HEMICELLULOSES OF THE CEMENTING LAYER AND OF SOME CELL WALLS OF THE CORN KERNEL[1]

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ABSTRACT

Hemicellulose fractions of the cementing layer and of various cell walls in the corn kernel were analyzed by quantitative paper chromatography. Dilute acid hydrolysis of the cementing layer showed that it contained 60% polysaccharide composed of 55% arabinose, 31% xylose, and 14% glucose. Total nitrogen analysis indicated a protein content of 30%.

Cell walls of the scutellar parenchyma, scutellar epithelium, and endosperm were found to contain 50, 30, and 20% hemicellulose, respectively. On hydrolysis, hemicellulose of the endosperm cell walls yielded 34% arabinose, 35% xylose, and 31% glucose in contrast to the hemicellulose of the cell walls of scutellar parenchyma and epithelium, each of which yielded about 60% arabinose, 30% xylose, and 5% galactose. In addition, a small amount of glucose was found in the hydrolysate of the cell walls of the scutellar parenchyma. No pectic substances were found in any of the cell walls examined.

The properties of cell walls of corn kernels are of considerable importance to the processor, because cell walls must be broken in either wet-milling for starch production or in dry-milling for the production of grits and meal.

Walls of the epithelial cells of the scutellum, as shown previously (9), are more easily degraded by enzymes than is the cementing layer
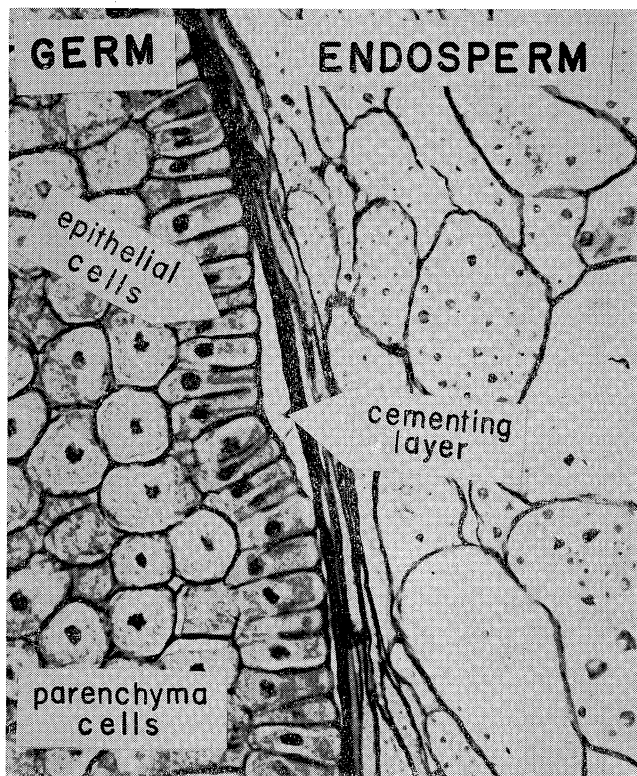
Fig. 1. Cross-section of the corn kernel through the region where the germ and endosperm meet, showing some of the cells which were analyzed. Mag. 250×.

between germ and endosperm. (These structures are illustrated in Fig. 1.) Even partial degradation of the epithelial cell walls helps to loosen the adhesion of the germ to the endosperm and facilitates degermination.

There are a number of well-known histochemical methods by which the chemical constituents of cell walls may be identified. A few of the methods can be used to obtain very rough estimates of the comparative amounts of the various constituents that are present, but histochemical tests are of chief value for qualitative identification of the constituents and determination of their locations *in situ*.

To study the quantitative composition of the cementing layer and adjacent tissues, methods were adapted for analysis of very small amounts of isolated cell walls. Emphasis was placed on the hemicellulose or pentosan fraction, because previous work had shown that this material was more readily degraded than other cell-wall constituents.

It is therefore of primary significance in degermination and other proc-
esses which involve cellular degradations. Methods were first tested on
cell walls that could be more easily isolated in larger quantity than
could material from the cementing layer region. These preliminary
studies, which were made on cell walls from the aleurone layer and
from the starchy endosperm, not only permitted refinement of the
methods, but also gave data of potential value to the processor. In this
paper, information thus obtained is combined with results of studies
on wall materials in the cementing layer region to provide a more
comprehensive view of cell-wall composition throughout the raw ma-
terial used by the corn milling industries.

Materials and Methods

Isolation of Cementing Layer. Yellow dent corn (U.S. 13 and Ill.
1277 varieties) was steeped in distilled water for 16 hours at room tem-
perature, then 120-μ cross-sections were cut on a freezing microtome.
Although the cementing material extends over the whole area between
the germ and endosperm, it is too thin in most places to isolate under
the dissecting microscope. However, masses of cementing material oc-
cur at gland formations or other irregularities of the epithelium (Fig.
1), and it was from these sites that about 200 γ of material were ob-
tained from some 5,000 selected sections.

Isolation of Cell Walls. A network of cell walls was easily obtained
by removing the contents from thin sections of tissue. The size and
shape of the cells determined how thick and in what direction to cut
the sections from the corn kernels. To aid in separation of starch and
protein from endosperm cell walls, 30-μ fresh sections were shaken in
a 0.5% solution of sodium hypochlorite for a few minutes. Residual
starch was gelatinized and solubilized by treatment with a malt alpha-
amylase preparation.

Cell walls of the scutellar parenchyma and epithelial cells were
obtained from 16-μ sections of germ cut perpendicular to the long axis
of the epithelial cells. After the network of cell walls had been defatted
in petroleum ether, it was inspected microscopically to confirm that
all protein and starch had been removed.

Cross cells were scraped from the inner surface of the pericarp and
extracted with hot 50% ethanol.

Aleurone cells were scraped from the outer surface of dehulled ker-
nels and defatted with hot 85% methanol.

The pericarp was hand-dissected, then extracted with hot 0.5% am-
monium oxalate to remove pectic substances, starch, and solubles.

Hydrolysis. Hydrolysis was carried out in a small, sealed tube con-

taining 40–110 γ of air-dried cementing material or cell walls and 0.2 ml. of 2% sulfuric acid. The tubes were autoclaved for 5 hours at 120°C., the hydrolysate was neutralized by first adding a saturated solution of barium hydroxide, and the pH was adjusted to 6.2–6.8 with powdered barium carbonate. The mixture was transferred to a strip of filter paper 4 by 15 cm. and eluted for 3 hours (4). This step removed the sugars while leaving the insoluble barium sulfate on the paper. The eluted volume (about 0.5 ml.) was evaporated to a small drop for spotting chromatograms.

Paper Chromatography. Both qualitative and quantitative paper chromatography was conducted by the descending method, with the use of known sugar standards and a solvent system of *n*-butanol, pyridine, water, 6:4:3 (2).

For quantitative studies, a single solution containing three sugars was applied on the chromatogram with a Gilmont[2] micropipet. The solution was spotted along the starting line in 0.5-μl. increments up to 2 μl., which deposited 2.5–10 γ of sugars. Three reference spots were used to determine the concentration curves; a fourth spot contained the entire neutralized hydrolysate. After 16 hours of solvent travel, the chromatograms were air-dried for 2–3 hours and then dipped into a silver nitrate solution similar to one used by Martin (5): it was prepared by dissolving 5.0 g. of silver nitrate in 3 ml. of pyridine, adding 197 ml. of acetone, and diluting the resulting solution 1:2 with acetone before dipping the chromatogram. After drying for a few minutes, the chromatogram was dipped into a sodium hydroxide reagent made by dissolving 0.60 g. of sodium hydroxide in 5 ml. of water and adding 95 ml. of ethanol. The reagent was diluted 1:2 with acetone before use. After the spots had developed for 15 minutes, the chromatogram was dipped into a 5% sodium thiosulfate solution containing 0.3% sodium sulfite. The chromatogram was held at room temperature until just damp (about 1 hour) and then dried at 105°–110°C. for 1 minute.

Quantitative estimation of sugars was made by measuring the optical density of the reduced silver spots with a Photovolt electronic densitometer (6). The cylinder lens was removed, and the entire oblong opening (4 cm.[2]) was used with a Wratten No. 45 blue filter. Slopes of the curves of optical density vs. concentration varied from sugar to sugar. Data from arabinose may be cited as typical of the results obtained. Usually a difference of 0.12 in optical density values was equivalent to an increment of 2.5 γ of arabinose. The recovery of known

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[2] Mention of firm names or trade products does not constitute endorsement or rejection by the U.S. Department of Agriculture over others of a similar nature not mentioned.

amounts of sugars was higher than 90%. Results are presented as percentage of sugar in the hemicellulose fraction.

With approximately 1-mg. quantities of air-dried cell walls, the sugars of the hydrolysate were determined by the Nelson modification of the Somogyi method as previously reported (10).

Nitrogen Determination. Total nitrogen was determined by the ultramicro-Kjeldahl method of Tompkins-Kirk (3).

Results

Qualitative Composition. Material chiefly obtained from cell walls by hydrolysis with 2% sulfuric acid was studied. This treatment affects the cell-wall hemicellulose and pectic substances. More drastic hydrolysis of the residues with 5% sulfuric acid yielded only glucose. This indicates that only cellulose remained unaffected by the original hydrolysis.

Arabinose and xylose were the main constituents of the hemicellulose fraction of all materials studied (Table I). Glucose was found in the hydrolysate of the cementing layer and in those of the cell walls of the endosperm, aleurone layer, and scutellar parenchyma.

TABLE I

ANALYSES OF THE HEMICELLULOSE FRACTIONS OF THE CEMENTING
LAYER AND CELL WALLS IN THE CORN KERNEL

| MATERIAL ANALYZED | SUGARS IN HEMICELLULOSE FRACTIONS | | | | | HEMI-CELLULOSE[a] |
	Xylose	Arabinose	Glucose	Galactose	Uronic Acid	
	%	%	%	%	%	%
Pericarp	+	+	0	+	+	(50)[b]
Cross-cells	+	+	0	+	+	..
Endosperm						
Aleurone layer	+	+	+	0	0	
Starchy portion[c]	35	34	31	0	0	21
Cementing layer	31	55	14	0	0	60
Germ						
Scutellar epithelium[d]	31	64	0	5	0	28
Scutellar parenchyma[e]	29	60	6	5	0	50

[a] Calculated from total weight of sugars in hydrolysate.
[b] Obtained by alkaline extraction (see reference 8).
[c] Average of nine analyses.
[d] Average of three analyses.
[e] Average of six analyses.

Galactose occurred in hydrolysates from the scutellar parenchyma and epithelial cell walls of the germ and also from the entire pericarp and cross-cells of the inner pericarp. Uronic acid was identified in the dilute acid hydrolysates from the pericarp and cross-cells, but not in those from any of the other cell walls. However, the fraction extracted from the pericarp with ammonium oxalate and hydrolyzed

with dilute acid contained no pectic material, as indicated by the absence of uronic acid.

Quantitative Composition. Because of the small quantity available, only 64 γ of cementing material were hydrolyzed. The hydrolysate yielded 21 γ of arabinose, 12 γ of xylose, and 5.3 γ of glucose (Table I). The recovered sugars were 60% of the air-dried weight of cementing material. Additional hydrolysis of the small amount of residue with 5% sulfuric acid yielded no additional carbohydrate material. This result confirmed an earlier observation that the cementing layer contains no cellulose (9).

Nitrogen determinations were made on two samples of cementing material; one weighing 35 γ had 30% protein (N $\times$ 6.25); the other sample weighing 103 γ contained 29% protein.

Cell walls of the scutellar parenchyma contained approximately 50% hemicellulose, those of the endosperm had only 21% (Table I), and scutellar epithelium cell walls averaged 28%. Previous work on the pericarp, with alkaline extraction methods, indicated a hemicellulose content of about 50% (8).

Of the four sugars identified in the hemicellulose fraction, xylose and arabinose represented the major components. About a third of the hemicellulose of the cell walls was xylose (29–35%), while the arabinose content varied from 34% (endosperm) to 64% (scutellar epithelium). The major difference in the composition of the hemicelluloses studied was the glucose content. Hemicellulose of the endosperm cell wall consistently showed a high glucose content in contrast to that of the cell walls of the scutellar epithelium, which had none. Only hemicellulose from cell walls of the scutellar parenchyma contained both glucose and galactose residues.

Discussion

Previous work showed that enzymatic separation of germ and endosperm of corn sections was effected by degradation of cell-wall pentosans and that tips of the epithelial cell walls of the scutellum were particularly susceptible to degradation (9). Consequently, the composition of the walls of the scutellar epithelium is of special interest. The present study shows that their hemicellulose content is relatively low. This fact is surprising in view of their comparative ease of degradation. Contamination with adjacent tissues is unlikely, since glucose would, in that case, have been found in the hydrolysate.

Examination of the epithelial cell walls with a polarizing microscope showed weak birefringence at the tips and a strong birefringence at the base. This difference in birefringence suggests a progressive in-

crease in cellulose content toward the base of the cells. Because of the method of isolation used, the wall material analyzed from these cells came mostly from the base, where the hemicellulose content may be low.

The hemicellulose from cell walls of the scutellar epithelium, unlike that from the other cells studied, was almost entirely pentosan (95%) and had the highest arabinose content of all hemicelluloses examined. Possibly the same chemical properties which render arabans more susceptible than other polysaccharides to acid hydrolysis may also account for the high enzyme susceptibility of these particular cell walls.

In the absence of pectic substances, it was assumed that the various types of cell walls studied were composed largely of hemicellulose and cellulose. Only qualitative determinations were made for cellulose (histochemical reactions, detection of birefringence, and further hydrolysis of residue left after removal of hemicelluloses). More work needs to be done, not only to elucidate the composition of the easily hydrolyzable hemicelluloses, but also to estimate the content of cellulose and other possible components such as protein and water-soluble polysaccharides.

The hemicellulose of the cementing layer is similar in composition to that of the endosperm cell walls. The presence of glucose, in particular, suggests strongly that the cementing material is of endosperm origin. This hypothesis appears to be reasonable since the germ in corn is known to develop partially at the expense of the endosperm. During the developmental period, there may be an infiltration of intercellular spaces over and around the epithelial cells by partial degradation products of the endosperm. On desiccation this material forms the carbohydrate-protein complex which becomes the so-called cementing layer between germ and endosperm.

Pectic substances are commonly regarded as important components of the primary cell wall and of the middle lamella, the bonding layer between adjacent cell walls. However, early work by Nanji and Norman (7) showed that only 0.59% of pectic material was present in corn kernels, and most of this was believed to be "in the middle lamella and cell wall of the aleurone layer." In the present study, as well as in previous work (9), no pectic materials were found in hydrolysates of any of the cells examined. Nor were pectic materials found in ammonium oxalate extracts of the pericarp. It has previously been shown, however, that corn pericarp contains a polyuronide hemicellulose with as much as 12% uronic acid (8). Possibly this has been mistaken by some workers for pectic material. Other workers

have reported low amounts of pectic substances in cereal grains. For example, Bishop *et al.* (1) found about 0.3% of pectic material (based on dry weight) in primary cell walls of the coleoptile of germinated oats.

Since pectic substances are virtually absent, the mechanism of tissue breakdown cannot involve pectic degradation. This concept agrees with previous results which showed that pectic enzymes had no obvious effect on cell walls of corn kernels (9). However, cell walls were degraded when treated with mixed pentosanase and cellulase in the absence of pectic enzymes.

The present work emphasizes the importance of hemicelluloses as structural components of cell walls of the corn kernel. Not only are they widely distributed in the kernel, but also they are easily solubilized, both enzymatically and chemically. They thus assume significance not only in controlling germ-endosperm separation, but also in weakening or degrading other cells within the kernel to aid in the release of starch, protein, and oil.

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