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Soybean (*Glycine max*) Cell Wall Composition and Availability to Feed Enzymes

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Defatted untoasted soybean cotyledons and hulls were fractionated as water solutes (WSc and WSh) and water unextractable (WUc and WUh). Further fractionation of WUc through deproteinization yielded the isolation of a water unextractable solid (WUS) fraction that was mainly composed (molar percent) of galactose (28.1%), glucose (27.8%), arabinose (13.3%), and uronic acids (17.6%), which accounted for 76% of the water insoluble polysaccharides in soybean cotyledons (WUc). The cell wall (WUS) was sequentially fractionated with chelating agents (chelating agent soluble solids, ChSS) and a gradient of agents (dilute alkali, DASS; 1 M alkali, 1MASS; and 4M alkali, 4MASS), which gave a final cellulosic residue. The ChSS and DASS extracts were characterized as pectin-rich fractions, whereas 1MASS and 4MASS were hemicellulose- and cellulose-rich fractions. Incubation in vitro of the WUc fraction with pectinase, cellulase, and xylanase resulted in the release of low amounts (not more than 5% bound basis) of monosaccharides, mostly uronic acids, xylose, and arabinose. Protein extraction hardly increased this release after enzymatic incubation (<7%). However, progressive fractionation of the cell wall matrix markedly increased the release of monosaccharides from pectin- (ChSS and DASS) and hemicellulose-rich fractions (1MASS and 4MASS). Significant degradation of cellulose (up to 20%) was achieved only after complete protein, pectin, and hemicellulose extraction.

KEYWORDS: Soybean meal; cell wall components; polysaccharides; enzymes

INTRODUCTION

Feed enzymes are widely used as additives, with an established role in animal nutrition. Although several cloned enzymes are available, they are applied only to improve cereal-based diets for broilers and, to a lesser extent, piglets. In fact, feed enzymes are used when profitable uses have been evidenced. For example, the availability of β-glucan- and arabinoxylan-degrading enzymes has fostered the incorporation of barley and wheat in poultry diets. They increase the metabolizable energy (ME) value of these diets, especially in batches with low nutritional value (*1*−*3*).

In general, feed enzymes have proved to be beneficial when effective enzymatic activities target a defined problem [e.g., glucanase and xylanase partial hydrolysis of water soluble glucans and xylans to reduce digesta viscosity (*4*)]. On the other hand, most efforts to improve the utilization of insoluble and otherwise unavailable plant cell wall components with feed enzymes have proved to be ineffective. The plant cell wall consists of a series of polysaccharides often associated with or replaced by proteins and phenolic compounds, such as the phenolic polymer lignin in some cells (*5*). The main polysaccharides of the plant cell wall are cellulose, arabinoxylans, mixed linked β-(1−3; 1−4)-D-glucans (β-glucans), xyloglucans, xylans, rhamnogalacturonans, and arabinogalactans (*6*, *7*).

Most of these carbohydrates are only partially digested or poorly utilized by the digestive enzymes. However, a disruption of these structures through processing [e.g., extrusion (*8*)] or degrading enzymes (*9*) can improve their nutrient availability. At present, the data available are contradictory. Zanella et al. (*10*) reported that a mixture of enzymes improved the nutritional value of corn−SBM diets for broilers. However, multienzyme preparations designed for soybean nonstarch polysaccharides have failed to improve the growth performance of broilers fed diets containing SBM as the main protein source (*8*, *9*, *11*, *12*). It is not clear whether the proper enzymes were missing or a complex structure remained insoluble and inaccessible.

The aim of this study was to evaluate the efficiency of cell wall degrading enzymes (pectinase, xylanase, and cellulase) in hydrolyzing soybean meal carbohydrates. Disruption of the cell wall polysaccharide network by sequential extraction may increase the degradability of extracts and residuals by enzymes, thus reflecting the influence of the structural network on the low cell wall degradability. We studied soybean meal, which is a major protein source for livestock and human feeding [world production is ∼101 million tonnes (*13*)]. We used a sequential extraction of cell walls proposed by Redgwell and Selvendran (*14*) in milder conditions, which prevent the chemical degradation of carbohydrates (*15*, *16*).

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MATERIALS AND METHODS

Plant Material. Cyclohexane solvent extracted untoasted soybean meal (53.9% CP and 0.7% crude fat) was obtained from a local market and physically separated on hulls and cotyledons by gradient sieving.

Sequential Fractionation of Untoasted SBM. Defatted solvent extracted cotyledons (1000 g) and hulls (100 g) were ground to pass through a 0.59-mm sieve. The meals were respectively suspended in 3.75 L and 375 mL of distilled water containing 50 mg of chloramphenicol/L for 2 h at room temperature and centrifuged at 11000*g* for 30 min. The recovered water unextractable (WU) fractions, from cotyledons (WUc) and hulls (WUh), were resuspended, and the procedure was repeated four times. The combined supernatants were freeze-dried (soluble water from hulls, WSh, and from cotyledons, WSc).

The residue was suspended in 3 L of solution containing 1.5% sodium dodecyl sulfate and 10 mM 1,4-dithiothreitol and stirred for 3 h at room temperature to extract the protein from the WUc fraction. After centrifugation (11000*g*; 30 min), this extraction was repeated three times. The final pellet was washed twice in distilled water.

Subsequently, starch was removed as follows: the pellet was suspended in 1 L of distilled water (pH 5.0) at 85 °C for 1 h and centrifuged at 11000*g* for 30 min. Following Huisman et al. (*17*) cell wall extraction procedure, the residue was suspended in 1 L of buffer solution (pH 6.5) containing 10 mM maleic acid, 10 mM NaCl, 1 mM $CaCl_2$, and 50 mg of chloramphenicol. Porcine pancreatic α-amylase (2 mg; EC 3.2.1.1, heat stable) was added, and the mixture was incubated at 30 °C for 19 h. After centrifugation (11000*g*; 30 min), the residue was washed in 1 L of hot water (65 °C) and centrifuged again. α-Amylase digestion and hot water washing were repeated. The combined supernatants, containing a negligible amount of nonstarch polysacharides (NSP) (*17*), were discarded, and the remaining unextractable residue was freeze-dried (WUS).

Soybean WUS was sequentially extracted with stirring following the method of Huisman et al. (*17*), with 0.05 M 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) and 0.05 M ammonium oxalate in 0.05 M NaAc buffer, pH 5.2 (8 times 600 mL) at 70 °C for 1 h (chelating agent soluble solids, ChSS) and washed in distilled water (twice 600 mL), and these extracts were added to the ChSS fraction, extracted with 0.05 M NaOH (3 times 600 mL) at 2 °C for 1 h (dilute alkali soluble solids, DASS), and extracted with 1 M KOH + 20 mM $NaBH_4$ (5 times 600 mL) at room temperature for 2 h (1 M alkali soluble solids, 1MASS) and with 4 M KOH + 20 mM $NaBH_4$ (3 times 600 mL) at room temperature for 2 h (4M alkali soluble solids, 4MASS). After each extraction, the solubilized fractions were separated from the residue [pellet 1 after ChSS extraction, pellet 2 after DASS extraction, pellet 3 after 1MASS extraction, and finally residual (RES) after 4MASS extraction] by centrifugation at 19000*g* for 30 min. All dialyzable fractions and pellets were acidified to pH 5.2 by glacial acetic acid, dialyzed (Medicell International Ltd., membrane < 12000 Da), and freeze-dried.

Enzymatic Degradation of Soybean Polysaccharides. A small amount of substrate was weighed (30 mg) in 2 mL Kimax tubes with a screw cap, containing 0.05 M sodium acetate buffer (pH 5.0) and 0.05 mg/mL chloramphenicol, and incubated with specific enzymes at 40 °C for 12 h in a horizontal shaking water bath. The substrates obtained from the sequential fractionation (WUc, WUS, ChSS, DASS, 1MASS, and 4MASS) and the intermediary pellets (pellets 1, 2, and 3 and RES) were used for incubations. The enzymes tested were from Quest International Co.: biocellulase A concentrate (8750 cellulase units/g), biopectinase NKP 120P (endopolygalacturonase 830 units/g; containing also cellulase, 1400 units/g; xylanase, 13270 units/g; and β-glucanase 9650 units/g), and glucanase−xylanase (β-glucanase, 300000 units/g; and xylanase, 200000 units/g). Enzyme addition was standardized to 1% DM^{-1}. At the end of each incubation, enzymes were inactivated by boiling (100 °C for 10 min) and centrifuged (4000*g*; 10 min), and the supernatant was collected and frozen for subsequent free monosaccharide analysis.

Analytical Methods. Prior to the analysis of soybean fractions, samples were lyophilized to constant weight. Protein content was assessed according to the Kjeldahl method, using selenium as a catalyst, with a conversion factor of 6.25. Fat was determined by the Soxhlet method.

Solvent-extracted SBM, WSh, WSc, WUh, WUc, WUS, ChSS, DASS, 1MASS, 4MASS, and RES were analyzed for their neutral sugar composition following the method of Theander (*18*) using inositol as internal standard. After treatment with 72% w/w H_2SO_4 (1 h, 30 °C), water dillution (1:30 v/v), incubation in an autoclave (1 h, 125 °C), and immediate filtration (Poro 2), monosaccharides were reduced to alditols with KBH_4 and converted to their alditol acetates using 1-methylimidazole and acetic anhydride. Alditol acetates were separated on a GLC column (25 m × 0.25 mm × 0.25 μm; Hewlett-Packard 19091 C-131) using D-glucose, D-galactose, D-xylose, l-arabinose, D-mannose, fucose, and rhamnose as standards. Free neutral monosaccharides in supernatants released after enzymatic incubations were also analyzed by GLC after direct derivatization to alditol acetates. The acidic cell wall polysaccharides released during enzyme incubations and total uronic acid in the cell wall polysaccharides were quantified as uronic acids, following the method of Theander (*18*).

Table 1. Yield and Composition of Soybean Meal and Fractions (Percent Weight)[a]

	soybean hulls		dehulled SBM			
fraction[a]	WSh	WUh	SBM	WSc	WUc	WUS
yield (%)	16.7	83.3	100	56.0	44.0	13.9
crude protein	26.0	10.7	53.9	57.2	49.6	5.2
crude fat	0.3	0.4	0.7	0.4	1.0	1.5
ash	12.8	2.6	6.9	10.5	2.4	2.9
total monosaccharides	37.2	63.5	24.9	17.9	37.5	90.3
sugar composition						
rhamnose	2.8	3.3	2.6	2.2	5.5	3.6
fucose	0.2	0.5	1.3	0	2.1	2.1
arabinose	3.7	7.3	10.4	1.8	13.3	13.3
xylose	1.3	12.6	4.3	0	5.9	5.9
mannose	36.2	4.5	3.5	5.1	2.2	1.5
galactose	20.4	3.0	31.9	39.6	29.6	28.1
glucose	26.9	66.3	37.7	49.4	28.2	27.8
uronic acids	8.4	2.6	8.2	1.8	13.2	17.6

[a] Sugar composition expressed as percent mole.

RESULTS AND DISCUSSION

Yield and Composition. Table 1 shows the composition of soybean meal hulls and cotyledons, as obtained after extraction with distilled water. Soybean hull solubility was 16.7% DM^{-1}, yielding a substrate (WSh) containing 26.0% crude protein (CP) and 37.2% carbohydrates. Soluble carbohydrates were mainly composed of mannose (36%), glucose (27%), galactose (20%), and uronic acids (8%), whereas the water insoluble fraction (WUh) contained low amounts of mannose (4.5%) and galactose (3.0%). Soluble polysaccharides in hulls consisted of galactomannans (*19*) or β-mannans, a mannose backbone linked to galactose units in a 2:3 ratio. Meanwhile, the higher glucose content in WUh carbohydrates (66%) may point to a large amount of cellulose (mainly β-1,4 glucosidic linkages). Soybean hulls contain two additional polysaccharides, pectins (linked α-1,4 D-galactopyranosyl uronic acids, most of which are extractable with water), and two hemicelluloses, unbranched xylan "hemicellulose A" and arabinoglucurunoxylan "hemicellulose B" (*20*).

Cotyledon water solubility was 56% DM^{-1}, yielding a substrate (WSc), containing 57.2% CP and 17.9% carbohydrates. Thus, cotyledon CP was mainly composed of water soluble proteins (59.4%), as reported by Huisman et al. (*17*) for dehulled and defatted untoasted soybean meal, in which most of the material was water soluble (59% DM^{-1} and 67% CP^{-1}). The dehulled soybean meal contains 24.9% carbohydrates (DM^{-1}), determined as the sum of neutral sugars and uronic acids. A

Table 2. Yield and Composition of Fractions Extracted from WUS (Percent Weight DM^{-1}, Sugar Composition Expressed as Percent Mole)

WUS fraction	ChSS	DASS	1MASS	4MASS	RES	recovery % of WUS[a]
yield (%)	33.7	8.0	13.2	18.5	10.7	84.1
protein content (%)	15.7	0	0	0	0	
total monosaccharides	54.0	68.3	71.1	83.1	71.8	
rhamnose	4.1	5.2	4.5	4.9	2.2	98.9
fucose	2.1	2.6	2.1	2.6	0.3	84.0
arabinose	14.2	16.7	13.4	17.1	4.2	85.7
xylose	4.3	5.0	15.6	7.1	2.9	93.2
mannose	1.2	0.3	1.8	0.3	2.2	64.9
galactose	33.0	31.9	27.1	39.1	4.8	88.2
glucose	18.3	12.4	25.6	15.7	76.3	77.8
uronic acids	22.7	25.8	10.0	13.1	7.3	80.8

[a] Sum of the amount of a given sugar found in all extracts expressed as a percentage of the amount measured in the unfractionated WUS.

relatively large fraction (40%) of carbohydrates was also recovered in the WSc fraction. Water soluble carbohydrates were mainly composed of mannose (5.1%), galactose (39.6%), and glucose (49.4%), which reveals a large amount of extracted α-galactosides (*21*) and *β*-mannan polysaccharides, which probably result from incomplete removal of hulls from the cotyledons. In fact, results obtained from our laboratory showed that the sucrose and oligosaccharide contents in the soybean meal were 72 and 53 g/kg of DM, respectively, and stachyose (±40 g/kg of DM) and raffinose (±14 g/kg of DM) were the major α-galactosides. Although WSc extracts contained high amounts of sucrose (88 g/kg of DM) and oligosaccharides (68 g of raffinose + stachyose/kg of DM), water extraction did not remove them all. In a study on soybean meal α-galactosides, Irish et al. (*22*) removed with water only 18% of oligosaccharides extracted by ethanol and water sequential extraction.

Protein extraction with sodium dodecyl sulfate (SDS) allowed 96.7% removal of WUc proteins and 24% of carbohydrates. However, the monosaccharide composition of the deproteinized residues (WUS) was similar to that observed in WUc, which suggests nonselective carbohydrate extraction of SDS. Thus, carbohydrates in the WUS fraction were considered to be representative of the total cell wall carbohydrates. Sugar composition (WUS) mainly consisted of galactose (28.1%), glucose (27.8%), arabinose (13.3%), xylose (5.9%), and uronic acids (17.6%), which reflects the galactan chains and the three structurally major domains in the cell wall [cellulose−xyloglucan framework and pectic polysaccharides (*23*)]. Most of the uronic acids contained in the native dehulled SBM (90%) and WUc (96%) were recovered in the WUS fraction, in agreement with Huisman et al. (*17*). Thus, pectins from soybean meal are less soluble than those from other plants such as onions (*14*), apples (*24*), and olives (*25*).

Table 2 presents the yield of substrates extracted from WUS based on the weight of the extracted material. Sequential extractions solubilized 33.7% ChSS, 8% DASS, 13.2% 1MASS, and 18.5% 4MASS, accounting for an 84% DM^{-1} recovery.

The fractions extracted with chelating agents (ChSS) and diluted alkali (DASS) had the same composition: 32−33% galactose, 12−18% glucose, 14−16% arabinose, 4−5% xylose, and 22−26% uronic acids. CDTA and ammonium oxalate solubilize pectic polysaccharides by abstracting Ca^{2+} from the cell walls and disrupting ionic cross-links. The galactose/arabinose (2.1:1−2.3:1) and uronic acids/rhamnose (5.5:1−5.0:1) ratios indicate the presence of arabinogalactan (*20*) and rhamnogalacturonan polysaccharides (*26*).

Extractions with stronger alkali (1 and 4 M KOH) further solubilize the amount of pectins and hemicelluloses. The 1MASS and 4MASS fractions contained, respectively, mainly galactose (27 and 39%), glucose (16 and 26%), arabinose (13 and 17%), xylose (7 and 16%), and uronic acids (10 and 13%). From the uronic acids content, it is suggested that certain pectins remained insoluble after chelating agents extraction, probably ester cross-linked within the wall matrix. However, the uronic acids/rhamnose ratio (2.2:1−2.6:1) was sligthly lower than those of ChSS and DASS. This may reflect the presence of higher amounts of rhamnogalacturonan containing many arabinosyl and galactosyl side chains, often referred to as the "hairy region" (*6*).

The final residue (RES) contained 76% glucose, probably in the cellulose framework, and low amounts of uronic acids (7.3%) and other neutral monosaccharides. The remaining rhamnose (2.2%) and uronic acids in the last residue suggest the presence of rhamnogalacturonan polymers tightly bound to the cellulose network.

Enzymatic Degradation of Cell Wall Polysaccharides. Previous assays showed no contaminant sugars were contained in the enzymatic preparations. This result allows us to estimate the carbohydrate hydrolysis from substrate incubations measuring saccharides released in the supernatant. Then, **Table 3** shows the amount of neutral sugars and uronic acids released (percent weight dry $matter^{-1}$) over a blank from the insoluble soybean meal (WUc) and cell wall polysaccharide (WUS) fractions, after incubation with three enzyme preparations containing an identified activity of pectinase, cellulase, and xylanase. Enzyme incubations of WUc and WUS fractions released an amount of monosaccharides lower than 7%. However, a slight increase was observed after the SDS protein extraction (WUS), especially of uronic acids with pectinase (11.4 vs 20.4%) and xylanase (6.3 vs 11.4%) incubations. It appears that a certain amount of pectins, likely protected or interacting with the SBM protein, is more accessible to feed enzymes after SDS extraction.

Marsman et al. (*27*) have also studied the in vitro accessibility of the water unextractable (WU) fraction from untreated, toasted, and extruded soybean meals for various enzyme activities. Proteolytic and cell wall degrading enzymes promoted the release of ∼15% of monosaccharides but nearly 50% of neutral sugars or polysaccharide fragments. After incubation with polysaccharide-degrading enzymes, 85% of the released polysaccharide fragments were composed of galactose, arabinose, and uronic acids, whereas limited amounts of glucose, mannose, and xylose were found. After an initial reaction over accessible material with the intermediate formation of soluble polymeric material (*28*), the enzymatic attack on insoluble substrates may occur by the direct and slow release of mono- or dimeric products.

Tables 4 and **5** present the free monosaccharides (percent bound $basis^{-1}$) released by the incubation of WUS, solubilized fractions (ChSS, DASS, 1MASS, and 4MASS) and their residual pellets (pellets 1, 2, and 3 and RES) with pectinase and cellulase, respectively. To evaluate the influence of structural disruption on enzyme efficiency, pectinase and cellulase incubations were focused on the fractions mostly containing pectins or cellulose: fractions WUS, ChSS, pellet 1, DASS, pellet 2, and 1MASS for pectinase incubations (**Table 3**) and fractions WUS, 1MASS, pellet 3, 4MASS, and RES for cellulase incubations (**Table 4**).

As mentioned above, pectinase incubation of WUS degraded the cell walls to a minor extent, because only some small neutral degradation products (3−7%) and uronic acid residues (∼20%)

Table 3. Amount of Solubilized Neutral Sugar (NS) and Uronic Acids (UA) (Percent Bound Basis) after 12 h of Incubation of Insoluble SBM Fractions with Pectinase, Cellulase, and Xylanase

eoybean fractions:		WUc			WUS	
enzymes:	pectinase	cellulase	xylanase	pectinase	cellulase	xylanase
∑ NS + UA[a]	3.8	5.4	5.0	6.9	6.8	6.3
specific sugars[b]						
rhamnose	6.8	nd[c]	nd	nd	nd	1.3
fucose	nd	nd	nd	nd	nd	nd
arabinose	3.7	2.6	18.6	5.8	2.6	19.6
xylose	1.5	1.5	6.3	4.0	2.3	9.6
mannose	nd	9.1	6.5	3.5	9.3	nd
galactose	1.3	1.0	0.8	4.4	1.1	nd
glucose	5.1	7.2	4.0	7.0	9.6	5.8
uronic acids	11.4	23.7	6.3	20.4	23.5	11.4

[a,b] Percentages calculated for the total monosacharides (∑ NS + UA) or a given monosacharide released in relationship to the total amount of that particular sugar in the incubated substrate. [d] nd, not detected.

Table 4. Amount of Solubilized Neutral Sugar (NS) and Uronic Acids (UA) (Percent Bound Basis) after 12 h of Incubation of WUS, ChSS, DASS, 1MASS, Pellet 1, and Pellet 2 with Pectinase

	WUS–SBM fractions					
	WUS	ChSS	pellet 1	DASS	pellet 2	1MASS
yield (% of WUS)[a]	100	33.7	76.3	8.0	58.3	13.2
∑ NS + UA[b]	6.9	15.7	10.8	21.9	8.7	19.0
specific sugars[b]						
rhamnose	nd[c]	nd	6.2	nd	nd	5.3
fucose	nd	nd	nd	nd	nd	nd
arabinose	5.8	16.4	19.1	21.5	13.6	10.4
xylose	4.0	nd	3.4	nd	3.6	6.6
mannose	3.5	4.4	nd	nd	nd	10.6
galactose	4.4	7.4	4.9	5.5	4.8	4.9
glucose	7.0	18.3	5.0	12.6	4.3	13.6
uronic acids	20.4	35.7	42.1	48.3	36.6	37.1

[a] Yields of pellet 1 and pellet 2 were determined as WUS − ChSS and WUS − (ChSS + DASS), respectively. [b] Percentages calculated for the total monosacharides (∑ NS + UA) or a given monosacharide released in relationship to the total amount of that particular sugar in the incubated substrate. [c] nd, not detected.

Table 5. Amount of Solubilized Neutral Sugar (NS) and Uronic Acids (UA) (Percent Bound Basis) after 12 h of Incubation of WUS, 1MASS, 4MASS, Pellet 3, and RES with Cellulase

	WUS–SBM fractions				
	WUS	1MASS	pellet 3	4MASS	RES
yield (% of WUS)[a]	100	13.2	45.1	18.5	10.7
∑ NS + UA[b]	6.8	15.4	5.7	10.6	19.7
specific sugars[b]					
rhamnose	nd[c]	2.5	nd	nnd	5.8
fucose	nd	nd	nd	nd	nd
arabinose	2.6	4.4	2.6	6.0	7.8
xylose	2.3	5.8	2.4	3.9	10.3
mannose	9.3	26.4	nd	nd	34.0
galactose	1.1	1.2	0.5	2.8	3.8
glucose	9.6	18.1	10.7	21.8	19.4
uronic acids	23.5	21.9	10.8	30.1	7.7

[a] Yield of pellet 3 was determined as WUS − (ChSS + DASS + 1MASS). [b] Percentages calculated for the total monosacharides (∑ NS + UA) or a given monosacharide released in relationship to the total amount of that particular sugar in the incubated substrate. [c] nd, not detected.

were released. Disruption of the cell wall network by sequential extraction increased the degradation of pectin-rich fractions (ChSS, DASS, and 1MASS). The release of uronic acids increased (20 vs 35−48%), as did that of arabinose (5.8 vs 16−21%), glucose (7 vs 12−18%), and, to lesser extent, galactose (4.4 vs 7.4%). This suggests that pectinase contained side activities, likely of endoarabinase, glucosidase, and galactosidase.

Pectin extraction increased the hydrolysis of the residual network (WUS vs pellets 1 and 2), especially of uronic acids (20 vs 42 and 36%) and arabinose (5.8 vs 19.1 and 13.6%, respectively). Pectic polysaccharide extraction (ChSS) enhanced the accessibility of the pectin and the arabinan chains remaining in the wall matrix. On the other hand, glucose was poorly released, which reflects that cellulose structures were still too complex or dense to be penetrated by the applied enzymes.

To evaluate the influence of cellulose complexity on the enzyme hydrolysis, we studied the degradation with cellulase of WUS and substrates obtained after further fractionation with a gradient of alkaly agents (hemicellulose/cellulose) of pellet 2 (**Table 5**). The fractions extracted (**Table 2**; 1MASS and 4MASS), mostly containing galactan, glucan, and arabinan chains, were more degraded by cellulase than WUS (9.2% glucose from WUS vs 18.1 or 21.8% from 1MASS and 4MASS, respectively). Cellulase degradation of cell wall polysaccharide residues (pellet 3 and RES) remained very low (10%), even after protein, pectin, and partial hemicellulose extraction (1MASS). Only after 4MASS extraction of xyloglucan components did cellulose degradation increase (10.7 vs 19.4% of glucose from pellet 3 and RES, respectively).

Our results show that enzymes hardly degraded the carbohydrates contained in intact soybean cell wall, as reported elsewhere (*27*, *29*, *30*). Although the crude enzymes used in the present experiment still contained nonidentified side activities, neither pure cloned enzymes (*29*) nor commercial mixtures (*27*) effectively hydrolyze cell wall carbohydrates to their component monosaccharides. However, the analysis of free monomers probably accounted for an incomplete index of cell wall degradation and chain size reduction. Huisman et al. (*29*) demonstrated that endoenzymes such as endogalactanase significantly reduce the volume of polymers, whereas exoenzymes release monomeric sugar residues from the polysaccharide without modifying hydrodynamic volume of heterogeneous polysaccharides. Incubation in vitro of WU−SBM with several commercial enzyme preparations (*27*) resulted in the solubilization of a large amount (up to 67%) of neutral sugars (fragments of neutral cell wall polysaccharides) but only 12−22% of monomers.

We used free monosaccharides as an index of carbohydrate hydrolysis to obtain information about the ability of the enzyme to reach hydrolysis sites. Our results indicate that sequential fractionation significantly increased enzyme degradation. However, the fractions clearly differed; pectins and arabinan chains were the most accessible carbohydrates, whereas xylans and

cellulose were practically inaccessible. We failed to obtain clear results on galactose accessibility, but Huisman et al. (*29*) revealed that combinations of endo- and exogalactanase, exoarabinase, and arabinofuranosidase promoted the release of high amounts of arabinose and galactose residues and a large number of oligosaccharides.

From a nutritional point of view, the activity of these enzymes in vivo or simulating the animal conditions in vitro (*30*) should be evaluated. In particular, the effective hydrolysis of carbohydrates in vivo depends on environmental digestive conditions and times of retention in the foregut. In monogastric animals in general, especially poultry, the times of retention are considerably short for an effective NSP hydrolysis (*4*, *31*). However, whether a potential release of cell wall monosaccharides within the small intestine causes an energetical advantage is unclear. Marsman et al. (*9*), Hughes et al. (*32*), and Kocher et al. (*33*) confirmed significant changes in the ileum digestibility of NSP in poultry but failed to observe significant increases in the animal performances. Although a complete hydrolysis of NSP to monosaccharides may expose monosaccharides to absorption in the gastrointestinal (GI) tract, selective absorption of monosaccharides in the GI tract of animals (*34*) may limit their utilization (35).

Galactose and glucose are efficiently absorbed, but mannose, arabinose, and xylose are absorbed at low rates (at only 20% of glucose). Nevertheless, nutritional effects other than the release of entrapped nutrients should be evaluated. In particular, oligosaccharides rather than monosaccharides are generated when polysaccharide-degrading enzymes are used as feed additives and may alter the microflora population of the digestive tract.

In conclusion, the present results show the difficulty of hydrolyzing intact cell wall carbohydrates by carbohydrase or protease enzymes. Nevertheless, in the search for ways to improve the utilization of indigestible carbohydrates in vegetable proteins, further research should focus on the protein hydrolysis and generation of soluble oligomers in vivo and in vitro.

ABBREVIATIONS USED

ChSS, chelating agent soluble solids; CP, crude protein; DASS, dilute alkali soluble solids; 1MASS, 1 M alkali soluble solids; 4MASS, 4 M alkali soluble solids; RES, residual; SBM, soybean meal; WSc, water soluble from cotyledons; WSh, water soluble from hulls; WUc, water unextractable from cotyledons; WUh, water unextractable from hulls; WUS, water unextractable solids.

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