

Effect on Buckwheat Protein Quality of Seed Germination and Changes in Trypsin Inhibitor Content

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ABSTRACT

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The effects of germination on trypsin inhibitor activity and on in vitro protein digestibility of buckwheat seed were studied. Trypsin inhibitor activity decreased substantially as germination proceeded. The susceptibility to peptic and pancreatic hydrolysis of the proteins of the

buckwheat seedlings was much greater than that of the ungerminated seeds. These findings indicate that the advantage of decreased trypsin inhibitory activity during germination may reflect better protein quality of the buckwheat seed.

Buckwheat (*Fagopyrum esculentum* Moench) is an important source of dietary protein in some areas of the world. Most buckwheat seed is milled for human consumption. Noodles made from buckwheat flour-water dough have long been popular in Japan. Buckwheat seedlings are also available for human consumption. Although the amino acid profile suggests that buckwheat protein may be of high biological value (Pomeranz and Robbins 1972, Pomeranz et al 1975), availability of the protein in animals is relatively low (Farrell 1978, Eggum et al 1981, Thacker et al 1983). The presence of some antinutritional factors in buckwheat seed would seem to affect the nutritive value. We present evidence for the occurrence of proteinaceous trypsin inhibitors in buckwheat seed (Ikeda and Kusano 1978) and discuss some properties of the purified inhibitors (Ikeda and Kusano 1983a).

Protease inhibitors, which are widely distributed in edible seeds, have been extensively investigated because of the adverse effects they may have on human nutrition. Inactivation or elimination of the protease inhibitors, without impairing the protein quality of edible seeds, is of particular interest to those concerned with public health and safety. In this connection, germination improves the nutritive value of several edible seeds. For example, when horse gram and moth beans are germinated, their protein digestibility is improved, and their trypsin-inhibitory activity decreases (Subbulakshmi et al 1976). Germination also improves the nutritive value of soybeans (Everson et al 1944) but does not substantially change the level of the trypsin inhibitor (Collins and Sanders 1976). In addition, the biological value of field pea proteins declines during germination without any significant change in the content of trypsin inhibitor (Chattopadhyay and Banerjee 1953). Thus, the effect of germination on the nutritive quality of edible seeds is not clear-cut. Moreover, the correlation between the nutritive value of edible seeds and their trypsin inhibitor content inherent during germination is also the subject of much controversy (Liener and Kakade 1980).

The present study was undertaken to clarify the changes of the buckwheat seed trypsin inhibitors during germination and to determine the effect of germination on the protein quality of buckwheat seed.

MATERIALS AND METHODS

Materials

Mature common buckwheat seeds (Japanese type), harvested in October, 1982, were obtained and stored at 4°C until used. Enzymes used in this study were obtained from the following companies: trypsin E.C.3.4.21.4; 2X crystalline, from bovine pancreas, 12,000 BAEE units/mg protein, Sigma Chemicals Co.; pepsin (E.C.3.4.23.1; from swine stomach mucosa), P-L Biochemical Inc.; and pancreatin NF, Difco Laboratories. *N*^ε-Benzoyl-D,L-arginine-*p*-nitroanilide (BAPNA) was obtained from

Boehringer Mannheim Co. Polybuffer exchanger PBE 94 and polybuffer PB 74 were products of Pharmacia Fine Chemicals. All other chemicals were of analytical grade.

Assay of Enzymatic and Inhibitory Activities

The activity of trypsin towards BAPNA as the substrate was determined according to the procedure of Erlanger et al (1961). The inhibitory activity against trypsin was estimated from the residual enzymatic activity as described previously (Ikeda and Kusano 1978, 1983b). One unit of inhibitory activity is defined as the amount of inhibitor that produces half the inhibition of 1 μg of trypsin.

Germination

Buckwheat seeds were immersed in 2% sodium hypochlorite and then soaked with deionized water. The seeds were spread thinly on trays, sprayed with deionized water, and kept at 30°C for germination studies. The seedlings were homogenized in a mortar with 10 volumes of 0.2M sodium chloride. The homogenate was stirred for 1 hr at 4°C and centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatants obtained were used for their trypsin-inhibitory activity and proteins.

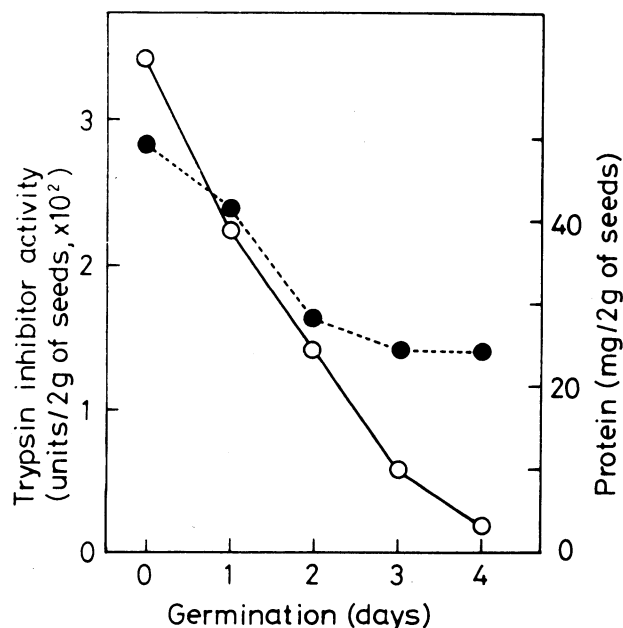


Fig. 1. Changes in the trypsin-inhibitory activity and in the 0.2M NaCl-soluble protein content of the aqueous extracts of buckwheat seeds during germination. —○— = trypsin-inhibitory activity, and - - -●- - - = 0.2M NaCl-soluble protein.

In Vitro Proteolytic Digestion

Proteolytic digestion was performed according to the procedure of Akesson and Stahmann (1964) but with a slight modification. Resting seeds or seedlings were homogenized in a mortar with 10-fold volumes of 0.1 M HCl. A pepsin solution was added to the homogenate. The ratio of enzyme to protein was 1:50. After incubation, the digestion mixture was adjusted to pH 8.0 with 2M Tris-HCl buffer. A pancreatin solution was then added to the digestion mixture. The ratio of enzyme to protein was 1:50. Pancreatic digestion was performed at 37°C for 20 hr. Sodium azide was added to the digestion medium to a final concentration of 0.025% to prevent growth of microorganisms. Immediately after digestion, the unhydrolyzed protein fraction was precipitated by the addition of trichloroacetic acid (TCA) in a final concentration of 6.6%. The suspension obtained was centrifuged at 3,000 × g for 10

min. The TCA-soluble fraction was assayed for nitrogen content by the micro-Kjeldahl method (AOAC 1980). Percent protein hydrolysis was calculated from the ratio of the TCA-soluble nitrogen to the total nitrogen of the seeds or seedlings.

Chromatofocusing

Thirty kernels of the seeds or seedlings were homogenized and were then extracted with 10 volumes of 0.2M NaCl. After extraction, the suspension was clarified by centrifugation (10,000 × g, 15 min). The aqueous extract was applied to a column of polybuffer exchanger PBE 94. Chromatofocusing on polybuffer exchanger PBE 94 was performed as the following procedure: the aqueous extracts from the resting seeds or the seedlings were applied to a column of polybuffer exchanger PBE 94 (35 × 1.0 cm), pre-equilibrated against 25 mM Tris-HCl buffer (pH 7.2).

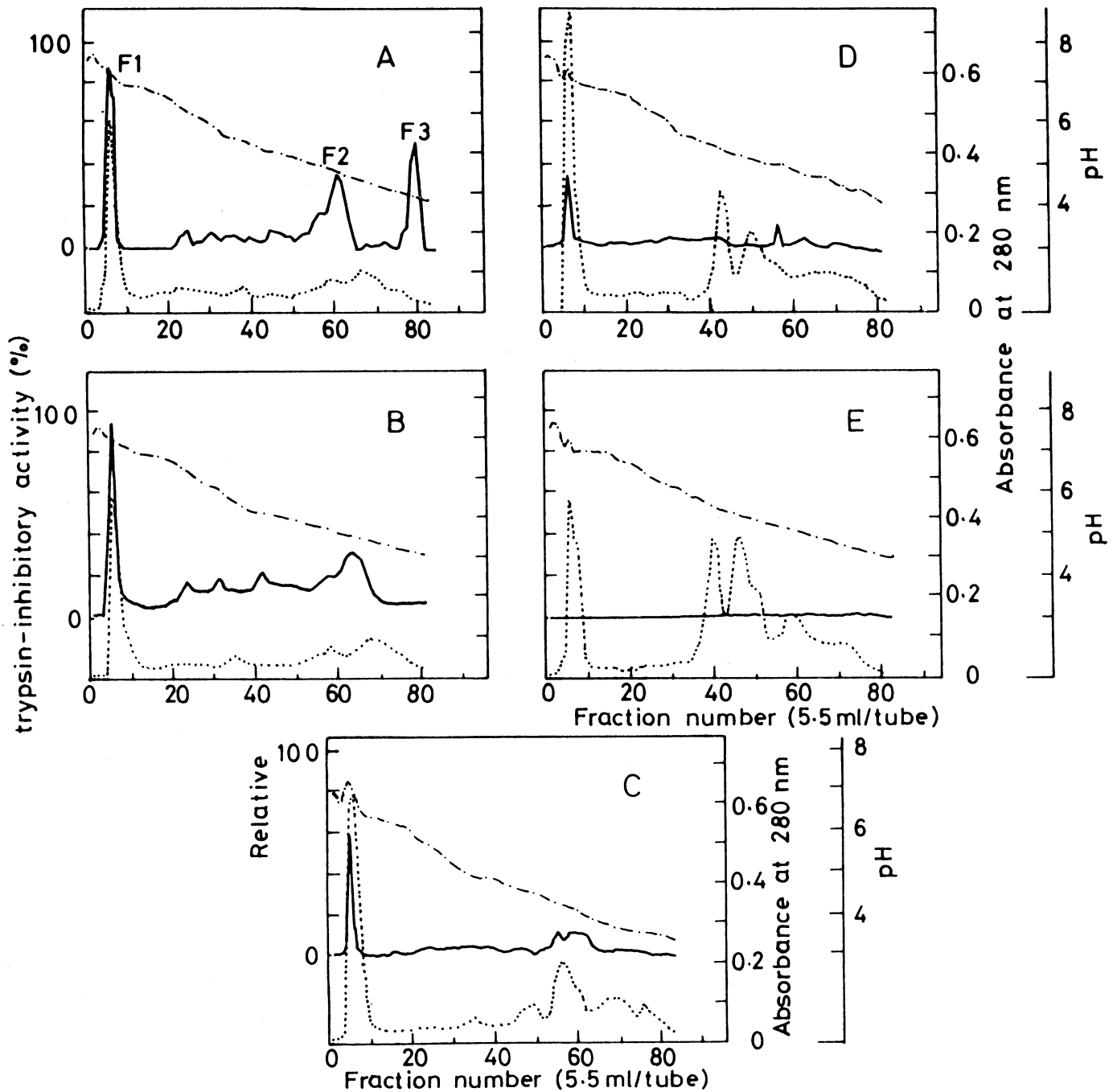


Fig. 2. Chromatofocusing of the aqueous extracts from the resting seeds or the seedlings on a polybuffer exchanger PBE 94 column. A, resting seeds; B, seedlings on the first day after germination; C, seedlings on the second day; D, seedlings on the third day; E, seedlings on the fourth day. — = trypsin-inhibitory activity, ····· = absorbance at 280 nm, and - - - = pH.

Polybuffer 74 at pH 4.0 (diluted from the stock solution), 5 ml, was applied to the column, followed by 5 ml of the aqueous extracts. Finally, polybuffer 74, pH 4.0, was added at a flow rate of 20 ml/hr.

Protein Estimation

The 0.2M NaCl-soluble protein content present in the aqueous extracts of the seeds or seedlings was estimated by the method of Lowry et al (1951). All other nitrogen analysis in this study was performed by the micro-Kjeldahl method (AOAC 1980).

RESULTS AND DISCUSSION

Changes in Trypsin Inhibitory Activities and Protein During Germination

Figure 1 shows the changes both in the trypsin-inhibitory activity and in the 0.2M NaCl-soluble protein content present in buckwheat seeds during germination. The trypsin-inhibitory activity rapidly decreased after germination. On the fourth day of germination, the seedlings had little or no detectable amounts of the trypsin inhibitor. This finding generally agrees with our preliminary report for which we used the heat-treated aqueous extract of buckwheat flour (Ikeda and Kusano 1978). There was also a rapid decrease in the NaCl-soluble protein content in the early stage of germination, but this was followed by a slight decline in the content of the soluble proteins. On the other hand, the total nitrogen in the seeds remained unchanged (dry weight basis) during germination (data not shown).

The trypsin inhibitor in buckwheat seeds consists of several active components on chromatography and electrophoresis (Ikeda and Kusano 1983a). Experiments were then run to determine what components were mainly responsible for the observed rapid decline in the antitryptic activity in the early stage of germination. The elution profiles on chromatofocusing of the aqueous extracts from the resting seeds or the seedlings are presented in Fig. 2. Three main inhibitor components, together with several minor components, were found in the aqueous extract of the ungerminated seeds (Fig. 2A). The three main inhibitor components were designated as the trypsin inhibitors F1, F2, and F3, according to their order of elution. As germination proceeded, the antitryptic activities of the inhibitors F2 and F3 declined rapidly and substantially disappeared on the second day of germination (Fig. 2A-E). On the other hand, the antitryptic activity of the inhibitor F1 declined slowly and was not detectable on the fourth day of germination (Fig. 2A-E).

Figure 2 also shows a drastic alteration of the seed proteins during germination. The seed germination resulted in the appearance of the three protein peak fractions eluted at pH 5.6, 5.3, and 5.0 (Fig. 2E). We are uncertain whether or not the observed changes in the protein components enhance the biological availability of the buckwheat seed proteins.

Protein Quality of the Resting Seeds and the Seedlings

Experiments were performed to evaluate the quality of proteins in the resting seeds and the fourth-day seedlings of buckwheat through *in vitro* proteolytic digestion. The susceptibility to peptic and pancreatic hydrolysis of the proteins in the seedlings was found to be significantly greater than that in the resting seeds. Analysis of nitrogen in the digestion mixture indicated that the percentage of the TCA-soluble nitrogen to the total nitrogen of the seedlings was 4.2 ± 0.5 times (means \pm SD, $n = 5$) as high as that of the resting seeds. This shows that germination improves the protein digestibility of buckwheat seeds and significantly decreases the inherent antitryptic activity.

The amino acid composition of the buckwheat seedling proteins differs from that of the resting seed proteins (*unpublished data*). The resting seed proteins were high in the acidic amino acids leucine, arginine, and lysine, and lower in methionine, tyrosine, and histidine. On the other hand, a high level of arginine, glutamic acid, lysine, valine, and phenylalanine and a low level of methionine, threonine, and isoleucine characterized the buckwheat seedling proteins.

Generally, the nutritive value of dietary proteins depends on the biological availability of their amino acids. The relative proportions of the constituent amino acids and the presence of antinutritional substances, including protease inhibitors, also affect the nutritive value of edible seeds. These factors can lead to impaired digestion, absorption, or utilization of dietary proteins.

Although buckwheat seeds are a source of well-balanced proteins (Pomeranz and Robbins 1972, Pomeranz et al 1975), their protein availability in animals is relatively low (Farrell 1978, Eggum et al 1981, Thacker et al 1983). The most likely candidate from our experimental works for the substance responsible for the poor protein availability was the proteinaceous trypsin inhibitor (Ikeda and Kusano, 1978 and 1983a). In buckwheat seeds, they nearly disappear during seed germination. Germination was also found to improve the *in vitro* protein digestibility of the seeds. These findings suggest that the advantage of decreased trypsin-inhibitory activity during germination may reflect an improvement in the protein quality of the seed proteins.

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