研究ノート

Composition and Pepsin Digestibility of Proteins Extracted from Microground Particles in Cooked Bean Paste (*Ann*)

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Abstract

In Japan, common beans are often processed to a cooked bean paste (*Ann*) for confectionary and bakery use. It is known that processing the beans to *Ann* paste causes formation of cell particles called *Ann* particles, which are highly resistant to human digestion. In this study, we microground freeze-dried *Ann* paste by using a jet mill; subsequently, proteins extracted from the cell particles were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and pepsin assays. The micrographs of *Ann* particles showed that they had been thoroughly disintegrated by the microgrinding process. The SDS-PAGE profile indicated that several polypeptides, including the acidic and the basic subunits of legumin, had been extracted from the microground *Ann* particles. The microground *Ann* particles had a low concentration of phaseolin, which is one of the major proteins found in common beans and is present in higher quantities in bean flour. Among the proteins, polypeptides that were presumably derived from legumin subunits showed significant resistance to pepsin. These results suggested that the traditional method of bean paste cooking is associated with the formation of *Ann* particles that trap pepsin-resistant polypeptides.

Key words: *Ann* particle, white common bean, legumin, pepsin-resistant protein, micro grinding

Abbreviations: SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Introduction

Among a wide variety of plant-derived foods, beans are one of the most important sources of human nutrition worldwide. Beans have a high protein content, low fat and sodium content, and are a good source of fiber, minerals, vitamins, and polyphenol antioxidants. However, in general, bean proteins have a low nutritional value because of their lower digestibility and deficiency in one or more essential amino acids[1, 2]. It is also known that heat processing of whole beans causes formation of cellular particles that are resistant to digestion in humans[3].

Previous studies have shown that phaseolin, the major storage protein in many common beans, is highly resistant to gastric enzymes in its native form, although its digestibility can be remarkably improved by prior heating[4, 5]. In our previous studies, we found that the basic subunit of legumin remained highly tolerant to pepsin digestion even after extensive heat processing and several enzymatic treatments[6, 7]. Legumin, one of the major storage proteins found in many legumes, had not been detected in common beans for a long time[8]. The tolerance of proteins to pepsin digestion could cause concerns because it is considered to reflect the possible risk of allergenicity[9]. In our previous study, the basic subunit of legumin was not detected in

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bean pastes prepared from whole beans, the cooking of which generates *Ann* particles during heat processing[10]. To elucidate the protein association of *Ann* particles, we prepared *Ann* paste from common beans through traditional Japanese cooking procedures, followed by freeze-drying and microgrinding of the paste. Then, proteins were extracted from the *Ann* particles and were examined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and pepsin digestion assays.

Materials and Methods

Preparation and microgrinding of freeze-dried *Ann* paste

Ann paste was prepared from the white common bean (*Phaseolus vulgaris* L. cv. Yukitebou), as described earlier[10]. Freeze-dried *Ann*-paste powder was microground using a jet mill (CO-JET system α, Seishin, Tokyo).

SDS-PAGE analysis

For SDS-PAGE analysis, proteins were extracted from 50 mg of each freeze-dried *Ann*-paste and its microground flour by using 500 μL sample buffer (62.5 mM Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromophenol blue) and boiled for 5 min. SDS-PAGE samples (10 μL), as well as extracts from bean flour (0.5 μg protein), were loaded onto a 5% to 20% polyacrylamide precast gel (NPG 520, Atto) and electrophoresed at 20 mA for 90 min. The resultant gel was stained with Coomassie Brilliant Blue (CBB R-250).

Pepsin digestion assay

Distilled water was added at 10 times (v/w) to 3 g of flour. The mixture was then homogenized using a Hiscotron homogenizer (NS-50, Nichi-On) for 1 min at 10,000 rpm. After centrifugation at 8,000 g for 20 min, the supernatant was collected and the protein concentration of the extracts was estimated using the microassay procedure with a protein assay reagent (BioRad). *In vitro* pepsin digestibility of the extracted protein was examined by using the method put forth by Astwood *et al*. (1996). The protein extracts were incubated in simulated gastric fluid (SGF, 0.32% pepsin from a porcine stomach [3300 U/mg, Wako], 30 mM NaCl, pH 1.2) for 0, 0.25, 1, 2, 4, 8, 15, or 60 min. SDS-PAGE samples were loaded onto a 5% to 20% polyacrylamide precast gel (Atto NPG 520) and electrophoresed at 20 mA for 90 min. The resultant gel was stained with silver staining methods (Silver Stain Plus, BioRad).

Results and Discussion

Protein composition of traditionally cooked and microground *Ann* paste

Ann paste prepared from common beans was found to be composed of particles derived from cotyledonary cells, which is similar to the findings of our previous study[10]. Since the preparation procedure for *Ann* paste included several rinsing steps, the background of the micrograph was clear of cell debris (Fig. 1 A). The *Ann* particles appeared to be thoroughly disintegrated by the microgrinding process, and their internal material was dispersed as shown in Fig. 1 B.

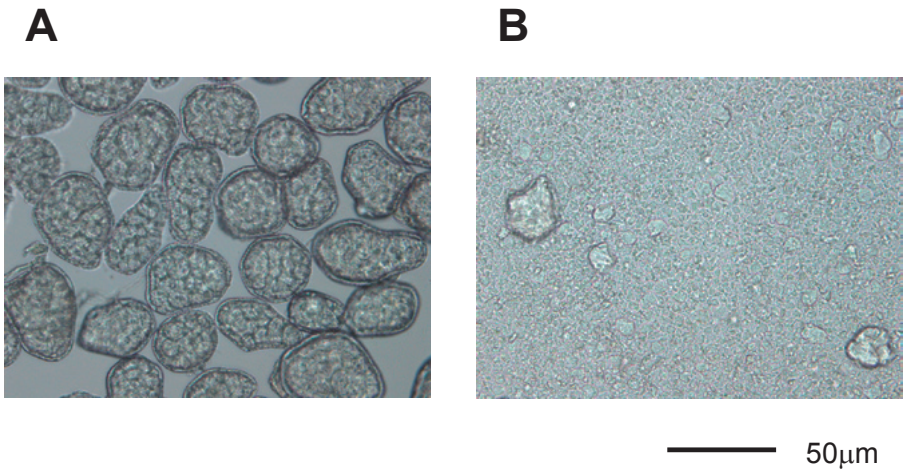

Fig. 1 Micrographs of *Ann*-paste preparation (left) and microground *Ann* particles (Right)

The SDS-PAGE profile (Fig. 2) indicated that the protein composition of microground *Ann* particles (*Ann* MP) was different from that of bean flour and the *Ann*-paste preparation (*Ann*). While small amounts of protein were extracted from intact *Ann* particles, several evident bands, including the ones representing legumin, were observed in the extract from microground *Ann* particles. Compared to bean flour, the microground *Ann* particles had lower concentrations of phaseolin and lectin, which are major proteins in common beans. The polypeptide with a molecular weight of 50 kDa was found to be the acidic subunit of legumin, determined with the help of previous studies[6, 8].

As mentioned earlier, legumin had not been detected in bean pastes prepared from whole beans, the cooking of which generates *Ann* particles during heat processing[10]. In the current study, acidic and basic subunits of legumin and several other polypeptides associated with *Ann* particles were detected. Noah *et al*. (1998) reported that approximately 17% of starch in the cotyledonary cell particles of cooked beans is a resistant type of starch and remains in the human ileum 3 h after ingestion[3]. Hence, we think that considerable amounts of proteins rich in legumin are "trapped" within the *Ann* particles, which are encapsulated by pepsin-resistant carbohydrates that are formed during the cooking procedure.

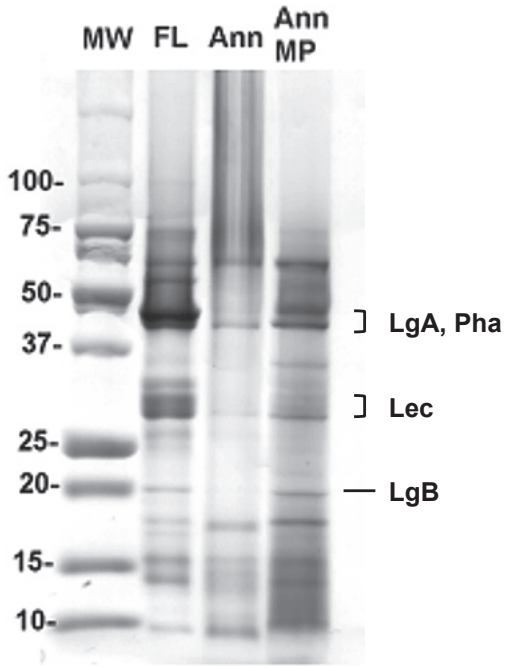

Fig. 2 SDS-PAGE profile of bean flour, *Ann* paste, and microground *Ann*-paste powder

M: molecular weight marker; FL, bean flour; Ann, *Ann* paste preparation; *Ann* MP, microground *Ann* particles.
The bands marked Pha, Lec, LgA and LgB indicate phaseolin, lectin, and acidic and basic subunits of legumin, respectively.

Pepsin digestibility of proteins in the microground powder of *Ann*-paste particles

Proteins extracted from the microground *Ann* particles were analyzed by pepsin-digestibility assays (Fig. 3). Two bands (indicated by * and ** in Fig. 3) were found to be remarkably tolerant to the pepsin digestion. Although the use of a molecular weight marker different from the one used in SDS-PAGE caused discrepancies in the estimation of molecular weights, the polypeptides with higher molecular weight (*) were estimated to be those derived from storage proteins (phaseolin and the acidic subunit of legumin) in common beans, judging from preliminary experiments (data not shown). Since it is known that phaseolin becomes susceptible to digestive enzymes after heat processing[4, 5], the resistant polypeptide was ascertained to be the acidic subunit of legumin. The smaller polypeptide (**), which is believed to be the basic subunit of legumin, showed

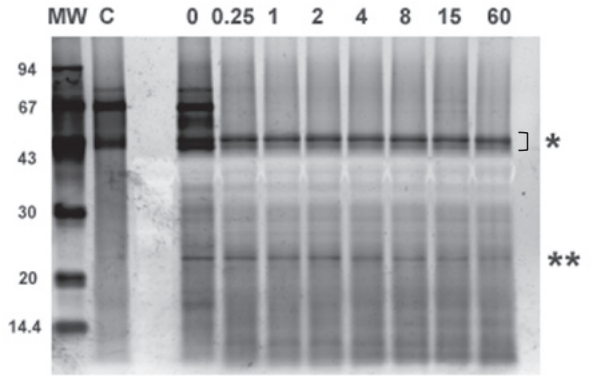

Fig. 3 Pepsin-digestibility assay results for proteins extracted from microground *Ann*-paste powder

MW, molecular weight marker; C, microground *Ann* particles without enzymatic treatment. The protein extracts were incubated in for 0, 0.25, 1, 2, 4, 8, 15, or 60 min. The marks on the right indicate phaseolin and the acidic subunit of legmin (*) and basic subunits of legumin (**).

significant tolerance to pepsin digestion.

It is considered that substantial nutritional losses occur while cooking bean paste because of the formation of indigestible granules[3]. It has also been presumed that bean paste cooking could prevent exposure to a pepsin-resistant protein, which is a possible allergen in common beans, during ingestion[10]. The results of the current study supported this in that they indicated that proteins, including the pepsin-resistant legumin subunit associated with *Ann* particles, are presumably trapped within the particles. These proteins likely aggregate or interact with other components during the extensive heat processing[11, 12].

Common beans are one of the most important sources of human nutrition, and their novel health benefits are drawing attention[13]. Further studies on the behavior and interaction of bean proteins and their other nutritional components such as carbohydrates would be necessary for more efficient use of common beans as food.

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References

1) Porzucek, H., Larsson-Raznikiewicz, M. and Miroslawa Klepacka. (1991). *In vitro* protein digestibility of flours and protein isolates form seeds of some Leguminous plants. *Swedish J. Agric. Res.*, **21**, 49-53.

2) Friedman, M. (1996). Nutritional value of proteins from different food sources: a review. *J. Agric. Food. Chem.*, **44**, 6-29.

3) Noah, L., Guillon, F., Bouchet, B., Bulleon, A., Molis, C., Gratas, M., Champ, M. (1998). Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L.) in healthy humans. *J. Nutri.*, **128**, 977-985.

4) Despande, S.S. and Nielsen, S.S. (1987). *In Vitro* enzymatic hydrolysis of phaseolin, the major storage protein of *Phaseolus vulgaris L. Journal of Food Science.*, **52**, 1326-1329.

5) Nielsen, S. S., Deshpande, S. S., Hermodson, M. A. and Scott, M. P. (1988). Comparative digestibility of legume storage proteins. *J. Agric. Food Chem.*, **36**, 896-902.

6) Momma, M. (2006). A pepsin resistant 20 kDa protein found in red kidney bean (*Phaseolus vulgaris* L.) was identified as basic subunit of legumin. *Biosci. Biotech. Biochem.*, **70**, 3058-3061.

7) Momma, M. (2007). *In vitro* protein digestibility of flours and cooked pastes prepared from white common bean (*Phaseolus vulgaris* L. cv. Yukitebou). *Food Science and Technology Research*, **13**, 166-168.

8) Muhling, M., Gilroy, J. and Croy, Ronald R.D. (1997). Legumin Proteins from Seeds of *Phaseolus Vulgaris L. J.Plant Physiol.*, **150**, 489-492.

9) Astwood, J. D., Leach, J. N. and Fuchs, R. L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotech.*, **14**, 1269-1273.

10) Momma, M. Sasaki, K., Ohba, K., Isobe, S. (2012). Effect of cooking procedure and roasting on the protein composition and *in vitro* digestibility of common bean proteins. *Rep. Nat'l Food Res. Inst.*, **76**, 17-22.

11) Carbonaro, M., Nicoli, S. and Musci, G.(1999). Heat-induced Aggregation of Phaseolus vulgaris L. Proteins: An electron spin resonance study. *J. Agric. Food Chem.*, **47**, 2188-2192.

12) Venkatachalam, M. and Sathe, S. K. (2003). Phaseolin *in vitro* pepsin digestibility: Role of acids and phenolic compounds. *J. Agric. Food Chem.*, **51**, 3466-3472.

13) Gallegos-Infante, J.-A., Bello-Perez, A. B., Rocha-Guzmann. E., Gonzalez-Laredo, R. F. and Avila-Ontiveros, M. (2010). Effect of the addition of common bean (*Phaseolus vulgaris* L.) flour on the *in vitro* digestibility of starch and indigestible carbohydrates in spaghetti. *J. Food Sci.*, **75**, 151-156.

いんげん豆あん粒子中のたんぱく質組成ならびにそのペプシン消化耐性について

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要　約

いんげん豆はあん等，全粒で加熱加工し，菓子やパン等に利用されることが多い．いんげん豆を全粒加熱したとき，細胞由来の難消化性の粒子が形成されるとともにペプシン抵抗性のレグミン塩基性サブユニットが検出されなくなることが明らかとなっている．本報告では，凍結乾燥したあんをジェットミルで微粉砕し，内部に含有されるたんぱく質組成を調べた．あん粒子微粉砕物からはレグミン塩基性サブユニットを含む数種類以上のポリペプチドが抽出され，その組成はいんげん豆たんぱく質とは異なり，主要たんぱく質であるファゼオリンの含有量が低かった．あん粒子から抽出したたんぱく質のペプシン消化性を測定したところレグミンサブユニットに由来すると見られるポリペプチドが高いペプシン抵抗性を示した．以上のことから，いんげん豆を全粒で加工するとペプシン消化耐性たんぱく質を包含する粒子が形成されることが示された．