In Vitro Accessibility of Untreated, Toasted, and Extruded Soybean Meals for Proteases and Carbohydrases

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The *in vitro* accessibility of the water unextractable solids (WUS) from untreated, toasted, and extruded soybean meals toward different enzyme activities was studied. WUS was incubated with seven commercial enzyme preparations. Two enzyme preparations were selected for further research. Upon addition of Neutrase, the extruded sample yielded considerably more solubilized protein compared with the toasted and untreated soybean meals. Energex solubilized high amounts of neutral sugars after heat treatments compared with the untreated meal. Cell wall polysaccharides solubilized by the enzymes were released as small oligosaccharides and monosaccharides. SDS−PAGE analysis showed that after enzyme addition to the extruded sample, proteins were more rapidly and completely degraded compared with enzyme addition to the toasted and untreated soybean meals. Neutrase degraded both β-conglycinin and glycinin. Energex could only, partly, degrade β-conglycinin. The basic polypeptide from glycinin showed the highest resistance against proteolytic activity.

Keywords: *Soybean meal; toasting; extrusion; enzymes; protein; cell wall components*

INTRODUCTION

Due to a good nutritional value and abundant availability as byproduct of the oilseed industry, soybean meal has received considerable attention for the replacement of other crops in feed. However, the nutritional value of soybean meal is limited by the presence of antinutritional factors (ANFs), e.g. trypsin inhibitors and lectins, which negatively affect protein digestion (Liener, 1994). Also, the compact structure of the most important proteins of soybean meal, β-conglycinin and glycinin, may hinder hydrolytic enzymes in the intestinal tract (Nielsen et al., 1988). The presence of a high content of cell wall components may also limit the nutritional value of legumes (Irish and Balnave, 1993; Melito and Tovar, 1995).

To improve the nutritional value of soybean meal, heat treatments such as toasting and extrusion are frequently used. Heat-labile ANFs, e.g. trypsin inhibitors, are effectively inactivated (Van der Poel et al., 1993), and proteins will denature to a certain extent, which makes them more susceptible to enzymic degradation. During extrusion, shear forces may play an important role in enhancing the *in vitro* protein digestibility (Marsman et al., 1993). However, too much heat (Araba and Dale, 1990) and/or shear force (Marsman et al., 1995a) may result in a decreased nutritional value of soybean meal.

Another approach for a further valorization of raw and processed materials is the application of feed enzymes. The main objectives are supplementation of the endogenous enzymes, removal of ANFs, and to render certain nutrients more readily available for absorption (Chesson, 1993). Nonstarch polysaccharides (NSP) are known to exhibit an antinutritional effect, partly because monogastric animals lack the appropriate enzymes to hydrolyze them (Walsh et al., 1993). Also, the metabolic utilization of the sugars from NSP is limited in monogastric animals. Legumes contain numerous NSPs, and they are more complex in structure than NSP from cereals. This makes it difficult to target the legume polysaccharides for feed enzyme supplementation (Annison and Choct, 1993). The complexity of the polysaccharides and proteins in soybean meal makes it necessary to use multienzyme preparations for solubilization rather than addition of a single enzyme (Lyons and Walsh, 1993). In most studies, the main attention has been focused on *in vivo* effects as a result enzyme addition (Chesson, 1993; Wenk and Boessinger, 1993).

In this research, untreated, toasted, and extruded soybean meals were studied for their *in vitro* accessibility toward different enzyme activities. First, seven commercial enzyme preparations (proteases and carbohydrases) were studied for their ability to solubilize proteins and cell wall polysaccharides from untreated and toasted soybean meals. The differences between toasting and extrusion with respect to the *in vitro* accessibility toward a protease and a carbohydrase were studied in more detail.

MATERIALS AND METHODS

Materials. Commercial solvent-extracted, toasted (85 °C, 20 min) soybean meal (TSBM) was used. From the same batch of soybean meal, after oil extraction, a part was not toasted but air-dried at room temperature, yielding untoasted soybean meal (USBM). The protein content of both meals was 51% (N × 6.25), and both were supplied by Cargill (Amsterdam, The Netherlands). The nitrogen solubility index (NSI) in potassium hydroxide was 98 and 73% for USBM and TSBM, respectively. Esperase, Neutrase, Bio-Feed Pro, Bio-Feed Plus, SP-249, and Energex were obtained from Novo Nordisk (Bagsvaerd, Denmark), and Driselase was from Sigma (St. Louis, MO).

Extrusion Experiments. Extrusion trials were performed with a Battenfeld single-screw extruder. USBM with an initial

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moisture content of 25% was extruded using a screw of a constant pitch, a compression ratio of 1.15, and a die diameter of 7 mm. At the end of the screw a torpedo element with four rows of flights was assembled. Temperature control was performed with one cooler, four heaters, and eight thermocouples, which were connected in the different sections of the barrel. The final product temperature, manually measured at the die using a thermocouple, was kept at 120 °C. The screw speed was set at 100 rpm. Moisturization was performed with a Sunther-Papenmeier mixer a day before extrusion. The premoisturized meal was stored overnight at 4 °C and brought to room temperature prior to extrusion. The extruded soybean meal (ExUSBM) was dried at 45 °C and ground to pass a 0.2 mm screen.

Preparation of Water Unextractable Solids (WUS). To avoid the disturbance by mono- and oligosaccharides, e.g. sucrose, raffinose, and stachyose, which are present in soybean meal during sugar analysis, USBM, TSBM, and ExUSBM were extracted with water to yield the WUS. Ten grams of soybean meal was extracted with 200 mL of water at room temperature for 2 h under continuous stirring. After centrifugation (20 min, 10000*g*), a residue and a supernatant were obtained. The residue was resuspended in 100 mL of water and centrifuged again. This procedure was repeated three times. The residues were resuspended in a small amount of water, dialyzed, and freeze-dried.

Enzyme Incubations. In a first experiment, several commercial enzyme preparations were screened for their ability to solubilize proteins and to release fragments of neutral and acidic cell wall polysaccharides from USBM and TSBM. Esperase, Neutrase, and Bio-Feed Pro (proteolytic enzymes) and Bio-Feed Plus, SP-249, Energex, and Driselase (cell wall degrading enzymes) were added to 10% soybean meal WUS suspensions in a 0.05 M acetate buffer (pH 5.0). With Esperase incubations were also performed in a carbonate buffer (pH 9.0), the optimum pH of this enzyme preparation. The enzyme/substrate ratios were 0.025 and 0.25% (protein/protein basis) for all the enzymes. Incubations were performed under continuous stirring at 37 °C for 0 (blanks) and 24 h. After incubation and centrifugation (10 min, 3000*g*), the supernatant was directly used for determination of the amount of soluble proteins and neutral and acidic cell wall polysaccharides fragments analyzed as neutral sugars and uronic acids, respectively.

Second, Neutrase and Energex were used to study the accessibility of USBM, TSBM, and ExUSBM toward these enzymes in more detail. Also, combinations of both enzymes were used. Three hundred milligrams of WUS was weighed into 10 mL Kimax tubes with screw caps. Three milliliters of 0.05 M sodium acetate buffer (pH 5.0) containing the enzymes was added. The enzyme addition was standardized to 750 μg of protein for the separate incubations with Neutrase and Energex and 2 × 375 μg of protein for the combined incubation with these enzymes. The tubes were rotated at 37 °C for 0, 15, 60, and 240 min and 24 h, then quickly cooled to 0 °C, and centrifuged (10 min, 3000*g*) at 4 °C. For the nitrogen determination 0.8 mL of the supernatant was pipetted in Kjeldahl tubes. For the molecular weight distribution of the carbohydrate fraction and for anion exchange chromatography analysis, 0.8 mL of supernatant was transferred to Eppendorf cups, which already contained 0.4 mL of 20% trichloroacetic acid (TCA). After precipitation of the proteins (20 min at room temperature) and centrifugation (10 min 3000*g*), 1 mL of supernatant was transferred from these cups to HPLC vials and subjected to analysis. Half of a milliliter of the supernatant was transferred to Eppendorf cups, heated for 10 min at 100 °C, and centrifuged (10 min, 3000*g*), after which time the supernatant was used for determination of the neutral sugar and uronic acid content. The residue was washed four times with excess water (0 °C), freeze-dried, weighed, and ground. The residue was analyzed for sugar composition and subjected to SDS−PAGE analysis to study protein breakdown.

Molecular Weight Distribution. The molecular weight distribution of solubilized cell wall polysaccharide fragments was studied using high-performance size exclusion chromatography (HPSEC), which was performed on a SP8800 HPLC (Spectra Physics, San Jose, CA) equipped with three Bio-Gel TSK columns (each 300 × 7.5 mm) in series (40XL, 30XL, and 20XL; Bio-Rad Labs, Hercules, CA) in combination with a TSK XL guard column (40 × 6 mm) and eluted at 30 °C with 0.4 M acetic acid/sodium acetate (pH 3.0) at a flow rate of 0.8 mL/min. The eluate was monitored using a Shodex SE-61 refractive index detector (Showa Denko K.K., Tokyo, Japan). The system was calibrated with dextran standards (10−500 kDa).

High-Performance Anion-Exchange Chromatography (HPAEC). Chromatographic analysis of the mono- and oligomeric fragments in the samples was performed using a Dionex Bio-LC system (Sunnyvale, CA) equipped with a CarboPac PA-1 column (250 × 4 mm) in combination with a CarboPac PA quard column (25 × 3 mm). Samples were injected using a Spectra Physics SP 8800 autosampler, and chromatograms were recorded with a Spectra Physics Winner system. The effluent was monitored using a pulsed electrochemical detector with a gold working electrode and an Ag/AgCl reference electrode (Schols et al., 1994). To elute monosaccharides and oligosaccharides, the following 0.1 M NaOH gradient was used: 0−26 min, 15 mM; 26−30 min, 15−100 mM; 30−59 min, 100−75 mM; 59−59.1 min, 75−0 mM; 59.1−64 min, 0 mM; 64−64.1 min, 100 mM; 64.1−70 min, 100 mM; 70−80 min, 100−15 mM. The simultaneous gradient of 1 M NaOAc in 0.1 M NaOH was as follows: 0−30 min, 0 mM; 30−59 min, 0−250 mM; 59−59.1 min, 250−1000 mM; 59.1−64 min, 1000 mM; 64−64.1 min, 1000−0 mM; 64.1−80 min, 0 mM. Samples (20 μL) were injected at 80 min.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed using the Protean II electrophoresis system from Bio-Rad. To reduce the protein, in order to obtain subunits, disulfide bonds were cleaved by β-mercaptoethanol. In general, the method of Laemmli (1970) was followed with minor modifications. From the residue an amount of sample corresponding to 2 mg of protein was dissolved in 1 mL of buffer solution (62.5 mM Tris-HCl, pH 6.8, 1.25% SDS, 10% glycerol, 0.00125% bromophenol blue, and 2.5% β-mercaptoethanol). Reduction and solubilization of the proteins were obtained after 3 h of mixing head over tail in Eppendorf cups at 37 °C. Every 90 min the samples were treated in a ultrasonification bath at 60 °C for 15 min. Runs were performed in homogeneous slab gels (T = 12.5%, C = 2.6%). Gel slabs were fixed and stained in a solution of 40% methanol, 10% acetic acid, and 50% water containing 0.1% Coomassie Brilliant Blue R-250. The staining solution was filtered just before use. The excess of Coomassie Brilliant Blue was removed by diffusion in a destaining solution containing 40% methanol, 10% acetic acid, and 50% water.

Thermal Behavior Studies. Differential scanning calorimetry (DSC) was used to study the thermal behavior of the main proteins in USBM, TSBM, and ExUSBM as well as the WUS obtained from these materials. Samples of 10% dispersions in water were sealed in high-pressure aluminum pans. A pan filled with distilled water was used as reference. The samples were analyzed in a Setaram Model Micro Disc III calorimeter. Heating was performed from 20 to 40 °C at a rate of 3 °C/min, which was followed by a 30 min stabilization at 40 °C and then a second heating from 40 to 120 °C at a rate of 0.5 °C/min.

Sugar Composition Analysis. NSP were analyzed for neutral sugar composition according to the method of Englyst and Cummings (1984) using inositol as internal standard. After pretreatment with 12 M H_2SO_4 for 1 h at 30 °C, followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C, the monosaccharides were reduced to alditols with $NaBH_4$ and converted into their corresponding alditol acetates using 1-methylimidazole and acetic anhydride. The alditol acetates were separated on a glass column (3 m × 2 mm i.d.), packed with Chrom WAW 80−100 mesh coated with 3% OV275 in a Carlo Erba Fractovap 2300 GC operated at 200 °C and equipped with a FID detector set at 270 °C.

Protein Determination. Total nitrogen in the supernatants after enzyme incubation and in the starting materials was determined by a semiautomated micro-Kjeldahl method

Table 1. Yield and Composition of WUS (Percent Weight) Isolated from USBM, TSBM, and ExUSBM

material	USBM	TSBM	ExUSBM
yield[a]	40.0	67.4	70.7
moisture	5.5	5.7	5.5
protein	44.4	63.6	69.0
nonstarch polysaccharides	43.0	22.8	18.5
rhamnose	1.3	0.6	0.5
fucose	0.6	0.3	0.2
arabinose	5.0	2.6	2.1
xylose	1.2	0.7	0.6
mannose	1.5	1.0	0.9
galactose	12.1	6.0	5.1
glucose	11.2	6.5	5.1
uronic acid	10.1	5.1	4.0
analyzed	92.9	92.1	93.0

[a] Expressed as weight percentage (as is basis) of USBM, TSBM, and ExUSBM.

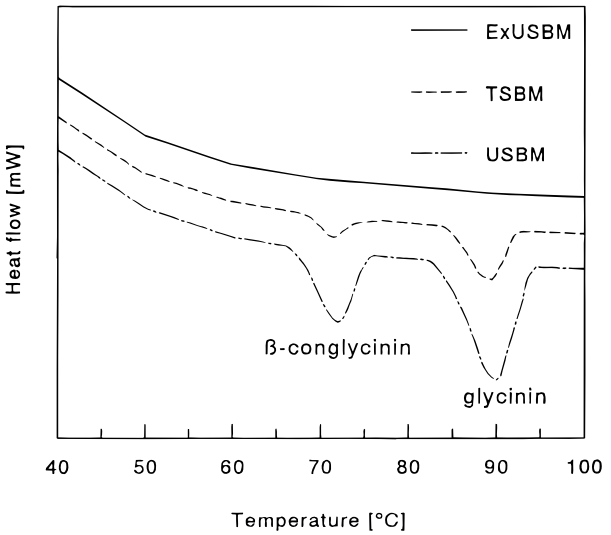

Figure 1. Thermal behavior, using DSC analysis, of β-conglycinin and glycinin in USBM, TSBM, and ExUSBM.

using a Cu/Ti catalyst. Protein content was estimated as total nitrogen × 6.25.

Neutral Sugars. Fragments of neutral cell wall polysaccharides released during enzyme incubations and total neutral sugars in the cell wall polysaccharides were analyzed, as neutral sugars, with the automated orcinol method (Tollier and Robin, 1979) using D-glucose and D-galactose (50:50 w/w) as standards.

Uronic Acids. Fragments of acidic cell wall polysaccharides released during enzyme incubations and total uronic acid content in the cell wall polysaccharides were analyzed, as uronic acids, with the automated *m*-hydroxydiphenyl assay according to the method of Thibault (1979). Sodium tetraborate (0.0125 M) was added to the 96% (w/w) H_2SO_4 to quantify glucoronic acid as well as galacturonic acid residues.

RESULTS AND DISCUSSION

Composition and Yield of WUS. Table 1 shows the yield and composition of the WUS obtained from USBM, TSBM, and ExUSBM. Total yields of WUS were higher after toasting and extrusion of soybean meal compared with the native meal. Moreover, protein content in WUS from the heat-treated samples was considerably higher compared with USBM. Previous studies have revealed that heat treatment of USBM sharply decreased nitrogen solubility in USBM (Marsman et al., 1993). Protein insolubility due to denaturation could also be seen from DSC analysis (Figure 1). The area under the peak is directly proportional to the enthalpic change, and its direction indicates whether the thermal event is endothermic or exothermic. Denaturation is an endothermic process. At high protein concentrations denaturation is mostly followed by aggregation, which is generally considered as an exothermic process (Biliaderis, 1983). Energies involved in aggregation are low (Donovan and Ross, 1973). If one neglects the energies involved in aggregation, a rough approach of the degree of denaturation can be made by calculating the surfaces of the peaks and with the assumption that proteins in USBM are native. Compared with the peaks in USBM, in TSBM about 90% of β-conglycinin and about 50% of glycicin were denatured during toasting, while after extrusion both proteins were completely denatured. A complete denaturation of the proteins in soy protein isolate after extrusion was also found by Kitabake and Doi (1992).

Table 2. Amounts of Solubilized Protein, Neutral Sugar (NS), and Uronic Acid (AUA) (Percent) after Incubation of WUS from USBM and TSBM with Different Commercial Enzyme Preparations

	USBM			TSBM		
	protein	NS	AUA	protein	NS	AUA
Esperase (pH 9.0)						
0.025%	46.6	0.4	<0.1	40.8	0.9	<0.1
0.25%	59.2	4.5	<0.1	59.7	5.6	<0.1
Esperase (pH 5.0)						
0.025%	16.7	2.1	<0.1	17.0	7.4	<0.1
0.25%	29.6	4.3	0.2	29.6	10.2	0.1
Neutrase						
0.025%	19.1	9.2	0.9	18.5	14.0	1.1
0.25%	32.4	16.6	1.1	30.7	23.3	2.0
Bio-Feed Pro						
0.025%	18.4	4.3	0.2	10.4	8.8	<0.1
0.25%	29.5	6.1	0.8	32.4	11.6	2.6
Bio-Feed Plus						
0.025%	3.1	2.5	0.1	2.6	17.2	2.3
0.25%	10.1	16.2	2.3	10.8	43.7	7.3
SP-249						
0.025%	9.3	11.5	8.6	4.7	12.6	8.7
0.25%	22.0	55.3	21.6	13.6	47.4	34.4
Energex						
0.025%	8.2	9.0	7.8	3.8	12.1	8.4
0.25%	20.0	50.6	30.3	8.3	48.9	32.9
Driselase						
0.025%	13.5	13.3	8.5	11.3	22.3	5.9
0.25%	29.3	52.4	21.6	24.6	49.8	22.1

The main sugars in the cell wall polysaccharides in WUS are galactose, glucose, and arabinose. Also, considerable amounts of uronic acids were found. Despite the lower NSP content in the heat-treated samples, the composition among the different soybean meal samples shows only minor differences. The relative mannose and glucose contents are higher in the WUS after heat treatment compared with USBM, while the uronic acid content has the tendency to decrease.

Effect of Different Enzymes on the Solubilization of Soybean Carbohydrates and Proteins. In Table 2 the proportions of solubilized proteins, neutral sugars, and uronic acids after incubation of WUS from USBM and TSBM with seven commercial enzyme preparations are given. The results were corrected for the blanks, which did not exceed 5% for any given component, except for the protein blank of WUS from USBM at pH 9.0, which was 13.4% (no further results shown). It can be concluded that Neutrase, Bio-Feed Pro, and especially Esperase were all three powerful proteolytic enzyme preparations, while Energex, SP-246, and Driselase were the most active cell wall degrading enzymes. To study the differences between toasting and extrusion for their *in vitro* accessibility

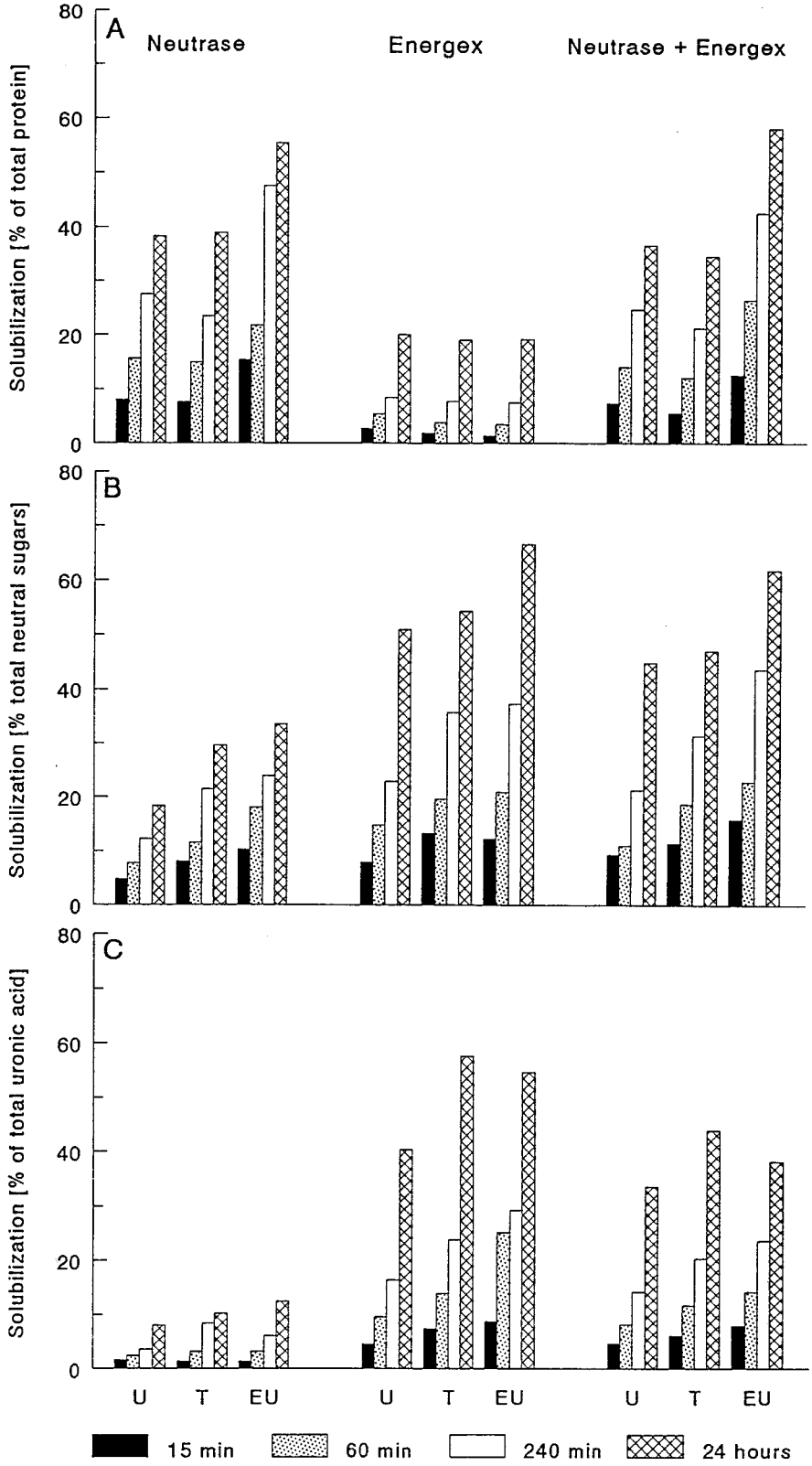

Figure 2. Proportion of solubilized (A) proteins, (B) neutral sugars (NS), and (C) uronic acids (AUA) (percent of total protein, neutral sugars, and uronic acids, respectively) after incubation of USBM (U), TSBM (T), and ExUSBM (EU) with Neutrase, Energex, and the enzyme combination at different times.

toward enzymic activity in more detail, Neutrase (as protease) was chosen, because the high pH optimum of Esperase limits its industrial application. From the cell wall degrading enzymes Energex was chosen, because it showed the most favorable ratio between a high ability in solubilizing neutral sugars and uronic acids and relatively low activity in solubilizing proteins compared with SP-246 and Driselase. In previous research, both enzyme preparations were also used for an *in vivo* experiment with broiler chickens (Marsman et al., 1995b).

Proportion of Solubilized Proteins. The amounts of protein released by Neutrase, Energex, and the combination of both enzymes are given in Figure 2A. The solubility was corrected for the soluble protein in the blank, which did not exceed 5%. It can be seen that

Neutrase, as expected, was very effective in solubilizing proteins. In USBM and TSBM about equal amounts of protein were solubilized, which was in line with the results from the previous experiment (Table 2). However, the amount of protein released by Neutrase sharply increased after extrusion. Energex was also able to solubilize up to 20% of the proteins, but limited effects were found among the different soybean meal samples. Work from others (Cone et al., 1994) also showed that typical cell wall degrading enzyme preparations, e.g. Viscozyme and Multifect, were able to solubilize 35−70% of the nitrogen in whole soybean meal after 25 h of incubation at 40 °C. In Energex, a considerable amount of protease activity, as determined with the azocaseine assay (results not shown), may explain why from all materials proteins were solubilized (Figure 2A). If the combination of both enzymes was applied, it was shown that about equal amounts of protein were solubilized compared with the separate Neutrase addition, despite the fact that Neutrase was added at only half the concentration compared to the separate incubation with Neutrase. This suggests a slight synergistic effect between Neutrase and Energex.

Process conditions can explain the differences in protein solubility obtained after extrusion and toasting. During extrusion, at intense shear forces and an excess of specific mechanical energy (SME), noncovalent interactions as well as covalent disulfide bonds between proteins are easily broken compared with toasting (Arêas, 1992). Upon cooling, the denatured proteins will aggregate rather than renature, because it is statistically unlikely that after extrusion the original bonds will be re-formed. After toasting, however, most disulfide bonds remain unaffected, which means that upon cooling the protein can return to its native conformation (Jaenicke, 1965). Therefore, the intensity of the thermal treatment may explain the increased proportion of solubilized proteins after enzyme incubation in ExUSBM compared with TSBM.

Proportion of Solubilized Neutral Sugars and Uronic Acids. The effects of incubation with Neutrase, Energex, and the combination of both enzymes on neutral sugar and uronic acid solubility are given in parts B and C of Figure 2, respectively. From Figure 2B it can be seen that Energex was able to solubilize large amounts of neutral sugars, up to 67% of all neutral sugars present from ExUSBM. Extrusion significantly increased the proportion of solubilized neutral sugars for all enzyme treatments compared with TSBM and USBM, while in the latter the lowest release of sugars was noticed. Treatment with the combination of both enzymes showed that the proportion of solubilized neutral sugars was lower compared with the separate Energex incubation. For the uronic acids it appeared that after incubation with Energex the proportions of solubilized uronic acids in both toasted and extruded soybean meal were increased compared with USBM (Figure 2C). In this case the solubility in the extruded samples after 24 h had the tendency to decrease again. Also, uronic acid solubility was lower after treatment with the combination of both enzymes compared with the separate incubation with Energex (Figure 2C).

Neutrase, a well-known proteolytic enzyme preparation, was also able to release considerable amounts of neutral cell wall polysaccharide fragments, measured as neutral sugars (Figure 2B). After extrusion, slightly more neutral sugars were analyzed compared with TSBM. Especially in the heat-treated samples, breakdown of protein aggregates may lead to a release in cell wall polysaccharide fragments. However, a nonstandardized amount of β-glucanase activity, present in Neutrase, may also explain the presence of soluble cell wall polysaccharide fragments. Only small amounts of acidic cell wall polysaccharide fragments, measured as uronic acids, were found after incubation with Neutrase (Figure 2C). Limited differences were found between toasting and extrusion.

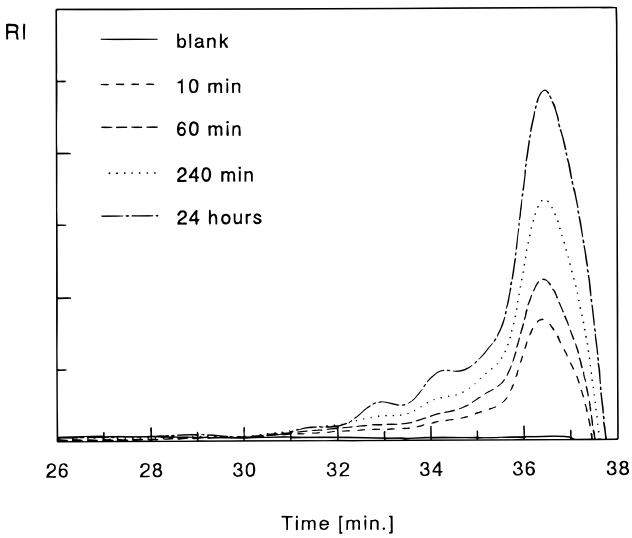

Figure 3. Molecular weight distribution of the soluble carbohydrate fraction of TSBM after incubation with the combination of Neutrase and Energex.

When the results from Figure 2 are combined, it can be concluded that, to solubilize considerable amounts of both protein and cell wall components, it is more effective to add a mixture of a protease and a cell wall degrading enzyme preparation in lower concentrations rather than to add each of the preparations separately in higher, e.g. double, concentrations.

Molecular Weight Distribution and Mono-/Oligosaccharide Ratio. HPSEC was used to study the molecular weight distribution of the solubilized cell wall polysaccharide fragments after enzyme incubation. It appeared that for all enzyme incubations the same HPSEC pattern were obtained. A typical example is given in Figure 3 for TSBM incubated with the combination of Neutrase and Energex. From the dextran standards used it can be calculated that the large peak at 36 min corresponds with a monosaccharide, while at 33 and 34 min small oligomers up to a degree of polymerization of 5 were eluted from the column. Therefore, it can be concluded that if cell wall components were solubilized, they also were degraded to small oligomers and monomers.

The degradation to small oligomers and monomers was also confirmed by HPAEC. Despite the fact that several oligomer standards were used, it was not possible to identify the various oligomers, because a high number of different oligomers resulted in much overlap between peaks. If it is assumed that monomers and oligomers have similar PAD response, the ratio between monomers and oligomers in the soluble fractions after the different enzyme incubations can be calculated. The results are given in Figure 4. After incubation with Energex for 15 min, 50−55% of the solubilized sugars were monomers, but after longer incubations relatively more oligomers, up to 55−70%, were released. After treatment with Neutrase, the relative amount of oligo-

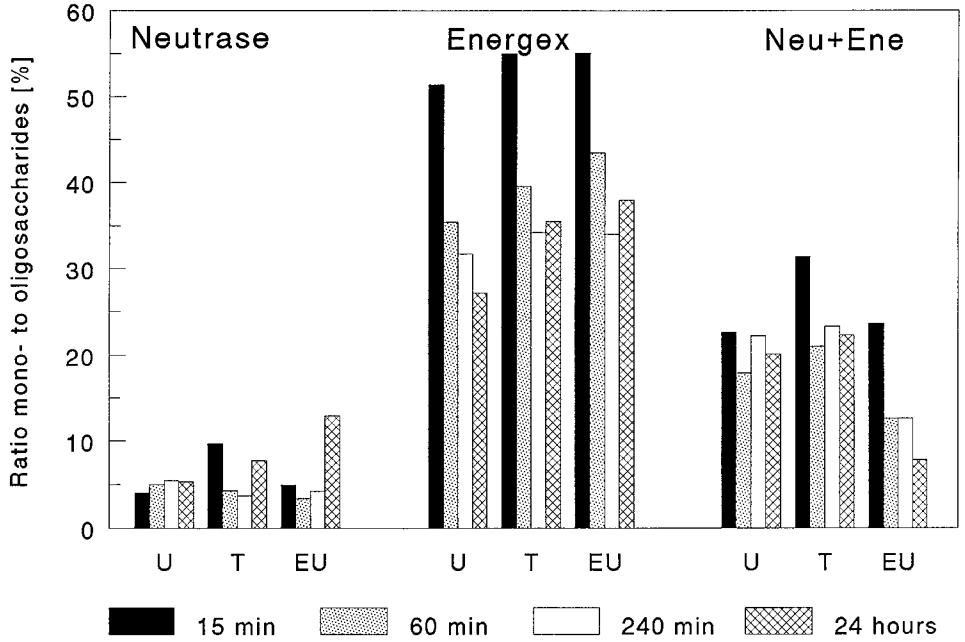

Figure 4. Monosaccharide/oligosaccharide ratio (percent of the soluble fraction) of USBM (U), TSBM (T), and ExUSBM (EU) after incubation with Neutrase, Energex, and the enzyme combination at different times.

mers represented >90% of the total solubilized cell wall constituents, indicating the lack of proper enzyme activities in Neutrase to degrade these oligomers to monomers as was seen for Energex. While the total amount of neutral sugars released by the combination of Neutrase and Energex was slightly lower than the amount released by Energex alone (Figure 2B), the ratio between monomers and oligomers was much lower for the enzyme combination (Figure 4). This indicates that enzyme activities present in Energex can further degrade fragments released by Neutrase. Only small differences in monomer/oligomer ratios between the native, toasted, and extruded soybean meals were found.

Breakdown of cell wall constituents to small oligomers and monomers was also found by Schols et al. (1993), who incubated a deproteinated untreated soybean meal WUS with several crude enzyme preparations. Although monomers were released by some enzyme preparations, the question is if these amounts would be sufficient to increase the metabolizable energy value (ME) of the feed (Chesson, 1993). If the amount of monomers obtained after HPAEC analysis were expressed on WUS basis, it appeared that after 24 h of incubation with Neutrase, Energex, and the enzyme combination, 2−5, 12−22, and 5−10%, respectively, of the total cell wall components in the WUS were degraded to monomers. An estimation of the ME increase after extrusion and incubation with Energex is made, using the following assumptions: 22% sugar release in the WUS, a yield of WUS of 70%, all sugars regarded as glucose and fully absorbed (17 kJ/g) and SBM for 30% incorporated in the diet. The increase in ME can be calculated as 790 kJ/kg. Normally, the ME of a chicken's diet is about 13 000 kJ/kg, which means that the ME may increase by 6%.

Sugar Composition. The molar sugar compositions of the soluble sugar fraction after 24 h of incubation with Neutrase, Energex, and the enzyme combination are given in Table 3. Despite the fact that after extrusion enzyme activity resulted in a higher amount of solubilized neutral sugars and uronic acids compared with TSBM (Figure 2B), limited differences in the molar sugar composition were found between toasting and extrusion (Table 3). After incubation with Energex, 80−85% of the soluble carbohydrate fraction consisted of galactose, arabinose, and uronic acids, while limited amounts of glucose, mannose, and xylose were found (Table 3). Neutrase was able to solubilize significant amounts of glucose, most likely due to the β-glucanase activity in Neutrase. Up to 25% of the soluble carbohydrates consisted of glucose and 60−65% of galactose, arabinose, and uronic acids, while also small amounts of xylose and mannose were found. The composition of the soluble fractions after incubation with both Neutrase and Energex showed a composition which was about the average of the separate incubations with these enzymes.

Table 3. Molar Sugar Compostion of the Soluble Sugar Fraction Obtained after 24 h of Incubation of WUS from USBM, TSBM, and ExUSBM with Neutrase (Neu), Energex (Ene), and Neu+Ene (Combination of Both Enzymes)

	USBM			TSBM			ExUSBM		
sugar	Neu	Ene	Neu+ Ene	Neu	Ene	Neu+ Ene	Neu	Ene	Neu+ Ene
rhamnose	4.7	5.5	4.4	4.9	5.5	4.8	3.6	5.3	3.7
fucose	0.7	0.9	0.9	1.0	1.2	0.8	1.0	1.1	0.6
arabinose	13.0	17.8	17.2	15.7	23.1	19.2	14.4	23.4	17.4
xylose	0.5	<0.1	0.5	<0.1	<0.1	<0.1	1.6	<0.1	0.6
mannose	4.7	1.4	3.8	4.6	<0.1	4.1	4.8	<0.1	3.8
galactose	26.2	38.5	33.7	31.0	49.6	38.3	32.9	49.5	35.6
glucose	25.0	10.1	17.0	21.5	2.2	13.4	21.7	<0.1	17.4
uronic acid	25.2	25.7	22.5	21.2	18.3	19.3	20.0	20.6	20.9

In chickens, glucose and galactose are known to be the best absorbed and utilized, while xylose, arabinose, and uronic acids can be absorbed but are utilized less efficiently compared with glucose (Longstaff et al., 1988; Cone et al., 1994). It can be questioned if the energy obtained after absorption and utilization of some monomeric sugars is high enough to justify the use of cell wall degrading enzymes. Energy obtained after fermentation of cell wall components by the hindgut flora should also be considered (Chesson, 1993). In this study, Neutrase seems to be the best choice because it is able to break down high amounts of protein, especially after extrusion, but also due to its ability to release substantial amounts of glucose components. Also, the

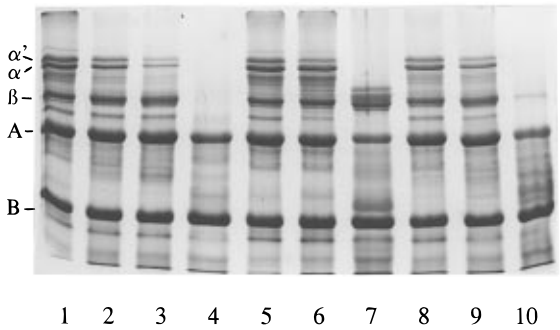

Figure 5. SDS−PAGE analysis of the residue of USBM after enzyme incubation: (lane 1) WUS; (lanes 2−4) incubation with Neutrase; (lanes 5−7) incubation with Energex; (lanes 8−10) incubation with the enzyme combination after 15 and 60 min and 24 h of incubation, respectively.

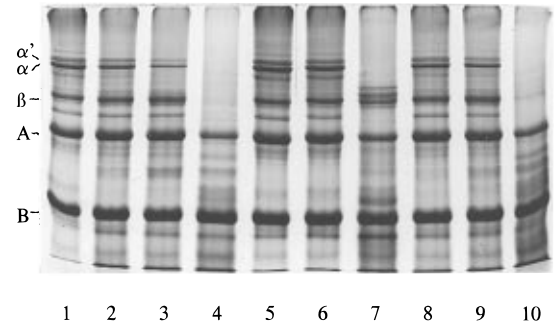

Figure 6. SDS−PAGE analysis of the residue of TSBM after enzyme incubation: (lane 1) WUS; (lanes 2−4) incubation with Neutrase; (lanes 5−7) incubation with Energex; (lanes 8−10) incubation with the enzyme combination after 15 and 60 min and 24 h of incubation, respectively.

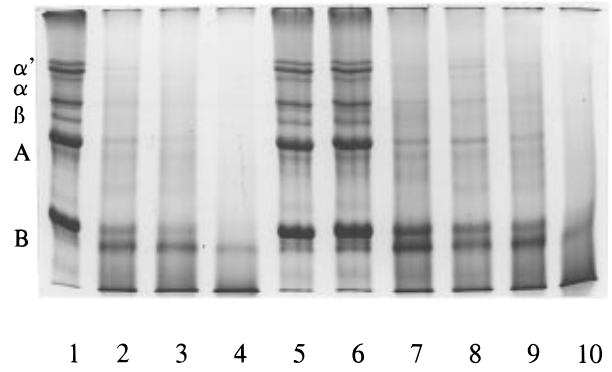

Figure 7. SDS−PAGE analysis of the residue of ExUSBM after enzyme incubation: (lane 1) WUS; (lanes 2−4) incubation with Neutrase; (lanes 5−7) incubation with Energex; (lanes 8−10) incubation with the enzyme combination after 15 and 60 min and 24 h of incubation, respectively.

combination of enzymes will result in a high release of both proteins and carbohydrates, but the feed quality of the released cell wall fragments may be lower, due to the different nutritional values of the released sugars: galactose, uronic acid, and arabinose, instead of glucose.

Protein Breakdown. To study the effect of the different enzymes on protein breakdown in the native and heat-treated soybean meals, SDS−PAGE was performed on the residues. The results are given in Figures 5−7 for USBM, TSBM, and ExUSBM, respectively. In these figures, lane 1 shows the starting material (WUS), followed by the results obtained after incubation with Neutrase (lanes 2−4), Energex (lanes 5−7), and the combination of both enzymes (lanes 8−10) after 15 and 60 min and 24 h of incubation, respectively. The main attention was focused on the two main storage proteins β-conglycinin and glycinin. β-Conglycinin shows three components, the α, α', and β subunits, while glycinin consists of the acidic (A) and basic (B) polypeptides (Romagnolo et al., 1990).

In the residue from USBM and TSBM (Figures 5 and 6) it can be seen that after 15 min (lane 2, 5, and 8) and 60 min (lane 3, 6, and 9) no substantial protein breakdown could be noticed for all enzyme incubations. After 24 h, Neutrase and the enzyme combination were able to degrade all of the subunits from β-conglycinin (lanes 4 and 10, respectively). Energex could also degrade the α and α' subunits but failed to degrade the β subunit (lane 7). New bands were noticed in the area of the β subunit (MW $\pm$ 60 kDa) and just above the B polypeptide (MW $\pm$ 25 kDa). The A and B polypeptides from glycinin could resist enzymic breakdown even after 24 h.

After extrusion, degradation of protein by the different enzymes was significantly increased when compared with toasting (Figure 7). After 15 min of incubation with Neutrase (lane 2) or the combination of both enzymes (lane 8), the three subunits from β-conglycinin and the A polypeptide from glycinin were fully degraded. Only small residual amounts of the B polypeptide could be noticed, but the B polypeptide was further degraded after longer incubations. A part of the degraded proteins appeared in a large band just below the B subunit (Figure 7). However, no protein breakdown was noticed after 15 and 60 min of incubation with Energex. After 24 h, Neutrase and Neutrase combined with Energex showed a complete breakdown of β-conglycinin and glycinin, while Energex was only able to degrade β-conglycinin and the A polypeptide from glycinin. It is concluded that after extrusion both β-conglycinin and glycinin were effectively degraded by all enzymes compared with toasting, after which only the β-conglycinin was (partly) degraded. These results correspond with the overall protein solubility as shown in Figure 2A.

In general, it can be concluded that β-conglycinin is easy to degrade, whereas glycinin showed more resistance against proteolytic activity. These findings are in agreement with work from others (Kim et al., 1990). Within the glycinin fraction it appeared that the B polypeptide was degraded much more slowly than the A polypeptide. These results were also found by Romagnolo et al. (1990), who incubated soybean meal with ruminal fluid. Proteins in USBM showed an extremely high resistance toward proteolytic activity, which can be explained by its compact native structure (Deshpande and Damodaran, 1989). In a previous study it was concluded that mainly noncovalent interactions were broken during the toasting process and that disulfide bonds remained more or less intact (unpublished data). In β-conglycinin two S−S bonds were found, whereas glycinin contains 18−20 S−S bonds (Fukushima, 1991). This explains why only β-conglycinin could be degraded in TSBM. Both noncovalent interactions and disulfide bonds were broken during extrusion (unpublished data), which means that β-conglycinin as well as glycinin were degraded in ExUSBM. The relatively high resistance of the B polypeptide against proteolytic activity compared with the A polypeptide can also be explained by the fact these polypeptides have the tendency to form large insoluble complexes, which make them less susceptible to enzyme hydrolysis (Yamauchi et al., 1991).

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