various trichothecene mycotoxins alone or in conjunction with other *Fusarium* mycotoxins.

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Comparison of Grain Composition and Nutritional Quality in Wild Barley (*Hordeum spontaneum*) and in a Standard Cultivar

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Hordeum spontaneum (or *Hordeum vulgare ssp. spontaneum*), which is considered the wild progenitor of barley, had grain protein contents of approximately 21.5% (N × 5.7), 65% greater than that of a standard Israeli barley cultivar Ruth with 13% protein content. Additional comparative compositional studies of the two varieties revealed that the free amino acid, mineral, carbohydrate, trypsin and chymotrypsin inhibitor, and hemagglutinin (lectin) contents of the two barley genotypes differed greatly. A short-term nutritional study suggests that an equal weight of *H. spontaneum* would stimulate approximately 50% greater weight gain in mice than *H. vulgare*. *H. spontaneum* merits evaluation as a plant-breeding resource for the production of new barley varieties with high protein contents.

Natural populations of wild barley (*Hordeum spontaneum* = *Hordeum vulgare ssp. spontaneum*) in Israel contain large amounts of untapped genetic material for improving the protein contents of new varieties of barley (Nevo et al., 1985). This is an important objective since barley is the world's fourth most commonly used grain. Quality has been an important practical goal in breeding barley for brewing but not as much for animal feed, although the latter is by far the largest use of barley grain.

At present, the protein in barley cultivars is of poor nutritional quality. *H. spontaneum* has been shown (Ahokas, 1982) to have higher grain protein content than available barley cultivars, though protein contents of different accessions varies.

Since *H. spontaneum* and cultivated barley (*H. vulgare*) intercross readily and their hybrids are fully fertile, we are investigating the content and inheritance of protein and other nutrients and antinutrients in the parent genotypes and in their crosses. In this paper we report the composition and nutritional value of two potential parent genotypes. Such compositional studies provide a rational basis for breeding new barley varieties with improved nutritional and agronomic properties.

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MATERIALS AND METHODS

Trypsin, chymotrypsin, benzoyl-DL-arginine *p*-nitroanilide hydrochloride (BAPNA), *N*-acetyl-L-tyrosine ethyl ester (ATEE), and the other reagents were obtained from Sigma Chemical Co., St. Louis, MO.

The locally bred cultivar Ruth is two-rowed high-yielding barley with ca. 50-mg grain weight and 12% protein in the grains, under normal field conditions. The *H. spontaneum* line was an advanced selfed generation of accession No. 297 from a collection supplied by Dr. E. Nevo (Haifa University) with 35–40-mg grain weight and 25% protein in the grain under normal conditions. Seeds of both varieties for the detailed analyses were collected from the same environment (neighboring field plots) and received identical water and nutritional regimens. The *H. spontaneum* brittle heads were manually threshed, and seeds were partially peeled to remove extra rachis and glume parts. These parts were removed from the cultivar seeds by ordinary threshing.

Proximate Analyses. Kjeldahl nitrogen, moisture, carbohydrate, fat, fiber, and mineral contents were measured by standard techniques (AOAC, 1980).

Amino Acid Analyses. Amino acid analyses were carried out with about 5 mg of protein hydrolyzed in evacuated tubes with 6 N HCl for 24 h at 110 °C. The hydrolysates were then evacuated to dryness with the aid of a water aspirator. Aliquots of the dry residue dissolved in pH 2.2 citrate buffer were analyzed on a Model D 500 Durrum amino acid analyzer under the following condi-

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tions: single-column ion-exchange chromatographic method; resin, Durrum DC-4A; citrate buffer, pH 3.25, 4.25, 7.90; photometer, 590 nm; column, 1.75 mm × 48 cm; analysis time, 95 min. Norleucine was used as an internal standard.

Half-cystine and methionine were assayed separately as cysteic acid and methionine sulfone, respectively, after performic acid oxidation of the barley flour (Moore, 1963; Friedman et al., 1979).

Two different assays were used to measure tryptophan contents (Friedman et al., 1984). In the first, the barley flours were hydrolyzed with barium hydroxide and the tryptophan content was measured by ion-exchange chromatography on an amino acid analyzer. In the second, the tryptophan content was analyzed by an acid ninhydrin method. This spectrophotometric procedure does not require hydrolysis of the protein.

Analysis for Free Amino Acids. Free amino acids were determined by a procedure adapted from Talley et al. (1958). Barley flour samples (200 mg each) were extracted with 100 mL of 70% ethyl alcohol in a Soxhlet apparatus on a steam bath for 4 h. The extraction was repeated twice with 100-mL portions of 70% ethyl alcohol. The combined extractions were evaporated to dryness, and the residue was redissolved in 50 mL of 70% ethanol. The ethanol solution was transferred to a separatory funnel to which was added 50 mL of chloroform. The funnel was shaken vigorously. The chloroform layer containing pigments and lipids was separated, and the alcohol–water solution was evaporated to dryness. The residue was dissolved in 5 mL of pH 2.2 sodium citrate buffer. A sample (50 μL) of this solution was injected into the amino acid analyzer for estimation of free amino acid content.

Trypsin Inhibitor Assay. Inhibition of trypsin activity was measured at pH 8.2 and 37 °C, with BAPNA as substrate (Mikola and Suolinna, 1969; Friedman et al., 1982). Specifically, 100 mg of barley flour was shaken for 1 h at room temperature in 15 mL of 0.5 M Tris-HCl buffer, pH 8.5. The material was then centrifuged, and 0.5 mL of the supernatant was diluted with 4.5 mL of 0.05 M Tris–0.02 M $CaCl_2$ (1:10), pH 8.2. A trypsin solution was prepared by dissolving 2.5 mg of twice-crystallized bovine trypsin in 25 mL of 1 mM HCl. A substrate solution was prepared by dissolving 30 mg of BAPNA in 1 mL of dimethyl sulfoxide (DMSO) and diluting to 100 mL with 0.05 M Tris-HCl buffer, pH 8.2, containing 0.02 M $CaCl_2$. Next, 0.25 mL of the enzyme solution was added to 0.5 mL of the barley extract solution, the control, and a blank at 15-s intervals. The three solutions were then incubated for 5 min. Then, 3 mL of the substrate solution was added to the barley extract solution, the control, and the blank at 15-s intervals to start the reaction. The reaction was stopped after 5 or 10 min by adding 0.5 mL of 30% acetic acid.

A trypsin unit (TU) is defined as the amount of trypsin that catalyzes the hydrolysis of 1 μmol of substrate/min and a trypsin inhibitor unit (TIU) as the reduction in activity of trypsin by 1 TU.

Chymotrypsin Inhibition Assay. The same barley extract used for the trypsin inhibitor assay was used for the chymotrypsin inhibition assay. Inhibition of chymotrypsin was determined titrimetrically, with 0.01 M ATEE as substrate. The procedure was adapted from that of Wilcox (1970). A chymotrypsin solution was prepared by dissolving 1.4 mg of chymotrypsin in 2 mL of 1 mM HCl. A substrate solution was prepared by dissolving 25 mg of ATEE in 0.5 mL of 100% ethyl alcohol. The barley solution (0.5 mL) was incubated in a Radiometer pH-stat with chymotrypsin (10 μL of 0.7 mg/mL) solution and then equilibrated for 10 min at 25 °C in 1.1 mL of H_2O. After the base-line uptake of titrant (0.1 N NaOH) was recorded, 100 μL of 0.2 M ATEE was injected into the solution. Initial reaction rates were determined from the linear portion of the plot of base uptake against time. The chart speed was 2 cm/min. The nonenzymatic breakdown of ATEE and the effect of order of adding ATEE and chymotrypsin (no inhibitor) were also measured and used to correct assays of inhibition.

One chymotrypsin unit (CU) is defined as the amount of chymotrypsin that catalyzes the hydrolysis of 1 μmol of substrate/min and a chymotrypsin inhibitor unit (CIU) as the reduction in activity of chymotrypsin by 1 CU.

Hemagglutination Assay. Barley flour (150 mg) was mixed with 1.5 mL of the *N,N*-bis(2-hydroxyethyl)glycine (BICINE; 0.2.M, pH 8.5) buffer. The lectin was extracted for 1 h with stirring at room temperature. After extraction, the resulting slurry was immediately chilled and centrifuged at 9000 *g* for 5 min in a Beckman Microfuge (Beckman Instruments, Palo Alto, CA). When necessary, the extracts were diluted with isotonic phosphate buffer (PBS, 0.05 M NaH_2PO_4 and 0.15 M NaCl, pH 7.2, isotonic to red blood cells) prior to plating so that incipient activity would fall midrange in the plated series. A 100-μL sample of this extract was placed in the second U-shaped well of a microtiter plate (TITERTEK microtitrator; Flow Laboratories, McLean, VA) and 50 μL aliquots of PBS were added via 50-μL dilutors (Flow Laboratories).

To the 50-μL aliquots of serially diluted extracts and to one "blank" well containing only 50 μL of PBS was added an equal volume of glutaraldehyde-stabilized human group A blood cells diluted with PBS to 3.3% hematocrit or percent of red blood cells in the buffer immediately before use. Agglutination was observed visually after 1 h (Lis and Sharon, 1981; Wallace and Friedman, 1985). Agglutination in the blank wells indicated that a fresh supply of blood cells was needed.

Activity is expressed as the minimum amount (in micrograms) of barley flour required to cause agglutination of blood cells under these test conditions. The results of four separate assays conducted on each sample were averaged.

Nutritional Evaluation. The two barley samples were fed to mice in a growth assay (Friedman and Gumbmann, 1988). Small sample size limited the study to 8 days. Weight gain and protein efficiency ratio (PER), defined as weight gain divided by protein intake, were compared to that of casein.

Table I. Proximate Composition (%) of *H. spontaneum* Line 297 and *H. vulgare* Cv. Ruth

material	*H. spontaneum*	*H. vulgare*
nitrogen[a]	3.64, 3.70	2.25, 2.33
total carbohydrates	69.9	76.4
starch	29.6	47.7
fiber	6.75	4.09
fat	2.49	2.18
water	10.93	11.67
ash	3.39	2.45

[a] Results from two separate analyses.

RESULTS AND DISCUSSION

Proximate Composition. Table I shows that the average grain nitrogen content from two separate determinations was 3.67% for *H. spontaneum* and 2.29% for *H. vulgare*. With a factor of N × 5.7, the corresponding

Table II. Mineral Contents (ppm) of *H. spontaneum* Line 297 and *H. vulgare* Cv. Ruth

mineral	*H. spontaneum*	*H. vulgare*
cadmium	0.9	0.9
calcium	810	600
chromium	0.6	0.6
copper	32.1	18.4
iron	69.2	43.2
magnesium	1430	1260
manganese	25.2	18.6
potassium	7080	6640
sodium	273	239
zinc	69.2	43.2

protein contents were approximately 21.5 and 13.0%, respectively; i.e., the protein content of *H. spontaneum* was about 65% greater than that of *H. vulgare*.

Table I also shows that *H. spontaneum* contained about 9% less total carbohydrate, 38% less starch, 65% more total fiber, 14% more fat, and 38% more ash than *H. vulgare*.

Table II lists the mineral contents of the two cultivars. The data show that *H. spontaneum* has about 35% more calcium, 74% more copper, 13% more magnesium, 35% more manganese, 60% more iron and zinc, and about the same amounts of cadmium, chromium, potassium, and sodium. The higher values of some of the trace elements in *H. spontaneum* might be due to the higher protein content that they may be associated with or chelated to.

Amino Acid Analyses. Table III lists the amino acid composition of the two barley varieties in two commonly used terms for cereals (Cavins and Friedman, 1968): (a) millimoles/100 g of flour; (b) grams/16 g of N. The first value is strikingly higher for all amino acids in *H. spontaneum* than in *H. vulgare* because the former has a higher protein content.

Tryptophan, one of the limiting amino acids in cereals (Mertz, 1975; Pomerantz, 1975), cannot be analyzed along with other amino acids by standard amino acid analysis techniques, since it is destroyed by the HCl used (Friedman and Cuq, 1988). Numerous attempts have been made to devise improved methods for measuring tryptophan in complex foods (Sarwar et al., 1983). These include hydrolysis by barium hydroxide and a new acid–ninhydrin method (Friedman et al., 1984). Table III shows that these two methods gave equivalent results for the barley samples. Additional studies are needed to measure precisely the tryptophan contents of barley and other grains.

Any improvement in growth response of animals fed equivalent weights of *H. spontaneum* and *H. vulgare* would be largely due to the higher protein content of the former.

Free Amino Acid Content. Table IV lists the free amino acid contents of the two barley genotypes. Comparison of Tables III and IV shows that the free amino acids in *H. vulgare* contribute about 1% to the total amino acid content and of *H. spontaneum* about 1.5%. Table IV shows that (a) the free amino acid content of *H. spontaneum* is nearly 3 times that of *H. vulgare*; (b) on a weight basis, the combined serine, glutamine, and asparagine contents as well as the lysine and arginine contents are significantly greater in *H. spontaneum* than in *H. vulgare*; (c) *H. spontaneum* contains several unknown amino acids or other ninhydrin-positive compounds; (d) both genotypes contain γ-aminobutyric acid, about 10 times more in *H. spontaneum* than in *H. vulgare*. The possible nutritional significance of this difference is not known.

Trypsin and Chymotrypsin Inhibitors. Enzyme inhibitors are known to have antinutritional and possibly other adverse effects when fed to rats over long periods of time (Gumbmann et al., 1986). Table V lists the trypsin inhibitor content of the two barley genotypes. The values are nearly identical. This might mean that the same genes coding for trypsin inhibitors are in both species so they both make the same amount of inhibitor protein.

Table V shows the chymotrypsin inhibitor content of the two varieties. In this case, the values for *H. spontaneum* are greater than those for *H. vulgare*.

In absolute values, the inhibition of both trypsin and chymotrypsin in the barley tested is about 20–25% of the corresponding values observed with raw soy flour (Rackis et al., 1986; Table V). Nevertheless, our results show that both barley genotypes contain significant amounts of inhibitors of digestive enzymes (Boisen et al., 1981).

Table III. Amino Acid Compositions of *H. spontaneum* Line 297 and *H. vulgare* Cv. Ruth

amino acid[a]	*H. spontaneum*		*H. vulgare*		ratio *H. spontaneum* to *H. vulgare*	
	mmol/100 g	g/16 N	mmol/100 g	g/16 g N	*B*[f]	*A*[g]
Asp	10.7 (0.27)	5.5 (0.14)	6.6 (0.17)	5.5 (0.15)	1.6	1.0
Thr	6.5 (0.27)	3.0 (0.12)	4.3 (0.11)	3.2 (0.08)	1.5	1.0
Ser	9.8 (0.10)	4.0 (0.04)	6.5 (0.20)	4.2 (0.12)	1.5	1.0
Glu	48.7 (1.56)	27.8 (0.90)	28.4 (1.06)	25.8 (0.97)	1.7	1.1
Pro	30.4 (0.42)	13.6 (0.18)	16.7 (0.32)	12.0 (0.23)	1.8	1.1
Gly	11.4 (0.36)	3.3 (0.11)	8.0 (0.29)	3.7 (0.14)	1.5	0.9
Ala	9.8 (0.38)	3.4 (0.13)	6.7 (0.17)	3.7 (0.09)	1.5	0.9
Cys[b]	3.9 (0.35)	1.8 (0.17)	2.7 (0.21)	2.0 (0.16)	1.4	0.9
Val	9.1 (0.92)	4.2 (0.41)	6.7 (0.34)	4.8 (0.25)	1.4	0.9
Met[c]	2.8 (0.16)	1.6 (0.10)	1.8 (0.13)	1.7 (0.13)	1.5	1.0
Ile	6.9 (0.15)	3.5 (0.08)	4.5 (0.36)	3.7 (0.29)	1.5	1.0
Leu	13.2 (0.08)	6.7 (0.05)	8.7 (0.47)	7.1 (0.38)	1.5	1.0
Tyr	4.4 (0.08)	3.1 (0.06)	2.8 (0.35)	3.2 (0.49)	1.6	1.0
Phe	9.0 (0.15)	5.7 (0.09)	5.4 (0.15)	5.5 (0.15)	1.7	1.0
His	3.7 (0.04)	2.2 (0.03)	2.4 (0.09)	2.2 (0.09)	1.6	1.0
Lys	4.8 (0.11)	2.7 (0.08)	3.6 (0.24)	3.2 (0.22)	1.3	0.8
Arg	6.7 (0.33)	4.5 (0.22)	4.6 (0.11)	4.9 (0.13)	1.5	0.9
Trp[d]	0.5 (0.15)	0.42 (0.12)	0.38 (0.18)	0.49 (0.23)	1.4	0.9
Trp[e]		0.45		0.54		

[a] Listed values are averages from three separate determinations with standard deviations shown in parentheses. [b] Determined as cysteic acid after performic acid oxidation. [c] Determined as methionine sulfone after performic acid oxidation. [d] Determined by ion-exchange chromatography after basic hydrolysis. [e] Determined spectrophotometrically by the acid ninhydrin method. [f] Millimoles/100 g values. [g] Grams/16 g of N values.

Table IV. Free Amino Acid Contents (mmol/100 g) of *H. spontaneum* Line 297 and *H. vulgare* Cv. Ruth

amino acid	*H. spontaneum*	*H. vulgare*
Asp	0.219	0.218
Thr	0	0.023
Ser[a]	1.05	0.292
Glu	0.258	0.239
Pro	0.309	0.085
Gly	0.074	0.055
Ala	0.163	0.105
Val	0.064	0.043
Ile	0.036	0
Leu	0.032	0.016
Tyr	0	0.017
Phe	0.034	0.019
His	0.089	0.024
Lys	0.066	0.020
Arg	0.346	0.032
X1[b,c]	0.048	0
X2[d]	0.036	0
X3[e]	0.027	0
X4[f]	0.238	0.029
total	3.081	1.218

[a] Values for serine include those for asparagine and glutamine, which elute in the same position on chromatograms as serine. [b] Unknown peaks are designated by X. [c] Elutes before aspartic acid. [d] Elutes between serine and glutamic acid. [e] Elutes before lysine. [f] Elutes in the same position as γ-aminobutyric acid (GABA).

The fact that the tested barley genotypes contain both trypsin and chymotrypsin inhibitors suggests that the inhibitor(s) are of the double-headed or Bowman–Birk type rather than of the single-headed Kunitz type (Laskowski, 1986). Another possibility is that there are two different inhibitor species, one for trypsin and the other for chymotrypsin.

Hemagglutinins (Lectins). Lectins are glycoproteins present in many plant foods (Lis and Sharon, 1981). Normally, lectins are thermally unstable and are partly or fully denatured during cooking of foods, although lectins from different sources may differ in heat stability. Inadequately cooked legumes, however, may cause gastrointestinal disturbances and adverse nutritional effects in humans (Pusztai et al., 1981; Reaidi et al., 1981; Begbie and Pusztai, 1989).

The activity of lectins is measured by the least amount of flour required to precipitate red blood cells (Liener, 1974). Table VI shows that the lectin activity of *H. vulgare* is about 2.3 times (0.23/0.10) that of *H. spontaneum*. Thus, in terms of lectin content, *H. spontaneum* appears to have a nutritional and food safety advantage over *H. spontaneum*. Table VI also shows that the lectin activity of *H. vulgare* is similar to that found in raw soybeans. The possible sensitivity of the lectins in the two barley varieties to inactivation by baking, cooking, fermentation, and germination awaits further study.

Nutritional Evaluation. Table VII lists the basal diet used to evaluate weight gain of mice fed the two barley genotypes and a standard animal protein casein, included for comparison.

Table VIII lists the observed weight gains and PER values, measures of protein nutritional quality. The results show that (a) there was no statistically significant difference in growth or PER among proteins from the two genotypes, although the absolute values for *H. spontaneum* were lower than for *H. vulgare*, and (b) the nutritional quality of the proteins from either source was lower than for casein.

The smaller weight gain that *H. spontaneum* had on a protein basis could be due to its lower content of the essential amino acid lysine compared to *H. vulgare* (2.7 vs 3.2 g/16 g of N; Table III). However, since the former contains 65% more protein (and 33% more lysine) per unit weight, it can be reasonably predicted that on a grain weight basis, *H. spontaneum* would promote about a 50% greater growth than *H. vulgare* (5.2/5.8 × 1.65; Table VIII). Since lysine is nutritionally the first limiting amino acid in cereals, clearly the goal should be to breed new barley varieties derived from high-lysine-containing parents (Eggum, 1978) with high-protein-containing *H. spontaneum* so that the new cultivars will have high contents of both lysine and protein.

Although the PER values from the short-term mouse bioassay cannot be compared with those from a standard 28-day PER in rats, mouse bioassays have been shown to correlate highly with rat bioassays for protein quality (Cossack and Weber, 1983). Mouse bioassays have a major advantage. They require about one-fifth of the test material compared to the rat animal model.

Potential for Barley Breeding. An effort has been made to increase the protein content of wheat by crosses with one of its wild progenitors, *Triticum dicoccoides* (Avivi et al., 1983; Levy and Feldman, 1987). This progenitor, like that of barley, is characterized by significantly higher protein content in the grain and variation in that respect between various isolates (Avivi, 1978, personal communication). Neither the genes responsible for the high-protein content nor their interaction with the alien genetic background of the cultivated species is known. However, it is already clear that in *T. dicoccoides* these genes cause an impressive increase in protein content in segregants of the interspecific crosses (Avivi, 1978, personal communication). Although it is not yet known whether the inheritance is relatively simple or quantitative, polygenic selection for grain protein content was proven effective for wheat. One of our objectives is to obtain information as to whether a similar mechanism operates in the crosses of *H. spontaneum* and cultivated barley.

Protein content of cereal grains is greatly affected by growing conditions. Torp et al. (1981) found that protein content could vary between 8.1 and 14.7% in the same genotype grown at different locations, even at similar nitrogen fertilization levels. In our tests, we found comparable differences between locations that had roughly the same nitrogen levels. As mentioned earlier, since the two

Table V. Trypsin and Chymotrypsin Inhibitor Content of *H. spontaneum* Line 297 and *H. vulgare* Cv. Ruth and of Raw Soy Flour

extent of inhibition[a]	*H. spontaneum*	*H. vulgare*	raw soy flour[b]
TIU inhibited/g flour	9.96 ± 0.05	8.30 ± 0.46	47.7 ± 1.5
TIU inhibited/g N	271 ± 1.4	362 ± 20.1	597 ± 12
mg trypsin inhibited/g flour	4.0 ± 0.02	3.3 ± 0.2	19.0 ± 0.6
CTU inhibited/g flour	1321 ± 265	638 ± 159	4125 ± 530
CTU inhibited/g N	35994 ± 7220	27860 ± 6943	51562 ± 6625
mg chymotrysin inhibited/g flour	4.4 ± 0.06	2.2 ± 0.1	11.9 ± 0.6

[a] Average from two separate determinations ± standard deviation. [b] Determined as described by Friedman et al. (1982) and Rackis et al. (1986).

Table VI. Hemagglutinating Activity (μg Flour Protein/50 μL) of Barley and Soybean Lectins Measured as the Minimum Amount of Flour Causing Hemagglutination in 1 h.

flour	act.
H. vulgare cv. Ruth	0.10
H. spontaneum line 297	0.23
soybean, Williams 82 variety	0.24

Table VII. Diet Formulation

ingredient	% in diet
protein[a]	10
corn oil	8
water	5
fiber (Alpahcel)	3
vitamin mixture[b]	2
mineral mixture (AIN-76)[c]	5
choline chloride	0.2
cornstarch	20
dextrose	44.8
total	100

[a] The test substance was added at the expense of cornstarch and dextrose (see Table VIII). [b] Friedman and Gumbmann (1988). [c] American Institute of Nutrition (1977).

Table VIII. Weight Gain in Mice Fed Barley Samples for 8 Days[a]

diet	protein source	wt gain, g	PER
1	*Hordeum vulgare*	5.8[bc]	1.72[c]
2	*Hordeum spontaneum*	5.2[c]	1.47[c]
3	ANRC lactic casein	7.8[a]	2.25[b]
	pooled SEM	±0.5	±0.16

[a] Mean values are for five mice per group. Means with no letter in common are significantly different, $P < 0.05$ (Ducan's multiple-range test). Initial body weight 11.3 g.

barley genotypes were grown under identical conditions, soil conditions are not a factor in the differences reported here. However, it is well established by us as well as by others that *H. spontaneum* shows wide genetic variation in grain composition. This variation will be used in choosing potential parents for breeding purposes, including barley genotypes that contain genes for the biosynthesis of high-lysine proteins (Balasaraswathi et al., 1984; Tallberg and Eggum, 1986).

There is a general negative correlation between yield and protein content in cereals (Scholz, 1984). The high harvest index of elite cultivars (Kramer, 1979) suggests that low yield productivity of many wild relatives may partly account for their high-protein content. However, Levy and Feldman (1987) reported significant protein yield gains in wheat lines bred from *T. dicoccoides*. Frey et al. (1984) showed that high-yielding lines of barley with good agronomic traits can be bred from *H. spontaneum*. Although transferring high-protein characteristics to barley cultivars may be difficult, the cited studies suggest that such efforts are expected to be fruitful. Our studies on the composition and nutritional quality of wild and cultivated barley genotypes provide information needed in choosing parental lines and selection traits.

In conclusion, *H. spontaneum* is easily crossed with the cultivated *H. vulgare*. The hybrids are fully fertile due to the full homology between the chromosomes of the two species. These considerations and the cited findings suggest that it may be worthwhile to explore the utility of increasing both the protein and lysine contents of barley through a breeding program based on its wild progenitor—*H. spontaneum*.

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Registry No. Chymotrypsin, 9004-07-3; trypsin inhibitor, 9035-81-8; starch, 9005-25-8; cadmium, 7440-43-9; calcium, 7440-70-2; chromium, 7440-47-3; copper, 7440-50-8; iron, 7439-89-6; magnesium, 7439-95-4; manganese, 7439-96-5; potassium, 7440-09-7; sodium, 7440-23-5; zinc, 7440-66-6; Asp, 56-84-8; Thr, 72-19-5; Ser, 56-45-1; Glu, 56-86-0; Pro, 147-85-3; Gly, 56-85-9; Ala, 56-41-7; Val, 72-18-4; Ile, 73-32-5; Leu, 61-90-5; Tyr, 60-18-4; Phe, 63-91-2; His, 71-00-1; Lys, 56-87-1; Arg, 74-79-3.

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Amino Acid Concentrations and Comparison of Different Hydrolysis Procedures for American and Foreign Chestnuts

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Amino acid concentrations were determined in chestnut meats of the American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea molissima*), an American hybrid chestnut grown in the United States, and a European chestnut (*Castanea sativa*) grown in Italy. A comparison is made of two hydrolysis methods using sealed tube and reflux hydrolysis procedures. Performic acid oxidation with acid hydrolysis was used for liberation of the sulfur amino acids. Alkaline hydrolysis was used to free tryptophan. Chestnuts, which have high carbohydrate levels, gave low amino acid recoveries. Slightly higher amino acid recoveries were obtained from the reflux procedure. Performic acid oxidation produced greater recoveries of the sulfur amino acids than either the sealed tube or reflux hydrolysis procedure.

Chestnuts have been used for food and timber since ancient times in northern China (Payne et al., 1983). The American chestnut (*Castanea dentata*) was once the most important hardwood species in the Eastern United States. However, the chestnut blight in the early 1900s caused the destruction, in less than 40 years, of every major stand of American chestnut (Anagnostakis, 1978). Currently, less than 160 ha of commercial chestnut orchards are in existence in the United States (Payne et al., 1983). In the United States the lack of availability of chestnut meats has limited their use as a food. Chinese chestnuts (*Castanea molissima*), which are blight resistant, are now being sold in place of the American chestnut for orchards and the home grower (Jaynes, 1979). As chestnuts from these new plantings become available, this nut should achieve wider acceptance from the American consumer.

Amino acid data have been published on the Japanese chestnut by Taira and Taira (1964), Manabe (1975), and the Food and Agricultural Organization (1972) and on the European chestnut by Souci et al. (1981). Data on the amino acid composition of American chestnuts are not available.

This paper compares the amino acid and ammonia concentrations obtained from two hydrolysis procedures of three chestnut species *C. denta*, *C. molissima*, and

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