

# Hypoallergenic buckwheat flour preparation by *Rhizopus oligosporus* and its application to soba noodle

Tri Handoyo <sup>a</sup>, Tomoko Maeda <sup>b</sup>, Atsuo Urisu <sup>c</sup>, Taiji Adachi <sup>a</sup>, Naofumi Morita <sup>a,\*</sup>

<sup>a</sup> Graduate School of Life and Environmental Sciences, Laboratory of Food Chemistry, Osaka Prefecture University,  
Gakuen-cho 1-1, Sakai, Osaka 599-8531, Japan

<sup>b</sup> Department of Life and Health Sciences, Hyogo University of Teacher Education, 942-1, Shimokume, Yashiro, Hyogo 673-1494, Japan

<sup>c</sup> Department of Pediatrics, School of Medicine, Fujita Health University, Toyoake, Aichi 470-1192, Japan

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## Abstract

The effect of *Rhizopus oligosporus* on the structure and functional properties of buckwheat grain during fermentation was investigated and the production of buckwheat hypoallergenic flour was studied. The spores of *R. oligosporus* were mixed with steamed buckwheat grains, and they were incubated at 30 °C and 85% relative humidity (RH) for 0, 24, 48 and 72 h. The changes of structure, free amino acids, minerals, phytic acid and allergenic proteins in the grains were determined during the time course of incubation. The 48-h-fermented buckwheat (FeB) was found to be involved in formation of amino acids with higher amounts of total amino acids, and some of them increased 50 times more; including isoleucine, leucine, lysine, valine, glycine, histidine, tyrosine and  $\gamma$ -amino butyric acid (GABA). SDS–PAGE and Western Blot analyses showed that *R. oligosporus* was very effective to reduce allergenic proteins of buckwheat and could produce the hypoallergenic buckwheat flour. In its application to noodle making, the FeB-substituted wheat flour noodle was softer and had more elasticity than the control without FeB flours (1CW), except for the 10% substitution of FeB. Uncooked and cooked noodles were brownish compared to those of the 1CW. The *R. oligosporus* could possibly be used to produce hypoallergenic buckwheat with higher functional and good rheological properties, and thus produced FeB flour was considered to become a big prospect for development of new food in the future.

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**Keywords:** Fermented-buckwheat; GABA; Allergenic protein; *Rhizopus oligosporus*; Soba noodle

## 1. Introduction

Buckwheat (*Fagopyrum esculentum*, Moench) is known to be used as a basic material for noodle, pasta, blended bread and other types of flour products. In Japan, soba noodle is usually prepared by the substitution of buckwheat flour for wheat flour (Udesky, 1988). Buckwheat grains contain a large amount of protein, starch and vitamin. The protein consists of well-balanced amino acids with a high biological value (Pomeranz, 1983) and is excellent supplement for cereal grains, although the digestibility is relatively low (Ikeda & Khisida, 1993). In addition, the level

of phytic acid is high in grains, which might inhibit the bio-availability of some elements in food (Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001). However, buckwheat contains allergen, and the allergy reaction is a critical health problem in most countries that have been in the custom of consuming buckwheat products. The major symptom of allergy is eczema or urticaria that would occur shortly after a buckwheat product was eaten, usually emerging as skin eruption and itching (Urisu et al., 1994).

Recently, it is a new issue to develop new foods with the aims to improve health and well-being, and to reduce the risk of certain diseases. Several food-processing techniques have been applied to foods in order to increase the functional property and to reduce the levels of allergenic proteins. Watanabe, Maeda, Tsukahara, Kayahara, and Morita (2004) have

\* Corresponding author. Tel.: +81 72 2549459; fax: +81 72 2549921.  
E-mail address: [morita@biochem.osakafu-u.ac.jp](mailto:morita@biochem.osakafu-u.ac.jp) (N. Morita).

reported that pre-germinated brown rice contained higher amount of GABA than the control sample, and brown rice has been already applied for breadmaking. Furthermore, GABA is considered to be a functional ingredient for blood pressure control, (Aoki, Furuya, Endo, & Fujimoto, 2003), relief of sleeplessness, depression and autonomic disorders (Okada et al., 2000). More than 2% of the adult populations in the developed countries suffer from allergy caused by foods such as cereal products (Simonato et al., 2001). The allergenic protein in buckwheat is one problem on the production of foods; many researches have been trying to obtain a food with lower or free from allergenic agents, but the allergen levels of thus prepared products were not clarified and still suspected. Previous studies have reported that enzymatic modification (Watanabe, Watanabe, Sonoyama, & Tanabe, 2000) and microbial fermentation (Kobayashi, Hashimoto, Taniuchi, & Tanabe, 2004) could eliminate the allergenic proteins in wheat.

Our research tried to modify buckwheat grains using a fungi, *Rhizopus oligosporus* to increase the functional properties and eliminate allergenic proteins. The *R. oligosporus* is one of the common microbes in East Asia region, and usually is used for the production of fermented soybean (tempeh). Tempeh has been a favorite food and staple source of protein, and it is now rapidly becoming more popular all over the world, as people look for ways to increase their intake of soybeans, and enjoy tempeh's versatility and delicious taste. Especially, vegetarians and vegans find tempeh as an interesting food because of the good functional properties. Our previous work reported that *R. oligosporus* has been a beneficial agent to produce fermented food with a higher amount of functional property (Handoyo & Morita, in press).

In the present study, at first, the effect of *R. oligosporus* on the functional, structural and rheological properties of buckwheat is studied. The second objective of the work is to propose a fermentation method using *R. oligosporus* for production of hypoallergenic buckwheat flours that would be tolerated by most patients who are allergic to buckwheat which is used for manufacturing soba noodle.

## 2. Materials and methods

### 2.1. Materials and chemicals

Buckwheat grains of Mancan variety as dry solid were used, and a hard-type wheat flour of 1CW (No. 1 Canadian Western Red Spring) was obtained from Miyake Flour Milling Co., Ltd. (Osaka, Japan). Dried culture of *R. oligosporus* was obtained from LIPI (Bandung, Indonesia). Other analytical chemicals used were reagent grade without more purification.

### 2.2. Preparation of fermented-buckwheat (FeB)

The FeB was prepared from buckwheat using the modified method for preparing Indonesian tempeh as shown in

Fig. 1: (a) buckwheat grains (500 g) were soaked with 1 L of water for 15 min; (b) after soaking, the samples were rinsed gently with approximately 0.5 L of tap water, drained, and cooked in 2 L of tap water at 100 °C for 45 min with an uncovered pan; (c) drained and allowed to cool at room temperature; (d) the cooked samples were inoculated with tempeh starter (dried culture of *R. oligosporus*) at a level of 1 g/kg of dry seeds, put in dish (diameter 15 cm, height 5 cm), and incubated at 30 °C and 85% relative humidity (RH) for 24, 48 and 72 h. After the end of fermentation process, thus prepared FeB grains were freeze-dried for 24 h, milled using a milling machine (Retsch, Germany) with 75 mesh screen, and used for the following experiments.

### 2.3. Noodle preparation

The noodle samples were prepared using a Haussler noodle apparatus (Haussler PN100, Germany) with various amounts of FeB for 48 h as described above (Table 1). The 1CW was substituted with the 10–30% of FeB, and five hundred grams of the substituted flours were mixed with 2% of salt (w/w sample) dissolved in 34% (w/w) of water (30 °C) at 10 rpm for 10 min. The pasta strand was extruded with 1.45 mm of diameter at 40 °C and cut with the length of 20 cm.

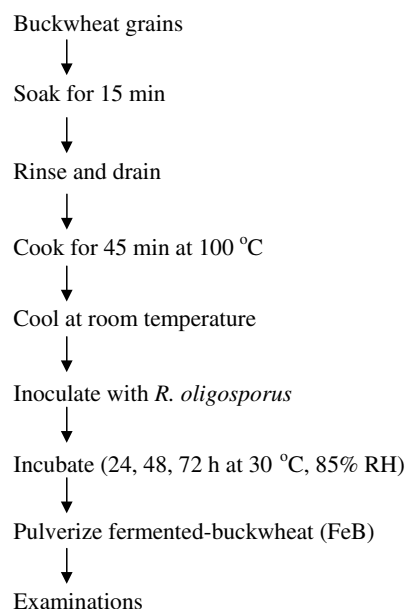


Fig. 1. Flow diagram of fermented-buckwheat.

Table 1  
Summary of ingredients for noodle making

| Ingredient        | Substitution of FeB (%) |    |    |    |
|-------------------|-------------------------|----|----|----|
|                   | 0                       | 10 | 20 | 30 |
| Wheat flour (1CW) | 100                     | 90 | 80 | 70 |
| FeB               | 0                       | 10 | 20 | 30 |
| Water             | 34                      | 34 | 34 | 34 |
| Salt              | 2                       | 2  | 2  | 2  |

1CW, a hard-type commercial wheat flour; FeB, fermented-buckwheat.

## 2.4. Analyses

### 2.4.1. Free amino acid components

The procedure to determine free amino acids was the same as the method reported by Saikusa, Horino, and Mori (1994). One point six grams of FeB flour with 4 ml of 8% trichloroacetic acid solution in test tubes ( $2 \times 16$  cm) were homogenized using a homogenizer for 5 min and then shaken (100 strokes/min; 5 cm amplitude) at 30°C for an hour. The suspension was centrifuged at 4°C and 14,000g for 15 min. The supernatant was filtered through a 0.45 µm membrane filter (Advantec Co., Ltd., Tokyo, Japan), and then the 20 µL of filtrate was injected into the column of LC-11 Amino Acid Analyzer (Yanaco Co., Kyoto, Japan).

### 2.4.2. Mineral content

Mineral composition in FeB flours was analyzed by using a Vista-MPX Simultaneous ICP-OES with Vista MPX's ICP-Expert software (Seiko Instrument Co., Ltd., Osaka, Japan). Two grams of flour samples were put in a well-glazed porcelain dish and burned at 550°C for 4 h. After samples became ash, they were cooled in a desiccator until they reached room temperature, the 50 ml of 0.1 N HCl was added, and then the solution was filtered using 0.45 µm membrane paper (Advantec Co., Ltd., Tokyo, Japan). An aliquot of the filtrate was diluted to 200–500 times, and 5 ml of the diluted samples was injected into the ICP-OES apparatus.

### 2.4.3. Phytate content

Extraction of phytate from FeB flours was carried out using the modified procedure of Latta and Eskin (1980). One gram sample was extracted with 25 ml of 24% HCl aqueous solution. Ten ml of the sample solution was passed through a 200–400 mesh AG1-X8 chloride anion exchange column, followed by elution of phytate with 0.7 M NaCl. Three ml of eluate was collected immediately and 1 ml Wade reagent was added for phytate measurement.

### 2.4.4. Extraction of crude protein from FeB flours

Extraction of crude protein from buckwheat flours was performed according to Weiss, Vogelmeier, and Gorg (1993) with some modifications. Ten ml of 50 mM Tris-HCl buffer (pH 8.8) containing 5 mM magnesium chloride, 10% (w/v) PVP, 10% (w/v) lauryl sulfate and 1 mM β-mercaptoethanol was added to three grams of each fraction, and they were mixed and stood at room temperature (RT) for 20 min. The mixture sample was centrifuged for 10 min at 14,000g at RT, and the supernatant was collected and dialyzed to remove salts, and the inner solution was freeze-dried. Thus prepared dry sample was used for the following experiments.

### 2.4.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a Mini Protean II electrophoresis system (BIO-RAD Laboratories) according to Laemmli (1970) using FeB flours for 12, 16, 24 and 48 h as described above. Protein samples of 10–15 µg were

dissolved in either reducing or non-reducing agent containing sample buffer and were applied to the homogeneous 12% gels. After electrophoresis, the gels were stained with coomassie brilliant blue R-250. For calibration, molecular weight markers were used ranging from 6.5 to 205 kDa (Molecular Probes, Eugene, Oregon): myosin (205,000 Da), β-galactosidase (116,000 Da), phosphorylase b (97,000 Da), transferrin (80,000 Da), bovine serum albumin (66,000 Da), glutamate dehydrogenase (55,000 Da), ovalbumin (45,000 Da), carbonic anhydrase (30,000 Da) trypsin inhibitor (21,000 Da), lysozyme (14,000 Da) and aprotinin (6500 Da).

### 2.4.6. Allergen assay by immunodetection

After SDS-PAGE, the proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane in a Trans-Blot Cell (Bio-Rad, USA) using Tris-buffer saline (TBS, pH 7.5). Transfer was performed at 250 mA for 2 h. The membrane was washed three times with TBST (TBS+0.05% Tween 20, pH 7.5) and blocked with 1% PVP (polyvinyl pyrrolidone) at 4°C for 12 h. The membrane was further incubated with primary sera (Immunoglobulin E specific for allergenic proteins of buckwheat from human) (Yoshioka, Ohmoto, Urisu, Mine, & Adachi, 2004) diluted in TBST at the ratio of 1:20 at room temperature for 4 h. It was washed three times and incubated for 2 h at room temperature with 1:1000 diluted anti rabbit IgE, followed by the second wash with TBST and AP (alkaline phosphatase) buffer three times, then immersed in a buffer solution AP direct labeling with CDP-Star (Boehringer Ingelheim, Germany). After 30 min, the membrane was scanned by a Luminescent image analyzer LAS 1000 Plus (Fuji Film, Co., Tokyo, Japan).

## 2.5. Characteristics of buckwheat noodle

### 2.5.1. Appearance of buckwheat noodle

Color ( $L^*$ ,  $a^*$ , and  $b^*$ ) of noodles made from FeB-substituted wheat flours was measured using Minolta Chromameter (Model CR-13, Osaka, Japan). The  $L^*$ ,  $a^*$  and  $b^*$  values indicated lightness, hue and chroma ( $a^*$  from green to red;  $b^*$  from blue to yellow), respectively. The noodle samples were put into a plastic dish (diameter 160 mm) and the results were shown from average of color parameter for all area. Three test samples were used for the same noodle samples (Pedreschi, Moyano, Kaack, & Granby, 2005).

### 2.5.2. Rheological properties of buckwheat noodle

Instrumental texture of noodles made from FeB-substituted wheat flours was tested using a texture analysis with a V-shaped blade (Fudoh Rheometer, Rheotech Co., Ltd., Tokyo, Japan). A cube of sample ( $1 \times 1 \times 1$  cm<sup>3</sup>) was oriented perpendicularly to the blade on smooth platform. The crossing speed was 6 mm/s and the plunger was stopped at 98% in depth of sample thickness. The data were processed using a computer program, Rheosoft TR-06

(Rheotech Co., Ltd., Tokyo, Japan) (Watanabe et al., 2004). Three replicates were taken within 1 min for each sample.

### 2.5.3. Pasting properties of starch in substituted flours

Pasting properties of substituted flours were determined according to the Approved AACC Method 22–10 (2000) with some modifications, using a Brabender Viscoamylograph. Sixty grams of flour were dispersed in 450 ml distilled water and the flour slurry was heated from 30 to 93 °C at a rate of 1.5 °C/min, held at 93 °C for 15 min and then cooled to 30 °C at the same rate and held for 15 min.

### 2.6. Scanning electron microscope (SEM) observation

The samples for scanning electron microscope (SEM) were prepared according to Morita, Nakata, Hamazu, and Toyosawa (1996). The samples (50 × 50 × 10 mm<sup>3</sup>) of FeB grains and noodles were oxidized using 2% OsO<sub>4</sub> in water for 12 h, and the osmium gas was completely removed under reduced pressure over NaOH as a desiccant. Thus fixed samples were put on the sample stage and coated with Pt–Pd for 4 min and observed by SEM apparatus (Hitachi S-8000, Osaka, Japan).

### 2.7. Statistical methods

The results were processed by the SPSS software (V.11.0 for Windows, SPSS, Chicago IL) using a one-way analysis of variance and then Duncan's multiple-range test or Student's *t* test. The resultant data were considered to be significant at *P* < 0.05.

## 3. Results and discussion

### 3.1. Analytical data of minerals and amino acids in buckwheat flours

Large amounts of minerals were liberated from buckwheat during fermentation processes, but some of them decreased as shown in Table 2. The time course of fermentation distinctly increased in the amount of potassium (729.9 mg/100 g), phosphorus (601.8 mg/100 g), magnesium (548.7 mg/100 g) and manganese (2.4 mg/100 g). The growing process of mold increased several minerals including sodium (684.8 mg/100 g), sulphur (1049.8 mg/100 g), zinc (203.1 mg/100 g), boron (143.4 mg/100 g), aluminum (127.9 mg/100 g) and iron (11.7 mg/100 g) on 24 h fermentation, while these minerals decreased after fermentation for 48 h or more. In contrast, the amounts of calcium and copper decreased during the time course of fermentation.

Phosphate is stored in seeds with the form of phytin as the Ca and/or Mg salts of phytic acid. Phytic acid is called as an anti-nutritious compound, because it binds with some minerals, such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, Zn<sup>2+</sup> and K<sup>+</sup>. The unavailability of phytic acid is very important, because it inhibits mineral absorption in food if its amount is very high (Lott, Greenwood, & Batten, 1995). The amount of

Table 2

Effect of time course of fermentation on mineral content of buckwheat (mg/100 g dry sample)

| Mineral | Time course of fermentation (h) |        |        |        |
|---------|---------------------------------|--------|--------|--------|
|         | 0                               | 24     | 48     | 72     |
| S       | 980.2                           | 1049.8 | 975.4  | 977.6  |
| K       | 395.4                           | 489.0  | 666.0  | 729.9  |
| P       | 326.4                           | 373.6  | 512.2  | 601.8  |
| Ca      | 3462.3                          | 3323.0 | 3155.8 | 2950.1 |
| Na      | 454.7                           | 684.8  | 475.4  | 476.7  |
| Mg      | 407.8                           | 470.9  | 505.8  | 548.7  |
| Zn      | 180.8                           | 203.1  | 188.4  | 154.0  |
| B       | 107.7                           | 143.4  | 115.8  | 119.2  |
| Al      | 89.5                            | 127.9  | 99.3   | 100.4  |
| Fe      | 9.8                             | 11.7   | 6.9    | 7.9    |
| Mn      | 2.1                             | 2.1    | 2.0    | 2.4    |
| Cu      | 1.5                             | 0.8    | 0.5    | 1.2    |

Values of SD are lower than 0.01 (SD < 0.01) (*n* = 3).

phytic acid was reduced approximately to 70–90% in FeB, suggesting its degradation by *R. oligosporus* during fermentation process. Previous study reported that phytic acid was degraded to inorganic phosphates during production of tempeh (Sutardi & Buckle, 1988; Hachmeister & Fung, 1993). Significantly positive correlation was found between phytic acid and some minerals (Ca, *r* = 0.98; Fe, *r* = 0.70) indicating their proportional increase in phytic acid content. However, Mg (*r* = −0.99) and K (*r* = −0.99) showed significantly negative correlation with phytic acid content (data not shown).

The formation of free amino acids in FeB is shown in Table 3. The *R. oligosporus* dramatically increased total and several amounts of amino acids during the length of fermentation time at the ratio of more than 50-fold as large as those of the control without fermentation. Non-essential amino acids, especially alanine and asparagin rapidly increased with the course of fermentation, and this result suggested that glutamic acid was catalyzed to form alanine and asparagin by amino transferase. Also, GABA increased during the growth of mold, showing that GABA synthesis depends on the amount of glutamic acid. GABA is commonly synthesized from 2-oxoglutarate via glutamate, and then decarboxylated to GABA by glutamate decarboxylase (Baum, Chen, Arazi, Takatsuji, & Fromm, 1993).

### 3.2. Degradation of allergenic protein in buckwheat flours during fermentation

SDS–PAGE patterns of buckwheat flours during fermentation are shown in Fig. 2(A). The pattern of protein in the control sample varies with various molecular weights, but both of high (HMW) and low molecular weight (LMW) proteins were degraded by *R. oligosporus* during the time course of fermentation. These results assumed that the mold could rapidly degrade all kinds of proteins, and utilize the produced amino acids or LMW peptides for its own growth. Sparringa and Owens (1999) suggested that the intermediate-sized protein products degraded were used

Table 3  
Composition of free amino acids in buckwheat during fermentation (mg/100 g dry sample)

| Amino acid                       | Time course of fermentation (h) |       |       |        |
|----------------------------------|---------------------------------|-------|-------|--------|
|                                  | 0                               | 24    | 48    | 72     |
| <i>Essential amino acid</i>      |                                 |       |       |        |
| Ile                              | 0.2                             | 14.7  | 53.0  | 64.7   |
| Leu                              | 0.2                             | 8.3   | 56.5  | 85.6   |
| Lys                              | 1.2                             | 13.5  | 74.1  | 90.9   |
| Met                              | 0.4                             | 2.0   | 2.4   | 2.2    |
| Phe                              | 0.7                             | 1.4   | 18.0  | 45.8   |
| Thr                              | 0.3                             | 0.1   | 0.1   | 70.2   |
| Val                              | 0.1                             | 2.9   | 48.9  | 64.5   |
| <i>Semi essential amino acid</i> |                                 |       |       |        |
| Arg                              | 2.3                             | 26.9  | 53.5  | 67.7   |
| Gly                              | 0.3                             | 2.2   | 28.0  | 53.4   |
| His                              | 0.6                             | 10.1  | 51.3  | 54.3   |
| Tyr                              | 0.7                             | 4.0   | 82.8  | 108.8  |
| <i>Non essential amino acid</i>  |                                 |       |       |        |
| Ala                              | 4.2                             | 70.6  | 186.3 | 234.1  |
| Asn                              | 10.7                            | 38.3  | 192.6 | 0.0    |
| Asp                              | 3.0                             | 2.3   | 15.5  | 8.4    |
| Cys                              | 1.4                             | 0.7   | 19.9  | 55.8   |
| Gaba                             | 1.1                             | 22.2  | 96.9  | 94.0   |
| Glu                              | 15.3                            | 21.1  | 47.0  | 236.1  |
| Orn                              | nd                              | 8.3   | 46.7  | 44.1   |
| Pro                              | 0.2                             | 9.2   | 9.6   | 24.1   |
| Ser                              | 1.5                             | 0.4   | 32.9  | 56.9   |
| Total                            | 40.3                            | 188.6 | 929.7 | 1227.5 |

nd, not determined.

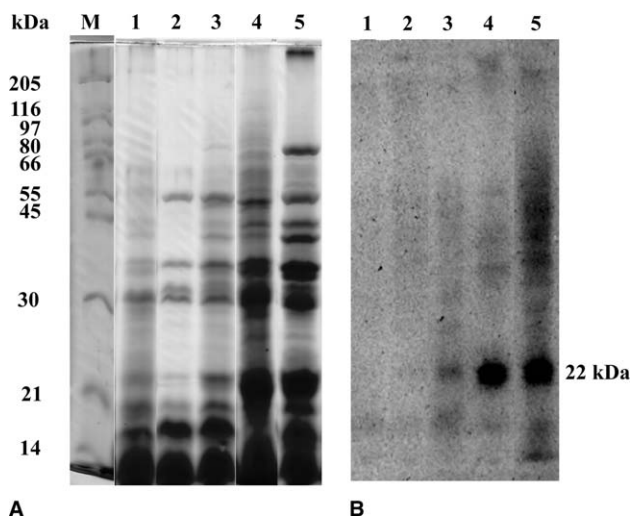


Fig. 2. SDS PAGE of fermented-buckwheat proteins (A) and western blot analysis of allergenic protein in FeB during fermentation (B). Total protein applied is 10 µg/lane. M, marker for standard protein. Lane numbers of 1, 2, 3, 4 and 5 are FeB for 48, 24, 16, 12 and 0 h (control; without fermentation), respectively.

for mold biomass production, and then was oxidized. But some protein bands at 48 h were stronger than at 16 h, because the mold was also considered to produce new proteins by metabolism during fermentation. The proteins produced by mold might have a similar molecular weight to

that of buckwheat protein. Therefore, the strength of some bands at 48 h was appeared to increase, as compared with 16 h. In addition, several investigators reported the total protein content during tempeh fermentation Nowak and Szebiotko (1992) observed a slight increase in total protein from 45.1% to 48.8% (w/w of dry matter) and Van der Riet, Wight, Cilliers, and Dattel (1987) reported the total protein increased from 40.1 to 49.3% (w/w of dry matter).

Immunoblotting analysis described the level of specific IgE in relation to buckwheat allergy as shown in Fig. 2(B). The strongest IgE binding with 22 kDa protein in the control (buckwheat without fermentation) meant that the molecular weight of major allergenic protein of buckwheat was 22 kDa. According to the report by Yoshioka et al. (2004), the 22 kDa protein has been believed to be a major allergenic protein of Mancan buckwheat. Western blot analysis showed that the allergenic proteins appeared in the control (lane 5 in Fig. 2(B)), but it disappeared during the time course of fermentation (lanes 1–4 in Fig. 2(B)). Allergenic proteins in buckwheat were almost degraded after 16 h fermentation and completely degraded to LMW (amino acids or small peptides) after 24 h fermentation (lanes 1–2 in Fig. 2(B)). The *R. oligosporus* is one of the safe microorganisms for human, and has many benefits for production of fermented foods, because it produces some enzymes to hydrolyze and eliminate allergen in the buckwheat grains.

### 3.3. Changes of buckwheat structure during fermentation

Appearances of buckwheat grains after cooking and fermentation were observed using SEM (Fig. 3). As to the image of the control (buckwheat grain without treatment), many raw starch granules with clear round shape were distinctly observed (Fig. 3(A)). After cooking, the round shape of raw starch granules was broken and the surface was somewhat viscous with paste produced by the gelatinization (Fig. 3(B)). Some gelatinized starches were connected and the network structure appeared after 24 h fermentation (Fig. 3(C)), and after 48 h fermentation, the appearance became irregular and typical image of gelatinized cereal products was obtained (Fig. 3(D)), where starch granules could not be distinguished. But, 72 h fermentation made different structure with big hole spaces like vacuole in the grains (Fig. 3(E)). During fermentation, some of the gelatinized starch granules were hydrated by enzymes such as lipases, amylases and proteases in *R. oligosporus* and the buckwheat structure was apparently more swollen and gelatinized than the cooked samples without fermentation. In addition, thus hydrolyzed substrates were considered to provide or supply the energy for the own growth of *R. oligosporus*.

### 3.4. Pasting properties of FeB-substituted wheat flour

Pasting properties of FeB-substituted wheat flours shows significant difference from those of the 1CW

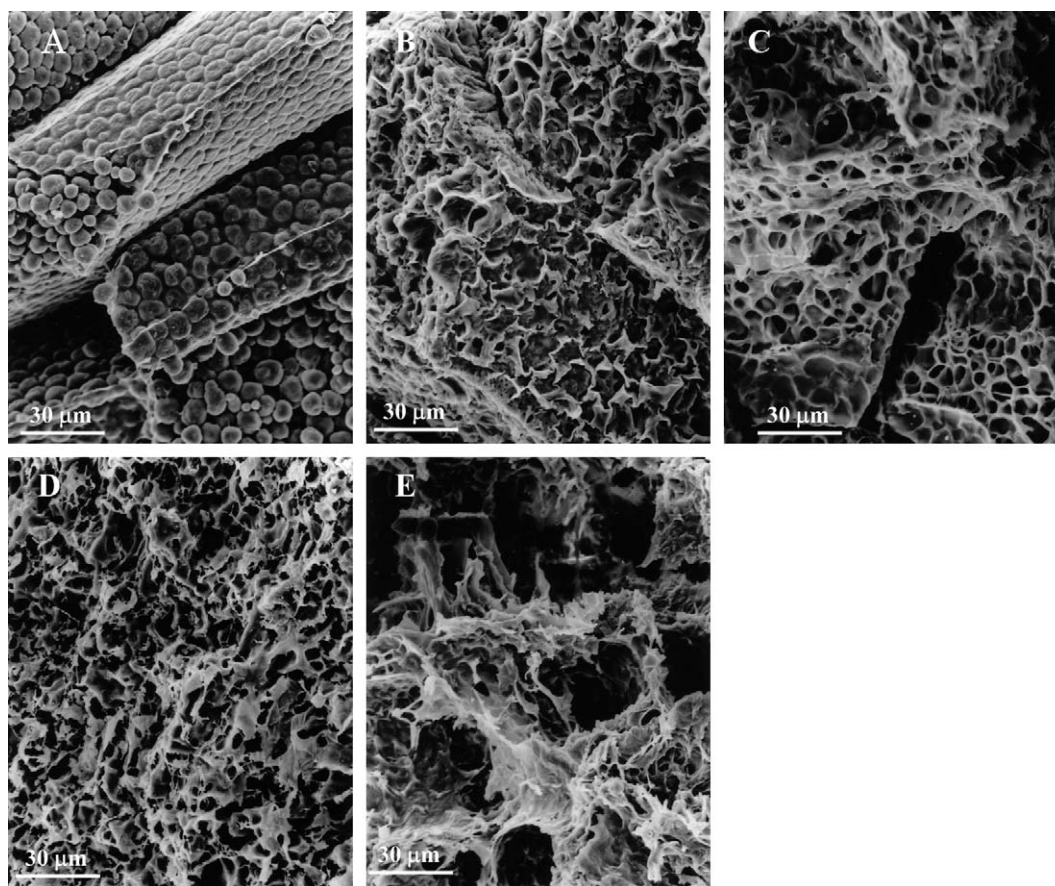


Fig. 3. SEM images of buckwheat grains cooked or fermented by the procedure as shown in Fig. 1. (A) control without treatment; (B)–(E) are samples after cooking and fermentation for 24, 48 and 72 h, respectively.

Table 4  
Pasting properties of wheat flours containing fermented-buckwheat flours

| Sample  | GT (°C) | PVT (°C) | PV (BU) | BV (BU) | FV (BU) | SB (BU) |
|---------|---------|----------|---------|---------|---------|---------|
| Control | 66.8 a  | 93.0 a   | 720 a   | 160 a   | 2000 a  | 1440 a  |
| 10% FeB | 84.0 b  | 91.5 b   | 520 b   | 180 b   | 845 b   | 505 b   |
| 20% FeB | 83.2 c  | 91.4 b   | 485 c   | 265 c   | 710 b   | 490 b   |
| 30% FeB | 79.5 d  | 91.5 b   | 440 d   | 260 c   | 510 c   | 330 c   |

GT, gelatinization temperature; PVT, peak viscosity temperature; PV, peak viscosity; BV, breakdown viscosity; FV, final viscosity; SB, setback; BU, brabender unit.

Control, 100% of 1CW; 10% FeB, 1CW: FeB = 90:10; 20% FeB, 1CW: FeB = 80:20; 30% FeB, 1CW: FeB = 70:30. Abbreviations are the same as in Table 1.

Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

$n = 3$ .

(control) as shown in Table 4. The control showed lower gelatinization temperature (GT) and breakdown viscosity (BV) than that of the substituted flours. For the substituted flours, peak viscosity temperature (PVT) was the relatively similar among samples, but the GT and peak viscosity (PV) were significantly different depending on the substituted amounts. Also, there was no significant difference on BV between 20% and 30% substitutions of FeB. But, the 30%-substituted FeB flour had significantly lower values of final viscosity (FV) and setback (SB) as com-

pared with other samples. The application of FeB as the substituted flour to gelatinized products affected the pasting properties, showing that the FeB has improving effect on the physical properties of normal flour during cooking process. The 30% of FeB substitution was the best choice for noodle manufacturing because it had lower peak viscosity of about 440 BU, which was suitable for Japanese noodle such as soba (Shuey & Tipples, 1980).

### 3.5. Characteristics of noodles made from FeB-substituted wheat flours

#### 3.5.1. SEM results

The control noodle made from 1CW showed the linking starch granules (large and small size) performing the conformation of network (Fig. 4(A) and (C)), but the surface between gluten and starch were clearly seen and the raw starch granules had smooth appearance as indicated by the arrows. Noodle substituted with 30%-FeB flour (Fig. 4(B) and (D)) had different appearance as compared with the control in Fig. 4(A) and (C), and contained swollen starch granules that were buried in viscous gluten sheet as indicated by arrows. The substance was not obtained for 1CW noodle (Fig. 4(A) and (C)) but distinguished only in the FeB-substituted noodle sample.

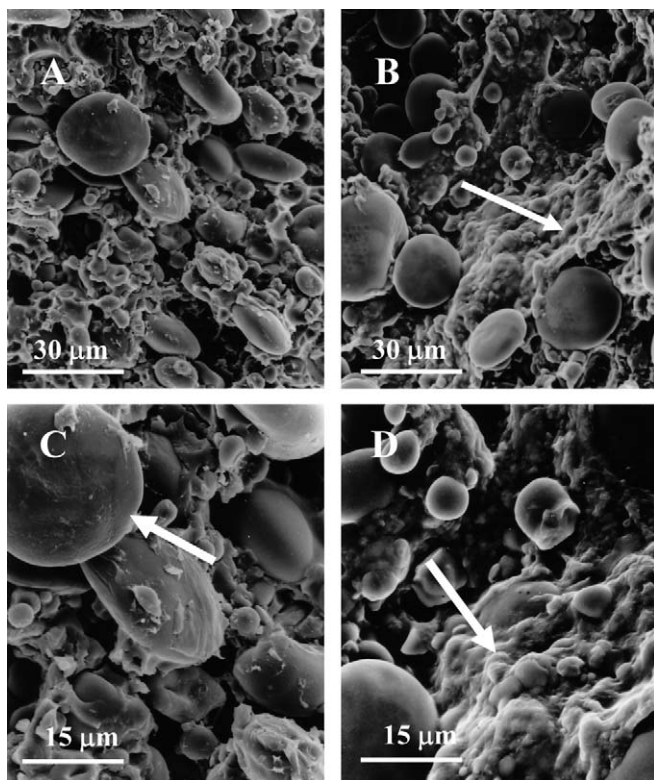


Fig. 4. SEM images of control (A and C) and 30% FeB-substituted noodles (B and D). Abbreviation are the same as in Table 1.

### 3.5.2. Appearance and rheological properties

The noodle substituted with 20% and 30% of FeB for wheat flour showed significant higher elasticity than that of the control, but not significant for that with 10% substitution (Fig. 5). From the cutting value, the noodle substituted with 30% FeB flour was significantly softer than that of the control.

Uncooked and cooked noodles substituted with 10%, 20% and 30% of FeB flour showed significantly different color values from the control (Table 5). The  $L^*$  value of the

Table 5

Color properties of noodle samples containing fermented-buckwheat flours

| Sample  | Uncooked |       |        | Cooked |       |        |
|---------|----------|-------|--------|--------|-------|--------|
|         | $L^*$    | $a^*$ | $b^*$  | $L^*$  | $a^*$ | $b^*$  |
| Control | 87.8 a   | 0.7 a | 11.0 a | 79.2 a | 0.3 a | 12.5 a |
| 10% FeB | 83.6 b   | 1.5 b | 8.7 b  | 69.0 b | 2.4 b | 11.9 b |
| 20% FeB | 83.9 c   | 1.3 c | 9.0 c  | 67.8 c | 2.3 c | 12.8 c |
| 30% FeB | 83.8 d   | 1.2 c | 10.1 d | 65.6 d | 2.3 c | 14.1 d |

Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

Abbreviations are the same as in Table 4.

$n = 3$ .

uncooked control noodle was significantly higher than those of substituted flour noodles. In addition, the control had significantly lower values of  $a^*$  and  $b^*$  than the noodle substituted with FeB. The substituted noodles had higher values of  $a^*$  near to red and  $b^*$  near to yellow, regardless of cooking. But, the cooked noodle samples had lower index values than uncooked ones.

Though many kinds of low molecular components in the fermented-buckwheat noodle are eluted in the solution during boiled cooking, normally some people have had a custom to drink the hot water after boiling with the noodle for a long time. Because the soluble nutritious components eluted from buckwheat noodle are included in the hot water, less nutritional benefit for the consumer are not thought on the eating buckwheat noodle. Also, GABA is quite stable in the healthy condition (stable for 1 h at 100 °C), like free amino acids. Therefore, the authors suggest that there are not so many disadvantages on the existence of low molecular materials derived from the fermentation process during cooking.

## 4. Conclusions

Based on these results, the FeB had important characteristics such as higher amount of amino acids and minerals,

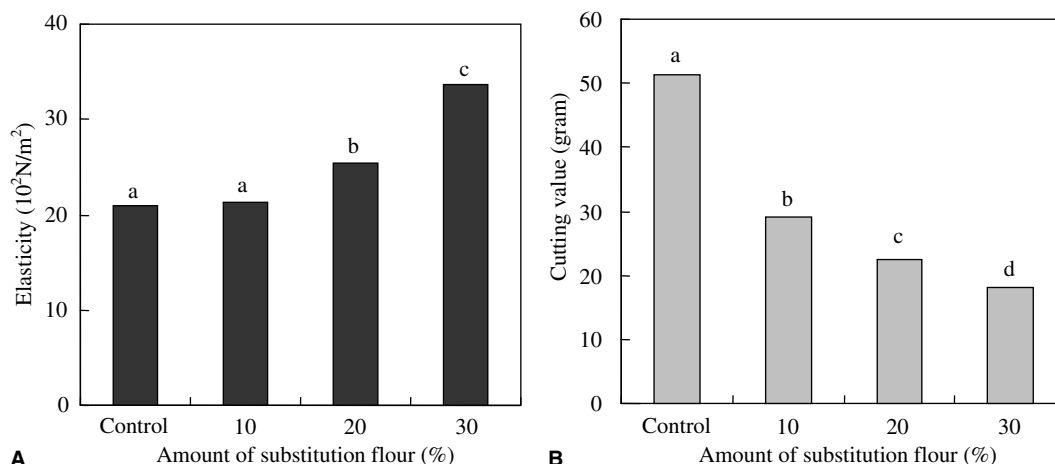


Fig. 5. Textural properties of noodle samples after cooking. Values indicated by the same letters in the same parameter are not significantly different ( $P < 0.05$ ).

but lower amount of allergenic proteins than the buckwheat sample without fermentation. The *R. oligosporus* was found to assimilate proteins as an energy source for its own growth. The hydrolyzed protein remained in the FeB as amino acids and peptides, and the mold was considered to use some kinds of allergenic proteins as a source of energy for assimilation and oxidation of mold biomass. GABA is one of the targeted amino acids, because it was a functional ingredient for blood pressure control, relief of sleeplessness, easing depression and autonomic imbalance. Moreover, the amount of phytic acid which is called anti-nutritional agent with chelating potency was reduced approximately to 70–90% in FeB, suggesting its degradation by *R. oligosporus* during fermentation process. As to the processing properties of FeB flours, pasting properties of FeB-substituted flour were lower than those of the control except for gelatinization temperature and breakdown viscosity. The cooked noodles with the FeB flours became more elastic and softer, and the colors were more brownish than those of the 1CW.

From the present results, the authors conclude that fermented-buckwheat should be a big prospect as a food ingredient, which contains valuable nutritive substances and helps in the development of hypoallergenic buckwheat as a new foodstuff.

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