JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enzymatic Degradation of Oligosaccharides in Pinto Bean Flour

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The use of dry edible beans is limited due to the presence of flatulence factors, the raffinose oligosaccharides. Our objective was to investigate the process for the removal of oligosaccharides from pinto bean using enzymatic treatment and to compare it to removal by soaking and cooking methods. Crude enzyme preparation was produced by six fungal species on wheat bran- and okara-based substrates with soy tofu whey. The loss of raffinose oligosaccharides after soaking pinto beans for 16 h at the room temperature was 10%, after cooking for 90 min was 52%, and after autoclaving for 30 min was 58%. On the other hand, the treatment using crude α-galactosidase (60 U mL^{-1}) produced by *Aspergillus awamori* NRRL 4869 from wheat bran-based substrate with soy tofu whey on pinto bean flour for 2 h completely hydrolyzed raffinose oligosaccharides. These results supported that the enzymatic treatment was the most effective among various processing methods tested for removing the raffinose oligosaccharides, and hence, crude α-galactosidases from fungi have potential use in the food industry.

KEYWORDS: Raffinose oligosaccharides; α-galactosidase; flatulence; pinto bean flour

INTRODUCTION

Dry edible beans (*Phaseolus vulgaris*) are highly nutritious and healthy foods. A recent epidemiological study supported that the consumption of beans and lentil is related to a lower incidence of breast cancer (*1*). One of the obstacles for the utilization of dry beans is the presence of flatulence factors, the raffinose oligosaccharides (raffinose, stachyose, and verbascose). Raffinose oligosaccharides in beans are well-known to produce flatulence in man and animals (*2*−*4*). Since human bodies lack the enzymes to hydrolyze these oligosaccharides, they are fermented to produce gas by bacteria in the large intestine. The flatulence causes some social problems in adults and may cause discomfort in infants and young children (*5*, *6*). The elimination or reduction of the flatulence factor(s) present in beans suggests an increased acceptability by consumers. This subsequently contributes to human health.

The traditional method for home preparation of dry beans consists of a water-soaking period (usually overnight) followed by cooking of the soaked beans after discarding the soaking water. Beans become edible after a long time of boiling in water or after a short time of pressure-cooking. Although the primary purpose of the cooking process is to render the bean texture palatable and to reduce trypsin inhibitor activity, the cooking process also decreases the raffinose oligosaccharide content to some extent (*7*−*13*). The raffinose oligosaccharide content can also be decreased by germination (*14*−*16*) or fermentation with microorganisms capable of degrading raffinose oligosaccharides (*17*−*19*). However, germination and fermentation produce a change in bean aroma that may not be desirable for all consumers (*20*, *21*).

Treatment of foods with preparations rich in α-galactosidase produced by microorganisms can reduce the raffinose oligosaccharide content (*22*, *23*−*29*). The fungal enzymes are relatively easy to cultivate and the most suitable for technological applications mainly due to their extracellular localization, acidic pH optima, and broad stability profiles (*29*,*30*). Using enzymes to remove oligosaccharides in legume flour can avoid the production of strong fermentation flavor (*21*). The present investigation was to compare the effectiveness of soaking, cooking, autoclaving, and enzymatic treatment for eliminating oligosaccharides in pinto bean flour.

MATERIALS AND METHODS

A. Materials. Pinto beans (*Phaseolus vulgaris*), the UI 114 cultivar, were obtained from a local bean handling company (Agri Sale, Inc., Casselton, ND). All beans were hand-sorted to remove split, wrinkled, and moldy ones. All experiments were conducted at least in duplicate.

B. Methods. 1. Home Processing. Pinto beans were soaked in tap water at 1:5 (w/v) bean-to-water ratio for 16 h at room temperature (22 ± 1 °C). A portion of the drained soaked beans was placed in tap water at 1:5 (w/v) bean-to-water ratio and boiled (99−100 °C) for 30, 60, 90 min, respectively. A second portion of the soaked beans was cooked in an autoclave (MIRRO Co., Manitowoc, WI) at 10 lb of pressure (115 °C) for 10, 20, 30 min, respectively. All treated beans were drained, freeze-dried, and ground to pass through a 60-mesh screen for further analysis.

2. Screening the Fungi and Properties of Crude Enzymes. Crude microbial enzymes were produced from six fungi species including *Aspergillus niger* NRRL 3, *A. niger* NRRL 326, *Aspergillus oryzae* NRRL *447*, *A. oryzae* NRRL *1989*, *Aspergillus awamori* NRRL *4869*, and *Rhizopus oligosporus* Saito NRRL 2710 (obtained from the USDA

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10.1021/jf0517041 CCC: $33.50
Published on Web 01/20/2006

ARS National Center for Agricultural Utilization Research, Peoria, IL). The solid-state fermentation method (*37*) was used for the production of α-galactosidases from fungi. Okara and soy tofu whey (2:1) or wheat bran and tofu whey (1:1) were used as substrates. Okara and soy tofu whey are byproducts of soy from the preparation of soy drink (soymilk) and tofu. These byproducts and wheat bran are regarded as industrial waste, but they still have high nutritional content and can be used as substrates for fermentation. The procedures of Mansour and Khalil (*28*) were followed to prepare crude enzyme extract and to characterize their molecular stability and properties, including optimal pH, temperature, pH stability, and thermostability. The six food fungi species were screened for their capability to produce α-galactosidases. The fungi that produced the highest α-galactosidase activity were chosen for further enzyme preparation for hydrolyzing oligosacchrides in pinto bean flour.

2.1. Production of Crude Extracellular Fungal α-Galactosidase. The stock cultures were grown and maintained on potato dextrose agar (PDA, Difco, Detroit, MI) slants at 30 ± 1 °C for 7 days and stored in a refrigerator at 4 °C with monthly subculturing.

Sterile, distilled water (3 mL) was added to each PDA slant and shaken gently. The spore suspension from each tube was poured into a 50-mL presterilized flask and mixed. This spore suspension was used as the inoculum for crude enzyme preparation. Viable spore count (cfu mL^{-1}) of the inoculum was determined by the serial dilution plate count method (*31*), using plate count agar. Duplicates of flasks with substrates for each type of culture were autoclaved (121 °C and 20 min) and cooled to 25 ± 2 °C. Each flask was inoculated with a spore suspension (1×10^7 spore mL^{-1}) from 7-d-old cultures of the fungi and incubated at 30 °C for 6 d.

At the end of the incubation period, 100 mL of distilled water was added to the flasks and thoroughly mixed to extract the crude enzymes. After 1 h at 4 °C, the mixture was filtered through Whatman No. 1 filter paper under vacuum, and the activities of α-galactosidases were determined in the filtrate.

2.2. Determination of Enzyme Activity. The rate of hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside was assayed by spectrophotometric measurement of the liberated *p*-nitrophenyl at 405 nm (*32*). One galactosidase activity unit (Gal U) was defined as the amount of the enzyme that liberated *p*-nitrophenol at the rate of 1 μmol min^{-1} under the given assay conditions.

2.3. Determination of Optimal pH and Temperature. The optimal pH for α-galactosidase activity was determined in the assay mixture over the pH range of 2.0−7.5 using 30 mM sodium acetate buffer and 30 mM sodium phosphate buffer. The optimal temperature of enzyme activity was determined by performing the standard assay for 15 min at the temperatures ranging from 30 to 70 °C.

2.4. pH Stability and Thermostability. For pH stability, enzyme preparations were preincubated at pH values in the range of 2.5−6.5 for 30 min at optimum temperature for each strain. For thermal stability determination, enzyme preparations were preincubated at a temperature range of 40−70 °C with 5 °C intervals for 30 min at the optimum pH for each strain. The residual enzyme activity was determined at pH 5.5 by a previously described method in *Food Chemicals Codex* (*32*).

3. Treatment of Pinto Bean Flour with Crude Extracellular Fungal α-Galactosidase. The raw beans were ground to pass through a 60-mesh screen. The bean flour was treated by heating at 121, 115, or 100 °C (SYBRON C3768 steam sterilizer, Rochester, NY) for 25 min, respectively. Then, bean flours (4 g) were treated with 40 mL of 60 U mL^{-1} enzyme preparations. (The crude enzymes were concentrated by ultrafiltration on Diaflo ultrafilters Amicon membrane PM 10.) The enzyme-treated bean flours were incubated for 0.5, 1, 2, and 3 h in a rotary shaker (250 rpm) at optimum conditions of pH 5.0 and temperature 55−60 °C. For the control, the volume of enzymes was replaced with distilled water (pH 5.0). After incubation, the contents were filtered through Whatman No. 1 filter paper under vacuum. The residues were dried in the vacuum oven at 70 °C for 20 h and ground to produce enzyme-treated bean flour. The oligosaccharides in the untreated control and enzyme-treated bean flours were extracted (*33*) and determined by HPLC as described in the following paragraphs.

4. Extraction Sugar from Bean Flour. A portion of 200−300 mg bean flour was placed in a 20-mL test tube. A 10-mL portion of 80% ethanol containing 5.5 mg of mannitol (internal standard) was added and vortexed for 2 min and further extraction was carried out for 30 min in a shaker at 72−75 °C. After centrifugation (Beckman J2-HS, Fullerton, CA) at 10 000*g* for 5 min at 10 °C, 6 mL of clear supernatant was taken to dryness with a rotary evaporator (Brinkmann, Westbury, NY). The dry extract was dissolved in double-deionized H_2O (2.5 mL) and then passed through a column of CM 52 (1 mL) overlaid by DE 52 (1.5 mL) to remove ionic substances (*33*). A total of 5.5 mL of eluent was collected for each flour sample and filtered through a 0.2 μm nylon membrane prior to HPLC analysis.

5. Determination of Oligosaccharides Content by Liquid Chromatography (HPLC). Sugar solutions extracted and purified by the above procedures (10 μL) were analyzed by HPLC, with a Model 720 system controller, two pumps, and a 746 data module (Waters Associates, Denver, CO). The separation of sugars was achieved by a Prevail carbohydrate ES column (250 × 4.6 mm, Rocket, Alltech, Deerfield, IL). The column was maintained at 30 °C using a column heater (temperature control unit model III). The elution was monitored by a Varex MK III ELSD (Alltech, Deerfield, IL). The mobile phase consisted of a gradient with 20% water at the beginning and 40% water at 16 min with a flow rate of 1.0 mL min^{-1}. The identification of sugars was achieved using raffinose and stachyose standards (Sigma Chemical Co., St. Louis, MO). The quantitation of sugars was based on the response factor relative to that of the internal standard (mannitol) added to the sugar solutions.

6. Statistical Analysis. An analysis of variance (SAS 1988) was conducted to analyze the oligosaccharide content in the bean flour as influenced by various processing methods. When a significant main effect was detected, the differences among the means were analyzed by the Duncan's multiple range test using $P < 0.05$.

Table 1. Reduction (%) of Raffinose Oligosaccharides in Pinto Beans by Home Processing[a]

home processing	raffinose	stachyose	raffinose + stachyose
soaking	6.0 ± 0.7	10.3 ± 0.9	9.8 ± 0.9 a
cooking, 30 min	29.2 ± 1.5	46.2 ± 1.2	44.4 ± 1.3 b
cooking, 60 min	32.6 ± 1.6	48.2 ± 0.4	46.6 ± 0.2 bc
cooking, 90 min	42.3 ± 0.1	53.5 ± 0.4	52.4 ± 0.3 de
autoclaving, 10 min	22.4 ± 0.7	50.1 ± 2.8	46.9 ± 4.1 bc
autoclaving, 20 min	28.4 ± 3.0	51.8 ± 1.1	49.5 ± 1.3 cd
autoclaving, 30 min	51.8 ± 0.4	59.0 ± 0.5	57.6 ± 0.4 f

[a] All values are means of duplicate determinations. Values followed by the same letters in the same column are not significantly different at $p<0.05$.

RESULTS AND DISCUSSION

1. Home Processing. HPLC revealed the presence of sucrose, raffinose, and stachyose in pinto beans. Raw pinto beans contained 0.38% raffinose and 3.34% stachyose on a dry weight basis. The results revealed that soaking and cooking reduced the level of raffinose oligosaccharides (**Table 1**). For example, soaking for 16 h at the room temperature reduced raffinose oligosaccharides by 9.8%. The extent of reduction increased when cooking time increased. Boiling for 30, 60, and 90 min effectively reduced raffinose oligosaccharides by 44.4%, 46.6%, and 52.4%, respectively. Autoclaving was more effective than open-kettle cooking in reducing oligosaccharides. Autoclaving for 10, 20, and 30 min significantly reduced raffinose oligosaccharides by 46.9%, 49.4%, and 57.6%, respectively.

In the literature, the percent removal of oligosaccharides by soaking, cooking, or their combination varied widely. Bean variety, the length of soaking and cooking period, and heat processing method (*8*, *9*, *11*) affected the extent of removal of oligosaccharides. Soaking cowpea decreased the amount of raffinose and stachyose by 39% and 18%, respectively, whereas soaking followed by boiling resulted in decreases of 58% and

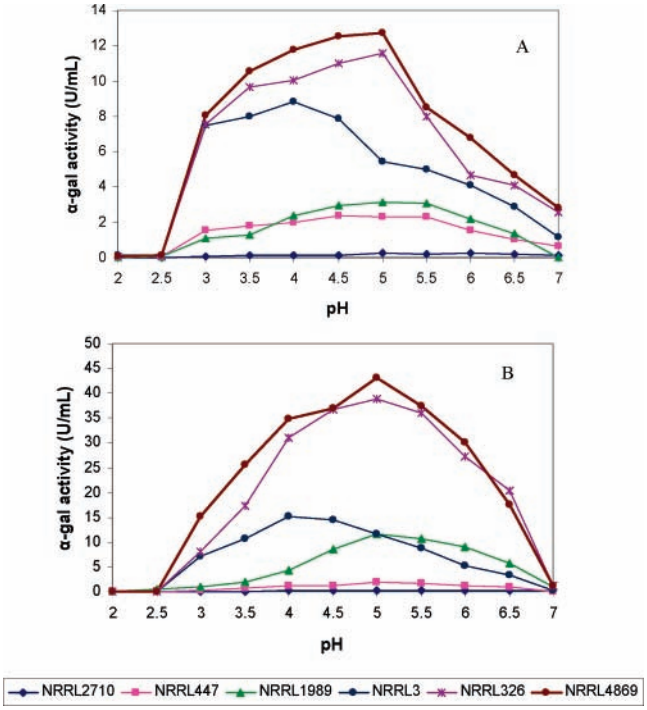

Figure 1. Optimal pH of the crude enzyme preparations produced by six fungi on (**A**) okara-based substrate with tofu whey and (**B**) wheat bran-based substrate with tofu whey.

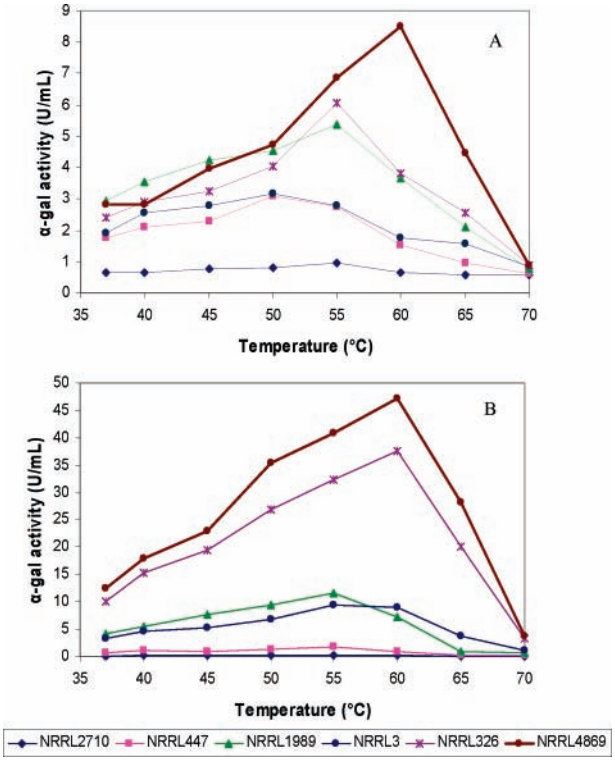

Figure 2. Optimal temperature of the crude enzyme preparations produced by six fungi on (**A**) okara-based substrate with tofu whey and (**B**) wheat bran-based substrate with tofu whey.

49%, respectively (*19*). Soaking of Great Northern, kidney, and pinto beans and their subsequent boiling for 90 min decreased the amount of raffinose and stachyose by 70−80% (*34*). In marked contrast, sucrose, raffinose, stachyose, and verbascose contents increased following the cooking of red, Bengal, black, and green grams (*17*, *35*). In this study, retorting (115 °C, 30 min) was the most effective method among various processing conditions tested in reducing oligosaccharides, but the reduction of raffinose oligosaccharides only reached to approximately 58%. Therefore, further research was needed to develop method for eliminating all raffinose oligosaccharides in bean products.

2. Screening the Fungi and Properties of Crude Enzyme Preparation. *2.1. The Optimal pH and Temperature of Crude Enzymes Produced by Six Fungi.* **Figure 1** summarizes the crude enzyme activities at various pH values. The crude enzymes produced by *A. awamori* NRRL 4869 exhibited the highest activity among six fungi on both types of substrates. The optimal pH of the crude enzymes was pH 5.0. We also obtained the optimal pH (range 4.0−5.0) of the crude enzymes produced by other fungi. Apparently, the enzymes produced on wheat bran-based substrate exhibited higher activity than that produced on okara-based substrate. The activity of the enzymes produced by NRRL 4869 at pH 5.0 was 13 U mL^{-1} on okara-based substrate, whereas on wheat bran-based substrate, the activity of the enzymes reached to 45 U mL^{-1}. The higher activity of the enzymes produced on wheat bran-based substrate may be due to more enzymes being formed on it. Since wheat bran contains more essential nutrients, e.g., B vitamins (*26*), the growth of fungi on wheat bran was more profuse than their growth on okara. **Figure 2** summarizes the effect of incubation temperature on crude enzyme activities. The enzymes produced by *A. awamori* NRRL 4869 exhibited the highest activity among six fungi on both types of substrates. The optimal temperature of the crude enzymes was 60 °C. We also obtained the optimal temperature (range 50−60 °C) of the crude enzymes produced by other fungi. The activity of the enzymes produced by NRRL 4869 at 60 °C was 8 U mL^{-1} on okara-based substrate, whereas on wheat bran-based substrate, the activity of the enzymes reached to 46 U mL^{-1}. The difference in the optimal pH and temperature of these crude enzymes may be due to different forms or quantities of the enzymes being produced. *A. niger* produced three forms of α-galactosidases (*36*, *37*). The three types (AglA, AglB, and AglC) displayed very different molecular composition, amino acid residues, respectively.

The α-galactosidase produced by NRRL 4869 exhibited its maximum activity at pH 5.0 and was stable in that pH range. The optimum temperature was 60 °C. The α-galactosidase reported here appeared quite similar to an α-galactosidase from *A. saitoi* (*23*). Both enzymes exhibit maximum activity at pH 5.0 and are inactivated at 70 °C. Other fungi (*9*, *23*−*26*, *28*, *38*−*40*) reportedly produced α-galactosidase, but direct comparisons of activity with *A. awamori* were impossible, because assays of the enzyme activity were conducted with different methods, which included the hydrolysis of *p*-nitrophenyl-α-D-galactoside, the amount of glucose released from melibiose, or the amount of reducing sugar liberated from raffinose or stachyose. From **Figures 1** and **2**, *A. awamori* NRRL 4869 and wheat bran-based substrate with tofu whey were chosen for the further enzyme preparation.

Table 2 summarizes the optimal pH and temperature of crude enzymes produced by the six fungi. Generally, the enzymes produced on both substrates had similar optimal pH and temperature. For example, the enzymes produced by *R. oligosporus* Saito NRRL 2710 had the optimal pH 5 and temperature 55 °C, respectively. However, there was a little difference in the case of *A. oryze* NRRL 447 and *A. niger* NRRL 326. Literature reported similar pH and temperature optima for *A. oryze* at pH 4.5 and 50 °C (*26*) and for *A. niger* at pH 4.5−5 and 50−60 °C, respectively (*9*, *29*, *37*). The crude enzymes produced by *A. niger* NRRL 3 were active at pH 4, which means this enzyme preparation could be used in a more acidic

Table 2. Optimal pH and Temperature of the Crude Enzyme Preparations

	okara and tofu whey		wheat bran and tofu whey	
species	pH	temp, °C	pH	temp, °C
R. oligosporus Saito NRRL 2710	5.0	55	5.0	55
A. oryzae NRRL 447	4.5	50	5.0	55
A. oryzae NRRL 1989	5.0	55	5.0	55
A. niger NRRL 3	4.0	55	4.0	55
A. niger NRRL 326	5.0	55	5.0	60
A. awamori NRRL 4869	5.0	60	5.0	60

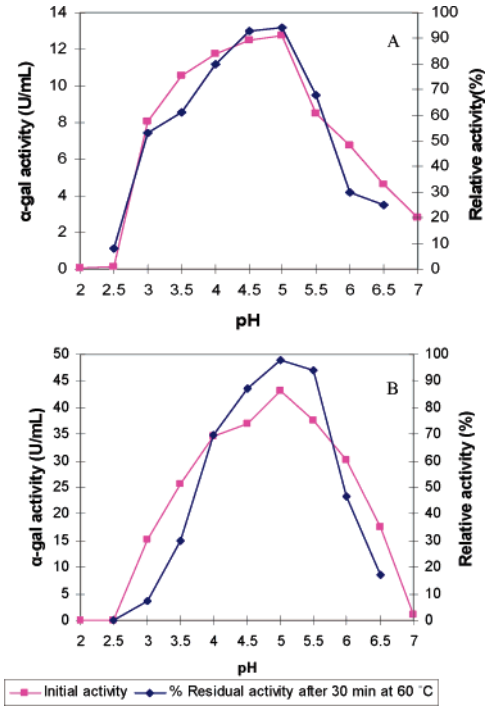

Figure 3. pH activity profile and pH stability of the crude α-galactosidase produced by *A. awamori* NRRL 4869 on (**A**) okara-based substrate with tofu whey and (**B**) wheat bran-based substrate with tofu whey.

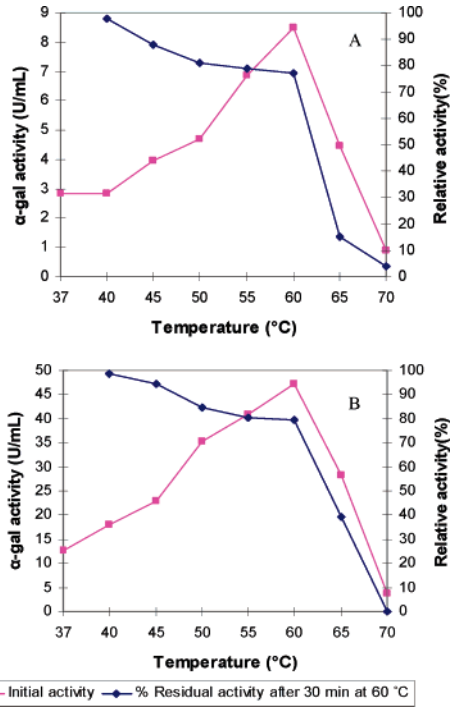

Figure 4. Thermal activity profile and thermostability of the crude α-galactosidase produced by *A. awamori* NRRL 4869 on (**A**) okara-based substrate with tofu whey and (**B**) wheat bran-based substrate with tofu whey.

Table 3. Reduction (%) of Raffinose Oligosaccharides by Enzymatic Treatment as Compared to the Controlled Flour Treated with Distilled Water[a]

time (h)	121 °C	115 °C	100 °C
0.5	10.6 ± 1.0 e	26.8 ± 4.9 d	32.2 ± 3.4 d
1	54.6 ± 1.2 b	47.6 ± 0.9 c	28.6 ± 2.5 d
2	57.1 ± 0.5 b	100.0 ± 0.0 a	98.0 ± 2.8 a
3	100.0 ± 0.0 a	100.0 ± 0.0 a	95.2 ± 6.8 a

[a] All values are means of duplicate determinations. Values followed by the same letters are not significantly different at $p < 0.05$.

environment, such as in beverage. The crude enzymes produced by *A. awamori* NRRL 4869 exhibited the highest activities at pH 5.0 and temperature 60 °C, which implies enzymes have better thermostability. However, the optimum temperature for the α-galactosidases for *A. awamori* reported by McGhee et al. (*23*) was 50 °C at pH 5.0.

2.2. The pH Stability and Thermostability of Crude Enzyme Preparation Produced by Six Fungi. **Figure 3** summarizes the pH stability profile of the crude enzymes produced by *A. awamori* NRRL 4869 on both substrates. For pH stability, the enzyme solution was preincubated at pH values in the range of 2.5−6.5 for 30 min at the optimal temperature. The residual activity, the activity of the residual enzyme, was expressed as relative activity (%) as compared to the original enzyme without preincubation. The pH stability of the crude enzymes was the best in the range of 4.5−5.0 for enzymes produced on okara-based substrate and 5.0−5.5 for enzymes produced on wheat bran-based substrate. When the pH remained at 5.0, the enzymes exhibited the largest stability, 95% relative activity for the residual enzymes produced from okara-based substrate and 98% relative activity for the residual enzymes produced from wheat bran-based substrate.

Figure 4 summarizes the thermostability of enzymes produced by *A. awamori* NRRL 4869 on both substrates. For thermostability, the enzyme solution was preincubated in the range of 40−70 °C with 5 °C intervals for 30 min at the respective optimal pH condition. The residual activity was expressed as relative activity (%) as compared to the original enzyme without preincubation. The optimal temperature of crude enzymes produced on both substrates was 60 °C with a wide range of stability (up to 60 °C). There were approximately 20% activity decreases during this range on both substrates. The enzymes exhibited the highest activity when the temperature remained at 60 °C. At 70 °C for 30 min, the crude enzyme solutions were almost inactive.

From **Figures 1**−**4** above, α-galactosidase activities were optimal at pH 5.0 and temperature within a 55−60 °C range on the artificial substrate *p*-nitrophenyl-α-D-galactopyranoside (PNPG). Therefore, pH 5.0 and 55−60 °C were chosen for the enzymatic incubation conditions with bean flours.

3. Screening Processing Conditions for Treating Bean Flour. From **Table 3**, temperature (115 °C > 100 °C > 121 °C, 25 min) and incubation time (3 h > 2 h > 1 h > 0.5 h) have significantly effects on the reduction the raffinose oligosaccharides. After heating treatment at 115 °C, the pinto bean flour treated with the crude enzymes only for 2 h can completely eliminate the raffinose oligosaccharides. The lower reduction of the raffinose oligosaccharides by 121 °C heat treatment may

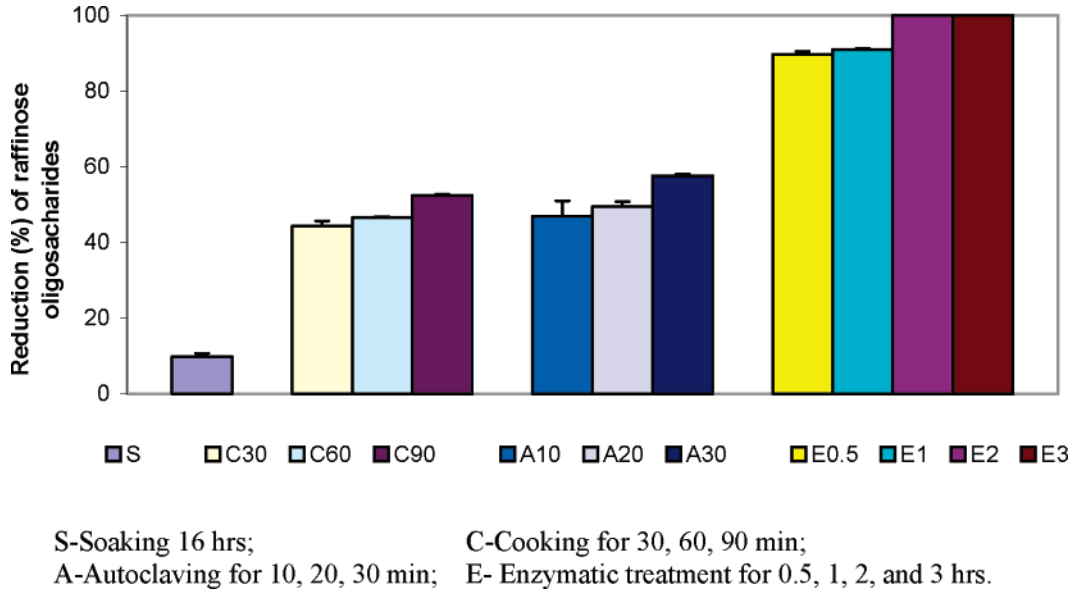

Figure 5. Reduction (%) of raffinose oligosaccharides by selected processing conditions.

be caused by the heat-induced physical−chemical changes on the bean particulates to prevent some oligosaccharides from contacting with the enzymes during incubation. After cooking at 121 °C, the bean flour became much darker than that heated at lower temperatures.

Figure 5 supported that the enzymatic treatment was the best processing method for eliminating raffinose oligosaccharides among various procedures tested. Although this was the first report on the use of fungal α-galactosidases from *A. awamori* NRRL 4869 for the hydrolysis of pinto bean flour oligosaccharides, there are reports on the use of this partially purified enzyme from *A. awamori* (*23*, *38*), *A. oryzae* (*25*), *A. saitoi* (*22*), *A. fumigatus* (*39*), *Cladosporium cladosporides* (*24*, *28*), *Gibberella fujikuroi* (*40*) for hydrolysis of oligosaccharides in soymilk. The optimum pH and temperature for α-galactosidases were in the similar range of pH 4.5−5.5 and 50−60 °C for the reported fungi. There were several reports available in the literature on the use of these enzymes for hydrolysis of oligosaccharides present in legume flours (*9*, *26*, *28*). Crude preparations of α-galactosidase (64 U μg^{-1} protein) from *A. niger* reduced raffinose and stachyose by 93% and 82% in cowpea flours of 20-mesh particle size after 2 h of treatment at pH 5.0 and 50 °C (*9*). Crude extracelluar α-galactosidases from *C. cladosporides*, *A. oryzae*, and *A. niger* (290, 210, and 130 U mL^{-1}, respectively) at the optimum conditions of each strain (pH 5.0 and 40 °C for *C. cladosporides*, pH 4.5 and 50 °C for *A. oryzae*, and pH 5.0 and 50 °C for *A. niger*, respectively) for 3 h reduced the raffinose and stachyose content by 100% in chickpea flours of 40-mesh particle size (*28*). Several bean flours, including Michigan black, red, and navy bean flour, incubated with commercial purified α-galactosidase at the room temperature for 1 h reduced raffinose oligosaccharides by 30−51% (*41*). This was a lower energy and shorter time processing method than that we used (115 °C preheating bean flour for 25 min and then incubation with crude enzymes at 60 °C for 2 h). However, the reduction of the raffinose oligosaccharide was obviously greater in our study than that reported in the study using purified enzymes (*41*). Also, our low flatulence flour was a precooked product. In addition, the cost of using commercially purified enzyme may be a challenge for commercial feasibility.

Considering that treatments such as germination and fermentation can change the physicochemical and sensory properties of legumes (*20*, *21*) and the high catalytic power of enzymes, enzymatic methods have the greatest potential as a technique to control the flatulence-inducing activity of pinto beans and probably other legume flours or soymilk.

Wheat bran and soy tofu whey are byproducts. Treating tofu whey to avoid water pollution is becoming a critical factor for tofu manufacturing. Utilizing tofu whey will reduce waste treatment cost. Fungi propagation and crude enzyme preparation can be easily processed. Without purification, crude enzymes can eliminate all raffinose oligosaccharides in bean flour. These advantageous features may contribute to the practical and economic feasibility of the methods using crude enzymes. Enzymatic treatment was effective for eliminating the raffinose oligosaccharides, and hence, crude α-galactosidases from fungi have potential use in the food industry. However, the crude enzymes also contain other hydrolytic enzymes, which may affect palatability, functionality, acceptability, and storage properties of enzyme-treated products. Therefore, the quality characteristics of the enzyme-treated bean products must be studied before commercial applications of the process. Further studies using semipurified enzymes may be necessary to reduce potential side effects and in the meantime to retain economic feasibility.

ACKNOWLEDGMENT

Special thanks to S.W. Peterson at the USDA National Center for Agricultural Product Utilization in Peoria for providing fungi species.

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Received for review July 15, 2005. Revised manuscript received October 19, 2005. Accepted November 4, 2005. The Northarvest Bean Growers Association and USDA NRI CGP Fund No. 2001-10853 provided funding for this research.

JF0517041