Lactic acid fermentation of black beans (*Phaseolus vulgaris*): microbiological and chemical characterization

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Abstract: Legumes, and particularly *Phaseolus vulgaris*, are an important source of nutrients, especially in developing countries. In spite of being part of the staple diets of these populations, their consumption is limited by the flatulence they produce. Natural lactic acid fermentation has proved to be an effective method to decrease flatulence-producing compounds. However, in order to use this method as a process on a large scale, it is fundamental to identify the microbial flora involved. When fermented seeds of *Phaseolus vulgaris* (black bean) were analysed microbiologically, it was found that the microorganisms present were *Lactobacillus casei* and *Lactobacillus plantarum*. On performing back-slopping or induced fermentation with different inocula, a 63.35% decrease was found for the soluble fibre and 88.6% for raffinose, one of the main flatulence-producing compounds. When cooking under atmospheric pressure was applied to the fermented samples, a significant diminution of the trypsin inhibitors and tannins was found as well as an increase in the *in vitro* and *in vivo* digestibility of the beans. All these results demonstrate that the lactic acid bacteria used for the induced fermentation can lead to a functional food with improved nutritional quality.

Keywords: *Phaseolus vulgaris*; natural fermentation; micro organisms; flatulence compounds; nutritional quality

INTRODUCTION

Phaseolus spp are the most widely consumed bean in Central and South American countries and in those located in Central and East Africa.[1] In Venezuela, they are a component of the diet, as they constitute part of the food habits of its inhabitants and represent a good source of proteins, carbohydrates, fibre, minerals and vitamins.[2] However, they also contain antinutritional compounds such as protease inhibitors, polyphenols and flavonoid compounds, among others, which are naturally present and are capable of decreasing the bioavailability and limiting the digestibility of the nutrients.[3]

Another factor that limits legume consumption is the intestinal discomfort (flatulence) produced after their ingestion. Flatulence is produced by the colonic fermentation of the α-galactosides, raffinose, stachyose and verbascose.[4] Additionally, the soluble fibre has been identified as a fermentable compound.[5]

Recent studies have shown that the application of natural fermentation processes to legumes increases their nutritional quality, decreasing the antinutritional compounds through the action of the microbial flora responsible for the fermentation process.[6,7] Additionally, Frías *et al*[8] and Granito *et al*[9] found that the application of natural fermentation to *Lens culinaris*, Magda variety, and to *Phaseolus vulgaris*, Victoria variety, respectively, decreases α-galacto-oligosaccharides (raffinose, stachyose and verbascose) contents, soluble fibre and resistant starch, the main flatulence-producing factors in legumes.

The natural fermentation process is performed with the endogenous flora. However, the beans contain aerobic microorganisms from air, water, soil, containers, etc, on their surfaces that can be more numerous than the fermentative species. Fermentation failures may often be attributed to the preponderance of undesirable organisms as well as to the delay in growth of the desirable organisms; however, the microorganisms responsible for the fermentation of any food are nearly always present. The fermentations can basically be performed either by spontaneous fermentation by back-slopping, or by addition of starters cultures.[11] The great majority of fermented foods produced throughout the world still depend upon a natural inoculation with the desired organisms. The microorganisms essential for a satisfactory fermentation may be introduced to food and gain dominance in the fermentation.[10]

In back-slopping, a part of a previous batch of a fermented product is used to inoculate the new batch. This procedure brings about a higher initial number of beneficial microorganisms than are found in raw material and ensures a faster and more reliable fermentation than occurs in spontaneous fermentation.[11] Spontaneous fermentation can be optimized through back-slopping, which results in dominance of the best adapted strains.[12]

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Contract/grant sponsor: FONACIT; contract/grant number: 2001000856
(*Received 11 February 2005; revised version received 1 May 2005; accepted 9 November 2005*)
Published online 26 April 2006; DOI: 10.1002/jsfa.2490

At the onset of a process of natural fermentation, different microorganisms can coexist simultaneously. During the course of the fermentation, in a sequential manner, part of the flora increases its population until the dominant microflora are defined. The species commonly identified as the dominant flora in natural fermentation processes belong to the *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus* families.[13]

Traditional fermentations of vegetables, fruit and grains most often include a lactic acid fermentation involving many different species of lactic acid bacteria (LAB), that are active at different stages of the fermentation process.[11]

LAB produce several antimicrobials, including organic acids (lactic acid, acetic acid, formic acid, phenyllactic acid and caproic acid), carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterin and reutericyclin.[14] Additionally, LAB improve the natural texture of products like yoghurts, ice cream and sour cream due to exopolysaccharidase production[15] and contribute to the aroma and flavour of fermented products. They acidify the food, resulting in a tangy lactic acid taste and produce aromatic compounds from, for instance, amino acids upon further bio-conversion.[16]

Given that flatulence is one of the main limiting factors for the consumption of this important foodstuff, the implementation of processes which allow for nutritious and non-flatulence-producing beans to be obtained would be interesting. Natural fermentation has been shown to be effective in this respect;[9] however, given the variability inherent in the bacteria present in the beans at a given time, it is fundamental to identify the microbial flora responsible for the process.

The objective of this work was to identify the microbial flora responsible for the natural fermentation process of a black variety of *Phaseolus vulgaris* beans, in order to suggest the implementation of back-slopping or induced fermentation processes that can be strictly controlled. Likewise, the effect of induced lactic acid fermentation of the beans with subsequent cooking on the chemical and nutritional quality of the beans and on the concentration of the flatulence-producing compounds was evaluated.

MATERIALS AND METHODS

Seeds

Black beans (*Phaseolus vulgaris*) were purchased at a local market to carry out natural fermentation. Freshly harvested black beans of *Phaseolus vulgaris* L-140 variety, on the process of certification, from the Instituto Nacional de Investigaciones Agropecuarias (INIA, Venezuela) were used to carry out the induced lactic acid fermentation.

Microbiological characterization

Once natural fermentation was carried out, the inoculum was obtained and the microbial population present identified and quantified. Duplicate dilution series were prepared and colony counts are reported as CFU g^{-1}. Bacterial counts were performed by surface plating onto plate count agar (PCA; Oxoid). Total coliforms, lactobacillus, streptococcus, moulds and yeasts were quantified. The microorganisms that presented greater population growths were transferred to rich solid culture media to ensure their complete recuperation and isolation. The colonies were differentiated based on their morphology. Specific culture media for each type of micro organism were used as follows: aerobic mesophiles (agar of standard recount, Merck); total coliforms (Cromocult, Merck); *Streptococcus* (KF, Pronadisa); *Lactobacillus* (culture broth and agar Man Rogosa-Sharpe MRS, Merck); moulds (agar of potato glucose, Merck); and yeasts (agar of potato glucose, Merck).[12]

Total coliform colonies were replicated in a rich solid medium to ensure total isolation, based on morphological characteristics such as pigments, shape and consistency, and later were characterized according to the IMViC (indol, methyl red, Voges-Proskauer and citrate) biochemical tests.

To identify the different lactobacillus populations, API 50 CH gallery and an API 50 CHL (Biomériux SA) media were used. The determinations of the study of the fermentation of 49 sugars present in the gallery were made in duplicate. To identify the coliforms, IMViC (indol, methyl red, Voges-Proskauer and citrate) tests were used. In raw *Phaseolus vulgaris* L-140 variety, aerobic mesophiles and total coliforms were quantified.

Initial natural fermentation

Commercial black beans (*Phaseolus vulgaris*) were fermented naturally (with only the microorganisms present on the seeds) in a proportion 1:4 (w/v) at 42 °C for 48 h. Afterwards, the seeds were aseptically drained and the fermentation water was characterized microbiologically and labelled as the initial culture.

Induced lactic acid fermentation

To optimize the back-slopping or induced lactic acid fermentation, different dilutions from the initial culture were prepared in sterile distilled water under aseptic conditions, and four different inocula were prepared with increasing concentrations of the initial culture: [inoculum 1] < [inoculum 2] < [inoculum 3] < [inoculum 4]. The induced fermentation process was finished after 48 h. Then, the fermentation liquid was drained, collected and characterized microbiologically, as described previously.

Fermentation process

Natural and induced fermentation was carried out in a fermentor Microferm New Brunswick Scientific Co Inc (Edison, NJ, USA) for 24 and 48 h at 42 °C. Samples were also taken at 24 h for chemical analysis (pH, acidity and SDF). Fermented grains

were drained off and part of them freeze-dried for further chemical analysis.

Cooking process

Part of the fermented grains was cooked in tap water (1:12, w/v) for 120 min at atmospheric pressure and subsequently drained, discarding the cooking liquid. After cooking, grains were freeze-dried for chemical analysis.

Analytical methods

Nominal acidity was determined in the natural and induced fermentation liquids according to AOAC[17] by titration with 0.1 mol L^{-1} NaOH and expressed as a percentage of lactic acid (g lactic acid kg^{-1} dry matter).

During the fermentation process, pH was measured on homogenated samples using a Coleman pH meter, model 39 (Coleman Instruments, a Division of the Perkin-Elmer Corporation Maywood, IL, USA).

Soluble (SF) and insoluble (IF) fibre of the raw, fermented and fermented–cooked samples were quantified according to Prosky *et al*.[18]

Soluble carbohydrates (glucose, galactose, sucrose and raffinose) were determined in raw, fermented and fermented–cooked samples by HPLC according to Frías *et al*[8] modified by Granito *et al*.[7]

Available starch (DS) was analysed in raw, fermented and fermented–cooked samples by the enzymatic-colorimetric method of Holm *et al*.[19] This method includes incubation with α-amylase (Sigma A-3306, Steinheim, Germany) for 15 min at boiling temperature, digestion with amyloglucosidase (Sigma A-9913, Steinheim, Germany) at 60 °C (30 min) and free glucose measurement using the combined glucose oxidase–peroxidase colorimetric assay. Resistant starch was determined in raw, fermented and fermented–cooked samples according to Champ *et al*.[20]

Nitrogen content was determined in raw, fermented–cooked beans according to the AOAC micro-Kjeldahl method 960.52[17] and a conversion factor of 6.25 was used. The fat content was determined in raw and fermented–cooked beans according the AOAC method 920.30.[17] Ash content was determined in raw and fermented–cooked beans according to the AOAC method 923.03.[17] Trypsin inhibitor activity (TIA) was determined in raw and fermented–cooked beans as in Kakade *et al*.[21]

Specific minerals present in raw and fermented–cooked beans were analysed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES), according to AOAC method 984.27.[17] Minerals were determined by atomic absorption spectrometry from ash residue dissolved in acid, using an Ar Spectroflame D (Ar ICP).

Tannins were determined in raw and fermented–cooked beans as in Nacz *et al*.[22] Available lysine was determined in raw and fermented–cooked beans as in Kakade and Liener.[23] Digestibility *in vitro* was determined in raw and fermented–cooked beans by the method of Hsu *et al*.[24]

Biological analysis

In vivo *digestibility (TD)*

The experimental diets of fermented–cooked beans were prepared by adding the appropriate amount of the legume at the expense of maize starch to provide 10% protein in the diet. Minerals (3.5%), maize oil (5.0%), vitamins (1.0%), choline bitartrate (0.2%) and starch were added up to 100%.

Six-week-old Sprague–Dawley rats, weighing about 50 g, were selected at random by weight into three groups of six animals (three male and three female) and housed in individual cages in an environmentally controlled room. The animals were fed a control diet from weaning until the study began. During a 14-day testing period, two experimental diets and a protein-free diet and water were supplied. Every other day, the animals and their feed intake were weighed. In order to calculate protein digestibility, the faeces from each animal were collected for the last 7 days of the experiment; the nitrogen consumed by each animal for this period was quantified. The faeces were oven-dried at 100 °C for 24 h. The dried samples were ground to 20 mesh. The experimental diet and faecal powder were analysed for protein (N × 6.25) using the micro-Kjeldahl method.[17]

Statistical analyses

The results of the chemical and microbiological characterization of the initial inoculum and of the back-slopping fermentation are expressed as the means ± standard deviations of four determinations (two samples of two fermentations). The rest of the data are expressed as means ± standard deviations of three determinations. All the data were subjected to a one-way analysis of variance (ANOVA) and Duncan multiple comparison tests. Statistical analyses were performed using the Statgraphic/PC 4.1 software for Windows.

RESULTS AND DISCUSSION

Preliminary trials of natural fermentation of *P vulgaris* L-140 at 42 °C for 48 h were carried out, and no variations in either pH or acidity were obtained during fermentation. The microbiological analysis of the raw grains of *P vulgaris* L-140 showed negative microbial counts for aerobic mesophiles and total coliforms. Therefore, it was considered that such grains were suitable for induced fermentation.

Figure 1 shows results obtained from the microbiological characterization of natural fermentation liquid of commercial black beans at 42 °C for 48 h. Total counts of moulds and yeasts yielded negative results. However, total coliforms and lactobacillus represented 62 and 36%, respectively, of the total aerobe population. Coliform colonies were characterized and it was found that microorganisms identified were an

J Sci Food Agric **86**:1164–1171 (2006)
DOI: 10.1002/jsfa

enterobacter of the *Klebsiella* species. For the identification of the *Lactobacillus* species, the results showed a pattern for carbohydrate metabolism typical of *Lactobacillus casei* and *Lactobacillus plantarum*, the former being a probiotic microorganism. At the end of this fermentation, a pH of 4.02 was obtained and a titratable acidity of 9.7 g lactic acid kg^{-1} dm. This demonstrates that lactic acid bacteria, besides the presence of coliforms, conducted the natural fermentation of these beans. These results are in agreement with Jay,[25] who reported that coliforms can grow in a pH range of 4.4–4.9 and that they have a wide range of growth temperatures, from −2 to 50 °C. At the same time, Sanni *et al*[26] characterized the microbial flora present in natural fermentations of maize flours and found diverse species of microorganisms such as *Lactobacillus, Leuconostoc, Saccharomyces, Debaryomyces, Candida, Bacillus, Micrococcus, Klebsiella, Escherichia* and *Aspergillus*, with *Lactobacillus* being the dominant species. Recent studies of characterization of microorganism populations present in assorted naturally fermented foodstuffs typical of India and Africa, such as Adai, Busaa, Kishk and Koko, showed the presence of mainly *Lactobacillus casei* and *plantarum*.[27] Likewise, *Lactobacillus casei* has been found to be the dominant species together with *Lactobacillus brevis* and *Lactobacillus plantarum* in natural fermentation of green olives.[28]

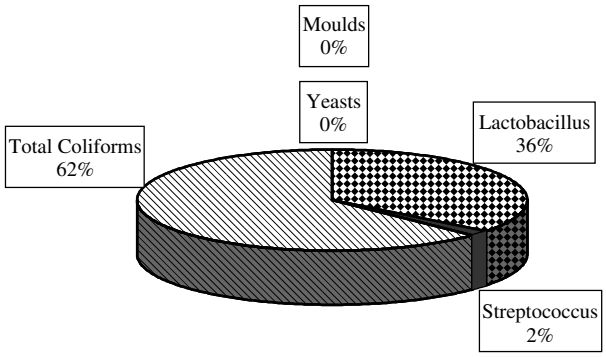

Figure 1. Percentage composition of microbial population present in the initial culture.

Phaseolus vulgaris L-140 variety was fermented with different dilutions of the initial culture in duplicate batches (inocula 1–4). Table 1 collects the evolution of the pH and the titrable acidity at the initial time and after 24 and 48 h of the four different inocula used. Fermentation carried out with inoculum 1 showed a pH higher than 4.5, which is characteristic for lactic acid fermentation;[8] therefore, it was not selected for the induced fermentation. Lactic acid concentration decreased after 48 h of fermentation and the largest amount was obtained with inoculum 4. Additionally, fermentation performed with inoculum 4 conducted a reduction of SDF up to 60%, which is recommended to avoid flatulence problems.[7] Based on this, inoculum 4 was chosen for induced fermentation of *Phaseolus vulgaris* L-140 and microbiological and chemical analysis were carried out.

Figure 2 shows the microbial flora and percentage of acidity of the selected induced fermentation. A decrease of 98% in the total coliforms was observed after a 48 h fermentation period. This decrease was probably caused by the increase in the acidity produced by the metabolism of the lactic acid bacteria. Similarly, lactobacilli underwent a slight diminution after 24 h, but they suffered a noticeable rise after 48 h, reaching a population equal to 10^7 CFU g^{-1}. These results coincide with those of Idris *et al*,[28] who reported values of 10^9 CFU g^{-1} of heterofermentative lactic acid bacteria after 72 h of fermentation of 'Awaze' and 'Datta', naturally fermented condiments used in some African countries. Similarly, Sanni *et al*[26] observed a decrease in the population of enteric bacteria down to 10 CFU g^{-1} and increments in the final population of lactic acid bacteria of up to 10^9 CFU g^{-1} in maize flours naturally fermented for 48 h.

When lactobacilli were characterized it was shown that the flora responsible for the natural fermentation of the sample of *Phaseolus vulgaris* L-140 was genus *Lactobacillus*, species *plantarum* and *casei*, which can be isolated and lyophilized for subsequent use as initiating microorganisms for further back-slopping fermentation processes.

Table 1. pH, titrable acidity and soluble fibre produced by different inoculum in induced fermentation of *P vulgaris* L-140

		Inoculum			
	Time (h)	I_1	I_2	I_3	I_4
pH	0	$4.79^{a1} \pm 0.17$	$4.36^{a2} \pm 0.00$	$4.39^{a2} \pm 0.08$	$4.43^{a2} \pm 0.17$
	24	$5.75^{b1} \pm 0.17$	$4.56^{b2} \pm 0.17$	$4.78^{b3} \pm 0.17$	$4.95^{b4} \pm 0.17$
	48	$5.78^{b1} \pm 0.17$	$4.31^{a2} \pm 0.17$	$4.88^{b3} \pm 0.17$	$4.47^{a2} \pm 0.17$
Lactic acid*	0	$6^{a1} \pm 0.17$	$4^{a1} \pm 0.17$	$16^{a2} \pm 0.02$	$12^{a3} \pm 0.17$
	24	$12^{b1} \pm 0.17$	$18^{b2} \pm 0.17$	$18^{c2} \pm 0.17$	$21^{b3} \pm 0.17$
	48	$12^{b1} \pm 0.17$	$20^{c2} \pm 0.17$	$17^{b3} \pm 0.17$	$28^{c4} \pm 0.17$
Soluble fibre	0	$16^{a1} \pm 0.12$	$16^{a1} \pm 0.12$	$16^{a1} \pm 0.12$	$16^{a1} \pm 0.12$
	48	$13^{b1} \pm 0.12$	$12^{a1} \pm 0.12$	$11^{b1} \pm 0.9$	$6^{b2} \pm 0.22$

I_1 = inoculum 1; I_2 = inoculum 2 ; I_3 = inoculum 3; I_4 = inoculum 4.
Data expressed as g kg^{-1} on a dry matter basis.
For each analysis, different numbers in same row mean significant differences ($P \leq 0.05$). For each analysis, different letters in same column mean significant differences ($P \leq 0.05$).

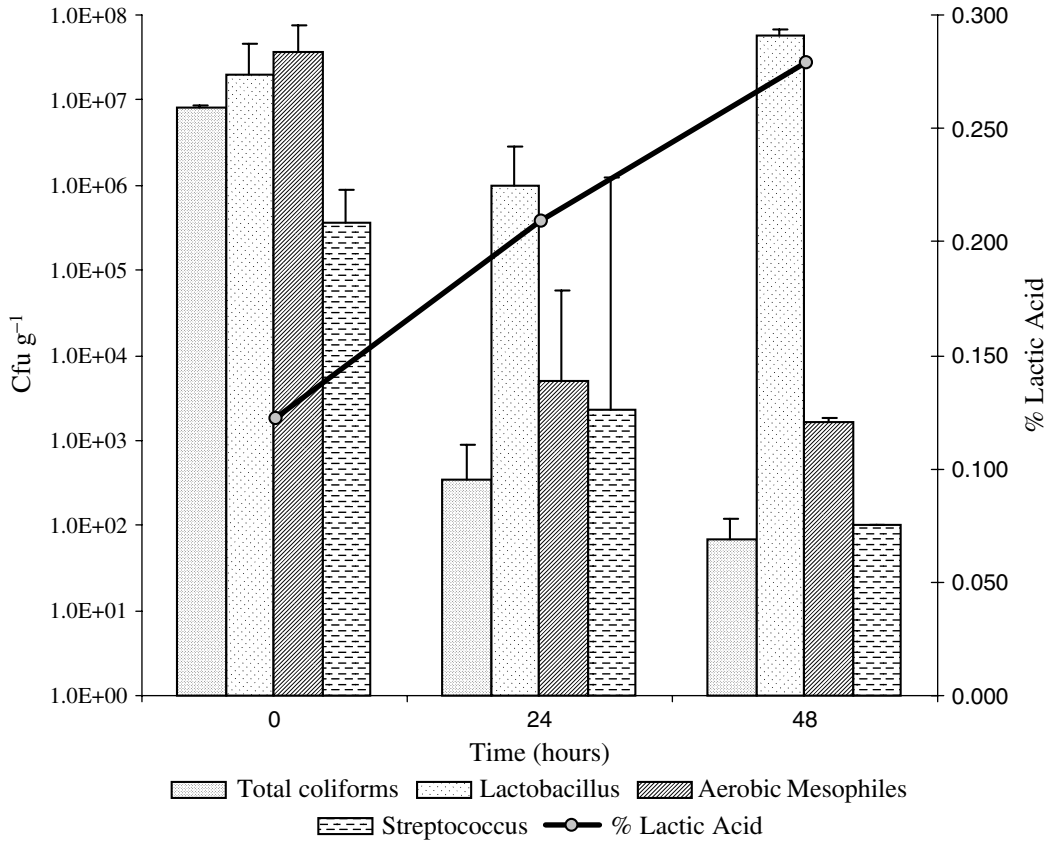

Figure 2. Microbial flora and percentage acidity of induced fermentation of *Phaseolus vulgaris*, L-140 variety.

Once the induced fermentation process of *Phaseolus vulgaris* variety L-140 was microbiologically characterized, the quantification of the flatulence-producing compounds such as soluble fibre, resistant starch and raffinose present in the raw and fermented beans was performed, in order to study the effect of the induced fermentation on the removal of these chemical compounds. Additionally, the available starch, disaccharides and monosaccharides were determined. Induced fermented black beans showed an insoluble dietary fibre content of 109.9 g kg^{-1} and a soluble dietary fibre content of 5.9 g kg^{-1}, which represented a decreased of 39.05 and 63.35%, respectively (Table 2). These results agree with those reported by Granito *et al*[9] and Martín *el al*[29] for fermented *Phaseolus vulgaris* seeds. Some authors have demonstrated that the decrease in soluble and insoluble fibre during fermentation processes in legumes could be attributed to the hydrolysis of pectic compounds and the use of cellulose and hemicellulose as substrates by the microorganisms responsible for the fermentation.[7,29,30] Likewise, it has been reported that the hydrolysis produced on the fibre during fermentation can originate an increment in the concentrations of glucose and maltose, which are later transformed into organic acids or alcohols through the microbial activity.[28,30,31]

When the concentrations of glucose, galactose and sucrose of raw and fermented beans were measured, it was found that induced fermentation produced a significant increase in the concentrations of glucose (51.43%), galactose was detected (1.8 g kg^{-1}) and sucrose underwent a sharp reduction (86.34%; Table 2). Granito *et al*[7] also observed the detection of galactose and an increase of glucose after natural fermentation of beans. Blandino *et al*[27] stated that the diminution of sucrose during the fermentation processes was due to its use as a source of energy by the lactic acid bacteria.

Table 2. Content of fibre, starch, di-monosaccharides and α-galactosides present in raw and fermented beans of *Phaseolus vulgaris* L-140 variety

	Raw beans	Fermented beans	Variation (%)
Insoluble fibre[a]	$180.3^{b} \pm 1.88$	$109.9^{a} \pm 0.12$	−39.05
Soluble fibre[a]	$16.1^{b} \pm 0.08$	$5.9^{a} \pm 0.02$	−63.35
Glucose[b]	$1.7^{a} \pm 0.17$	$3.5^{b} \pm 0.35$	+51.43
Galactose[b]	ND	$1.8^{a} \pm 0.18$	+18
Saccharose[b]	$22.7^{b} \pm 0.08$	$3.1^{a} \pm 0.02$	−86.34
Resistant starch[a]	$125.0^{a} \pm 1.88$	$143.9^{b} \pm 0.12$	+13.13
Available starch[a]	$222.6^{a} \pm 0.85$	$341.9^{b} \pm 0.85$	+34.89
Raffinose[b]	$22.0^{b} \pm 2.27$	$2.5^{a} \pm 0.31$	−88.64

[a] Data expressed as g kg^{-1} on a dry matter basis.
[b] Data expressed as mg kg^{-1} on a dry matter basis.
ND, not detected; (+) increment; (−) diminution. For each analysis, different letters in same row mean significant differences ($P \leq 0.05$).

Table 2 also shows the content of resistant and available starch. Regarding resistant starch values of 125 and 143.9 g kg^{-1} were found for the raw and fermented beans, respectively, data which agree with the results reported by Granito *et al*[7,9] for a white

J Sci Food Agric **86**:1164–1171 (2006)
DOI: 10.1002/jsfa

variety of *Phaseolus vulgaris*. In general, raw *Phaseolus vulgaris* beans were characterized by presenting high values of resistant starch, particularly of the RS_1 type, which are little affected by natural fermentation.[28] The available starch, on the other hand, increased by 35% with the induced natural fermentation process.

The concentration of raffinose diminished significantly ($P \leq 0.05$) by 88.64% after 48 h of fermentation. Frías *et al*[8] and Granito *et al*[5] reported similar results when *Lens culinaris*, Magda variety, and *Phaseolus vulgaris*, Victoria variety, were fermented with the endogenous microflora.

From the previous results, it can be concluded that the back-slopping or induced lactic acid bacteria fermentation significantly reduced ($P \leq 0.05$) the concentration of the flatulence-related compounds of *P vulgaris* beans.

Table 3 collects the chemical compositions of raw and fermented–cooked *P vulgaris* L-140 variety. Raw bean seeds presented values of 286, 46.2 and 23.2 g kg^{-1} for proteins, ash and fats, respectively, which are within the ranges stated by Berrios *et al*[32] and Sammán *et al*[33] for black varieties of raw *P vulgaris*. During the combined processes of induced fermentation and cooking, the content of proteins, ash and fats decreased significantly by 4.51, 66.88 and 37%, respectively. Siebenhandl *et al*[30] and Enujiugha[34] reported an increment of raw protein during the fermentative process of 'Tape Ketan' and *Pentaclethra macrophylla* Benth variety. According to these authors, the increment of raw protein in the fermented products could be due to the protein hydrolysis produced by extra cellular enzymes of fermentative micro organisms, such as proteases, promoting an increase in the total nitrogen content caused by the release of amino acids and short-chain peptides. However, given that both liquids (fermentative and cooking water) were discarded, it could be possible that soluble protein was removed.

Table 3. Nutritional quality of raw and fermented beans of *Phaseolus vulgaris* L-140 variety

	Raw beans	Fermented–cooked beans	Variation (%)
Protein[a]	286.0[b] ± 0.15	273.0[a] ± 0.20	−4.51
Fat[a]	23.0[b] ± 0.04	14.6[a] ± 0.07	−37.07
Ash[a]	46.0[b] ± 0.01	15.3[a] ± 0.07	−66.88
Ca^{2+}[b]	1555.0[b] ± 2.57	1307.5[a] ± 8.30	−15.93
Mg^{2+}[b]	752.0[b] ± 1.04	569.9[a] ± 0.94	−24.18
Zn^{2+}[b]	139.0[b] ± 1.06	94.3[a] ± 0.26	−32.21
Na^{+}[b]	175.0[b] ± 3.55	97.0[a] ± 0.81	−44.67
K^{+}[b]	466.0[b] ± 1.69	135.0[a] ± 0.88	−70.94
Fe^{2+}[b]	1655.0[b] ± 3.6	817.0[a] ± 3.5	−50.63
Available lysine[a]	71.0[b] ± 0.03	55.0[a] ± 0.09	−22.22
Trypsin inhibitors (TIU mg^{-1} db)	17.2[b] ± 0.20	7.3[b] ± 0.20	−57.41
Tannins[b]	369.0[a]	61.0[b]	−83.43
In vitro digestibility	767.0[a] ± 1.12	863.0[b] ± 0.65	+12.55
In vivo digestibility	822.0[a] ± 4.29[c]	857.0[b] ± 1.21	+3.5

[a] Data expressed as g kg^{-1} on a dry matter basis.
[b] Data expressed as mg kg^{-1} on a dry matter basis.
[c] Unfermented cooked sample.
(–) Diminution; (+) increment compared with casein digestibility, 96.13. For each analysis, different letters in same row mean significant differences ($P \leq 0.05$)./

Regarding the mineral content of the raw beans, calcium and iron were the minerals with the highest concentrations (155.3 and 1655.5 g kg^{-1}, respectively), followed by magnesium and potassium (752 and 466 g kg^{-1}, respectively). Finally, it was observed that sodium and zinc were the minerals in the lowest concentration (175 and 139 g kg^{-1}, respectively). Maldonado and Sammán[35] report values of 159.0 and 35.2 g kg^{-1} for iron and zinc, respectively, in beans of *Phaseolus vulgaris* from the Argentinean northeast, which are very similar to those found in this research.

When thermal treatment was applied to the fermented beans, it was found that the mineral content decreased significantly in relation to the raw samples. Iron and potassium diminished by 71 and 51%, respectively; magnesium, zinc and sodium decreased by 24, 32 and 45%, respectively; and calcium showed a small but significant ($P \leq 0.05$) decrease (16%). According to Fennema,[36] sodium and potassium are free ions that possess a high solubility in water and thus could be lost through the processes of lixiviation or physical separation during the preparation of the product.

Available lysine was determined in raw and fermented–cooked beans and the whole process brought about a significant ($P \leq 0.05$) reduction, and a retention of 78% was observed. These results were probably due to the effect of the heat treatment applied, in agreement with Bhatty *et al*,[37] who reported a diminution of lysine of about 46% in cooked *Phaseolus vulgaris*.

Trypsin inhibitor activity and tannin content decreased by 57 and 83%, respectively, when black beans were fermented and cooked. However, the *in vitro* digestibility increased by 12.55%. These results show that antinutritional compounds can be involved with *in vitro* protein digestibility. Similarly, a significant ($P \leq 0.05$) increment (3.5%) in the *in vivo* digestibility of the diet prepared with fermented–cooked beans compared with the unfermented-cooked diets was found. Although the content of essential amino acids is one of the main indicators of the protein quality, this also depends on the degree of protein digestibility they have. Previous studies of protein digestibility of legumes have shown interactions between antinutritional compounds such as trypsin inhibitors and tannins and the protein structure forming protein complexes less susceptible to proteolysis, which implies a diminution in the usage of proteins.[38,39] In diets prepared with naturally fermented and cooked light-coloured beans (*Phaseolus vulgaris* var. Victoria) Granito *et al* (personal communication) found that the *in vivo* digestibility increased by 4.7% compared with unfermented–cooked beans.

Table 4. Content of fibre, starch, di-monosaccharides and α-galactosides present in fermented and fermented–cooked beans of *Phaseolus vulgaris* L-140 variety

	Fermented beans	Fermented–cooked beans	Variation (%)
Insoluble fibre[a]	$109.9^{a} \pm 0.12$	$217.1^{b} \pm 0.58$	+97.54
Soluble fibre[a]	$5.9^{b} \pm 0.02$	$5.2^{a} \pm 0.03$	−11.86
Glucose[b]	$3.5^{b} \pm 0.35$	$0.7^{a} \pm 0.07$	−80
Galactose[b]	$1.8^{b} \pm 0.18$	$0.62^{a} \pm 0.06$	−65.56
Saccharose[b]	$3.1^{b} \pm 0.02$	$0.63^{a} \pm 0.03$	−79.68
Resistant starch[a]	$143.9^{b} \pm 0.12$	$3.5^{a} \pm 0.58$	−97.57
Available starch[a]	$341.9^{a} \pm 0.85$	$374.9^{b} \pm 1.58$	+9.65
Raffinose[b]	$2.5^{b} \pm 0.31$	$1.6^{a} \pm 0.06$	−36

[a] Data expressed as g kg^{-1} on a dry matter basis.
[b] Data expressed as mg kg^{-1} on a dry matter basis.
ND, not detected; (+) increment; (–) diminution. For each analysis, different letters in same row mean significant differences ($P \leq 0.05$).

The results show that the induced lactic acid fermentation process increases the proportion of nitrogen absorbed during ingestion, which leads to an increment in the protein quality.

Table 4 shows the effect of cooking on carbohydrates of previously induced lactic acid fermented beans. The insoluble fibre increased by 97.5%, probably due to the formation of complex molecules and retrograded starch, which are quantified as insoluble fibre when the methodology of Prosky *et al*[18] is used. Soluble fibre decreased by 12%, probably due to the hydrolysis and subsequent solubilization of some pectic compounds in the cooking water. There seems to be a similar occurrence with the disaccharide, monosaccharide and oligosaccharide concentrations, which decreased significantly ($P \leq 0.05$).

Resistant starch, which in legumes is basically of RS_1 type,[5] also decreased, which could be caused to gelatinization and solubilization in the cooking water. Based on the results found, it can be concluded that induced lactic acid fermentation of *Phaseolus vulgaris* beans decreases flatulent compounds and increases the nutritional value of the beans. Therefore, the lactic acid bacteria involved in the bean fermentation, which include *L casei* as a probiotic, could be used as functional starter cultures in the food industry. Likewise, the cooking applied after induced fermentation produced an additional diminution of the compounds related to flatulence.

ACKNOWLEDGEMENTS

This work was sponsored by FONACIT (National Fund for Science and Technology) through project no. 2001000856. The authors wish to thank Ing Alberto Sali of The Agronomic Research Institute of Venezuela (INIA, Maracay) for supplying the samples of *Phaseolus vulgaris* L-140.

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