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A method for softening beans with coats

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Abstract

Conventionally cooked beans are not suitable for individuals with dysphagia, because the coats are still firm. Herein, we report a new method of softening beans with coats entirely. Dry soybeans sank in water (Material), Material boiled in water (W), W treated with enzyme (W+E), Material boiled in citric acid solution (CA), and CA treated with enzyme (CA+E) were prepared. The physical properties of W, W+E, CA and CA+E were within the range suitable for individuals with dysphagia. Especially, CA+E met the range of the severest dysphagia. In penetration test, the maximum force of CA+E was the least, and the coat of CA+E was smoothly collapsed, unlike that of W, W+E and CA. The coat of CA+E was the most fragile as seen in scanning electron microscopy. Distinctive constituents, thought to be fragments of the coat, were observed in CA by GC-MS, but were not in W. These results suggested that the softened beans including coats were obtained by boiling in citric solution and treating with enzyme, and could be provided with people with dysphagia. Boiling beans in citric acid solution was considered to decompose the coats partly and enabled enzyme to destroy the coats effectively.

Introduction

Beans are nutritious and contain nutrients such as carbohydrates, fiber and protein. In particular, soybeans are a unique source of the isoflavones genistein and diadzein, which have numerous biological functions (Anderson *et al.*, 1999; Wang *et al*., 2011) Also, the frequency of consumption of beans increased with age in both sexes in Japan (Iso *et al*., 2005). Thus, in hospitals and nursing homes, beans are often provided to elderly people due to the nutritious value of beans and the preference of these individuals.

Elderly people tend to have oral problems due to aging or diseases. Some of these individuals have difficulties in swallowing and report issues such as "food sticking" and "lump in the throat" during a meal, which are symptoms of dysphagia (Cook, 2008). Due to the decreased ability to swallow, residues of food remain in the throat of these individuals and can fall into the airway during breathing, which may cause aspiration pneumonia (Langmore *et al*., 1998). Therefore, food for dysphagic people has to be easy to crumble completely against food sticking and mis-swallowing.

Boiling beans is a generally used process to soften the beans. An enzyme-infusion method for controlling the firmness of food materials is also applied to white flower beans containing hard tissue (Shibata *et al*., 2006). With an autoclave at severe condition (115°C / 20 min), soft carioca beans were obtained (Siqueira *et al*., 2013). In other study, beans were treated with two or more enzymes, including cellulase, hemicellulase, and pectinase, to make beans soft (Hayashi, 2013). Also, it was reported that the softening rate constants of coats of soybeans were much smaller than those of the cotyledons during cooking (Yasui *et al*., 2014). From these results, it was considered that, even if the cotyledons of the beans became soft by the above methods, the coats still retained some firmness and remained in the mouth or throat during chewing, which was dangerous for people with dysphagia.

In this study, we developed a new method for softening whole beans including coats for elderly people and patients with dysphagia, and investigated the softening process. Soybeans were used as the representative for various types of beans.

Materials and methods

Sample preparation

Dried soybeans produced in Hokkaido, Japan (Kadoyabeikoku Co., Ltd, Japan) were purchased. The soybeans were soaked in water at room temperature for 5 hours (weight increased to more than 180% of the dry weight), and used as "Material." Material was placed in water that was twice its weight, heated with saturated steam cooker (Miura

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Co., Ltd, Japan) at 130°C for 30 minutes, removed from the water, cooled at room temperature, and used as "W." W was added to an enzyme solution including 1% (w/w) hemicellulase (Amano Enzyme inc., Japan) that was twice its weight, heated with steam convection oven (Rational AG, Germany) at 55°C for 40 minutes for the enzyme reaction and at 85°C for 5 minutes for inactivation, removed from the solution, and used as "W+E." Material was added to 1% (w/w) citric acid solution that was twice its weight, heated with the saturated steam cooker at 130°C for 30 minutes, removed from the solution, cooled at room temperature, and used as "CA." CA was added to the same enzyme solution as W+E that was twice its weight, heated in the same method as W+E, and used as "CA+E." Some of the grains (10 to 30%) lost their coat during the procedure; however, only grains with remaining coats were used in the following experiments.

Test for food for people with dysphagia

The test was performed using a Creepmeter RE2-33005B (Yamaden Co., Ltd, Japan) at 20 ± 2°C based on a method of testing food for people with disyphagia (Ministry of Health, Labor and Welfare, Japan 2009). A grain of sample was vertically compressed twice with a plastic plunger (20 mm in diameter) to a depth of 66.7 % of its original height. The speed of the plunger movement was 10 mm/s. From the curve obtained, hardness, adhesiveness and cohesiveness were calculated. The test was repeated 10 times.

Penetration test

A Creepmeter RE2-33005B was used in the measurements. The grain of W, W+E, CA and CA+E was laterally placed on a stage with 2.5 mm groove, and vertically pierced by stainless steel plunger (1.5mm in a diameter) under the following conditions: plunger movement, 1 mm/s; maximum strain, 150%; temperature, 20 ± 2°C. The maximum force and a penetration force curve were obtained. The test was repeated 10 times.

Scanning electron microscopy (SEM)

Samples were cut to about 5 mm^3 and fixed in 2.5% glutaraldehyde in 0.1 M PBS for 2h at 4°C (Ichinose *et al*., 2013). Fixed sections were washed overnight at 4°C in the same buffer and postfixed in 1% OsO_4 buffered with 0.1 M PBS for 2h. Sections were then dehydrated in graded ethanol series and dried in a critical point drying apparatus (HCP-2; Hitachi) with liquid CO_2. The sections were then spatter-coated with platinum and examined by scanning electron microscopy (S-4500; Hitachi).

Gas chromatography-mass spectrometry (GC-MS) analysis

W and CA samples (50 g) were immersed in 50 mL of chloroform for 30 s (Shao *et al.*, 2007; Greer *et al*., 2007). Chloroform extracts were evaporated under vacuum at 40°C. Concentrates were analyzed using a GC-MS QP-5050A (Shimadzu) connected to DB-5ms (30 m x 0.25 mm ID, 0.25 μm, Agilent Technologies). Samples were injected in splitless mode. The initial temperature of the column oven was kept at 40°C for 3 min and increased (10°C min-1) to 235°C. MS spectrometry was performed in scan mode.

Statistical analysis

The maximum forces in penetration tests were evaluated using an analysis of variance (ANOVA; Tukey–Kramer HSD tests, JMP 8.0 software). The statistical significance level was set at $p < 0.05$.

Results and Discussion

Suitability for people with dysphagia

The physical properties of the samples were examined, and compared with the ranges of Dysphagia Diet Level (Table 1) (Ministry of Health, Labor and Welfare, Japan, 2009; Cichero *et al*., 2013). While the hardness for Material was 330 x 10^3 N/m^2, those for the other samples decreased to equal to or below 15 x 10^3 N/m^2. W, W+E, CA, and CA+E were within the range of Dysphagia Diet Level in terms of hardness. Softer carioca beans were obtained by an autoclave at 105-115°C for 10-20 minutes than by a boiling water bath (98°C for 30-60 minutes) (Siqueira *et al.*, 2013). On the other hand, Güzel and Sayar (2012) reported that the hardness of some beans processed by atmospheric pressure cooking (boiling for 55-85 minutes) or high pressure cooking (120°C for 15-20 minutes) were not significantly different. We thought that the softening of W, W+E, CA, CA+E was mainly attributed to the common heating with saturated steam cooker at 130°C for 30 minutes. The cohesiveness of the samples were a little bit less than or equal to the lower limit value of Dysphagia Diet level 1 and 2. In regards to adhesiveness, all samples were included in the range of Dysphagia Diet Level. The averages of physical properties of W, W+E and CA met Dysphagia Diet Level 3, and those of CA+E did Disphagia Diet Level 1. The lower Dysphagia Diet Level is, the severer dysphagia it is suitable for people with (Ministry of Health, Labor and Welfare, Japan, 2009; Cichero *et al*., 2013). Therefore, it was

Table 1. Physical properties of the samples, compared with the ranges of dysphagia diet level*

	Hardness (x 10^3 N m^{-2})	Cohesiveness	Adhesiveness (x 10^2 J m^{-3})
Material	330 ± 57	0.18 ± 0.028	0.68 ± 1.0
W	15 ± 2.8	0.18 ± 0.017	3.5± 1.5
W+E	14 ± 2.7	0.19 ± 0.015	2.4 ± 0.96
CA	11 ± 1.5	0.18 ± 0.012	2.3 ± 0.40
CA+E	8.5 ± 1.5	0.20 ± 0.018	1.7 ± 0.40
Dysphagia Diet Level 3	0.30 – 20	–	≦15
Dysphagia Diet Level 2	1.0 – 15	0.2–0.9	≦10
Dysphagia Diet Level 1	2.5 – 10	0.2–0.6	≦4

n=10, mean ± SD. * Ministry of Health, Labor and Welfare, Japan, 2009; Cichero *et al*., 2013

Table 2. Maximum forces observed in penetration tests

	W	W+E	CA	CA+E
Maximum force (N)	0.32 ± 0.048 a	0.27 ± 0.037 b	0.27 ± 0.030 b	0.15 ± 0.029 c

n=10, mean ± SD. a, b, c: Values with different letters are significantly different ($p< 0.05$).

thought that though the W, W+E, and CA could be provided for dysphagic people, CA+E was the most suitable.

Penetration test

The penetration test was conducted to determine the detailed textures of W, W+E, CA and CA+E. The maximum forces of the samples were shown in Table 2. The force for W+E (0.27 N) was significantly less than the force for W (0.32 N). The primary cell wall of plant foodstuff is composed of an organized network of pectic substances, hemicelluloses, cellulose and protein (Ilker and Szczesniak, 1990). The process of softening soybeans with hemicellulase could be due to the breaking down of a part of the primary cell wall of the soybeans. The force for CA (0.27 N) was also significantly less than that for W (0.32 N). This could be caused by the acid and chelating effects of citric acid. Toews (2001) reported that the firmness of green and yellow peas was reduced further by tempering the peas in a solution containing 1% (w/w) citric acid and 2% (w/w) ascorbic acid and cooking, compared with tempering in distilled water and cooking. The addition of citric acid could be effective to soften beans not only during tempering but also during boiling. The force of W+E was not significantly different from that of CA. Even though the processes of W+E and CA were different, the damages might be the same extent. The maximum force for CA+E was 0.15 N, which was the lowest force observed. The difference between CA and CA+E (0.12 N) was more than that between W and W+E (0.05 N). Thus, boiling soybeans in citric acid solution was thought to be a more effective process before enzyme treatment than boiling in water.

Shibata *et al.* (2006) reported that the hardness of white flower beans decreased to 7.5 x 10^4 N/m^2 (compressed with a plunger (3 mm in a diameter) to 70% of its original height at plunger movement of 10 mm/s) by boiling at 95°C for 30 minutes, freezing, treating with enzyme, and autoclaving at 120°C for 60 minutes. Fresh grains of Carioca beans were softened to 0.77 N (= 2.5 x 10^5 N/m^2) (compressed with a plunger (2 mm in a diameter) to 90% of its original height at plunger movement of 1 mm/s) by autoclaving 110°C for 15 minutes (Siqueira *et al.* 2013). The hardness of soybeans reduced to 4.9 x 10^4 N/m^2 (compressed with a plunger (3 mm in a diameter) to 70% of its original height at plunger movement of 10 mm/s) by treating with enzyme, peeling, and boiling at 130°C for 60 minutes (Hayashi, 2013). From these reports, it was considered that, though CA+E (0.15 N = 8.5 x 10^4 N/m^2) (compressed with a plunger (1.5 mm in a diameter) to 150% of its original height at plunger movement of 1 mm s^{-1}) might be not the softest, the process of CA+E could be relatively simple and effective.

Curves in penetration tests

The representative curves for W, W+E, CA and CA+E in penetration tests are illustrated in Figure 1. There were two peaks in the ranges from 20% to 40% strain and from 100% to 120% in W, W+E, and CA curves. The two peaks were assumed to be the force needed to break through two sides of coats, and one of the peaks was the maximum of force. The firmness

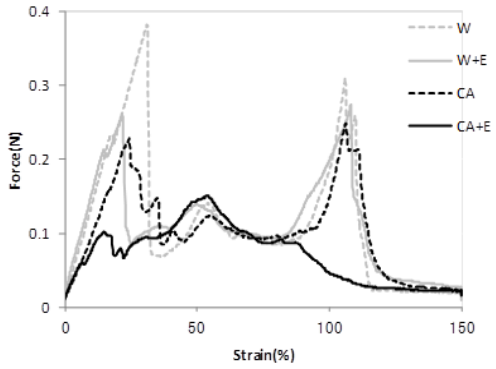

Figure 1. Representative curves from penetration tests

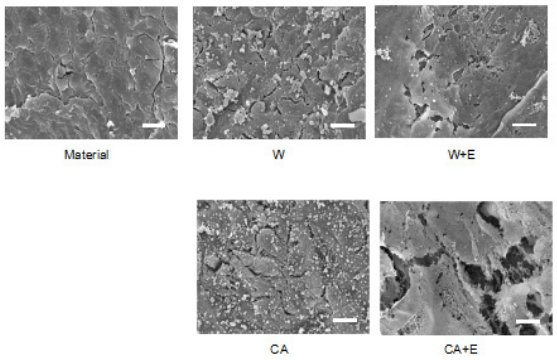

Figure 2. Scanning electron microscopy of coats from treated soybeans
Bars 3 μm

of the CA coat was lower than that of the W coat. Brief treatment with hot alkali created holes in the coat of soybeans (Shao *et al.*, 2007). The coats of soybeans could be affected by acid as well as alkali. The CA+E curve only had gradual peaks in the range from 30% to 80% strain, without the two peaks seen in other samples. CA+E coats were anticipated to be easy to crumble in the mouth. In the range from 40% to 80% strain, the forces for all samples were equivalent. These results show that the differences in maximum force between the samples were caused by the firmness of the coats of the samples and not that of the cotyledons. Also, the relative firmness of soybean coats to the cotyledons increased during cooking (Yasui *et al*., 2014). Hence, it was considered that, though the softening of coats of W, W+E and CA was not very different from conventional cooking, that of CA+E could be superior to the cooking.

Scanning electron microscopy

The coats of all samples were observed by SEM (Figure 2). The coat of Material had overlapping cuticles and a rigid structure, which resembled the image taken by Shao *et al.* (2007). For the coat of W, cuticles were thinner and the spaces between cuticles were wider than that of Material. The coat of W+E did not consist of cuticles but, instead consisted of torn film. Xylanase, which is an enzyme that degrades a certain type of hemicellulose (xylan), partially destroyed the lingocellulosic structure of seed coat of cotton (Csiszár *et al*., 2006). The enzymatic treatment of W+E might destroy the structure of cuticles in coats. Though there were small particles in the surface of CA, which were thought to be crystals of citric acid or citrate, CA coats were similar to W coats. In results from penetration tests, CA was the same as W+E and not W. These results suggest that acid hydrolysis caused by boiling in citric acid solution decomposed the coats differently from enzymatic treatment. The coat of CA+E had large tears and was the weakest structure. Enzymatic decomposition after boiling in citric acid solution could destroy the structure of the coat more than the enzymatic treatment after boiling in water.

GC-MS analysis

The constituents of W and CA coats were determined by GC-MS analysis to examine the difference between boiling soybeans in water and citric acid solution. The GC-MS chromatogram in Figure 3 shows a larger number of peaks in CA than in W, and some of these peaks were observed only in CA. Some of the specific peaks for CA (peak No.1-7) were identified. Soybean seed coats include uronic acid, pectin, hemicellulose, protein, cellulose and lignin (Mullin and Xu, 2001). From jute fiber, 2,3-dihydro-5-methylfuran-2-one and 5,6-dihydropyrane-2,5-dione as carbohydrate-derived compounds, and 3,4-dihydroxybenzaldehyde and 4-vinylphenol as lignin-derived compounds were found (del Río *et al.*, 2009). Thus, it was thought that 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Peak No.2), 2,3-dihydroxy-6-methyl-4H-pyran-4-one (Peak No.3), 5-hydroxymethyl-2-furancarboxaldehyde (Peak No.5) were derived from carbohydrates such as cellulose and hemicelluloses of soybean coats, and benzoic acid (Peak No.4), 2-methoxy-4-vinilphenol (Peak No.6) and 4-methyl-2,5-dimethoxybenzaldehyde (Peak No.7) were derived from lignin of the coats. 2,3-dyhydroxy-5,6-dimethyl-1,4-dioxin (Peak No.1) not including an benzene ring might be derived from carbohydrates. Acid pretreatment is also a process that breaks the rigid structure of lignocellulosic material, in which hydronium ions breakdown and attack intermolecular and intramolecular bonds among cellulose, hemicelluloses, and lignin in a biomass hierarchy structure (Lee *et al.*, 2014). Therefore, it was anticipated that the constituents of soybean coats were decomposed to the specific compounds

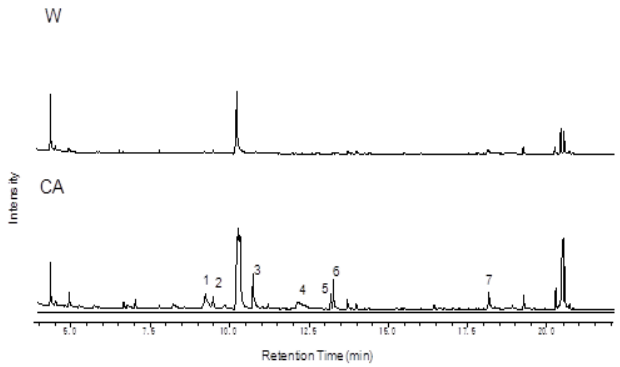

Figure 3. GC-MS chromatogram

Peak legend: (1) 2,3-dihydroxy-5,6-dimethyl-1,4-dioxin; (2) 2,5-dimethyl-4-hydroxy-3(2H)-furanone; (3) 2,3-dihydroxy-6-methyl-4H-pyran-4-one; (4) benzoic acid; (5) 5-hydroxymethyl-2-furancarboxaldehyde; (6) 2-methoxy-4-vinylphenol; (7) 4-methyl-2,5-dimethoxybenzaldehyde.

by boiling in citric acid solution. The appearance of W was similar to that of CA in Figure 2. The decomposition of CA could occur in part of the coats. However, the maximum force of CA+E was significantly lower than that of W+E. Hence, these findings indicated that the decomposition of boiling in citric acid solution was not strong but enough to prompt enzyme to destroy the coats in the process of CA+E.

Conclusions

These results show that it is possible to soften entire beans, including coats, by fragmenting a part of the coats with citric acid solution and treating the beans with enzymes. We expect that this method will be helpful in providing beans for individuals with dysphagia, since the beans with coats could be easy to crumble completely in the mouth.

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References

Anderson, J. W., Smith, B. M. and Washnock, C. S. 1999. Cardiovascular and renal benefits of dry bean and soybean intake. The American Journal of Clinical Nutrition 70 (3 Suppl.): 464S-474S

Cichero, J. A., Steele, C., Duivestein, J., Clavé, P., Chen, J., Kayashita, J., Dantas, R. and Lecko, C. 2013. The need for international terminology and definitions for texture-modified foods and thickened liquids used in dysphagia management: foundations of a global initiative. Current Physical Medicine and Rehabilitation Reports 1: 280-291

Cook, I. J. 2008. Diagnostic evaluation of dysphagia. Nature Clinical Practice Gastroenterology and Hepatology 5: 393-403

Csiszár, E., Losonczi, A., Koczka, B., Szakács, G. and Pomlényi, A. 2006. Degradation of lignin-containing materials by xylanase in biopreparation of cotton. Biotechnology Letters 28: 749-753

del Río, J. C., Gisela, J. R., Li, M. J., Gellerstedt, G., Jiménez-Barbero, J., Martínez, A. T. and Gutiérrez, A. 2009. Structural characterization of the lignin from jute *(Corchorus capsularis)* Fibers. Journal of Agricultural and Food Chemistry 57: 10271-10281

Greer, S., Wen, M., Bird, D., Wu, X., Samuels, L., Kunst, L. and Jetter, R. 2007. The cytochrome P450 enzyme CYP96A15 is the midchain alkane hydroxylase responsible for formation of secondary alcohols and ketones in stem cuticular wax of Arabidopis. Plant Physiology 145: 653-667

Güzel, D. and Sayar, S. 2012. Effect of cooking methods on selected physicochemical and nutritional properties of barlotto bean, chickpea, faba bean, and white kidney bean. Journal of Food and Science Technology 49: 89-95

Hayashi, M. 2013. Method for softening beans. European patent EP2484228

Ichinose, S., Tagami, M., Muneta, T., Mukohyama, H., Sekiya, I. 2013. Comparative sequential morphological analyses during *in vitro* chondrogenesis and osteogenesis of mesenchymal stem cells embedded in collagen gels. Medical Molecular Morphology 46: 24-33

Ilker, R. and Szczesniak, A. S. 1990. Structural and chemical bases for texture of plant foodstuffs. Journal of Texture Studies 21: 1-36

Iso, H., Date, C., Noda, H., Yoshimura, T. and Tamakoshi, A. 2005 Frequency of food intake and estimated nutrient intake among men and women: the JACC Study. Journal of Epidemiology 15(Suppl. 1): S24-42

Langmore, S. E., Terpenning, M. S, Schork, A., Chen, Y., Murray, J. T., Lopatin, D. and Loesche, W. J. 1998. Predictors of aspiration pneumonia: how important is dysphagia? Dysphagia 13: 69-81

Lee, H. V., Hamid, S. B. and Zain, S. K. 2014. Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process. ScientificWorldJournal Article ID 631013

Ministry of Health, Labor and Welfare, Japan. 2009. Pharmaceutical and Food Safety, Department of food Safety, Notice No. 0212001 (in Japanese)

Mullin, W. J. and Xu, W. 2001. Study of soybean seed coat components and their relationship to water absorption. Journal of Agricultural and Food Chemistry 49: 5331-5335

Shao, S., Meyer, C. J., Ma, F., Peterson, C. A. and Bernards, M. A. 2007. The outermost cuticle of soybean seeds: chemical composition and function during imbibition. Journal of Experimental Botany 58: 1071-1082

Shibata, K., Ishihara, M. and Sakamoto, K. 2006. Development of a hardness control technique for white flower beans using an enzyme-infusion method. Nippon Shokuhin Kagaku Kogaku Kaishi 53: 566-571 (in Japanese)

Siqueira, B. S., Vianello, R. P., Fernandes, K. F. and Bassinello, P. Z. 2013. Hardness of carioca beans (*Phaseolus vulgaris* L.) as affected by cooking methods. LWT - Food Science Technology 54: 13-17

Toews, T. 2001. Effects of pretreatment and micronization on the cookability and chemical components of green and yellow filed peas. Manitoba, Canada: University of Manitoba, MSc Thesis.

Wang, S., Melnyk, J. P., Tsao, R. and Marcone, M. F. 2011. How dietary antioxidants in fruits, vegetables and legumes promote vascular health. Food Research International 44: 14-22

Yasui, T., Sasaki, T., Kohyama, K. and Hajika, M. 2014. Variation in firmness of whole beans, embryos, and testas of cooked soybean *(Glycine max)* cultivars. Cereal Chemistry 91: 419-424