Note on Trypsin Inhibitor Activity in the Acetate Extract of Cereal Samples[1]

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Trypsin inhibitors occur naturally in plants and animals. In plants, they are found chiefly in the reserve tissue of leguminous seed, and Solanaceae and Gramineae families also reportedly display trypsin inhibitor activity (Ryan 1973). The physiologic functions of trypsin inhibitors in plants remain mysterious and controversial (Richardson 1977). Distribution of trypsin inhibitor activity varies among fractions in plant tissue. Kirsi and Mikola (1971) found that barley has six times more trypsin inhibitor activity in the embryo than in the endosperm. Halim et al (1973) stated that corn endosperm has more inhibitor activity than does the embryo. Barber et al (1973) claimed that in rice, trypsin inhibitor activity accumulates in the bran fraction. Soy trypsin inhibitors have been studied extensively by Kakade (1973), Rackis (1974), but only a few reports concern cereal trypsin inhibitors (Kirsi and Mikola 1971, Barber et al 1973, Halim et al 1973, Xavier 1974). Madl and Tsen (1974) reported on the activities of trypsin and chymotrypsin inhibitors in extracts of triticale, wheat, and rye flours. They found that triticale apparently inherits its trypsin inhibitors from its rye parent, for rye flour has high but wheat flour has very low inhibitor activity. In this study we used a modified AACC method to determine the trypsin inhibitor activity in triticale and rye samples and its distribution among milling fractions (flour, shorts, and bran) of triticale and wheat samples.

MATERIALS AND METHODS

Samples

Eleven Mexican triticale samples and 17 Dakota rye samples were used to determine trypsin inhibitor activity, which was compared with that of two Kansas soybean samples. The whole grain samples were ground by Wiley experimental mill to a flour fine enough to pass through a 60-mesh screen.

We studied the trypsin inhibitor distribution pattern in milling fractions of triticale and hard red winter wheat from Tribune, KS. The triticale samples were produced in three groups, which differed relative to nutrient in the fertilizer applied during their growth. The amount of nitrogen-phosphate-potassium was 160-18-0, 160-0-0, and 0-0-0 for the designated triticale groups A, B, C, respectively. The grains were milled into three fractions—flour, shorts, and bran—by a pilot mill in the Department of Grain Science and Industry, Kansas State University.

Assay for Determining Trypsin Inhibitor Activity

Of the methods for determining trypsin inhibitory activity (Kakade et al 1969, Fritz et al 1974), the one developed by Kakade et al has been widely used. The official AACC method 71-10 (1976) was established largely according to the method of Kakade et al (Rackis et al 1972, Kakade et al 1974). The AACC method was designed primarily for determining the trypsin inhibitory activity of soy products. Several modifications made the method more suitable for determining the trypsin inhibitory activity of cereals in this study.

Inhibitor Extract. A sodium acetate buffer solution with a pH 3.8 and ionic strength of 0.02*N* was used as the extracting solvent (Madl and Tsen, 1974). Acetate buffer was more effective than 0.01*N* NaOH as a solvent for extracting cereal inhibitor. One gram of whole grain flour was dispersed in 15 ml of solvent and the content was mixed by an automatic shaker for 1 hr. Then the suspension was centrifuged at 30,000 RPM for 30 min at 4°C, and the supernatant was used for trypsin inhibitor activity determination. Soluble protein was determined by the Folin-Ciscalteu method (Bruening et al 1970).

Enzyme and Substrate Solutions. Trypsin (bovine pancrease Type III, twice crystallized) and *N*-benzoyl-DL arginine-*p*-nitroanilide HCl (BAPA) were obtained from Sigma Chemical Company, St. Louis, MO. The solutions were prepared according to Kakade et al (1969). The substrate solution was prepared daily. A stock solution in dimethylsulfoxide could, however, be prepared and stored in a refrigerator for a long time without formation of BAPA precipitate.

Assay. Inhibitor extract (0.5 ml) and enzyme solution (0.25 ml) were added to a 5-ml cuvette; 3 ml of BAPA was added after the enzyme-inhibitor mixture equilibrated at room temperature for 5 min. The mixture was thoroughly mixed by inverting the cuvette many times. Absorbance increment at 410 nm of the mixture was measured by a spectrophotometer and monitored on a recording chart. Reaction rate of trypsin was taken as the positive slope of

TABLE I
Trypsin Inhibitor Activity of Whole Flour Samples of Triticale, Rye, and Soybean

Variety	Designation	HIT[a]/ml	Protein (mg/ml)	HIT/mg of Protein
Mexican Triticale				
Inia-Arm. "S" X-1648-2N-OM	MT-1	10.0	5.6	1.8
Maya II-Arm. "S"				
X-2802-38N-3M-6N-5M-OY	MT-2	9.7	5.0	1.9
X-2802-38N-3M-6N-6M-OY	MT-3	10.6	4.4	2.4
X-2802-38N-7M-7N-5M-OY	MT-4	11.2	5.5	2.0
X-2802-38N-2M-6N-2M-OY	MT-5	10.4	5.2	2.0
Ciuuamon	MT-6	10.0	5.6	1.8
Maya II-Arm. "S"				
X-2832-13N-1M-3N-OM	MT-7	9.1	5.4	1.7
X-2832-24N-3M-7N-4M-OY	MT-8	11.1	5.1	2.2
X-2832-28N-5M-5N-3M-OY	MT-9	10.6	5.4	2.0
X-2832-41N-1M-5N-1M-OY	MT-10	7.7	4.3	1.8
Camel	MT-11	13.7	5.4	2.5
Rye (South Dakota)				
Cougar	R-1	32.0	5.6	5.7
Zelder	R-2	23.4	5.8	4.0
Kodiak	R-3	21.6	5.4	4.0
Coloma	R-4	18.8	6.5	2.9
Dominant	R-5	34.8	7.2	4.8
Antelope	R-6	34.1	6.2	5.5
Rymin	R-7	27.0	5.8	4.7
Sangaste	R-8	35.9	7.0	5.1
Von Lockow	R-9	30.6	6.6	4.6
Elk	R-10	28.0	5.8	4.8
Elbon	R-11	41.2	5.8	7.1
Balbao	R-12	33.7	5.7	5.9
Rye (North Dakota)				
Cougar	R-13	37.6	6.1	6.2
Pearl	R-14	26.7	6.5	4.1
Coloma	R-15	22.1	7.0	3.2
Caribou	R-16	31.2	7.0	4.6
Rymin	R-17	30.0	6.3	4.8
Soybeans				
Williams	S-1	1110.0	11.4	97.4
Cutler	S-2	1161.8	12.8	90.8

[a]HIT = half inhibition of trypsin.

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TABLE II
Distribution of Trypsin Inhibitor Activity in Triticale and Wheat Samples[a]

	Flour			Shorts			Bran		
	HIT[b]/ml	Protein (mg/ml)	HIT/mg of Protein	HIT/ml	Protein (mg/ml)	HIT/mg of Protein	HIT/ml	Protein (mg/ml)	HIT/mg of Protein
Triticale									
A	20.4	7.30	3.3	13.8	5.20	2.7	9.3	5.30	1.8
B	20.0	6.48	3.1	12.0	5.10	2.4	8.9	5.40	1.6
C	16.0	5.22	3.1	11.3	4.36	2.6	6.7	4.16	1.6
Wheat									
Eagle	5.2	5.10	1.0	4.5	4.62	1.0	...	...	...

[a]The extract was prepared by suspending 1 gm of sample in 10 ml of acetate buffer at pH 3.8.
[b]HIT = half inhibition of trypsin.

absorbance vs. time. A control reaction was conducted in the same manner except that 0.5 ml of inhibitor extract was replaced by 0.5 ml of water. The control reaction was run frequently during each inhibitor activity determination to prevent temperature fluctuation.

Expression of Trypsin Inhibitor Unit. Trypsin inhibitors exerted linear inhibition on trypsin by an increased amount of inhibitor up to 70–90% of the total enzymatic activity (Dixon and Webb 1964). To make the result reproducible, we arbitrarily defined one unit of trypsin inhibitor activity as the amount of inhibitor needed to inhibit 50% of the control trypsin activity (one-half of its slope value). The inhibitor unit was thus expressed as HIT (half inhibition of trypsin). HIT could be calculated by adjusting to 50% inhibition basis if the extent of inhibition was in the range of 40–65%. If the inhibition was outside that range, the amount of inhibitor extract had to be adjusted until 40–65% of inhibition was obtained.

The modified method is short, simple, and reproducible for determining the inhibitory activities of cereals. Furthermore, the exact timing of the enzyme reaction is not important in the modified method but is critical in the original method. The method has been expedient for our subsequent studies on the isolation and characterization of trypsin inhibitor from cereals.

RESULTS

Trypsin Inhibitor Activity in Triticale and Rye Samples

Table I lists the amount of trypsin inhibitor in each sample. The inhibitor activity (HIT per milliliter of extract) of soy flour tested ranged from 1,110 to 1,161.8, compared with 18.8–41.2 for rye flour and 7.7–13.7 for triticale flour. The average trypsin inhibitor activity of triticale flour was 1% of that of soy flour and the activity of rye flour was 2 or 3%. Rye flour had a trypsin inhibitory activity two or three times greater than that of triticale flour.

Among the 11 triticale samples, nine had similar trypsin inhibitor activity (9–11 HIT per milliliter). Inhibitor activity of sample MT-10 was significantly low (7.7 HIT per milliliter) and of sample MT-11 significantly high (13.7 HIT per milliliter). Sample MT-11 had a distinctly different breeding background from that of the other triticale samples.

The 17 rye flours had a wider range of trypsin inhibitor activity (18.8–41.2 HIT per milliliter) than did the triticale flour. Trypsin inhibitor activity of most of the rye flours ranged from 25 to 35 HIT per milliliter. Some rye samples (ie, Couger, Coloma, Rymin) from North and South Dakota, differed significantly in trypsin inhibitor activity because of environmental factors. Specific activity of the inhibitors (HIT per milligram of protein) in each sample changed similarly to that of their HIT values.

Distribution of Trypsin Inhibitor Activity

Distribution of inhibitor activity in flour, shorts, and bran portions of triticale and wheat samples is listed in Table II. In all samples tested, trypsin inhibitor activity decreased in the following order: flour, shorts, and bran. In triticale, shorts had 60–70% and bran had 42–46% of the inhibitor activity in flour. Application of nitrogen fertilizer could increase the trypsin inhibitory activity of triticale. In wheat, shorts had 87% of the inhibitor activity in flour, and bran fraction had no detectable trypsin inhibitor activity. The difference in the extent of protein and trypsin inhibitor activity of the three groups of triticale samples reflected the effect of environmental factors on the growing crops.

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