Endogenous Factors Affecting Protein Digestibility in Buckwheat

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ABSTRACT

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Endogenous factors affecting protein digestibility of buckwheat were studied. High levels of protease inhibitor and tannin, which are inhibitory factors of protein digestibility, were found in various buckwheat products. A significant difference in susceptibility to proteolytic action among buckwheat protein components was noted, with the globulins being more digestible by proteases than the albumins. These findings indicate that the protein digestibility of buckwheat is defined by two factors: the inhibitory potency of endogenous antinutrients, such as protease inhibitor and tannin, and the susceptibility of protein per se to proteolytic action.

Buckwheat (*Fagopyrum esculentum*) is an important crop in some areas of the world. Its potential value as a dietary source of protein is well recognized. The seed is used for a variety of dishes. Noodles made from a dough of buckwheat flour and water have long been popular in Japan. It is also common practice in many countries to prepare groats from buckwheat seeds, which are eaten as a cooked porridge.

Although buckwheat protein is of high biological value (Sure 1955), experiments with animals have shown that availability of buckwheat protein for gastrointestinal absorption is low (Farrell 1978, Javornik et al 1981, Thacker et al 1983). The revised Japanese Standard Tables of Food Composition show the protein digestibility of buckwheat by humans to be lower than that of other edible seeds such as soybean and wheat (Resources Council 1981). Despite the importance of buckwheat as a dietary source of protein, endogenous factors responsible for the poor availability of buckwheat protein are still not fully clarified.

Legumes and cereals contain a number of constituents that adversely affect the utilization of the protein present (Anderson 1985). Among the many deleterious factors present in edible seeds, protein protease inhibitors have been most extensively investigated (Liener and Kakade 1980, Rackis 1981). We presented evidence for the occurrence of a protein protease inhibitor in buckwheat seed (Ikeda and Kusano 1978) and discussed some properties of the inhibitor (Ikeda et al 1986). However, it is still not known whether the poor bioavailability of the buckwheat protein is attributable to the presence of endogenous antinutrients such as protease inhibitors or to reduced susceptibility of the buckwheat protein to proteolytic action.

The present study was designed to clarify endogenous factors involved in the protein digestibility of common buckwheat products.

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MATERIALS AND METHODS

Samples

Samples of fresh buckwheat seed were obtained from Takii Co. (Kyoto, Japan). Commercial buckwheat groats and whole straight flour were obtained at local markets and stored at $-35°C$ until use. Two different kinds of commercial dried buckwheat noodles made from whole flour, designated as noodles G and S, were also examined. The protein ($N \times 6.31$) contents of the commercial groats and the noodles G and S, as determined by the micro-Kjeldahl method (AOAC 1984), were 10.5 ± 0.1 g, 11.4 ± 0.2 g, and 14.3 ± 0.2 g per 100 g of dry matter, respectively.

In addition, two buckwheat inner-layer flour fractions from a commercial mill were provided by Masuda-ya Milling Co. (Kobe, Japan). On milling of buckwheat seeds, the inner endosperm is easily ground into flour, whereas the periphery is more difficult to grind into flour. Thus, on milling, flour fractions are successively obtained from the inner to outer layers. The two inner-layer flour fractions examined were prepared early in the process of successively milling dehulled buckwheat seeds: fraction 1 was superior flour (extraction yield 16%); and fraction 2, first flour (extraction yield 40%).

Two different kinds of noodles were made in our laboratory from the buckwheat whole flour and from the inner-endosperm flour, i.e., a combined flour mixture of the first flour with the superior flour (2:1). Buckwheat noodles and groats were cooked as usually prepared: noodles were cooked in boiling water for 5 min, followed by washing in cold water; groats were immersed in 1.5-fold (v/w) volume water for about 5 hr and then cooked in an electric rice cooker for about 20 min at about $95°C$.

The albumin, globulin, prolamin, and glutelin from buckwheat were isolated from the seeds according to the procedure of Javornik et al (1981).

Soybean and wheat products were selected as reference plant protein sources for this investigation. Soybean (*Glycine max* (L.) var. Tsurunoko) was from Takii Co. (Kyoto, Japan). Commercial soybean products examined were *yuba* (a protein-lipid film) and *nattō* (fermented whole soybeans). Soybean protein isolate was prepared from the aqueous extract of soybean meal through acid precipitation according to the procedure of Nash et al (1971). Wheat flour examined was a commercial product. Wheat gliadin (prolamin) was obtained from ICN Pharmaceutical Inc. (Cleveland, OH), and wheat glutenin (glutelin), from Nacalai Tesque, Inc. (Kyoto, Japan).

Analytical Methods

Trypsin inhibitor activity. Food samples were lyophilized, ground to pass through a 0.36-mm sieve, and then stored at $-35°C$ prior to analysis. The samples were extracted with eightfold (v/w) volumes of $0.2M$ NaCl for 2 hr at $4°C$ with stirring. After centrifugation of the suspensions at $10,000 \times g$ for 15 min, the supernatants obtained were assayed for trypsin inhibitory activity. The activity of trypsin (Type III, Sigma Chemical Co., St. Louis, MO) towards the substrate benzoyl-DL-arginine *p*-nitroanilide (BAPNA) (Nacalai Tesque, Inc., Kyoto, Japan) was determined by measuring the amount of *p*-nitroaniline liberated from the substrate via its spectrophotometric reading at 410 nm as described previously (Ikeda et al 1986). The enzymatic assay consisted of 1.0 nmol of trypsin, 4.0 μmol of BAPNA, 980 μmol of Tris-HCl buffer (pH 8.2), and 150 μmol of $CaCl_2$ in a total volume of 4.0 ml. The reaction was performed at $37°C$ for 15 min. One unit of enzyme activity was defined as the conversion of 1 μmol substrate per min. Inhibitory activity was determined from the residual enzymatic activity after preincubation of inhibitors with the enzyme for 10 min at $37°C$. One inhibitory unit (IU) was defined as the number of enzyme units inhibited under the assay conditions employed.

In vitro protein digestibility. Susceptibility of the buckwheat protein components (i.e., the albumin, globulin, prolamin, and glutelin) and other food proteins to pepsin (1:60,000, Sigma) action was examined by the method of Rick and Fritsch (1974). Susceptibility of these proteins to trypsin action was examined by

the method of Rick (1974).

Fractionation of protein. Fractionation of protein of the buckwheat whole flour and the inner-layer flour fractions was performed by the procedure of Javornik et al (1981). Protein ($N \times 6.31$) in solid samples was assayed by the micro-Kjeldahl method (AOAC 1984). Protein concentration was assayed by the method of Bradford (1976).

Determination of tannin. Tannin in the food samples was assayed by the colorimetric procedure with vanillin-HCl (Price et al 1978). The amount of tannin assayed was expressed as a catechin equivalent.

In vitro food digestion. Food digestion was performed using the procedure of Akeson and Stahmann (1964) but with a slight modification (Ikeda et al 1986). One gram each of the ground samples was at first incubated with α-amylase (Type XI-A, Sigma) at pH 6.8 at $37°C$ for 30 min. After incubation, the digestion mixtures were adjusted to pH 1.0 with $2M$ HCl and then incubated with pepsin for 3 hr at $37°C$. After incubation, the digestion mixtures were adjusted to pH 8.0 with $2M$ Tris and subsequently incubated for an additional 20 hr with pancreatin (NF, Difco Laboratories, Detroit, MI). The enzyme-to-protein ratio, except for 500 IU of α-amylase per one gram of food, was 1:10. Sodium azide was added to the pancreatin digestion mixture to a final concentration of 0.025% to prevent growth of microorganisms. After digestion, a 4-ml aliquot of the soluble digesta was added to a test tube containing 1 ml each of 10% sodium tungstate and 0.67N sulfuric acid (Scheffner 1967, Kan and Shipe 1984). Tubes were allowed to stand for 10 min, then centrifuged at 3,000 $\times g$ for 15 min. The supernatant obtained was assayed for peptide with 2,4,6,-trinitrobenzenesulfonate (Habeeb 1966). Percent protein hydrolysis was calculated from the ratio of the content of free peptide released upon the digestion to the original protein content of the foods.

Statistical Analysis

Data were subjected to analysis of variance and the significance of differences in means was determined by *t* test.

RESULTS AND DISCUSSION

Distribution and Characteristics
of Protein Components in Buckwheat Flour

Table I shows the distribution of protein components in buckwheat whole flour and the inner-layer flours. The buckwheat whole flour contained a high level of albumin and globulin and a low level of prolamin and glutelin. This finding agreed with that of Javornik et al (1981). Albumin and globulin were also major components of two inner-layer flours, as well as a high level of insoluble protein fraction. There was a difference in the albumin-to-globulin ratio (A/G ratio) among the buckwheat

TABLE I
Distribution of the Protein Components
in Buckwheat Whole Flour and Inner Layer Flours

Protein Fraction	Protein Content[a] (mg/1 g of flour)		
	Whole Flour	Superior Flour	First Flour
Total protein	121.0 ± 9.0	42.0 ± 2.0	37.2 ± 2.0
Albumin	15.1 ± 0.9	5.2 ± 0.1	3.9 ± 0.2
Globulin	78.0 ± 5.1	7.1 ± 0.4	4.8 ± 0.2
Prolamin	3.5 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
Glutelin	9.7 ± 0.3	1.9 ± 0.1	1.1 ± 0.1
Insoluble protein fraction[b]	14.7	26.5	26.3
A/G ratio[c]	0.19	0.73	0.81

[a] Values are means $\pm$ SD ($n = 4$).
[b] The insoluble protein fraction was estimated by subtracting the sum of the albumin, globulin, prolamin, and glutelin fractions from the total protein.
[c] Albumin-to-globulin ratio.

flours examined, with A/G ratio in the two inner-layer flours being markedly higher than that in the whole flour.

Table II shows susceptibility of buckwheat proteins and other food proteins to proteolytic action. The susceptibility of buckwheat globulin, a major protein component of buckwheat flour (Table I), to trypsin and pepsin action was significantly ($P < 0.05$) higher than those of the other proteins examined. Another major protein component of buckwheat flour, albumin, was significantly ($P < 0.05$) less susceptible to proteolytic action. Susceptibility of buckwheat prolamin to proteolytic action was very low compared with the other proteins examined.

Pepsin Plus Pancreatin Digestibility of Buckwheat Products

Table III shows the pepsin plus pancreatin digestibility of various buckwheat products. There were significant differences in protein digestibility among the various products examined. For both the raw and cooked samples, the protein digestibility of the commercial noodles G and S, which were made from buckwheat whole flour, and the noodle prepared in our laboratory from whole flour was significantly ($P < 0.05$) higher than that of the noodle made from inner-layer flour. In general, various types of buckwheat noodles are available in Japan, including those made from whole flour and those made from inner-layer flour alone. A possible explanation responsible for the low protein digestibility of the noodle made from inner-layer flour (Table III) may be its higher proportion of albumin and prolamin proteins, which were less susceptible to proteolytic action (Table II), and lower proportions of globulin and glutelin proteins, which were more digestible by proteolytic enzymes (Table II). The high level of insoluble protein fraction found in the inner-layer flours

may also contribute to low protein digestibility in the noodles prepared from it.

The increase in protein digestibility on cooking of noodle G and groats, but not of noodle S (Table III), is hard to explain, since larger decreases in antinutrients in noodle S with cooking did not result in increased protein digestibility. It is possible that different protein solubilities among the products (not determined) might account for some protein digestibility difference. In this connection, information on conformational changes of buckwheat proteins on heating would be helpful. Our preliminary experiments indicate that heating does not exert a pronounced effect on susceptibility of buckwheat globulin to proteolytic action (Ikeda et al 1991).

Trypsin Inhibitor Activity and Tannin Content of Buckwheat Products

Figure 1 compares trypsin inhibitor activity and tannin content in various buckwheat products and in wheat and soybean products. All the buckwheat noodles exhibited considerable trypsin inhibitor activity and had a high level of tannin even after cooking. The soybean products, with the exception of raw soybean meal, exhibited less trypsin inhibitor activity. Less or no tannin was found in the raw soybean meal and soybean products. Very low levels of trypsin inhibitor and tannin were found with wheat flour (Fig. 1).

Although buckwheat seed is a source of well-balanced protein (Pomeranz and Robbins 1972), the biological availability of the protein of buckwheat for humans is low (Resources Council 1981). One possible reason suggested for this poor availability of buckwheat protein is the presence of protease inhibitor. Considerable trypsin inhibitor activity was found in various buckwheat products in this experiment (Fig. 1). In addition, this study also indicated that a substantial amount of tannin was present in cooked buckwheat products (Fig. 1). We have shown that tannic acid exhibits noncompetitive inhibition against trypsin (Ikeda et al 1986). Others have shown that tannic substances inhibit the gastrointestinal absorption of dietary protein (Butler et al 1984, Reddy et al 1985). Much lower levels of trypsin inhibitor activity and of tannin were found in soybean products and wheat flour (Fig. 1). Experiments with human subjects have shown that wheat and various soybean products such as *yuba* and *nattō* exhibit higher protein digestibility than buckwheat products (Resources Council 1981). The present in vitro findings generally agree with those studies.

We conclude that there are two factors that contribute to the poor availability of buckwheat protein. First, the buckwheat-containing samples had high levels of the endogenous antinutrients, protease inhibitor and tannin, which persisted even after cooking (Fig. 1). Secondly, there was a significant difference in susceptibility of proteolytic action among the buckwheat protein

TABLE II
Susceptibility of Buckwheat Proteins and Other Food Proteins to Proteolytic Action

Substrate Protein	Enzyme Activity[a] (μg of peptide released/min per milliliter of digesta)	
	Pepsin	Trypsin
Buckwheat		
albumin	15.6 ± 2.3 c	13.9 ± 3.7 de
globulin	32.8 ± 1.0 a	27.5 ± 2.5 a
prolamin	2.9 ± 0.8 e	7.4 ± 2.1 e
glutelin	19.8 ± 1.3 b	24.0 ± 1.7 b
Wheat		
gliadin	19.2 ± 1.8 bc	18.0 ± 2.7 cd
glutenin	7.9 ± 1.3 d	22.8 ± 3.0 b
Soybean		
acid-precipitated protein	20.7 ± 2.2 b	18.2 ± 1.4 cd

[a] Values are means ± SD ($n = 4$). Means within the same column that are not followed by the same letter are significantly different at $P < 0.05$.

TABLE III
Pepsin Plus Pancreatin Digestibility of Various Buckwheat Products

Products Tested	Protein Digestibility[a] (%)	
	Raw Sample	Cooked Sample[b]
Commercial noodle G	53.2 ± 3.9 b	62.8 ± 6.0 a*
Commercial noodle S	56.2 ± 5.6 ab	55.5 ± 5.8 ab
Commercial groats	32.8 ± 6.0 c	49.8 ± 8.0 b*
Noodle prepared from whole flour	60.9 ± 3.9 a	59.0 ± 5.2 ab
Noodle prepared from inner-layer flour[c]	20.0 ± 3.4 d	12.9 ± 6.7 c

[a] Values are means ± SD ($n = 4$). Means within the same column that are not followed by the same letter are significantly different at $P < 0.05$.
[b] Means with asterisks are significantly different from the raw sample in the same row at $P < 0.05$.
[c] The noodle was prepared from a flour combined from two parts of the first flour and one part of the superior flour.
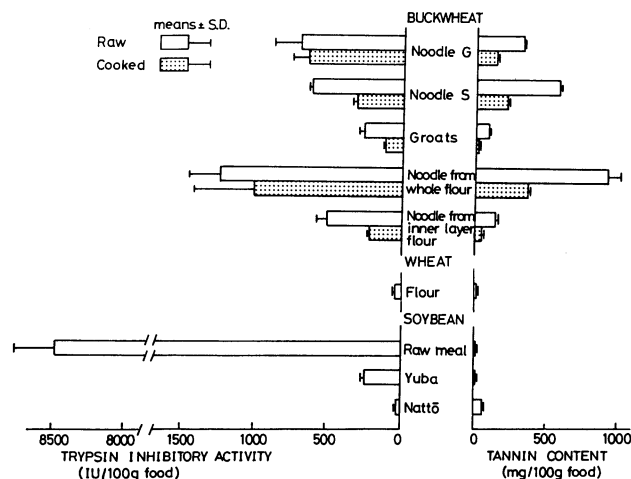

Fig. 1. Trypsin inhibitory activity and tannin content in various buckwheat, wheat, and soybean products.

fractions (Table II). The prolamin and albumin were less digestible with proteases than the globulin and glutelin fractions. The composition of protein in buckwheat products, as found in the noodle made from inner-layer flour (Tables I and III), substantially affected its digestibility. Thus the protein digestibility of buckwheat is affected by the inhibitory potency of the antinutrients and the susceptibility of protein per se to proteolytic action.

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