that a more complete study of the oxidative states of cysteine in the protein is warranted.

As previously reported (Finley and Lundin, 1980), when hydrogen peroxide reacts with peptide cysteine, a variety of products is formed. Excess hydrogen peroxide produces large amounts of GSO₂SG and GSO₂H. The sulfinic acid derivative does not yield dehydroalanine on alkaline hydrolysis, but we suspect that the disulfide dioxide does. This is based on the observation (Kice and Rogers, 1974; Savige et al., 1964) that the active dehydroalanine-producing disulfide monoxide form is a prominent intermediate in the alkaline hydrolysis of related disulfide dioxides. Lysinoalanine was determined after several proteins and protein isolates were oxidized with benzoyl peroxide, potassium bromate, or linoleic acid hydroperoxide followed by alkaline treatment (Table IV). With the exception of the safflower isolate, proteins oxidized by lipid hydroperoxide yielded the most lysinoalaanine. Results of Finley and Lundin (1980) indicate that lipid hydroperoxide caused formation of more cystine monoxide and cystine dioxide in oxidizing glutathione than did hydrogen peroxide. This suggests that the stronger, more rapid oxidant may oxidize cysteine residues directly to the sulfinic acid without going through the disulfide but that oxidation by lipid hydroperoxide probably goes through the disulfide intermediate. In Table IV the safflower samples which had oxidant added have lower lysinoalanine contents than control samples. This may be due to the fact that the safflower isolate was slightly oxidized. Although the amount of oxidized lipid present was probably small, it gave the safflower isolate a moderately rancid smell, indicative of some oxidation.

In conclusion, it appears that partial oxidation of cysteine residues in proteins to the disulfide or monoxide form increases the susceptibility of the residue to dehydroalanine formation. This dehydroalanine can yield a variety of products, including lysinoalanine. Cysteine residues that are oxidized to the sulfinic acid or cysteic acid stages appear to be more stable to alkali, at least to the extent that they do not form significant amounts of dehydroalanine or lysinolanine.

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Lysine Content of Triticale Protein Increased by Germination

Y. Victor Wu

Triticale, a cross between wheat and rye, was germinated for 1-8 days. Lysine content of germinated triticale increased after 8 days from 3.5 to 5.9 g/16 g of nitrogen. A large increase in water-soluble nitrogen (rich in lysine) and a large decrease in 70% ethanol soluble nitrogen (low in lysine) accompanied sprouting. The percent protein in triticale germinated for 3 days or more is greater than in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of protein per kernel is decreased.

Triticale, a cross between wheat and rye, is the first man-made cereal. The protein content of the best triticales has held at about 13% in CIMMYT since 1973 when their yields first closely approached those of the best bread wheats (CIMMYT, 1978). The average content of lysine in triticale protein was 3.4% in 1972 and 3.7% in 1973 (Villegas and Bauer, 1974). The comparable figures for most bread wheat would be 10-12% protein and 2.7%

lysine, whereas the average rye has 13% protein and 3.7% lysine (Bushuk, 1976). International triticale yield nursery testing data confirmed the high-yield potential of two triticales in 1979–1980, with up to 10% more average yield across all locations than the bread wheat check variety (CIMMYT, 1981). Lorenz (1974) reviewed the history, development, and utilization of triticale; Hulse and Laing (1974) reported the nutritive value of triticale protein; and Wu et al. (1978) summarized the food uses of triticale.

Although triticale has higher lysine content than wheat, it is still deficient in lysine—the first limiting amino acid. Dalby and Tsai (1976) reported an increase in lysine content expressed as percent of dry weight of triticale

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during germination. Robbins and Pomeranz (1971) found an increase in lysine content of malt and malt sprouts of triticale compared with that of the grain.

The objective of this work is to examine the potential for improving nutritional quality of triticale by sprouting. It is well-known that sprouting is affected by moisture content, temperature, composition of gases in atmosphere, and light. The present study is concentrated on the effect of length of germination period while holding other variables constant and is not designed as a comprehensive study of all the factors. Triticale was germinated for 1-8days. Dry matter and protein changes per 100 kernels, as well as protein, fat, and ash contents of germinated triticale was fractionated into different solubility classes, and essential amino acid compositions of the sprouted triticale and protein fractions were determined.

EXPERIMENTAL SECTION

Triticale. Fas Gro 385, a winter hexaploid grown in Kansas, was supplied by Farm Management Services, Inc., Wichita, KS. The grain was soaked in distilled water overnight, briefly surface-sterilized with 0.2% formaldehyde solution to retard mold growth during germination, and then thoroughly washed and soaked in distilled water to remove residual formaldehyde. The wet triticale was spread out thinly on Whatman filter papers saturated with distilled water in trays, allowing plenty of air space. The trays were sealed individually in plastic bags and placed in a dark 20 °C room for 1–8 days, with separate trays for each test condition. More details on treatment of grain were reported previously (Wu and Wall, 1980).

After 1 day 93% of the grain had sprouted, and after 3 days 96% had sprouted. No mold was observed after 3 days, but about one-third of the grain was moldy after 6 and 8 days. All moldy grains was discarded. The entire sprouted grains and incubated but unsprouted grains were freeze-dried separately and ground twice in a Wiley mill equipped with a sieve with $^{1}/_{16}$ -in. diameter holes. In addition, 100 kernels of sprouted triticale (in duplicate) were dried at 105 °C to constant weight and compared with weight of 100 kernels of untreated grains for dry matter loss during germination.

Protein Extraction. Each sample (10 g) was put in a stainless steel cup with 100 mL of solvent and blended for 5 min in a Waring Blendor. After being blended the sample was centrifuged at 10400g for 10 min, and the residue was extracted with the next solvent. The solvents used sequentially were water (twice), 1% NaCl, 70% ethanol (twice), and 0.1 N NaOH (twice). This series of solvents extracted albumins and nonprotein nitrogen, globulins, prolamins, and glutelins, respectively. The combined ethanol extracts were evaporated to dryness on a rotoevaporator. The combined water extracts, NaCl extract, combined NaOH extracts, and residue were all freeze-dried separately. A portion of freeze-dried combined water extracts (0.21-0.29 g) was dissolved in 5 mL of distilled water, and 5 mL of 20% trichloroacetic acid was added to precipitate protein. The mixture was centrifuged at 5900g for 30 min, and the supernatant was analyzed by micro-Kjeldahl. The supernatant nitrogen is nonprotein nitrogen, whereas the precipitated nitrogen is albumin.

Composition. Protein, fat, and ash were determined by micro-Kjeldahl, petroleum ether extraction, and heating to 600 °C according to procedure no. 46-13, 30-26, and 08-16 of AACC approved methods (American Association of Cereal Chemists, 1976), respectively. Protein is calculated from $N \times 5.7$ and includes also any free amino acids and peptides. Moisture was determined by heating sam-

Table I. Weight and Protein Changes of 100 Kernels of Triticale during Germination (Dry Basis) at 20 °C in the Dark

days sprouted	weight, g	weight loss, %	protein, g	protein loss, %
0	3.07		0.479	
3	2.75	10	0.440	8
6	2.61	15	0.426	11
8	2.37	23	0.412	14

Table II. Composition of Triticale at Different Stages of Germination (Percent Dry Basis)

treatment	protein, $N \times 5.7$	fat	ash	
none	15.8	1.5	1.8	_
soaked 1 day	15.2	1.5	2.2	
1-day sprouted	15.3	1.6	1.8	
3-day sprouted	16.1	1.5	1.8	
6-day sprouted	17.1	1.8	2.0	
8-day sprouted	18.2	2.3	2.5	
6-day incubation, unsprouted ^a	16.9			
8-day incubation, unsprouted	17.6			

^a The grains were treated identically with the sprouted grain in the same tray but did not sprout in the wet state in the time period mentioned.

ples at 105 °C to constant weight. Protein was determined in quadruplicate, because the emphasis of this paper is on protein and the average of four determinations is more reliable than two determinations. Fat and ash determinations were in duplicate.

Each sample for amino acid analysis was hydrolyzed for 24 h by refluxing in 6 N hydrochloric acid. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was dissolved in pH 2.2 citrate buffer. A portion of the acid hydrolysate was analyzed in a Beckman Spinco Model 121 amino acid analyzer, and the data were calculated automatically by the method of Cavins and Friedman (1968). Tryptophan was determined by the method of Concon (1975).

RESULTS AND DISCUSSION

Dry Matter and Absolute Amount of Protein. Weight and protein changes of 100 kernels of triticale during germination at 20 °C in the dark are listed in Table I. Dry matter and protein decrease as germination proceeds; however, the percentage of protein loss is smaller than dry matter loss and the difference is larger with increasing germination time. No dry matter loss of germinated triticale has been reported previously. For comparison, average dry matter loss for three varieties of wheat is 12% after 7 days of germination at 20 °C (Nielsen et al., 1978).

Composition. Protein, fat, and ash contents of triticale at different stages of germination are shown in Table II. Protein content of triticale decreased when soaked 1 day probably as a result of loss of soluble protein and other nitrogenous compounds, and remained at that level after 1 day of germination. After 3 days of sprouting, the protein content was slightly higher than original. The 6-day and 8-day sprouted triticales had increased percentage of protein. This can be explained by the data in Table I, which shows a widening difference between percents of dry matter and protein losses as germination proceeds. The 6- and 8-day incubated but unsprouted triticales (the grains that were treated identically as sprouted grains in the same tray but did not sprout in the wet state in the time period mentioned) also had increased protein content but at a lower level than sprouted ones. Fat and ash contents changed little in the first 6 days of sprouting, but

Table III. Nitrogen Distribution of Triticale as Germination Proceeds

	% total nitrogen of fraction at day of germination				
fraction	0	3	6	8	
nonprotein nitrogen	8	17	31	41	
albumin	17	18	18	17	
globulin	7	7	7	5	
prolamin	19	14	6	2	
glutelin	26	26	22	19	
residue	16	12	10	11	
total	93	94	94	95	

the 8-day sprouted triticale showed substantial increases for both fat and ash. Not enough unsprouted triticales were left for fat and ash determinations. The increase in protein, fat, and ash contents of sprouted triticales can be accounted for mostly by the loss in dry matter during germination (Table I). The increase in protein content of incubated but unsprouted triticales probably also results from loss in dry matter. However, the small amount of incubated but unsprouted triticales available was not sufficient to determine the dry matter loss accurately. Lemar and Swanson (1976) and Ranhotra et al. (1977) reported increases in lipid and ash contents of wheat during sprouting. Miller (1978) found that sprouting wheat for 7 days increased the protein content approximately 10%. Dalby and Tsai (1976) sprouted wheat, triticale, rye, barley, oats, and rice for up to 5 days at 28 °C in the dark and observed total protein (as percent of dry weight) increased steadily with time of sprouting except oats, which showed an initial increase in protein content that leveled off. The apparent increases in protein may reflect a loss of carbohydrates during respiration rather than an actual increase in protein. Although protein contents of grains usually increase during germination, Hwang and Bushuk (1973) reported a small loss in protein of flour from wheat soaked at 10 °C for 2 days and then germinated and sprouted for up to 8 days. The authors attributed the decrease in protein to the loss of low molecular weight nitrogen compound.

Protein Fractions. Triticale protein at various stages of sprouting was fractionated sequentially by a series of solvents into different solubility classes (Table III). The three largest protein fractions of triticale are albumin, prolamin, and glutelin. The 3-day sprouted triticale had higher nonprotein nitrogen but lower prolamin and residue proteins compared with triticale. As germination proceeded, the nonprotein nitrogen fraction continued to increase accompanied by decreases in prolamin and glutelin at both 6 and 8 days. After 8 days of sprouting, nonprotein nitrogen increased to more than 40% of the total nitrogen, prolamin almost disappeared, and glutelin and residue protein decreased. An increase in water-soluble nitrogen and a decrease in prolamin were also observed for sprouted sorghum (Wu and Wall, 1980). Dalby and Tsai (1976) found that prolamin contents of wheat, triticale, barley, rye, and oats decreased as time of sprouting increased from 1 to 5 days. Nielsen et al. (1978) observed the watersoluble proteins increased 3 and 6 times for two varieties of wheat after 10 days of sprouting. Hwang and Bushuk (1973) reported the most notable change in the solubility pattern of germinated wheat flour proteins was the marked increase in the amount of the acetic acid soluble fraction and a parallel decrease in the residue proteins. The gradual degradation of the residue protein is important because the bread-baking potential of wheat is directly related to residue proteins.

Amino Acid Composition. The essential amino acid composition of triticale at different stages of germination and the National Academy of Sciences (1980) pattern of high-quality protein for human consumption are listed in Table IV. Values in Table IV have been adjusted to 100% N recovery. The average N recovery was 91.3% and ranged from 85.3 to 101.8%. Triticale 385 has 15.8% protein and 3.5 g of lysine/16 g of N. Although the value of lysine may appear to be high for that protein content, it agrees with a value of 3.4 g determined previously (Wu et al., 1978). The lysine content of untreated triticale, although higher than that of wheat, is still deficient. The grain that was soaked 1 day did not differ greatly in its amino acid composition compared with triticale. The 1and 3-day sprouted triticales also had similar amino acid patterns compared with triticale, except the sprouted materials had higher levels of sulfur amino acids. A large increase in lysine contents of 6- and 8-day sprouted triticales was evident in Table IV, although other essential amino acids did not change substiantially. The large increase in lysine content of germinated triticale may ssem surprising at first glance. However, even larger increase in lysine content was observed for high-lysine sorghum germinated for comparable period (Wu and Wall, 1980). Both the 6- and 8-day triticales essentialy meet or exceed the National Academy of Science pattern for high-quality protein for human consumption. The 6- and 8-day incubated but unsprouted triticales also had a substantial increase in lysine content compared with triticale, although the increase in lysine was somewhat less than that of the corresponding sprouted grains. In addition to the changes in essential amino acids in Table IV, the 8-day sprouted triticale had higher aspartic and alanine but lower glutamic and proline compared with triticale, which is consistent with the changes in protein classes. The 8-day incubated but unsprouted triticale had higher histidine and alanine but lower glutamic and proline compared with triticale.

Tryptophan is not reported in most amino acid analyses, because the acid hydrolysis destroys tryptophan. Ahmed and McDonald (1974) reported the tryptophan contents of Fas Gro 203 and Fas Gro 204 triticales were both 1.1 g/16 g of nitrogen. Villegas and Bauer (1974) determined that the tryptophan contents of three triticales with the extremes and the average protein content for 2700 samples

 Table IV.
 Essential Amino Acid Composition of Triticale as Germination Proceeded (Grams per 16 Grams of Nitrogen Recovered)

			days sprouted			days unsprouted		NAS ^a	
amino acid	amino acid triticale	1 day	1	3	6	8	6	8	(1980)
Ile	3.9	3.9	3.6	4.0	4.3	4.3	4.1	4.2	4.2
Leu	7.5	7.1	6.9	7.2	7.2	7.0	7.2	6.9	7.0
Lys	3.5	3.9	3.3	3.6	4.7	5.9	4.3	5.0	5.1
Met + Cys.	3.1	2.8	3.9	3.7	3.0	3.3	3.5	3.3	2.6
Phe + Tvr^2	8.8	9.8	8.3	8.6	9.0	8.5	8.9	8.5	7.3
Thr	3.2	3.0	3.0	3.2	3.5	3.6	3.4	3.4	3.5
Val	4.7	4.4	4.6	5.0	4.9	5.1	4.9	4.9	4.8

^a National Academy of Sciences pattern of high-quality protein for human consumption.

Table V.Essential Amino Acid Composition of ProteinFractions from 3-Day Sprouted Triticale (Grams per 16Grams of Nitrogen Recovered)

amino acid ^a	water extract- able	glob- ulin	pro- lamin	glu- telin	resi- due
Ile	3.7	3.6	4.3	4.3	4.0
Leu	6.6	6.0	7.3	8.3	7.4
Lys	5.0	3.6	1.0	4.2	4.0
$Met + Cys_2$	3.7	3.0	1.4	1.8	3.1
Phe + Tyr	8.4	7.3	8.9	9.6	8.0
Thr	3.1	2.9	2.4	4.1	3.7
Val	4.5	4.9	3.8	5.5	6.0

^a Tryptophan not determined.

varied from 1.0 to 1.2 g/100 g of protein. The amino acid pattern for high-quality proteins for human consumption is 1.1 g of tryptophan/100 g of protein (National Academy of Sciences, 1980). Thus, tryptophan is adequate in triticale protein. Tryptophan contents of triticale Fas Gro 385 sprouted at 0, 1, 3, 6, and 8 days increased with time of germination, and an increase of about 50% was observed after 8 days of sprouting. Whereas the absolute values of tryptophan content of triticale 385 germinated at various times may be subject to adjustment, the trend is believed to be real. It may be concluded that the tryptophan content of triticale protein is adequate before germination and more than adequate after germination. Dalby and Tsai (1976) reported increases in lysine, tryptophan, and total protein but decrease in prolamin after germination of triticale for 1-5 days. Since their results were expressed as percent of dry weight and no dry weight loss was determined, no direct comparison can be made with data in Tables II and IV. Robbins and Pomeranz (1971) malted triticale for 5 days at 16 °C and found increases in lysine content for malt and sprouts. Since the percentage of malt and sprouts was not given, no comparison can be made with the data in Table IV.

Folkes and Yemm (1958) observed that lysine in germination barley increased an average of 65% over that in ungerminated grain during a 10-day germination period at 22.5 °C. Smith (1972) reported that germination increased lysine but decreased cystine in barley grain. Nielsen et al. (1978) found that germination increased nutritional quality in terms of lysine content as effectively in wheat grain as in barley and corn grain. Miller (1978) reported increases in lysine content of 15-27% after 7 days of sprouting wheat. Jones (1969) observed increase in concentration of lysine during the first 4 days of barley malting followed by a sharp decline on subsequent days. Tryptophan and methionine rose similarly in concentration during the same time period but remained constant thereafter. Concentration of lysine in barley malt increased with duration of malting for 2, 5, and 11 days (Pomeranz and Robbins, 1971; Robbins and Pomeranz, 1971).

The essential amino acid composition of protein fractions from 3-day sprouted triticale is listed in Table V. Water extract had the highest lysine content and prolamin the lowest. Prolamin and glutelin had the lowest sulfur amino acids contents. As germination proceeds, prolamin with low lysine content was replaced by water extractables with high lysine content, and an increase in lysine for the sprouted grain was observed in Table IV. The changes in glutelin and residue fractions are smaller and their lysine contents do not greatly exceed that of triticale. However, this simple replacement of prolamin by water extractables does not rule out a more complicated explanation and does not imply that water extractables have a constant composition during germination.

CONCLUSION

The significant increase in lysine content (expressed as grams per 16 g of nitrogen or per 100 seeds) of triticale after 6 days of sprouting appears to indicate a simple method to improve the apparent nutritive value of triticale protein. The real nutritive value of triticale protein after sprouting will have to await human feeding studies. The high percentage of sprouting grain and the increased lysine content of incubated but unsprouted grain make the removal of unsprouted triticale unnecessary while still realizing a large increase in lysine content of the triticale. The sprouted triticale can be used as vegetable or salad or can be dried and ground to flour in more tradiational food uses.

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