研究ノート

Effect of cooking procedure and roasting on the protein composition and *in vitro* digestibility of common bean proteins

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Abstract

We examined the effect of traditional nama-ann preparation and paste cooking from whole beans and bean flour with /without enzymatic treatment on the formation of cell granules and protein composition in order to improve the protein availability and quality of common beans. We found that a substantial amount of protein and other nutritional elements was lost in the traditional whole bean cooking procedure and that it could remove the basic subunit of legumin, a pepsin-resistant protein, from the available protein fraction. Roasting before milling improved the pepsin digestibility of phaseolin; trace amounts of phaseolin, lectin, and 15-kDa polypeptide remained tolerant to digestion. Legumin, a pepsin-resistant protein in common beans, was not detected in roasted bean flour.

Key words: common bean, legumin, pepsin-resistant protein, phaseolin, red kidney bean, roasting
Abbreviations: SGF, simulated gastric fluid

Introduction

Among a variety of plants foods, beans are one of the important resources for global human nutrition. They are high in protein, low in fat and sodium, and a good source of fiber, certain minerals, vitamins, and antioxidant polyphenols. Bean proteins in general, however, have a lower nutritional value due to their lower digestibility and deficiency in one or more essential amino acids (Porzucek *et al.,* 1991; Friedman, 1996). It is also known that heat processing of whole beans causes formation of cell granules which are resistant to human digestion (Noah *et al.,* 1998).

The digestibility of bean proteins has been studied extensively. It has been known that the digestibility of phaseolin, a major protein in common beans, is remarkably improved by heat processing, although it is highly resistant to gastric enzymes in the native state (Despande and Nielsen, 1987; Nielsen *et al.,* 1988). In contrast, we found that the basic subunit of legumin, a storage protein in beans, remained highly tolerant to pepsin even after extensive heating processing and several enzymatic treatments (Momma, 2006; Momma, 2007). The tolerance of proteins to digestive enzymes is also considered to reflect the risk of allergenicity, as it has been suggested that resistance to pepsin digestion is a significant and valid parameter that distinguishes a food allergen from a nonallergen (Astwood *et al.,* 1996).

In this study, first, we surveyed the protein composition of a traditionally cooked common bean paste (nama-ann). Then, we examined the effects of paste processing

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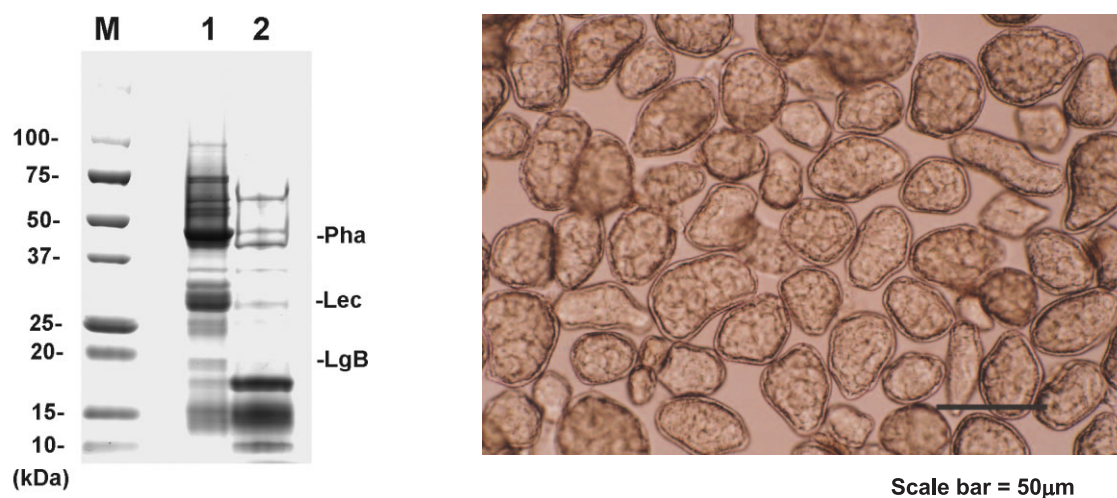e-mail: michiko@affrc.go.jp

Fig.1 Protein composition (left) and micrograph (right) of nama-ann prepared from white common beans

M: Molecular weight marker; lane 1, bean flour; lane 2, nama-ann extract. Marks on the right indicate phaseolin (Pha), lectin (Lec), and the basic subunit of legumin (LgB), respectively.

from bean flour and whole beans with/without enzymatic treatment and roasting of beans before flouring on the protein composition and behavior of pepsin-resistant proteins.

Materials and Methods

Preparation of traditional bean paste (nama-ann)

Nama-ann, a traditional bean paste, was prepared at the Tokachi Area Regional Food Processing Technology Center as follows. Beans were soaked overnight and boiled for 15 min. After cooling them by pouring water, beans were boiled again for 1 h, kept there for another 1 h, and strained through a 60-mesh to remove the hull. The process of washing with water and collecting the precipitated nama-ann was repeated 3 times. Nama-ann was put in a cloth bag, pressed to remove excess water, and freeze dried. Proteins in the freeze-dried powder or flour (50 mg) were extracted by adding 500 μL sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and boiling for 5 min.

Preparation of whole bean/flour paste

Whole beans (20 g) were soaked in 60 mL of water

overnight. Intact beans were milled, and 20 g of flour was mixed with 60 mL of water. The mixture was heated at 120℃ for 4 min by using an autoclave. After cooling down to 50℃ to 53℃, 100 mg of crude α-amylase (EC 3.2.1.1. from *Bacillus subtilis*, Wako 013-03732) was added, the mixture was kept at 50℃ for 1 h, and then boiled for 5 min. Control samples were incubated at the same temperature without adding amylase. Proteins were extracted from 200 mg of the pastes with 0.2 mL of SDS-PAGE sample buffer and analyzed by SDS-PAGE.

Roasting of red kidney beans

Red kidney beans (*Phaseolus vulgaris* cv. Taisho kintoki) were supplied by the Bean Fund Association of Japan. About 60 kg of beans were roasted at 152℃ for 20 min at a manufacture's plant in the Tokachi area of Hokkaido. Roasted beans were crushed by using a twin-screwed crusher (Suehiro EPM, Yokkaichi, Japan) at 200 rpm and subsequently milled by using a hummer mill (Type SK1, Retsch Gmbh, Haan, Germany) with a screen of 250 μm.

Pepsin digestion assay
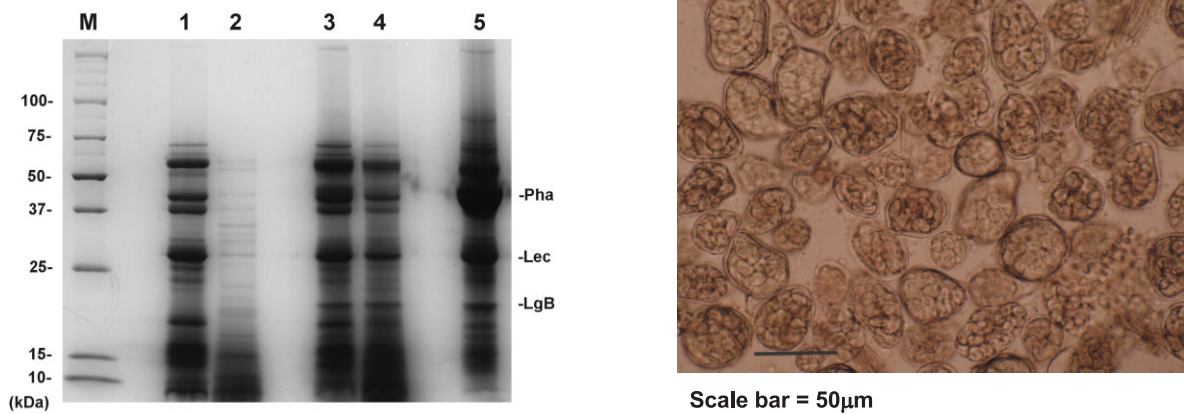
Distilled water was added at 10 times (v/w) of the

Fig.2 Protein composition (left) and micrograph (right) of cooked common bean pastes

Lane 1, whole bean paste without enzymatic treatment; lane 2, whole bean paste digested with crude amylase; lane 3, flour paste without enzymatic treatment; lane 4, flour paste with crude amylase digestion; lane 5, bean flour (raw). Marks on the right indicate phaseolin (Pha), lectin (Lec), and the basic subunit of legumin (LgB), respectively.

flour. The mixture was then homogenized by using a His-cotron homogenizer (NS-50, Nichi-On) for 1 min at 10,000 rpm. After centrifugation at 8,000g for 20 min, supernatants were collected. The protein concentration of the extracts was estimated by employing a microassay procedure using a protein assay reagent (BioRad). *In vitro* pepsin digestibility of the extracted protein was examined by using the method of Astwood *et al.* (1996). SDS-PAGE samples were loaded onto a 5% to 20% polyacrylamide precast gel (Atto NPG 520) and subjected to electrophoresis at 20 mA for 90 min. The resultant gel was stained with Coomassie brilliant blue (CBB R-250).

Results and Discussion

Protein composition of traditionally cooked bean paste (nama-ann)

White common beans (*Phaseolus vulgaris* L. cv. Yukitebou) were used for the nama-ann preparation since it is the most popular material for nama-ann preparation in Japanese confectionery. The protein composition and a micrograph (right) of nama-ann prepared from white common beans are shown in Fig. 1. As shown in the micrograph, the nama-ann paste prepared from whole beans was composed of cell granules associated with a proteinaceous matrix, derived from cotyledonary cells of beans (Noah *et al.,* 1998). Some debris was found, probably because of the washing procedure during the preparation. In

the SDS-PAGE pattern (Fig. 1, left), phaseolin was the major protein in common bean flour, and lectin and other minor proteins, including legumin, were also found in the flour. In contrast, the content of phaseolin, lectin, and other high-molecular weight polypeptides was very low, and legumin was not detected in the nama-ann paste (lane 2). Noah *et al.* (1998) reported that about 17% of starch in cotyledonary cell granules in cooked beans was resistant starch, and they showed that the cell granules remained in the human ileum at 3 h after ingestion. From these results, it was suggested that proteins and other substance in common beans were "trapped" in cell granules, which are covered by resistant carbohydrates during the cooking procedure. Though a more detailed examination is needed with regard to the composition of materials in the granules and their extractivity, the results implied that a substantial amount of proteins and other nutritional elements was lost in the traditional cooking procedure. On the other hand, it was also implied that the basic subunit of legumin, a pepsin-resistant protein and possible allergen in common bean, was removed or "trapped" in cell granules in the traditionally cooked nama-ann paste, which could be related to the low incidence of common bean allergies in Japan.

Protein composition of cooked pastes made of whole beans or bean flour

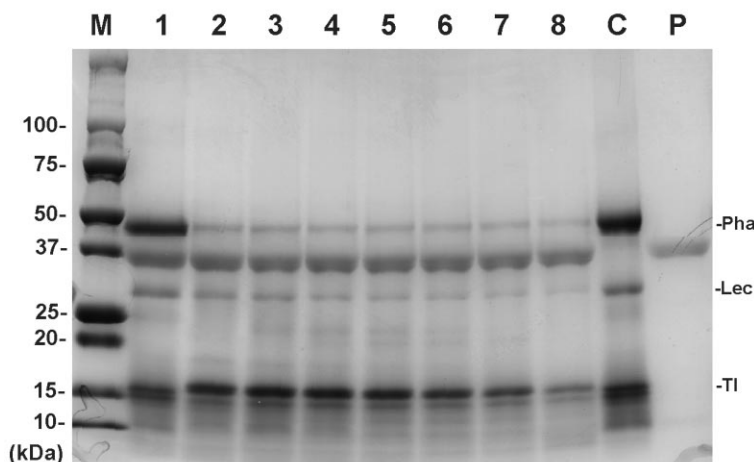In the previous studies, we found a 20-kDa polypep-

Fig. 3 Pepsin digestibility of proteins in roasted beans

M: Molecular weight marker. Protein extract of roasted beans was incubated in simulated gastric fluid (SGF, 0.32% pepsin from porcine stomach [3300 U/mg, Wako], 30 mM NaCl, pH 1.2) for 0, 0.25, 1, 2, 4, 8, 15, and 60 min, respectively (lanes 1-8). Extract and SGF were run alone (lanes marked C and P, respectively). Marks on the right indicate phaseolin (Pha), lectin (Lec), and trypsin inhibitor (TI), respectively.

tide in common beans, which was highly tolerant to pepsin digestion, and identified it to be the basic subunit of legumin (Momma, 2006, 2007). Since red kidney beans contain rather high amounts of legumin among the a series of bean samples examined, in this study, we used red kidney beans (*Phaseolus vulgaris* cv. Taisho kintoki) to examine the behavior of legumin and proteins in bean pastes prepared from bean flour and whole beans with/ without enzymatic treatments.

As shown in the micrograph (Fig. 2, right), the whole bean paste contained cell granules and a substantial amount of debris, because the paste preparation procedure did not include a washing process as compared to the nama-ann preparation. In the enzymatically processed whole bean paste (lane 2), almost no protein was detected, except for low-molecular-weight polypeptides. This result implied that most of the proteins outside the cell granules were degraded during paste preparation, probably by coexisting proteinases, and proteins in the cell granule were not extracted in the SDS-PAGE sample preparation.

In the result of SDS-PAGE, the paste prepared from bean flour appeared to contain more proteins than that prepared by cooking whole beans (Fig. 2, left, lanes 1 and 3). The amounts of phaseolin, lectin, and 60- and 37-kDa polypeptides in the flour paste decreased by incubation

with a commercial crude amylase in the course of paste cooking (lanes 3 and 4 in Fig. 2), probably because of coexisting proteolytic enzymes. In the pastes prepared from flour with/without enzymatic treatments, an evident band of the basic subunit of legumin was observed, while it was not detected in the paste made of whole beans (lane 1). The enzymatic treatment during paste cooking appeared not to affect the amount of the basic subunit of legumin.

These results indicated that the available protein content was increased by preparing the paste from flour, but this procedure resulted in the presence of legumin, a pepsin-resistant and possibly allergenic polypeptide, in the bean paste. The formation of cell granules in whole bean pastes might prevent the release of the basic subunit of legumin into the fraction of available proteins in the pastes.

Effect of roasting on the protein composition and pepsin digestibility of flour paste

In order to enhance the digestibility of bean proteins and prevent the presence of legumin in the products, we applied a roasting procedure to whole red kidney beans prior to flouring. Roasted bean flour contained phaseolin, lectin, and a trypsin inhibitor as major proteins (Fig. 3, lane 1). Minor proteins, including the pepsin-resistant le-

gumin subunit, were not observed in the SDS-PAGE profile. As explained above, though phaseolin, the most abundant protein in beans, is highly stable in the intact state, it is known that heating results in a drastic increase in its susceptibility to proteolytic enzymes (Despande and Nielsen, 1987). In the previous studies, we affirmed that the digestibility of phaseolin in red kidney beans was improved remarkably by boiling for 10 min with three times of water (Momma, 2006). The rapid decrease in phaseolin in the pepsin assay (Fig. 3) suggested that heat processing by roasting at 152℃ for 20 min, which was a similar procedure as the preparation of roasted soybean powder (Kinako), could improve the digestibility of phaseolin in red kidney beans. Notably, no evident band of the basic subunit of legumin was detected in the flour and pepsin digests after up to 60 min. It would be of interest to investigate whether legumin molecules interact with each other and/or other components (eg, starch molecules) during the roasting process.

However, it should be noted that there were trace amounts of phaseolin, lectin, and a 15-kDa polypeptide, which showed resistance to pepsin. Maleki *et al.* (2000) reported that Ara h1 and Ara h2, two major allergens in peanuts, were more resistant to digestion by gastrointestinal enzymes once they had undergone the Maillard reaction. Ara h1 belongs to the 7S globulin family (vicilin-type protein) in peanuts as phaseolin in common beans. We consider that similar mechanisms might be involved in the persistent resistance of phaseolin and other proteins in roasted red kidney beans. The amount of a 16-kDa polypeptide appeared to increase after the start of the pepsin assay (lane 2). This polypeptide, probably generated by cleavage of phaseolin (Venkatachalam and Sathe, 2003), also showed resistance to pepsin digestion. Further investigation is necessary to identify the optimal heating condition.

In conclusion, we found that a substantial amount of proteins and other nutritional elements was lost in the traditional whole bean cooking procedure, while it could remove the basic subunit of legumin, a pepsin-resistant protein, from the available protein fraction. From the experimental results of this paste preparation and roasting study, we concluded that it would be possible to enhance the availability of bean proteins and develop more innovative utilization processes by combining appropriate heating and flouring processing, though further quantitative analysis and optimization of heat treatment are necessary.

Acknowledgment

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いんげん豆加工処理が耐消化性粒子の形成，蛋白質組成ならびに試験管内消化性に与える影響について

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要　約

　いんげん豆蛋白質の有効利用を図るため，生餡加工，ペースト化における細胞粒子形成ならびに蛋白質組成への影響について調べたところ，いんげん豆の全粒加熱調理により耐消化性細胞粒子が形成され，蛋白質の利用効率が低下するが，一方でペプシンに耐性をもつレグミン塩基性サブユニットが除去されることが示された．いんげん豆主要蛋白質ファゼオリンの消化性を高めつつ，レグミンの混入を防ぐことを目的に，粉砕前に焙煎処理を行った．焙煎処理によってファゼオリンはペプシン分解を受けやすくなったが，痕跡量のファゼオリン，レクチンおよび15kDa タンパク質の残存が見られた．焙煎粉末ではペプシン耐性蛋白質であるレグミンのバンドは観察されなかった．本研究により，加工条件によって餡に含まれる蛋白質やアレルゲンの組成を制御できる可能性が示唆され，いんげん豆蛋白質の利用に関する有益な知見が得られた．