Chemical Composition of Potato Cell Wall

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Cell wall–middle lamella material free of starch granules was obtained by a purification process involving sonication to rupture unbroken cells followed by Miracloth filtration using a specially designed filtration apparatus. Potato tuber contained 1.2% (wet basis) of cell wall–middle lamella material. The material was composed of 55% pectic substance, 7% hemicellulose, 28% cellulose, and 10% protein. The isolated pectic substance contained 51% anhydrogalacturonic acid and 49% polysaccharide, while the hemicellulose contained 7% anhydrogalacturonic acid and 93% polysaccharide. The pectin polysaccharides were composed of 6.0% rhamnose, 0.6% fucose, 5.6% arabinose, 1.8% xylose, and 86.0% galactose. The hemicellulose polysaccharides were composed of 0.5% rhamnose, 2.1% arabinose, 23.1% xylose, 5.8% mannose, 12.0% galactose, and 56.7% glucose.

Although the cell wall and middle lamella of potato tuber tissue constitute only a minor portion of the total solids present in the tuber, this material has a profound effect on the textural properties of both the raw tuber as well as of the processed potato tissue. Sterling and Bettelheim (1955) succeeded in describing the principal factors determining the textural properties of cooked potatoes. Still, the detailed chemical composition of the cell wall–middle lamella has yet to be described. Such information is required for an understanding of the reactions that take place during storage, when unpredictable changes in textural properties may take place, and during processing when the tissue undergoes a pronounced softening resulting in either soggy or mealy potatoes depending upon the time-temperature history of the process as well as on the genetic and cultural background of the potato. The storage changes of texture are probably brought about by enzymatic reactions involving substrates in the cell wall and middle lamella. Similar enzymatic reactions may also be operative during part of the heating cycle in thermal processing (Unilever, 1966).

A prerequisite for evaluation of textural and quality changes is the existence of sufficiently detailed quantitative and qualitative information on the chemical composition of the cell wall. We here present results of such an investigation as a first step in that direction.

EXPERIMENTAL

Potato tubers of the variety Superior stored 1 to 2 months at 45° F. and weighing between 150 and 250 grams were peeled and cut in half along their long axis. Slices approximately 1 mm. thick were cut from the center faces and weighed portions of 20 grams rapidly suspended in cold water to wash away loose starch. The slices were ground in a 100-ml. Waring Blendor with 50 ml. of cold water and a few drops of antifoaming agent for a total period of 10 minutes interspersed by cooling periods to maintain the temperature below 10° C. The ground material (Figure 2*A*) was transferred quantitatively to the Miracloth filtration apparatus (Figure 1*A*, *B*) where all free starch grains were removed (Figure 2*B*).

The filter apparatus consisted of two flanged glass hemispheres arranged to hold a circular disk of Miracloth (Chicopee Mills, Inc., New York) at the equatorial plane by means of two rubber gaskets and two polystyrene rings furnished with bolts and wing nuts. The top hemisphere was provided with an inlet for sample loading, while the lower hemisphere had a side inlet for solvent (water) and a bottom outlet for drainage of suspended starch. In operation the apparatus was filled about $^3/_4$ full from the solvent supply line and the sample introduced from the top and evenly distributed in the upper hemisphere by gentle stirring or shaking of the whole apparatus. The liquid level was now allowed to drop rapidly to slightly above the filter surface by opening the bottom outlet,

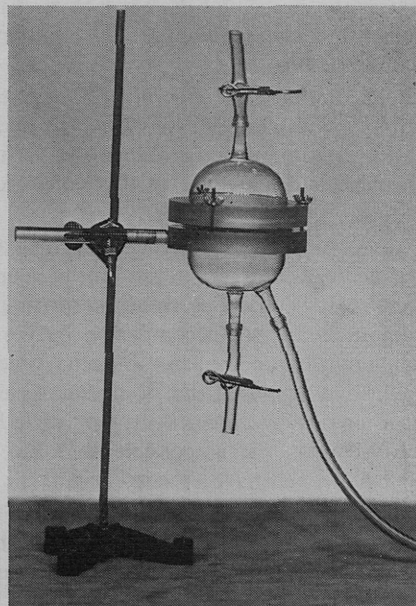

Figure 1*A*. Assembled filtration apparatus

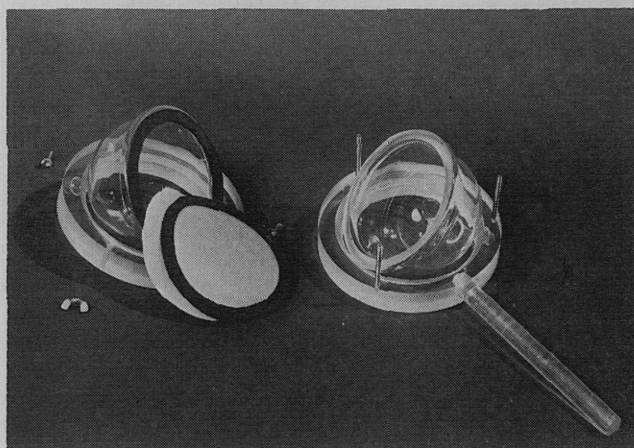

Figure 1*B*. Disassembled filtration apparatus showing Micracloth filter, gaskets, and polystyrene frame

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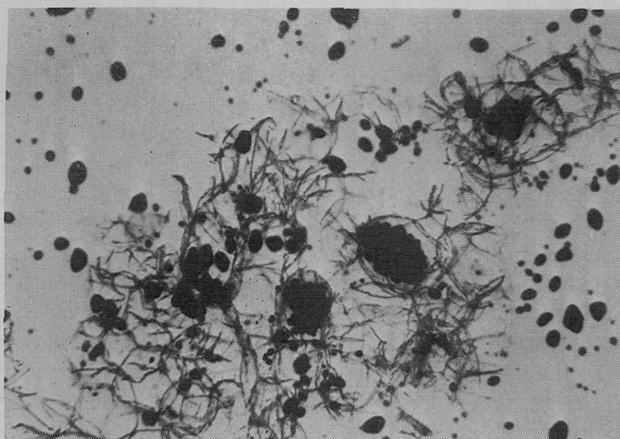

Figure 2*A*. Ground potato tuber. Starch grains stained with iodine. 50×

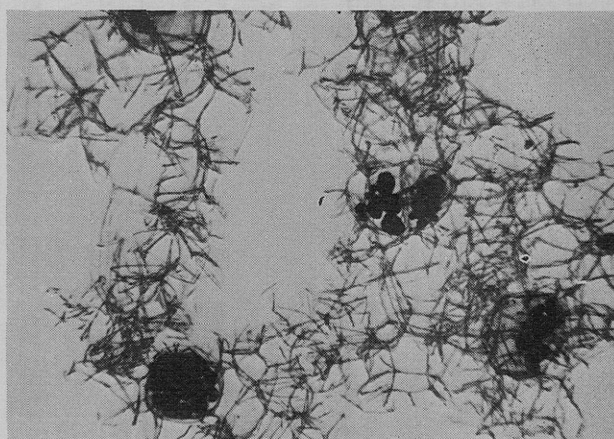

Figure 2*B*. Ground potato tuber filtered on Miracloth. All free starch grains removed. Some intact cells remain. 50×

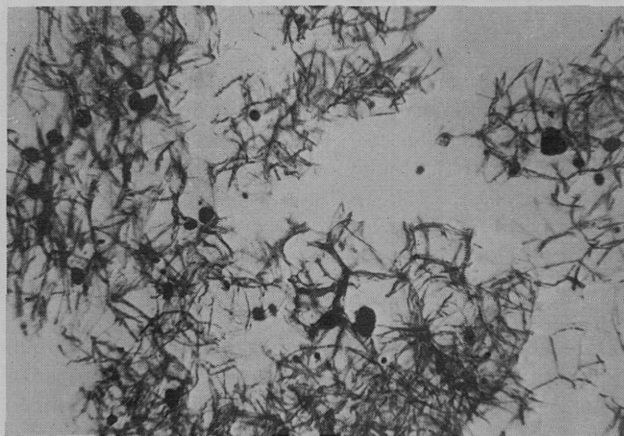

Figure 2*C*. Cell wall material following sonication. The remaining intact cells have been ruptured, releasing their starch grains. 50×

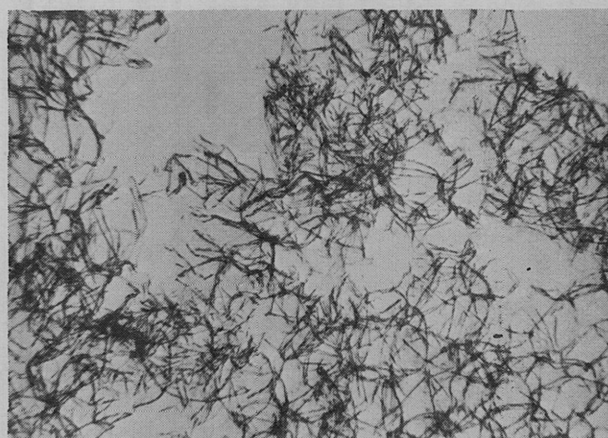

Figure 2*D*. Cell wall material after final Miracloth filtration. 50×

leaving the lower hemisphere filled preventing air from entering underneath the Miracloth filter. If this happened, the active filtering surface was drastically reduced in subsequent operations. The apparatus was next rapidly filled to the original level, thereby lifting the sample renewing the filter surface. The operations were then repeated until the desired degree of starch removal had taken place. In case air had accidentally entered the lower hemisphere, it was easily removed by clamping the sample entry port, inverting the apparatus and, while opening the solvent supply line, allowing the air to escape through the drainage line.

The sample still contained many unbroken cells. Since further grinding proved unprofitable, the sample was next transferred quantitatively to a sonic oscillator (Raytheon, Model DF 101) and sonified for 1 hour while maintaining the temperature below 20° C. The sonified sample (Figure 2*C*) was further purified by Miracloth filtration yielding the final essentially starch free cell wall material (Figure 2*D*). The material was transferred quantitatively to a small Miracloth filter, washed twice with absolute alcohol and twice with diethyl ether, and finally dried overnight in vacuum oven at 40° C.

The purified cell wall material was fractionated into three fractions consisting of pectic substance, hemicellulose, and lignin plus cellulose according to procedures described by Dever *et al.* (1968). The latter fraction was accepted as the residue left after extraction first with oxalic acid–ammonium oxalate, which solubilizes the pectic substance, and second by alkali, which solubilizes the hemicelluloses. Each of the fractions was washed and dried as described for the cell wall material.

Polyuronide was determined according to McComb and McCready (1952), methoxy groups according to a gas chromatographic procedure by Bartolome and Hoff (1969), and sugars by a procedure described by Albersheim *et al.* (1967). The latter involves hydrolysis of the polysaccharides with trifluoroacetic acid at 121° C. followed by reduction of the aldoses to alditols with sodium borohydride and finally formation of polyacetates in preparation for gas chromatographic determination.

RESULTS AND DISCUSSION

Recommended procedures (Joslyn, 1962) for the isolation of the intact pectic substances involve as a first step the isolation of the alcohol insoluble solids (AIS) under conditions which inactivate rapidly all pectin-degrading enzymes. Typical for such procedures is the immersion of plant tissue immediately after slicing in boiling alcohol. For the purpose of this investigation, such an approach has several disadvantages. Potato tuber tissue consists overwhelmingly of starch. For an accurate determination of the carbohydrate composition of the cell wall and middle lamella polysaccharide, it is therefore imperative that virtually all of the starch be removed. Complete physical separation of the starch grains is not possible after boiling in alcohol, since some starch grains will adhere to the cell wall, probably because of embedding in the coagulated cytoplasmic proteins. Starch removal by means of amylase was used by Le Tourneau (1956), but has in our

Table I. Tuber Cell Wall Content and Proximate Composition of Cell Wall

	%[a]
Cell wall content of tuber	
Wet basis	1.2 ± 0.1
Dry basis	5.6 ± 0.6
Proximate composition of cell wall (dry basis)	
Pectic substance	55.0 ± 7.5
Alkali-soluble hemicellulose	6.8 ± 1.4
Cellulose plus lignin	27.5 ± 2.5
Protein (N × 6.25)	9.8 ± 1.7

[a] Mean of nine determinations followed by standard deviation.

Table II. Composition of Pectic Material and of Constituent Sugars

	%[a]
Material solubilized by TFA[b]	98.1 ± 1.4
Polyuronide (as polygalacturonic acid, PGA)	51.4 ± 10.1
Sugars (by difference)	48.6 ± 10.0
Recovery of sugars	83.1 ± 16.7
Methoxyl content of PGA	6.2 ± 0.9
Composition of sugars[c]	
Rhamnose	6.0 ± 2.1
Fucose	0.6 ± 0.3
Arabinose	5.6 ± 3.5
Xylose	1.8 ± 1.1
Mannose	0.0 ...
Galactose	86.6 ± 5.3

[a] Mean of nine determinations followed by standard deviation.
[b] Trifluoroacetic acid.
[c] Glucose (7.1 ± 4.0%) was assumed to stem from starch contamination. The sugar composition was correspondingly corrected.

Table III. Composition of Hemicellulose

	%[a]
Material solubilized by TFA	72.5 ± 16.3
Recovery of solubilized material (as sugars)	83.2 ± 8.8
Polyuronide (as polygalacturonic acid)	6.6 ± 1.1
Composition of sugars	
Rhamnose	0.5 ± 0.1
Fucose	0.0 ...
Arabinose	2.1 ± 0.5
Xylose	23.1 ± 1.1
Mannose	5.8 ± 1.4
Galactose	12.0 ± 1.2
Glucose	56.7 ± 1.8

[a] Mean of nine determinations followed by standard deviation.

hands not been satisfactory because of incomplete starch hydrolysis. The procedure employed by us (see materials and methods) involved extensive treatment of the raw tissue in water and can be criticized on the grounds that no effort was made to inactivate pectolytic enzymes that might be present. However, we were unable to detect any pectolytic activity in a phosphate buffer extract of potato tuber (Morré, 1968). An effort was made to maintain the temperature as low as conveniently possible during the various manipulations in aqueous media, and the major portion of water-soluble compounds (including cytoplasmic proteins) was removed shortly after the grinding step. It is therefore unlikely that the pectic substances in the purified cell wall material (Figure 2*D*) have been significantly altered by enzymatic activity during the purification.

The amount of soluble pectic substances in raw potato tuber is relatively small (Le Tourneau, 1956; Sterling and Bettelheim, 1955). We made no effort to determine its value, and much of its was undoubtedly lost.

In spite of extensive sonication, it was not possible to break all cells. The resistant cells had thick cell walls and contained unusually small starch grains. We believe these to be sclereids, which occur in the potato tuber (Artschwager, 1924). The sclereid starch would in the subsequent operations be extracted with the pectic substances and later be determined as glucose. In view of the unusual variability in this analysis (7.1 ± 4.0%, Table II), roughly corresponding with a visual estimation of the number of intact cells remaining in the final preparation, we have assumed that all the glucose found in the pectic substance was due to starch contamination and have corrected the values for the other sugars accordingly.

The results of the analysis are presented in Tables I, II, and III. Each value is based on determinations of samples from nine different tubers. The estimated standard deviation therefore incorporates inter-tuber variation as well as the standard error of analysis.

The cell wall content of potato tuber as determined here is (Table I) in general agreement with determinations by others (Sterling and Bettelheim, 1955). Approximately one half of this material consists of pectic substance. About 25% of the material is cellulose and the remainder lesser quantities of protein and hemicellulose. The distribution of these fractions is similar to that found in young growing tissue of many plants (Roelofsen, 1965) and indicates that the parenchyma cells of potato tubers do not undergo the aging phenomenon of plant tissues, namely massive deposition of cellulose and lignin in the secondary cell wall at the cost of pectic substance and hemicellulose.

The composition of the pectic substances (Table II) indicates that only about one half of the material is composed of polyuronide, the carboxyl groups of which are esterified to an extent of only 40% in general agreement with previous reports (Sterling and Bettelheim, 1955). The polysaccharide content of this material is unusually large when compared with pectin isolated from various other sources (Joslyn, 1962), but probably reflects the method of isolation used and the extent and concentration of the aqueous alcoholic washings applied. Arabans and galactans, which are known to accompany pectins (Joslyn, 1962), are considerably more soluble in alcoholic solutions than pectin. Unknown quantities of these, and particularly of araban, may have remained in solution after precipitation with alcohol. This consideration is borne out by the change in the ratio of arabinose to galactose (w./w.) when comparing an analysis (not shown here) of the intact cell wall with that of the sum of the fractions of pectic substance and hemicellulose. The ratio changed from 1:4 before fractionation and precipitation to 1:15 following isolation of the two fractions. This indicates that appreciable quantities of arabans remained in solution and were not accounted for. The loss was estimated as approximately 5% of the dry cell wall material.

The sugars in the pectic substance (Table II) contain predominantly galactose. Other sugars are mostly rhamnose and minor quantities of xylose and fucose. Three or four other substances appeared, as judged from the gas chromatograms, to be present in trace quantities (less than 1% of the total sugars). We made no attempt to identify these unknown substances, but it is reasonable to believe that they comprise certain methylated sugars, as observed in other plant materials (Andrews *et al.*, 1959; Barrett and Northcote, 1965).

The major monosaccharides of the hemicellulose fraction (Table III) are glucose, xylose, and galactose. Lesser quantities of mannose and arabinose are also found and only traces of rhamnose and two unknown substances. Mannose

appears to be specific for the hemicellulose. Considering that a clear-cut separation of the two fractions, pectins and hemicellulose, is not to be expected, it is tempting to assign xylose exclusively to the hemicellulose, and rhamnose and fucose exclusively to the pectic material. We have already assumed that glucose is not present in the pectic substance and is, therefore, found only in the potato tuber hemicellulose. These assumptions can, of course, be made firm only by detailed studies of the isolated homogeneous and monodisperse components.

The chemical composition of the potato cell wall as given here is in qualitative agreement with earlier findings by Le Tourneau (1956), but disagrees sharply with a more recent report by Vechner and Prokazov (1967), who found 65 to 80% glucose in the cell wall of potato tubers. Galactose is, according to our findings, the major single monosaccharide component, while no mention of it was given by these workers. The fact that maltose was found by Vechner and Prokazov indicates that starch must have been a major contaminant of their cell wall preparation, and this underlines again the need to remove starch as quantitatively as possible before undertaking a chemical study of the cell wall.

The relatively large amounts of neutral sugars found to be present in the potato tuber cell wall indicate that they may exert profound effects on the physical properties of the constituent compounds. Thus, the isolated pectic material was soluble in 50% alcohol and the resulting solution formed a gel on standing, in sharp contrast to the alcohol solubility of pectinic acids in general, where 20% causes complete precipitation. The association of the araban-galactan fraction with the polyuronides is still a matter of conjecture—whether the association is the result of a physical admixture, or whether one is confronted with chemical covalent binding. As has been repeatedly shown (Joslyn, 1962), large quantities of araban —and to a lesser extent galactan—can be readily separated from the remaining pectic substance by repeated washings with aqueous alcohol. However, this is achieved only after the pectic substance has once been rendered soluble—*i.e.*, not with the protopectin in its insoluble state in the cell wall. It is likely that the solubilization process proper, for instance, the use of oxalic acid as in this study, hydrolyzes labile covalent linkages involving these polymers, and that the separation observed later is essentially an artifact due to manipulation of the material and does not reflect the conditions in the native state. This contention is strengthened by the fact that an arabanase-galactanase complex has been shown to be operative in carrot tissue (Hatanaka and Ozawa, 1965) solubilizing araban-galactan specifically with no apparent effect on the polyuronide chain. The possibility exists that such enzyme systems are also present in the potato tuber and contribute to the spontaneous or induced modification of the properties of the cell wall during storage and processing. The isolation of pure cell wall material and the composition of the isolated material as reported here should make possible systematic studies of such phenomena.

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