Use of Fermented Black Beans Combined with Rice To Develop a Nutritious Weaning Food

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Common beans are an important source of energy and nutrients, but have significant amounts of antinutritional factors and a limited digestibility. A nutritious weaning food was developed by combining fermented black beans and rice. Raw beans were coarsely ground, soaked, cooked, fermented with *Rhizopus oligosporus* for 15, 20, or 25 h, and then homogenized to obtain a supernatant and a precipitate. Raw, cooked, and fermented beans, and the precipitates were chemically characterized and the data statistically analyzed to choose an optimum fermentation time to develop the weaning food product. Ash and mineral contents of the beans decreased after soaking and in the precipitates. Cooking improved protein digestibility and decreased the levels of lectin and trypsin inhibitor. The oligosaccharide content of beans fermented 25 h was lower than in the other treatments. The weaning food product (27% 25 h fermented beans, dry weight/73% cooked rice, dry weight) had an in vitro protein digestibility of 86% and a very low content of oligosaccharides.

Keywords: *Dry beans; Phaseolus vulgaris; fermentation; tempeh-like products; weaning foods*

INTRODUCTION

The development of low cost foods with high levels of energy, protein, and other nutrients is necessary for the weaning food market. The high cost of animal protein foods, especially in developing countries, creates the need to combine various plant protein sources, to obtain foods of high protein quality. High nutritional quality foods are needed to help infants that suffer from malnutrition in these countries.

Legumes represent a major protein source consumed by a large section of the population of developing countries (Bressani, 1993). In Central America, rice and beans represent the basic diet of much of the population. However, rice is mainly deficient in the essential amino acid lysine and is relatively low in protein content (Bressani and Valiente, 1962). Beans are higher in lysine but deficient in methionine, and have low digestibility and other negative factors such as oligosaccharides, tannins, trypsin inhibitors, phytates, and hemagglutinins. Combining rice and beans has proven to improve protein quality (Bressani, 1987; Sgarbieri et al., 1979). Therefore, the development of a weaning food containing an optimum combination of these two plant foods, processed to have a higher protein quality and a lower content of antinutritional factors, is important for Latin American nations.

Fermented foods are consumed in many parts of the world, including Southeast Asia, Africa, and the Near East. These foods are important sources of energy, protein, and vitamins (van Veen and Steinkraus, 1970). Solid-state fermentations (SSF) offer a promising option in developing high nutritional quality foods (Paredes-López et al., 1990). During SSF, there is an increase in the synthesis of enzymes that hydrolyze some of the substrate constituents that can decrease or eliminate the antinutritional factors, thereby improving the nutritional quality of the bean food (Paredes-López et al., 1990).

The objective of this study was to create a nutritious weaning food by combining fermented black beans with rice. The weaning food was defined with regard to processing, formulation, and chemical characteristics.

MATERIALS AND METHODS

Sources of Beans and Rice. Common beans, *Phaseolus vulgaris* variety Talamanca, which are black beans typical of those consumed in Costa Rica, were provided by Dr. George Hosfield in the Department of Crop and Soil Science at Michigan State University, East Lansing, MI. Extra fancy long grain enriched rice marketed by North Arkansas Wholesale Co., Inc., (Bentonville, AR) was purchased at Sam's Club, Lafayette, IN.

Experimental Design. *Experiment 1.* Experiment 1 (Figure 1) was designed to define the fermentation process, by testing three different fermentation times (15, 20, and 25 h) and to study the composition of water soluble and insoluble fractions of the fermented beans. Experiment 1 was repeated two times.

Experiment 2. After an optimum fermentation time (optimum of the conditions tested) from compositional data obtained from experiment 1 was chosen, the objectives of experiment 2 were to develop a weaning food product using the chosen fermented bean and rice and to chemically characterize this final product (Figure 2). Experiment 2 was conducted once. The proportion of rice to beans used to develop the final product was calculated on the basis of the amino acid composition of the bean (Table 1) and rice (data not shown), and on the infant requirements for lysine and sulfur-containing amino acids (methionine and cysteine) (World Health Organization, 1985). The proportion was determined when ratios of content compared to requirements were the same for both sulfur-containing amino acids and lysine. Proportions used were 53% bean protein and 47% rice protein; which is equivalent to 27% fermented beans, dry weight, and 73% cooked rice, dry weight.

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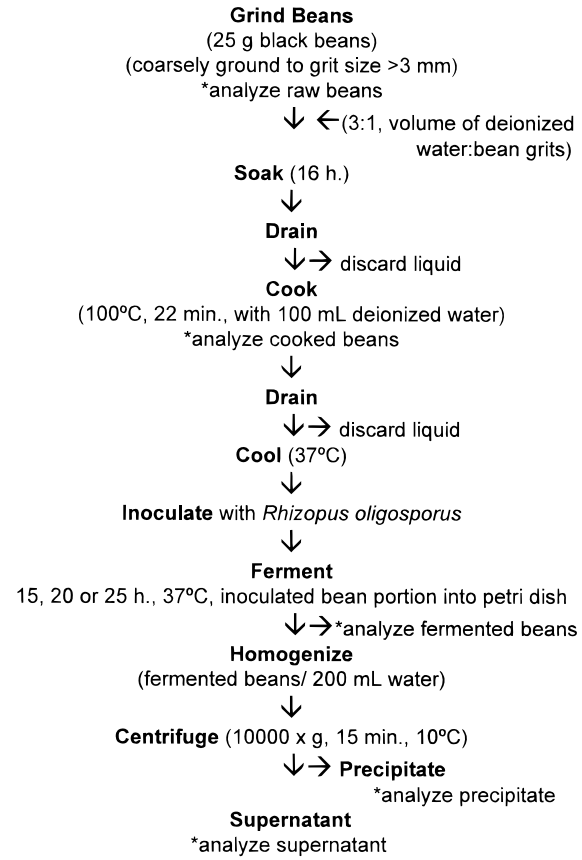

Figure 1. Flowchart for experiment 1, fermentation of dry beans.

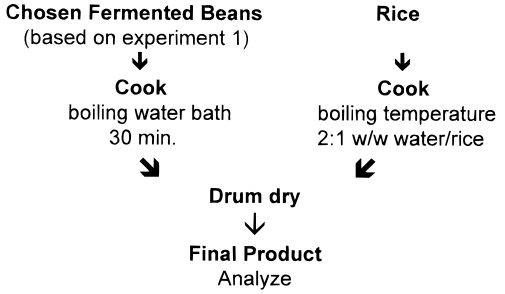

Figure 2. Flowchart for experiment 2, combining fermented dry beans and rice to develop weaning food product.

Inoculation Procedure. A *Rhizopus oligosporus* NRRL 2710 culture (provided by Kerry O'Donnell, Microbiologist, USDA, Northern Regional Research Lab, Peoria, IL) was propagated in potato dextrose agar slants for 7 days at 25 °C. The slants were stored at 5 °C. To ferment the beans, fresh slants were prepared, inoculated, and propagated at 25 °C for 5−7 days. The agar slant culture was suspended in 5 mL of sterile water. A 2-mL aliquot of the suspension was added to Petri dishes containing cooked grits from 25 g of raw beans, as described below.

Sample Preparation. Raw beans were coarsely ground to grit size with a Waring blender (Model 1002, Waring Products Co., Winsted, CT) for 25 s at the lowest setting. Small pieces of bean were separated and discarded from the grits using a 3-mm screen. Grits (25 g) were cooked at 100 °C for 22 min with 100 mL of deionized water. After fermentation of the cooked grits, samples were homogenized for 5 min at speed 5 using a Brinkmann Homogenizer (Model PT 10/35, Brinkmann Instruments Co., Westbury, NY). Raw grits, cooked grits, fermented beans, precipitates, supernatants (experiment 1, Figure 1), and cooked rice (experiment 2, Figure 2) were freeze-dried (Virtis Freezemobile 5SL, Gardiner, NY) then milled through a 0.5 mm screen (Tecator Cyclotec, 1093 Sample Mill, Tecator, Höganics, Sweden) prior to analyses. Final product (experiment 2, Figure 2) was drum dried (Bufloval, Model 175Br5, Buffalo, NY) under the following conditions: 250 °C surface temperature, 0.01-in. distance between drums, and 0.8 rpm.

Table 1. Amino Acid Content of Raw, Cooked, and Fermented Beans[a,b]

	amino acid content (g of amino acid/100 g of dry matter)				
			fermented beans		
amino acid	raw beans	cooked beans	15 h	20 h	25 h
Ala	1.10	1.21	1.24	1.17	1.21
Arg	2.01	1.81	1.62	1.44	1.46
Asp	2.91	3.06	3.22	3.40	3.36
Cys	0.24	0.22	0.24	0.22	0.22
Glu	3.98	4.08	4.12	4.12	4.19
Gly	1.09	1.18	1.13	1.09	1.13
His	0.85	0.86	0.86	0.84	0.90
Ile	1.24	1.34	1.35	1.43	1.43
Leu	2.20	2.40	2.35	2.40	2.43
Lys	1.96	2.12	1.91	1.73	1.98
Met	0.30	0.35	0.34	0.34	0.35
Phe	1.54	1.62	1.63	1.68	1.76
Ser	1.50	1.72	1.71	1.72	1.69
Thr	1.10	1.24	1.25	1.22	1.22
Tyr	1.04	1.01	1.03	0.96	0.97
Val	1.36	1.48	1.51	1.53	1.54

[a] See Figure 1. [b] Experiment was repeated one time for each treatment, and triplicate assays were performed.

Proximate Composition. The moisture, ash, and fat contents were determined in duplicate using AOAC Methods 925.09, 923.03, and 920.39C, respectively (AOAC, 1995). Protein was determined measuring the nitrogen content of duplicate samples and using a conversion factor of 6.25, by AOAC micro-Kjeldahl Method 960.52 (AOAC, 1995). Carbohydrate content in the final product was calculated by difference.

Total Dietary Fiber Determination. AOAC Method 985.29 (AOAC, 1990) was used to determine total dietary fiber content. The following modifications were used: (1) 0.05 M phosphoric acid was used for the starch gelatinization during treatment with heat stable α-amylase, (2) after this hydrolysis, the pH was adjusted to 7.5 ± 0.1 with a 0.171 N NaOH solution, and (3) after the incubation with protease, the pH was adjusted to 4.5 ± 0.2 with a 0.205 M phosphoric acid solution.

Starch Determination. Starch was determined in triplicate using the method described by Tomkinson (1986) with the following modifications: (1) a sample of 240 mg of flour was placed in a 50-mL Erlenmeyer flask and 5 mL of ddH_2O was added, (2) 18 mL of 0.125 N NaOH was added after gelatinization, (3) after the addition of the acetic acid and the homogenization, the sample was quantitatively transferred to a 100-mL volumetric flask, (4) an enzyme solution containing 5 mg/mL amyloglucosidase (EC 3.2.1.3) (A-7255, Sigma Chemical Co.) was used, (5) after addition of the thymol, samples in flasks were brought to volume with ddH_2O, and (6) glucose content of each flask was measured at 505 nm according to procedure no. 315, Sigma Diagnostics, Sigma Chemicals Co., using Glucose Trinder Reagent (315−100, Sigma Chemicals Co.).

Mineral Determination. Inductively coupled plasma−atomic emission spectroscopy (ICP-AES) was used to determine content of specific minerals (Fe, Zn, Ca, Mg, P, and K) according to AOAC Method 984.27 (AOAC, 1995).

Amino Acid Analysis. Amino acids (including sulfur-containing amino acids) were analyzed by AGP Limited (Courtland, MN) according to AOAC Method 994.12 (AOAC, 1995).

In Vitro Protein Digestibility Determination. The pH-stat procedure (Pedersen and Eggum, 1983) was used to determine in vitro protein digestibility. A 1-mL aliquot of an enzyme solution containing 22704, 186, and 0.052 units of trypsin (T-0134, Sigma Chemicals Co.), α-chymotrypsin (C-4129, Sigma Chemicals Co.), and peptidase (P7500, Sigma Chemicals Co.), respectively, was used for each sample. For experiment 1 (Figure 1), raw, cooked, fermented beans, and precipitates from the fermented beans were analyzed using this method. In addition, these samples and the supernatants were heated further for 20 min in a covered 30-mL beaker with 10 mL of water using a boiling water bath. After cooking, the sample was cooled to 22 °C and analyzed as a regular sample. Calculations were done for all protein sources using the regression equation reported by Pedersen and Eggum (1983).

Oligosaccharide Determination. The method used was a modification of the one described by Kuo et al. (1988). Ethanol (80% v/v) was heated to 70 °C in a Blue M shaking water bath (Magni Whirl, Blue Island, IL). Five samples in duplicate and two recovery standards (see below) were run at the same time. Approximately 1 g of sample was weighed accurately into a 50-mL Erlenmeyer flask. Standard recovery solutions [1 mL of raffinose (6.00 mg/mL), 1 mL of verbascose (6.00 mg/mL), and 1 mL of stachyose (15.0 mg/mL)] was added to both recovery standards. Then, 10 mL of preheated ethanol was added to each flask, and flasks were sealed with rubber stoppers and mixed at speed 10 for 30 min in the water bath at 70 °C. Flask contents were transferred quantitatively into 35-mL graduated screw-capped centrifuge tubes and were brought to 25 mL with 80% ethanol. Flasks were centrifuged at 10000*g* for 15 min at 10 °C, and 10 mL of the clear supernatant was placed into a test tube and dried with a nitrogen evaporator (Model 730, Cole-Parmer Instrument Co., Chicago, IL) at 40 °C. After drying, tubes with dry extract were stored at −20 °C.

Preswollen DEAE−cellulose (5 g) (DE 52, Whatman, Kent, England) was weighed into a beaker, and 75 mL of a 0.1 M phosphate buffer at pH 6.5 was added. The solution was stirred for 3 min, and the pH was adjusted to 6.5 ± 0.1 with one of the two components of the buffer. After the slurry settled, supernatant was removed to leave ∼35 mL of a soft resin slurry. The resin slurry in 1.5-mL aliquots was added to 35-mL centrifuge tubes. The dry extract sample was then dissolved in 1.5 mL of nanopure H_2O (Nanopure II, Barnstead, Dubuque, IA), and the solution was added to the centrifuge tube that contained the resin. Tubes with resin and extract were rinsed three times with 0.5 mL of nanopure H_2O for a total volume of 4.5 mL including the resin. Centrifuge tubes were shaken for 30 min using an automatic shaker and centrifuged at 10000*g* for 15 min at 10 °C. Carefully, 3 mL of the supernatant was transferred to a 10-mL volumetric flask and the flask was brought to volume using nanopure H_2O. The content of the volumetric flask was filtered through a disposable 3-mL syringe fitted with a 0.45-μm membrane (13-mm Acrodisc LC13 PVDF, Gelman Sciences, Ann Arbor, MI) into a clean test tube and the sample was stored at 4 °C.

The HPLC analysis was performed on a Dionex BioLC system using a Carbopac PA1 anion exchange column, a Dionex PAD-2 pulsed amperometric detector, and a Spectra-Physics 4400 integrator (Dionex Corporation, Sunnyvale, CA). Prior to injection, the column was washed with a 1 M NaOH solution for 5 min using a flow rate of 2 mL/min. It was then conditioned for 10 min with a 200 mM NaOH solution using the same flow rate. After injection of the samples and recovery standards, the oligosaccharides were eluted with a 300 mM NaOH solution at a flow rate of 1 mL/min for 25 min. Standard solutions of 0.0 0.10, 0.25, and 0.50 mg/mL standard solutions of raffinose (R-0250, Sigma Chemical Co.), stachyose (S-4001, Sigma Chemicals Co.), and verbascose (Megazyme, North Rocks, Australia) were prepared, filtered through a 0.45 μm filter, and 25 μL of the 0.1 mg/mL standard solution were injected.

Oligosaccharide content of each sample was calculated using the following equation, where 37.5 is the dilution factor. The recovery factor was calculated as the concentration of standard sugar for recovery, calculated from the standard curve, over the actual concentration of the standard sugar added to the recovery:

$$\text{milligrams of oligosaccharide/gram of sample} = \frac{\text{calculated sugar concentration (mg/mL)} \times 37.5}{\text{sample weight (g)} \times \text{recovery factor}}$$

Table 2. Yield Factors Calculated for Experiment 1 To Correct Results for Weight Losses during Processing

treatment	yield factor[a]
raw beans	1.00
cooked beans	0.81
fermented beans (15 h)	0.83
fermented beans (20 h)	0.80
fermented beans (25 h)	0.80
precipitate (15 h)	0.69
precipitate (20 h)	0.66
precipitate (25 h)	0.72
supernatant (15 h)	0.10
supernatant (20 h)	0.09
supernatant (25 h)	0.07

[a] Yield factors were calculated determining the moisture content and weight for each one of the treatments.

Trypsin Inhibitor Determination. Trypsin inhibitors were determined as described by Nielsen and Liener (1988) modified from Kakade et al. (1969), except that the bean solution was made with 20 mg of bean sample. Also, 1.93 mL of Tris buffer and 0.3 mL of the trypsin standard solution were used for the reaction.

Lectin Determination. The method of Liener (1955), with modifications based on methods used by Paredes-López et al. (1988) and Donatucci et al. (1987), was used to determine lectins. In centrifuge tubes, 100 mg of dry sample and 20 mL of phosphate buffer solution (PBS) (0.136 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) were stirred at 22 °C for 1 h and then centrifuged at 40000*g* for 30 min at 4 °C. Serial 2-fold dilutions of the protein solutions were made in test tubes to give a final volume of 2 mL. Each sample was run in duplicate with a series of 10 dilutions. Trypsinated erythrocyte suspension was adjusted to an absorbance reading of ∼1.0 at 650 nm. The erythrocyte suspension (2 mL) was added to each tube and then contents were vortexed and left undisturbed for 2.5 h. Carefully, absorbance was read at 650 nm in a Spectronic 20D (Milton Roy Co., Chicago, IL). Trypsinated erythrocyte suspension was prepared according to the procedure described by Donatucci et al. (1987). Rabbit blood with heparin was purchased from Pel Freez Biologicals (Rogers, AR).

Statistical Analysis. The data were subjected to analysis of variance (ANOVA) using general linear models procedure of the Statistical Analysis System (SAS) (SAS, 1988). Student−Newman−Keuls (SNK) sequential range test was used to perform a means comparison of the following data from experiment 1: protein, ash, fat, total dietary fiber, starch, minerals, in vitro protein digestibility, oligosaccharides, and trypsin inhibitors.

All the results, except for in vitro protein digestibility, were expressed per gram of dry raw bean for comparison purposes to account for weight losses during the fermentation procedure (Figure 1). To do that, a yield factor (Table 2) was calculated for each experiment 1 treatment, and then the results were multiplied by that factor to finally express them on a per gram of dry raw bean basis.

RESULTS AND DISCUSSION

For experiment 1, raw black beans were cooked and fermented for 15, 20, or 25 h, then homogenized and a precipitate and supernatant were obtained from a sample at each fermentation time. Samples were analyzed at each of the steps as described in Figure 1.

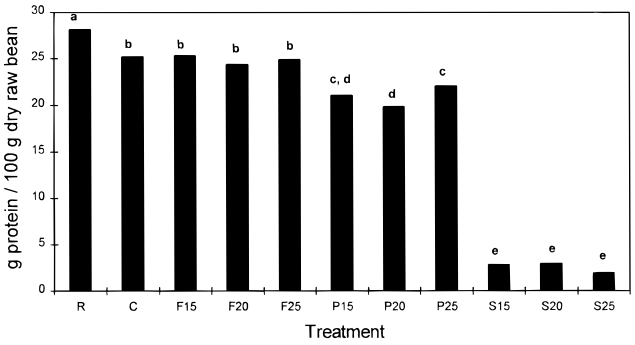

Figure 3. Protein content of raw (R), cooked (C), 15, 20, and 25 h fermented beans (F15, F20, F25), precipitates (P15, P20, P25), and supernatants (S15, S20, S25) (see Figure 1). Identical letters represent no statistical difference while different letters indicate a statistical difference within the same analysis (probability is 0.0001). Experiment was repeated two times for each treatment, and duplicate assays for each repetition were performed. Values are the average of the two repetitions.

Proximate Composition, Total Dietary Fiber, and Starch. Protein content results showed that the cooking process caused a 3% protein loss (Figure 3), perhaps because of losses in soluble protein. Protein levels were unaffected by fermentation time. Upon homogenization of the fermented beans, approximately 85% of the protein remained in the precipitate and about 15% went into the water extract. Because the protein content (as calculated from nitrogen content) of the cooked beans was the same as that of fermented beans, we can conclude that fermentation did not have an effect on the protein content, which is consistent with results reported by Wang et al. (1968). de Reu et al. (1995) reported an increase in proteolytic activity by the mold *Rhizopus oligosporus*. Our results do not contradict that observation, because proteolysis likely caused an increase in soluble protein and ammonia, but the content of nitrogen was unchanged.

Cooking had no effect on the fat content of beans (Figure 4A). Fermentation significantly reduced the fat content. Precipitates from beans fermented 15 or 20 h had a lower fat content compared to fermented beans. However, the precipitate from 25 h fermented beans had the same content of fat as the fermented beans. Results here seem consistent with a report by Wagenknecht et al. (1961) that *Rhizopus oligosporus* can produce lipases that hydrolyze lipids during fermentation.

Ash content decreased significantly from raw beans to cooked beans, but it was the same for cooked and fermented beans, and did not differ with time of fermentation (Figure 4B). This reduction in ash content is due to leaching of minerals into the soaking water prior to cooking and possibly also leaching into the cooking water. Ash content was lower in the precipitates than in the fermented beans due to losses of more minerals in the water extract from homogenization. Therefore, as reported by Wang (1968), fermentation does not affect ash content.

Starch content of cooked beans was lower than in raw beans (Figure 4C). Starch content of fermented samples was the same as for raw beans, and starch contents of 15 and 25 h fermented beans were also statistically grouped with cooked beans. Precipitates from all fermented beans had the same starch content as cooked beans. These results indicate that fermentation did not cause a decrease in the starch content of dry beans.

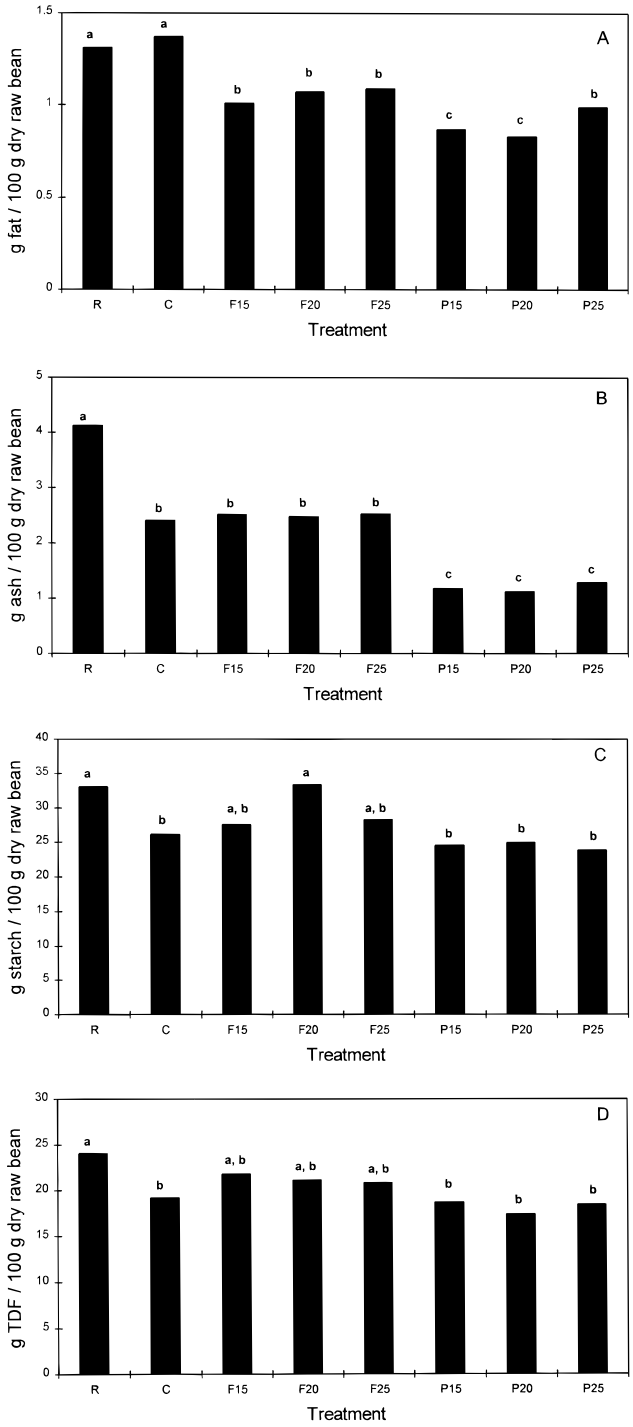

Figure 4. Fat (A), ash (B), starch (C), and TDF (D) content of raw (R), cooked (C), 15, 20, and 25 h fermented beans (F15, F20, F25), and precipitates (P15, P20, P25) (see Figure 1). Identical letters represent no statistical difference while different letters indicate a statistical difference within the same analysis (probabilities are 0.0001 for fat and ash, 0.0093 for TDF and 0.0079 for starch). Experiment was repeated two times for each treatment, and duplicate assays for each repetition were performed. Values are the average of the two repetitions.

Content of total dietary fiber (TDF) was higher in raw beans compared to cooked beans (Figure 4D). The differences observed in TDF content, of raw versus cooked beans, might be due to some losses in the soaking water. Cooked beans and the precipitates from 15, 20, and 25 h fermented beans were statistically grouped

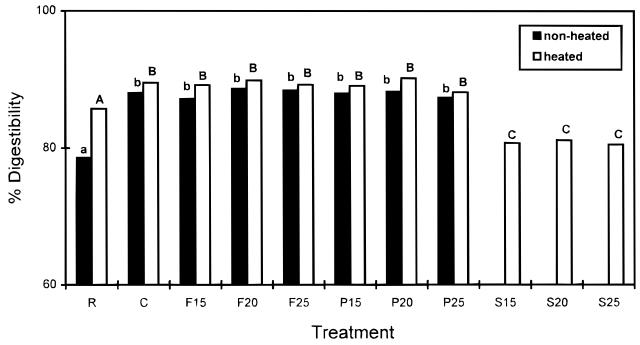

Figure 5. In vitro protein digestibility (heated and nonheated prior to determination) of raw (R), cooked (C), 15, 20 and 25 h, fermented beans (F15, F20, F25), precipitates (P15, P20, P25), and supernatants (S15, S20, S25) (see Figure 1). Identical letters represent no statistical difference while different letters indicate a statistical difference within the same analysis ($p = 0.0001$). Experiment was repeated two times for each treatment, and duplicate assays for each repetition were performed. Values are the average of the two repetitions.

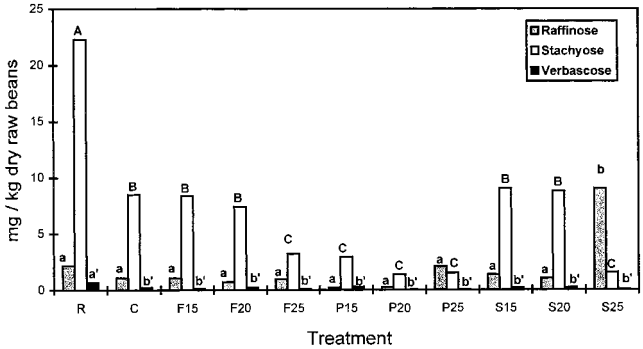

Figure 6. Oligosaccharide content of raw (R), cooked (C), 15, 20, and 25 h fermented beans (F15, F20, F25), precipitates (P15, P20, P25), and supernatants (S15, S20, S25) (see Figure 1). Identical letters represent no statistical difference while different letters indicate a statistical difference within the same oligosaccharide (probabilities are 0.01, 0.0001, and 0.004 for raffinose, stachyose, and verbascose, respectively). Experiment was repeated two times for each treatment, and duplicate assays for each repetition were performed. Values are the average of the two repetitions.

with both raw beans and cooked beans. The TDF contents of fermented beans at all fermentation times did not differ from those of the precipitates prepared from the fermented beans. This result shows that there is no decrease in the TDF due to fermentation. Wang et al. (1968) reported that fermentation had no effect on TDF content of soybeans.

Minerals. Mineral analysis results showed the same pattern found for ash content (data not shown). The K, Mg, and P contents of raw beans were higher than those of cooked beans. However, there was a further decrease in those minerals for precipitates from 15, 20, and 25 h fermented beans. These results are consistent with the results obtained by Rodríguez-Sosa et al. (1984) and Bressani (as cited in Bressani, 1993). Losses of minerals can be attributed to leaching of the minerals into the water, either the soaking water, cooking water, or extraction water.

The Ca content of cooked beans was lower than that of raw beans, but it remained the same for fermented beans and precipitates from fermented beans. Interestingly, Zn content was not lowered after cooking or fermentation, but it did decrease after homogenization. The Fe content decreased significantly from raw beans to 15 h fermented beans, but the decrease was small. The mineral that presented the highest reduction with cooking was K, followed by Mg, P, and Ca.

Amino Acid Content. There were no apparent changes in the individual amino acid levels due to any of the treatments tested (Table 1). However, no statistical analysis was performed on the amino acid content data because experiment 1 was only repeated once for this determination.

In Vitro Protein Digestibility. *In vitro* protein digestibility for raw beans was 78% (Figure 5). This value is similar to in vivo (77%) and in vitro (76%) protein digestibility results determined by Coelho and Sgarbieri (1995) for *Phaselus vulgaris*. Fermentation did not increase the *in vitro* protein digestibility of the cooked beans. An increase in the protein digestibility after fermentation was expected because Steinkraus et al. (1960) and Paredes-López and Harris (1989) reported an increase in the soluble protein with this type of fermentation for common beans. However, the soluble protein (supernatant) from 15, 20, and 25 h fermented beans had a statistically lower *in vitro* protein digestibility than the precipitates from the fermented beans (Figure 5). A further heating step after fermenting the beans failed also to increase the in vitro protein digestibility. These data indicate that the soluble protein had lower digestibility than the insoluble protein present in the precipitate. However, it is possible that this low in vitro protein digestibility was due to the presence of free amino acids that would not be detected by the method used.

Contradictory results on the changes of protein quality due to fermentation have been reported for legumes. Agosin et al. (1989) found no increase in the protein quality of lupine seeds, while Wang et al. (1968) reported higher protein efficiency ratios for fermented soybeans compared to cooked soybeans. Fermentation times used in our study might not have allowed for sufficient proteolysis to cause an increase in the digestibility. However, longer fermentation times are not feasible because of the reduction in palatability of the fermented product after prolonged fermentation times (Paredes-López et al., 1990).

Oligosaccharide Content. Raffinose content remained the same for all the treatments (Figure 6), except for the supernatant from 25 h fermented beans, in which there was an increase in the raffinose content. Stachyose decreased approximately 62% from raw beans to cooked beans due to the loss of this sugar during soaking. Fermenting beans for 15 or 20 h did not decrease stachyose content compared to cooked beans, but fermenting beans for 25 h reduced the stachyose level. Verbascose level in all samples was low and decreased from raw beans to cooked beans, but was not further reduced by fermentation. There was a high content of stachyose in the supernatant from 15 and 20 h fermented beans. Interestingly, stachyose content was decreased in the supernatant from 25 h fermented beans compared to 15 or 20 h. fermentation, but raffinose content increased by a similar amount. Hydrolysis of stachyose to raffinose might be the reason of these changes. No prior studies have reported the effect of fermentation on the oligosaccharides of black beans. However, a reduction in the oligosaccharide level of lupine seeds due to fermentation was reported by Agosin et al. (1989). Bau et al. (1994) explained the decrease in oligosaccharides during fermentation by their utiliza-

Table 3. Trypsin Inhibitor Units (TIU) and Hemagglutinating Units (HU) of Raw, Cooked, Fermented Beans, Precipitates, and Supernatants[a,b]

treatment[c]	TIU (TIU/g of dry raw beans)	HU (HU/mg of dry raw beans)
raw (mean)	62.8[A]	2.2
(SD)	(1.0)	(0.2)
cooked (mean)	1.1[B]	ND[d]
(SD)	(0.3)	
fermented 15, 20, 25 h	ND[d]	ND[d]
precipitate 15, 20, 25 h.	ND[d]	ND[d]
supernatant 15 h (mean)	0.8[B]	ND[d]
(SD)	(0.1)	
supernatant 20 h (mean)	0.6[B]	ND[d]
(SD)	(0.4)	
supernatant 25 h (mean)	1.1[B]	ND[d]
(SD)	(0.1)	

[a] For the mean values, identical capital letters represent no statistical difference while different capital letters indicate a statistical difference (probablility is 0.0001 for TIU). [b] Experiment was repeated two times for each treatment, and duplicate assays for each repetition were performed. First two values are the average of the two duplicates, third value is the mean value and fourth value, in parentheses, is the standard deviation (SD) of the two repetitions. [c] See Figure 1. [d] Not detectable.

tion for biosynthesis and production of energy for the mold growth.

Antinutritional Factors. There was a large decrease of trypsin inhibitor activity after cooking the beans, and levels were very low in cooked beans and in the supernatants from the fermented beans (Table 3). This large decrease in trypsin inhibitors was expected since trypsin inhibitors are known as heat-labile toxic components of legumes (DiPietro and Liener, 1989). Some residual antitryptic activity in soybeans has been reported after heat treatment (DiPietro and Liener, 1989).

After the beans were cooked, lectins were not detected (Table 3). Lectins such as trypsin inhibitors are heat-labile toxic components of beans. Antunes and Sgarbieri (1980) reported a 100% decrease in the activity of lectins in dry beans after 15 min of cooking at atmospheric pressures.

Choosing the Optimum Fermentation Time To Prepare the Weaning Food Product. After analysis of the results reported above, we chose 25 h fermented beans as the optimum product to be combined with rice to develop the weaning food. The selection of that fermentation time was based on maximizing the content of positive factors present in beans and on minimizing the content of antinutritional factors.

Ash and mineral contents were reduced after cooking and homogenization. Mineral levels need to be kept as high as possible because they are important in the development of children. Starch, which is high and an important source of energy in beans, showed some changes in content with the different treatments, but those changes were very small. On the basis of the protein content results, fermented beans would be preferred rather than precipitates, because of their higher protein content. The digestibility of protein did not increase with treatments beyond simply cooking. Therefore, protein digestibility was not an important decision factor in determining optimum fermentation time. However, a further treatment that would increase protein digestibility would be highly desirable. Fermentation or homogenization did not alter the essential amino acid content.

Ideally, the negative factors present in beans would be decreased, eliminated, or inactivated. Trypsin inhibitors and lectins were partially or completely inactivated after cooking. Therefore, fermentation did not play an important role in their inactivation. One of the most important decision factors for optimum fermentation time was the oligosaccharide level. Low content of stachyose, the most prevalent oligosaccharide in beans,

Table 4. Chemical Composition of the Final Product[a] Made with 53% Bean Protein and 47% Rice Protein (FP), 25 h Fermented Beans (FB), and Cooked Rice (CR)

A

	% protein (dwb)	% fat (dwb)	% ash (dwb)	% TC[b] (dwb)	% starch (dwb)	% TDF (dwb)
FP	15.0	0.21	1.11	83.7	63.0	6.8[c]
(SD)	(0.3)	(0.02)	(0.01)	(0.3)	(1.1)	
FB	31.1	1.36	3.15	64.4	35.3	26.0
(SD)	(0.8)	(0.10)	(0.12)	(0.8)	(4.1)	(0.4)
CR	10.0	0.51	0.69	88.8	69.2	2.6
(SD)	(0.2)	(0.06)	(0.03)	(0.3)	(4.4)	(0.1)

B

		oligosaccharide content (mg of oligosaccharide/g of dry matter)		
	% protein *in vitro* digestibility	raffinose	stachyose	verbascose
FP	86.37	0.18	0.00	0.04
(SD)	(0.02)	(0.05)	(0.00)	(0.05)
FB	88.43	1.18	3.98	0.60
(SD)	(0.70)	(0.47)	(1.55)	(0.68)
CR	85.11	*d*	*d*	*d*
(SD)	(0.55)			

C

	mineral content[3] (mg of mineral/kg of dry matter)					
	K	Mg	P	Ca	Zn	Fe
FP	2768	631	1582	394	48	47
FB	10474	1445	4474	1055	35	43
CR	788	372	1354	182	16	16

[a] See Figure 2. Values shown represent mean values. [b] Calculated by difference: % carbohydrate = 100 − (% protein + % fat + % ash). [c] Single determination. [d] Not determined.

was found in 25 h fermented beans, and all the precipitates from the fermented beans. Homogenization and centrifugation involve extra steps in processing, equating to higher costs in production and these steps may not be easy to implement. Because the level of stachyose was similar for 25 h fermented beans and precipitates, 25 h fermented beans was the choice for use in the weaning food product. Also, as mentioned above, the protein and mineral contents of fermented beans were higher than the levels in the precipitates. All those factors led to the choice of 25 h fermented beans as the optimum fermentation time.

Composition of the Weaning Food Product. The proportion of bean protein and rice protein used to develop the weaning food product was 53%/47%, which is equivalent to 27% fermented beans, dry weight/73% cooked rice, dry weight. The analyses performed on the final product were not analyzed statistically, because experiment 2 was only done once. Therefore, all the comparisons discussed in this section are not based on statistical analysis.

Data for the final product, 25 h fermented beans and rice, are reported in Table 4 and are expressed on a dry weight basis for comparison purposes. Protein, fat, ash, and total carbohydrate contents of the bean−rice mixture were 15.0, 0.21, 1.11, and 83.38%, respectively. All components decreased except for the carbohydrate percentage when compared to simply beans fermented for 25 h. This increase in the carbohydrate content is explained by the higher starch content of rice. The TDF decreased in the final product due to the lower content of TDF in the rice. In vitro protein digestibility decreased from 88% in 25 h fermented beans to 86% in the final product. Oligosaccharide content of the bean−rice weaning food was very low, due to the reduction of the oligosaccharides in the 25 h fermented beans as well as the dilution factor when rice was incorporated into the product. Trypsin inhibitors and lectins were not measured because experiment 1 showed that they were inactivated after the cooking step. The contents of K, Mg, P, and Ca decreased in the final product compared to 25 h fermented beans. This reduction was due, in general, to the low content of minerals in the rice. However, Fe and Zn levels did not decrease.

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