50% ethyl alcohol. Samples treated in this way were difficult to separate by filtration or centrifugation. After the extraction with water and all other extractions with aqueous solutions, the residue was finally washed with ethyl alcohol and then with ether and air-dried to facilitate future handling. Approximately 8% of the total crude polysaccharide was removed by aqueous extraction. Chromatographic separation of the hydrolyzates revealed the presence of galactose and arabinose. When large amounts of the hydrolyzate were examined, faint spots corresponding to those of known ribose and galacturonic acid appeared on the chromatograms. The small amount of galacturonic acid found is in accord with reports of other workers (*5*, *6*), who have found little, if any, cold water–soluble pectin in potato tubers.

The 50% ethyl alcohol–insoluble residue was gelatinized on a steam bath, cooled, and treated overnight with saliva (*9*). The insoluble material was filtered off and washed with water. The aqueous filtrates were combined and ethyl alcohol was added to yield a final concentration of approximately 80% ethyl alcohol. The mixture was allowed to stand several days at 40° F. and then separated by filtration through hardened filter paper, into an alcohol-soluble and an alcohol-insoluble fraction. The water-soluble, alcohol-soluble fraction of the saliva hydrolyzate comprised 80% of the total crude polysaccharide. When examined chromatographically, this fraction was found to contain small amounts of glucose, large quantities of maltose, and an unidentified material. In ethyl acetate–pyridine-water irrigant the $R_{glucose}$ of this material was 0.50. Maltose has an R_g of 0.70. From its position on the chromatogram and its reaction with the spray reagents, it appeared to be maltotriose. This sugar has been identified in salival hydrolyzates of both the amylose and amylopectin fractions of starch (*11*, *16*). In addition to these distinct spots, there was an undifferentiated streak near the origin of the chromatogram. This streak was

probably the result of dextrins (*16*) not precipitated by the alcohol.

The water-soluble, 80% alcohol–insoluble fraction of the salival hydrolyzate accounted for 1% of the total crude polysaccharide. When hydrolyzed and chromatographed, it yielded spots identical with those of known galacturonic acid, galactose, arabinose, and glucose. The glucose in the hydrolyzate probably was derived from incompletely hydrolyzed starch molecules still large enough to be precipitated by alcohol. Varying amounts of glucose were noted, depending on the length of the enzymatic hydrolysis. A second treatment with saliva usually decreased the amount of glucose in this fraction.

The insoluble residue following the enzymatic hydrolysis amounted to 11% of the total crude polysaccharide and contained 25% protein (Kjeldahl nitrogen × 6.25). This residue was twice extracted with 0.05*N* hydrochloric acid according to the method of Kertesz (*8*) to remove the pectin. After extraction, the insoluble residue was separated by filtration. The acid-soluble filtrates were combined and 2 volumes of 95% alcohol added. The mixture was allowed to stand for several days at 40° F. and filtered.

The 0.05*N* acid–soluble, alcohol-soluble fraction, representing 2% of the total crude polysaccharide, was concentrated. Chromatograms of hydrolyzates of this fraction revealed the presence of galactose, arabinose, rhamnose, and glucose. The glucose again appeared to be derived from incomplete enzymatic hydrolysis of the starch. Application of iodine–potassium iodide and microscopic examination of the residue following enzymatic hydrolysis have shown that the interior of unbroken cells may still give a reddish color, indicative of incomplete starch hydrolysis. From total solids and Kjeldahl analyses this fraction was found to contain 6.4% nitrogen.

The 0.05*N* hydrochloric acid–soluble, alcohol-insoluble fraction was 1% of the total crude polysaccharide. This fraction contained 1.6% nitrogen. Upon hydrolysis, spots identical with galacturonic acid, arabinose, and galactose appeared on the paper chromatograms.

The 0.05*N* hydrochloric acid–insoluble fraction represented 8% of the total crude polysaccharide and contained 3.8% nitrogen. This residue was treated with an excess of pepsin (*17*) and filtered. The pepsin-soluble fraction was 3% of the total crude polysaccharide. Hydrolyzates of this fraction gave spots indistinguishable from those of known galactose and arabinose. Judging from the amount of material chromatographed and the size and intensity of the spots, only a small quantity of polysaccharide was present in the fraction.

The pepsin-insoluble fraction, 5% of the total crude polysaccharide, contained 1% of nitrogen. This was extracted under nitrogen with 17.5% sodium hydroxide (*17, 19*). The alkaline filtrate and washings were neutralized to pH 5 with glacial acetic acid and 4 volumes of ethyl alcohol added. After standing at 40° F. for several days the precipitate was collected by filtration. This precipitate, containing 0.5% nitrogen, corresponded to 1% of the total crude polysaccharide and is probably potato hemicellulose (*2*). The precipitate did not represent complete recovery of the alkali-soluble fraction. Incomplete recovery of this fraction was also noted by Williams and Bevenue (*17*) working with tomato hemicellulose. Subsequent to hydrolysis this hemicellulose fraction was found to contain spots corresponding to those of known galactose, glucose, arabinose, galacturonic acid, xylose, and traces of rhamnose.

The alkali-insoluble fraction was 3% of the total crude polysaccharide. This fraction should represent pure α-cellulose; however, it still contained 0.3% nitrogen. Chromatograms of hydrolyzates showed glucose, arabinose, and traces of galactose and xylose.

Discussion and Summary

As expected, starch constitutes the major portion (80%) of the polysaccharide portion of the potato tuber. In addition to starch, other fractions identified included: (1) an araban-galactan soluble in 50% alcohol; (2) a pectin fraction; (3) an araban-galactan fraction extracted during pepsin hydrolysis; (4) a hemicellulose fraction; and (5) cellulose. In contrast to the glucose found in the starch, these other fractions are made up primarily of galactose and arabinose and smaller quantities of other sugars. It is possible that some or all of the glucose found in other fractions may be due to the incomplete removal of starch by enzymatic hydrolysis.

The results obtained in the present study are not to be considered as quantitative. No method of separating various polysaccharides from one another, or from other polymers, is, as yet, completely quantitative. In particular, such fractions as hemicellulose depend on the strength of the basic extractant and the temperature at which the extraction is carried out (*2*). In addition, starch comprises such a large portion of the polysaccharide fraction that chances for error are increased when fractions making up only 1% of the original sample are separated, particularly where procedures involved repeated extractions, precipitations, and filtrations. For these reasons all data in the present study have been rounded off to the nearest whole number.

Although the present study was not quantitative, certain results may be of value in developing routine methods of analyses. Enzymatic hydrolysis appears to be the most feasible method for starch removal. Other nonenzymatic methods have been attempted, but as yet they have not been as successful as enzymatic hydrolysis. For quantitative starch removal two enzymatic treatments may be necessary. This second enzymatic hydrolysis might be dispensed with if the method of grinding resulted in rupture of all the cells. Other methods of removing the starch by sieving methods (*14*) may be applicable. The present results corroborate earlier results, showing little, if any, pectin in the water-soluble fraction. Thus, starch removal by mechanical means should not affect pectin determination.

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