Enzymic Solubilization of Cereal Proteins by Commercial Proteases[1]

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ABSTRACT

Commercial sources of trypsin, α-chymotrypsin, pronase, papain, and thermolysin were tested for their ability to solubilize cereal protein from seven different cereals. Trypsin and α-chymotrypsin were relatively poor in this ability, whereas the remaining three enzymes were generally able to solubilize better than 90% of the total protein. Although pronase was the most effective overall, papain was superior in its ability to solubilize oat protein. Variations in the completeness of solubilization and degree of hydrolysis were noted between the various cereals and also among cultivars within a given cereal.

The presence of certain essential amino acids as well as availability determine the nutritive value of cereal proteins. Both of these characteristics have been tested through enzymic digestion studies with proteolytic enzymes. The availability of essential amino acids in a particular feedstuff is normally measured as the degree of utilization of these amino acids in a feeding study. However, several in vitro tests for prediction of the digestibility have been reported (Saunders and Kohler 1972, Büchmann 1979). These tests are based on the degree of solubilization of the protein in the feedstuff by certain proteolytic enzymes.

The presence, and more importantly, the quantitative level of most amino acids (including the essential ones) is commonly determined from a protein hydrolysate, using an amino acid analyzer. This technique requires the complete conversion of the protein to free amino acids before analysis. However, both the acidic and alkaline hydrolysis conditions normally used to hydrolyze the protein cause degradation of certain amino acids. Protein hydrolysis by proteolytic enzymes is a nondestructive alternative to these methods, and several procedures involving a combination of enzymes (Hill and Schmidt 1962, Bennett et al 1972) have been used to effect complete conversion to free amino acids.

When only one or two essential amino acids are to be estimated, an alternative to using an amino acid analyzer is analysis using selective chemical means. Certain chemical tests may not require the complete conversion of protein to free amino acids. If a selective chemical test for a particular amino acid can measure the free amino acid and its residue form in a soluble peptide equally well, then only solubilization of the protein component is required, not total hydrolysis. For tryptophan (Opienska-Blauth et al 1963) and lysine (Tsai et al 1972, Kakade and Liener 1969), such selective chemical tests have been reported.

The work presented in this article was part of a larger project to evaluate the potential of screening cereals on a single half-seed basis for improved levels of the amino acids mentioned above. An important requirement of such a screening procedure is the effective solubilization of the cereal protein before subsequent selective amino acid quantitation. Ideally, all of the cereal protein should be solubilized to ensure that the results represent the total sample protein, not just a solubilized component. Another factor that should be considered is the degree of contamination of the cereal protein by noncereal protein (ie, protein from protease enzymes). Thus, the present study was done to evaluate the effectiveness of several commercial protease preparations for solubilization of cereal proteins by proteolytic degradation.

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MATERIALS AND METHODS

Cereals

The hybrids and cultivars used (where cultivar refers to both named varieties and experimental lines) were obtained from seed stocks held in the Department of Plant Science, University of Manitoba. These included the maize hybrids A495 fl_2 × B8 fl_2, K_{26} × K_{52}, and $W63_{02}$ × $MS206_{02}$; oat cultivars Harmon, Hudson, and Terra; rye cultivars Gazelle, Prolific, and UC-90; sorghum cultivars Riosweet, Winner, and sorghum X; triticale cultivars Carman, Rosner, Welsh, and Cocorit 71 × UC-90; wheat cultivars Cocorit 71 and Neepawa; and barley cultivars Betzes, Herta, Karl, and Risφ 1508. Sorghum X was an unidentified high-tannin sorghum.

Enzymes

The enzymes tested were α-chymotrypsin (Worthington Biochemical Corp.), activity 67.8 units per milligram; trypsin, bovine type III (Sigma), activity 10,000 sodium benzoyl-L-arginine ethyl ester units per milligram of protein; Pronase B grade (Calbiochem), activity 45,000 Kunitz units per gram; thermolysin, protease type *X* (Sigma), 55 units per milligram solid; and crude papain powder (Sigma), 2.1 units per milligram. Trypsin, α-chymotrypsin, pronase, and thermolysin were prepared in 0.05*M* sodium borate buffer (pH 8.3) containing 8 m*M* calcium chloride. The crude papain powder, which was not totally soluble, was extracted (16 mg/ml) with 0.05*M* sodium borate buffer (pH 8.3) containing 1.5 m*M* potassium cyanide, and the insoluble material was removed by centrifugation. The potassium cyanide added as an activator was present at a lower concentration than that reported by Buchanan and Byers (1969) but was found to give maximum activation of this particular enzyme preparation.

Sample Preparation

All cereal samples were ground in a Udy cyclone mill equipped with a 0.5-mm screen. The ground samples (grists) were defatted by an 8-hr Soxhlet extraction with petroleum ether (40–60°C) and then allowed to air dry. After drying, the grists were stored at room temperature in a container maintained at 30% relative humidity.

Protein Solubilization Conditions

A fixed ratio of enzyme protein to cereal protein was used for each enzyme based on the enzyme's relative efficiency in solubilizing cereal proteins in a preliminary study. The ratios adopted for every 100 mg of sample protein were α-chymotrypsin, 10 mg; trypsin, 10 mg; papain, 10 mg; pronase, 8 mg; and thermolysin, 3.1 mg. The amount of enzyme used in any digest was thus determined from the protein concentration of the particular cereal grist and the weight of that grist in the digest. Samples of cereal grists (approximately 100 mg) were accurately weighed into centrifuge tubes, and a 5.0-ml aliquot of buffer containing the appropriate amount of enzyme was added to each tube. After being mixed to a uniform suspension, each digest was placed in a water bath at a predetermined temperature and incubated for the time

indicated. The digest was mixed five times during the first 3 hr and occasionally thereafter. To terminate the enzymic hydrolysis, the sample was removed from the bath and centrifuged at 27,000 × *g* for 10 min at 4°C. The supernatant was carefully decanted and immediately heated for 5 min in a boiling water bath to inactivate the enzymes. Heat inactivation followed centrifugation to prevent gelatinization of starch. The heat-treated supernatant was then stored at 4°C until analyzed. When the residue (pellet) was to be analyzed, it was washed twice with distilled water to remove soluble peptides and proteins trapped within the pellet. Since the washings contained an insignificant proportion (0.2–0.5%) of the total protein, they were discarded.

Determination of Protein

A 2.0-ml aliquot of the supernatant fraction was used to determine the amount of nitrogen by micro-Kjeldahl following the procedure reported previously (Nkonge and Ballance 1982). This value was corrected for nitrogen contributed by enzyme protein by subtraction of the appropriate enzyme blank. Where the residue was analyzed, the total washed residual pellet was used. Protein was reported as N × 5.7.

Estimation of Tannin

The modified vanillin procedure of Price et al (1978) was used to estimate in duplicate the tannin content of the three sorghum varieties (200-mg samples).

Amino Acid Analysis

For total amino acid analysis, samples were hydrolyzed in 6*N* HCl at 110°C for 24 hr and analyzed on a Beckman model 121 Amino Acid Analyzer following the procedure of Spackman et al (1958). Aspartic acid (asparagine) and glutamic acid (glutamine) were measured as aspartic acid and glutamic acid, respectively, whereas tryptophan and cysteine were not determined.

Degree of Hydrolysis

The degree of hydrolysis of the solubilized protein was determined according to a ninhydrin method (Cocking and Yemm 1954, Yemm and Cocking 1955), with the ninhydrin reagent formulation as reported by Mertz et al (1974). Ninhydrin solution (1.5 ml) was added to 0.5 ml of a digest supernatant that had been diluted 50 times. After mixing, the solution was heated for 20 min at 100°C and then cooled under tap water. Two milliliters of 50% (v/v) aqueous *n*-propanol was added, and the solutions were mixed before the absorbance was measured at 570 nm against a correspondingly diluted enzyme blank. Glutamate was used as the standard, with all values reported in glutamate equivalents.

Temperature Optima for Solubilization

To find the optimum temperature for each enzyme, we incubated the enzymes with two cereal grists. The cereal grists chosen were those from the maize hybrid $K_{26} \times K_{52}$ and the unidentified sorghum variety (sorghum X). The enzyme digests were incubated for various periods up to 24 hr before the reaction was stopped, and the amount of protein that had been solubilized was estimated.

Completeness of Solubilization of Individual Amino Acids

To determine which amino acids were most completely solubilized in the various enzyme-cereal digests, we selected for analysis one cultivar from each cereal: Herta barley, $K_{26} \times K_{52}$ maize, Terra oats, Prolific rye, sorghum X, Rosner triticale, and Neepawa wheat. Two duplicate sets of samples of each cereal were weighed and digested by each of the enzymes at their respective temperature optima for a period of 24 hr. The residues were isolated as described previously. One set of residues for each cereal-enzyme combination was subjected to micro-Kjeldahl digestion to estimate residual nitrogen, and the second set was pooled and used for amino acid analysis. Similar analyses were conducted on samples of the original defatted grist of each cereal to measure the total amount of each amino acid originally present. The percentage of each amino acid solubilized for each cereal-enzyme combination was calculated from the difference between the total amount of amino acid originally present and that remaining in a residue fraction derived from an equivalent weight of cereal grist. The percentage was then calculated as (total — residue)/total × 100. From these figures the most completely and least completely solubilized amino acids were identified.

RESULTS AND DISCUSSION

Temperature Optima for Solubilization

As indicated above, the ratio of enzyme protein to cereal protein was selected for each enzyme based on preliminary studies. To minimize the contamination of solubilized cereal protein by the soluble enzyme protein, a maximum ratio of 1:10 was arbitrarily chosen, and several of the enzymes were tested at this level. Trypsin and α-chymotrypsin were tested at this maximum ratio at 37°C but with maize only. After 4 hr, trypsin solubilized only 44% of the total protein, and the amount solubilized did not increase with longer incubation periods up to 24 hr. Alpha-chymotrypsin was not very effective either, as it solubilized 53% in the first 4 hr, and this value increased to 56% after 24 hr. The low efficiency of these enzymes may be a result of several factors. Both enzymes were relatively pure by comparison to other enzymes tested, and both have relatively narrow specificity. Thus, a low frequency of potentially cleavable sites in the cereal proteins may have contributed to the limited degree of solubilization. Another factor could be the presence of trypsin-specific and chymotrypsin-specific inhibitors, which have been reported in many plant tissues including cereal seed tissues (Ryan 1973). Also, although calcium was added to stabilize the enzymes against autolysis, the actual stability of the enzymes was not determined for the digest conditions used. Because the goal was to solubilize all of the protein and because these two enzymes were relatively ineffective

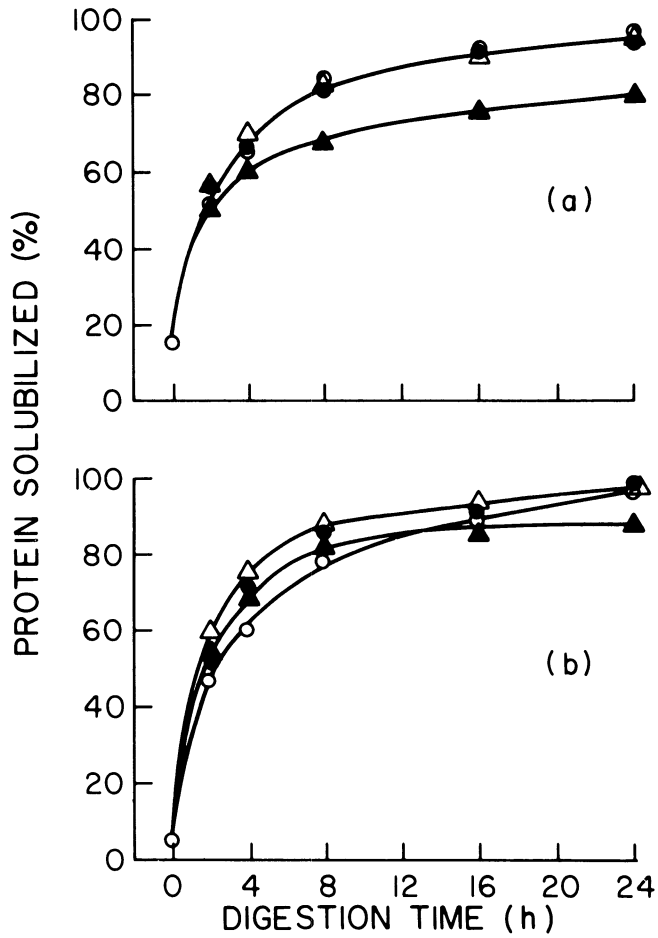

Fig. 1. Pronase solubilization of (a) maize and (b) sorghum proteins as a function of digestion time at various incubation temperatures. o—o = 37°C, •—• = 45°C, △—△ = 50°C, and ▲—▲ = 57°C.

compared to the other enzymes, no further work was done with trypsin and α-chymotrypsin.

Pronase was incubated with both maize and sorghum grists at four incubation temperatures. The results are shown in Fig. 1. In the maize sample, the enzyme was equally effective at 37, 45, and 50° C, whereas at 57° C it released less protein. This may be due to inactivation of one or more of the enzymes in the pronase preparation. The same results were also observed with the sorghum sample, except that at 37° C the protein was somewhat more slowly solubilized than at 45 or 50° C. The wide temperature range observed for maximum activity is in agreement with the previous findings (Nomoto and Narahashi 1959) and may be related to the multienzyme nature of pronase preparations (Narahashi et al 1968). Based on these results, 45° C was selected as the optimum temperature to be used for this enzyme in further studies.

Papain had previously been shown to solubilize leaf protein more effectively at 60° C than at 37° C (Byers 1967) and therefore was tested at higher temperatures than those used for pronase. The results (Fig. 2) indicate that the maize protein is solubilized in a similar manner at both 65 and 70° C. Although the 57° C digest solubilized the same amount of protein in 24 hr as those digests incubated at the higher temperatures, the amount solubilized with any shorter incubation time was significantly less. The papain digestion study with sorghum differed from that with maize in effectiveness of solubilization at 65 and 70° C. In sorghum, the incubation temperature of 70° C yielded considerably less soluble protein than that at 65° C. Thus, 65° C was selected as the optimum for papain.

Like papain, thermolysin is also thermostable (Drucker and Borchers 1971). With this enzyme, the maize and sorghum proteins were solubilized equally well at 65 and 70° C (Fig. 3). Lower temperatures caused a slower rate of solubilization, although the amounts solubilized at all four temperatures were very similar after 24 hr. The temperature optimum selected for further work with this enzyme was 65° C.

Solubilization of Cereal Proteins

Following the procedures described and using a 24-hr incubation period, we measured the extent of protein solubilization for all of the cereal samples. We used each of the three enzymes at the respective temperature optima selected above to measure extent of solubilization. The results presented in Table I represent the average of two duplicated experiments per enzyme-cereal combination. Comparison of the relative effectiveness of the three enzymes on each of the cereal crops (Table I) indicated that pronase and papain were equally effective ($P < 0.01$) on all crops, with the exception of sorghum, for which pronase was superior. Thermolysin was slightly inferior to one or both of the other enzymes on all crops, with the exception of wheat and sorghum, in which it was not significantly different from the most effective enzyme.

The ability of each enzyme to solubilize protein varied somewhat among the enzymes for the specific crops. Pronase was equally effective on most crops, with the exception of barley and oats. For barley, it was marginally less effective, whereas with oats it solubilized an average of only 80% of the protein.

Papain was also equally effective on five of the seven crops, with lower levels of protein being solubilized from sorghum and oats. Once again, oat protein was solubilized to the lowest extent. Sorghum protein was solubilized differentially depending upon the cultivar being examined. An obvious difference in seed color of the sorghum cultivars and the knowledge that certain enzymes are sensitive to tannins (Ramachandra et al 1977) led the authors to test the tannin content of the three cultivars (Table I, footnote b).

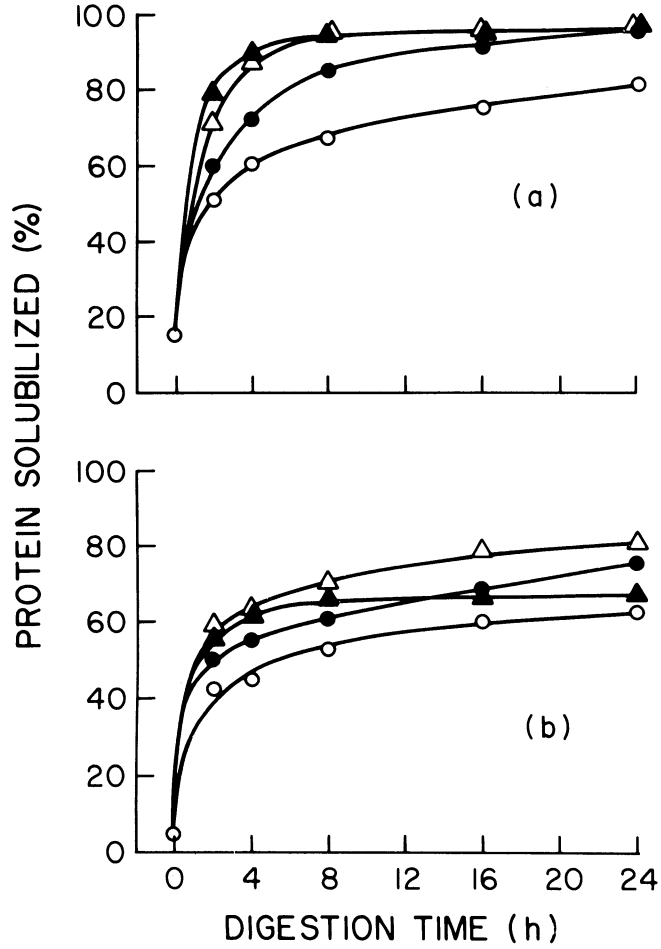

Fig. 2. Papain solubilization of (a) maize and (b) sorghum proteins as a function of digestion time at various incubation temperatures. o—o = 45° C, •—• = 57° C, △—△ = 65° C, and ▲—▲ = 70° C.

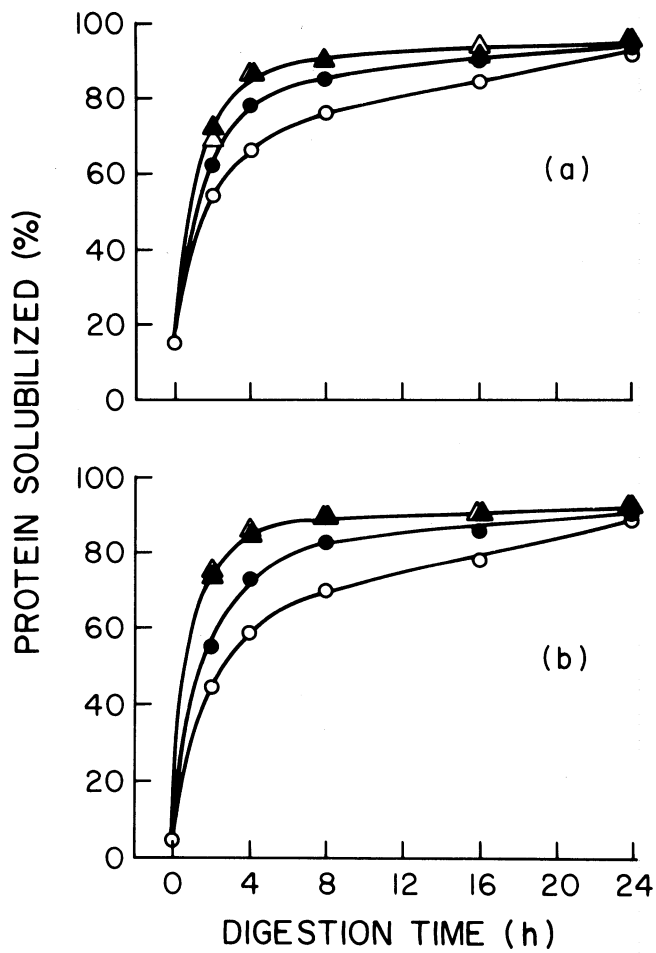

Fig. 3. Thermolysin solubilization of (a) maize and (b) sorghum proteins as a function of digestion time at various incubation temperatures. o—o = 45° C, •—• = 57° C, △—△ = 65° C, and ▲—▲ = 70° C.

Protein from the low-tannin cultivar, Winner, was significantly ($P < 0.01$) more susceptible to solubilization by papain than that from the cultivars higher in tannin. That tannin content is the cause of the observed differences with papain can only be suggested because of the small number of samples tested. Although pronase did not distinguish between the three sorghums, thermolysin was slightly more effective ($P < 0.05$) on the low-tannin cultivar.

TABLE I
Percentage of Total Protein Solubilized from Defatted Cereal Grists in 24-hr Enzyme-Cereal Digests

Sample	Protein Solubilized (%)[a]		
	Pronase	Papain	Thermolysin
Barley			
Betzes	96.9 ± 0.5	97.0 ± 0.4	93.4 ± 0.7
Herta	94.4 ± 0.4	93.9 ± 0.5	91.3 ± 0.3
Karl	94.6 ± 0.4	94.7 ± 0.2	90.8 ± 0.5
Risø 1508	92.3 ± 0.2	95.6 ± 0.6	88.6 ± 0.1
Mean	94.5 ± 0.5 r/b	95.3 ± 0.4 r/a	91.0 ± 0.5 s/d
Maize			
A495 fl_2 × B8 fl_2	96.7 ± 0.4	97.9 ± 0.3	93.7 ± 0.8
K_{26} × K_{52}	96.2 ± 0.5	95.5 ± 0.6	93.9 ± 1.0
$W63_{02}$ × $M206_{02}$	96.7 ± 0.4	98.1 ± 0.5	92.8 ± 0.3
Mean	96.5 ± 0.2 r/ab	97.2 ± 0.4 r/a	93.4 ± 0.4 s/bc
Oats			
Harmon	76.4 ± 0.3	75.6 ± 0.5	71.8 ± 0.5
Hudson	75.9 ± 0.3	75.2 ± 0.6	71.7 ± 0.6
Terra	87.2 ± 0.4	82.2 ± 0.5	75.6 ± 1.1
Mean	79.8 ± 1.6 r/c	77.6 ± 1.0 rs/c	73.0 ± 0.7 s/e
Rye			
Gazelle	97.0 ± 0.7	96.5 ± 0.1	95.0 ± 0.3
Prolific	95.9 ± 0.3	94.0 ± 0.6	93.9 ± 0.2
UC-90	97.0 ± 0.3	96.2 ± 0.3	94.6 ± 0.1
Mean	96.6 ± 0.3 r/ab	95.6 ± 0.4 rs/a	94.5 ± 0.2 s/ab
Sorghum[b]			
Riosweet	94.1 ± 0.5	83.6 ± 0.4	90.7 ± 0.2
X	95.7 ± 0.8	81.3 ± 0.4	91.0 ± 0.7
Winner	97.3 ± 0.5	96.3 ± 0.9	94.2 ± 0.9
Mean	95.7 ± 0.5 r/ab	87.1 ± 2.0 s/b	92.0 ± 0.6 rs/cd
Triticale			
Carman	97.7 ± 0.5	96.6 ± 0.6	96.3 ± 0.5
Cocorit 71 × UC-90	98.2 ± 0.6	97.2 ± 0.3	96.4 ± 0.4
Rosner	97.3 ± 0.3	96.1 ± 0.4	95.2 ± 0.6
Welsh	97.8 ± 0.3	97.0 ± 0.5	95.9 ± 0.8
Mean	97.8 ± 0.2 r/a	96.7 ± 0.2 rs/a	95.9 ± 0.3 s/a
Wheat			
Cocorit 71	98.7 ± 0.7	98.5 ± 0.3	97.2 ± 0.7
Neepawa	98.0 ± 0.3	96.6 ± 0.3	96.3 ± 0.8
Mean	98.3 ± 0.4 r/a	97.6 ± 0.4 r/a	96.7 ± 0.5 r/a

[a] Values in each row (across enzymes, within a crop) followed by the same letter (r,s) and values in each column (across crops, within an enzyme) followed by the same letter (a–e) are not significantly different by Tukey's multiple range test ($P < 0.01$).
[b] The tannin contents of the sorghum cultivars Riosweet, X, and Winner were 3.20, 5.40, and 0.10%, respectively, when determined on an "as-is" moisture basis.

TABLE II
Percentage of Total Protein Solubilized from Undefatted Oat Grist in 24-hr Enzyme-Cereal Digests

Undefatted Oat Samples	Protein Solubilized (%)[a]		
	Pronase	Papain	Thermolysin
Harmon	76.3 ± 0.2 b (76.4 ± 0.3) b	94.6 ± 0.2 b (75.6 ± 0.5) d	72.6 ± 0.4 ab (71.8 ± 0.5) b
Hudson	76.0 ± 0.2 b (75.9 ± 0.3) b	94.1 ± 1.1 b (75.2 ± 0.6) d	70.7 ± 0.1 b (71.7 ± 0.6) b
Terra	87.9 ± 0.2 a (87.2 ± 0.4) a	98.3 ± 0.1 a (82.2 ± 0.5) c	75.3 ± 0.3 a (75.6 ± 1.1) a

[a] Values in parentheses are for corresponding defatted oat grists. Values in each column followed by the same letter are not significantly different by Tukey's multiple range test ($P < 0.01$).

Thermolysin showed the greatest degree of difference among the crops. Wheat, triticale, and rye were the most effectively solubilized, and sorghum, barley, and oats were the least effectively solubilized. As with the other two enzymes, the oat cultivars were the least effectively solubilized. Analysis on individual oat cultivars showed that protein from the hulless cultivar, Terra, was solubilized more effectively ($P < 0.01$) than that from the hulled cultivars by all three enzymes.

All the samples used had been defatted, as previous reports had indicated that defatted samples were more extensively digested (Tsai et al 1972, Byers 1967). The lower level of solubilization of oat

TABLE III
Degree of Hydrolysis of Solubilized Protein from 24-hr Enzyme-Cereal Digests

Sample	Degree of Hydrolysis (glutamate equivalents × 10^{-2})[a]		
	Pronase	Papain	Thermolysin
Barley			
Betzes	506 ± 14	163 ± 2	201 ± 3
Herta	512 ± 6	164 ± 4	211 ± 3
Karl	527 ± 8	151 ± 2	213 ± 10
Risø 1508	574 ± 13	180 ± 5	259 ± 6
Mean	530 ± 9 a	165 ± 3 c	224 ± 6 b
Maize			
A495 fl_2 × B8 fl_2	618 ± 9	248 ± 4	277 ± 1
K_{26} × K_{52}	648 ± 10	207 ± 4	255 ± 3
$W63_{02}$ × $M206_{02}$	610 ± 14	230 ± 3	283 ± 4
Mean	625 ± 8 a	228 ± 5 c	272 ± 4 b
Oats			
Harmon	558 ± 4	179 ± 4	250 ± 4
Hudson	588 ± 17	172 ± 3	273 ± 3
Terra	584 ± 15	176 ± 2	199 ± 5
Mean	577 ± 8 a	176 ± 2 c	239 ± 10 b
Rye			
Gazelle	488 ± 8	187 ± 3	219 ± 4
Prolific	516 ± 10	186 ± 3	222 ± 3
UC-90	516 ± 4	160 ± 2	199 ± 3
Mean	507 ± 6 a	177 ± 4 c	214 ± 4 b
Sorghum			
Riosweet	679 ± 15	181 ± 3	277 ± 1
X	636 ± 8	173 ± 2	258 ± 4
Winner	671 ± 7	213 ± 3	321 ± 7
Mean	662 ± 8 a	189 ± 5 c	285 ± 8 b
Triticale			
Carman	517 ± 10	188 ± 4	214 ± 3
Cocorit 71 × UC-90	507 ± 7	190 ± 2	180 ± 4
Rosner	524 ± 6	171 ± 4	208 ± 2
Welsh	533 ± 8	172 ± 4	219 ± 3
Mean	520 ± 4 a	180 ± 3 c	205 ± 4 b
Wheat			
Cocorit 71	564 ± 4	185 ± 2	222 ± 4
Neepawa	530 ± 7	163 ± 2	166 ± 3
Mean	547 ± 7 a	174 ± 4 b	194 ± 11 b

[a] Values in each row (across enzymes) followed by the same letter are not significantly different by Tukey's multiple range test ($P < 0.01$).

TABLE IV
Relative Amounts of Lysine and Glutamic Acid Solubilized from Cereal Grists in 24-hr Proteolytic Enzyme Digests

Sample	Amino Acid Solubilization (%)					
	Pronase		Papain		Thermolysin	
	Lys	Glu	Lys	Glu	Lys	Glu
Herta barley	93.7	98.0	91.3	97.7	90.5	96.7
K_{26} × K_{52} maize	90.2	97.6	91.5	98.6	89.1	97.2
Terra oats (undefatted)	···	···	96.1	97.9	···	···
Prolific rye	94.3	98.7	90.6	98.0	91.6	97.6
Sorghum X	92.2	97.9	···	···	86.5	95.0
Rosner triticale	96.4	99.3	92.8	98.9	93.5	98.4
Neepawa wheat	96.3	99.3	92.9	99.0	93.2	98.6

protein relative to the other cereals led to the reexamination of the native grists before defatting. The conditions used were identical, except that undefatted oat grist replaced defatted oat grist in the digests. The results (Table II) indicate that defatting has no effect on the ability of pronase and thermolysin to solubilize oat protein but has a marked effect on the action of papain in this regard. The reason for this effect is not clear and was not examined further.

Degree of Hydrolysis

The degree of hydrolysis is a measure of the number of peptide bonds cleaved by the enzymes. The values reported in Table III are therefore a measure of the extent of hydrolysis of the solubilized protein. For all crops, the pronase enzyme preparation produced the greatest degree of hydrolysis, which might be expected , based on the multienzyme nature of this preparation (Narahashi et al 1968). (Several of the enzymes are exopeptidases.) Papain gave the lowest degree of hydrolysis, which was 2.5–3 times less than that of pronase, while thermolysin was intermediate.

Completeness of Solubilization of Individual Amino Acids

The percentage of each amino acid solubilized for each enzyme-cereal combination was calculated as described. Based on the average of all enzyme-cereal combinations glutamic acid, proline, tyrosine, and methionine were the most completely solubilized amino acids, whereas glycine, lysine, threonine, and arginine were the least completely solubilized. This pattern was generally true for most individual enzyme-cereal combinations as well.

An alternative way to have obtained this information would have been by comparing the levels of amino acids in the solubilized fraction to those in the whole grist. This procedure was not used because amino acids derived from the proteolytic enzymes are also present in this fraction, and the amino acid composition of the soluble fraction would have to be corrected for this contribution. Table IV gives the relative levels for one each of the most completely solubilized (glutamic acid) and least completely solubilized (lysine) amino acids for each of the enzyme-cereal combinations. The results are based on a single amino acid analysis for residue and for whole grist samples. Reproducibility of individual enzyme-cereal digestions is apparent from Table I, whereas reproducibility of amino acid analyses on similar material has indicated that individual values were within 4% of replicated means on all but minor amino acid constituents. Glutamic acid recoveries ranged from 95 to 99.3%, while lysine recoveries ranged more widely, with values from 86.5 to 96.4%.

Amino acid analysis results indicated that the basic amino acids were among the least completely solubilized amino acids in all the cereals. Of the endosperm proteins, the albumins and globulins are the richest in lysine, but these are readily soluble even without proteolytic degradation. Thus, the lower recovery of lysine is unlikely to be due to incomplete solubilization of these proteins. A more likely source of the nonsolubilized basic amino acids is the protein in the outer seed tissues. Analysis of those wheat mill fractions that are derived largely from outer seed tissues indicate that the proteins of these tissues are quite rich in basic amino acids (Kasarda et al 1971). In our study, a preliminary microscopic examination of the residue material (from several cereals), which had been stained for protein, indicated the presence of a source of unbroken aleurone cells. This may be one example of a source of nonsolubilized protein but may generally reflect that much of this nonsolubilized protein may be inaccessible to the enzymes.

Pronase, papain, and thermolysin are relatively effective in the solubilization of defatted cereal proteins, with oat protein as the exception. Although pronase was equal to papain for most cereals, it was notably superior on the two high-tannin sorghums tested. Papain, however, was the only enzyme of the three that was effective on undefatted oats. Thus, selection of the most efficient enzyme will be dependent on the particular cereal being examined.

An additional consideration, as mentioned previously, may be the amount of contaminating protein contributed by the enzyme. If this were a concern, thermolysin might be a better choice for those cereals on which it is effective. The relatively high recovery of even the amino acids that were the least completely solubilized means that the amino acid composition of the solubilized material fairly represents that of the total cereal protein. This means that these enzymes could be selectively used to screen for improved levels of those amino acids for which specific chemical tests are available.

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