JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Natural and Controlled Fermentation on Chemical Composition and Nutrient Dialyzability from Beans (*Phaseolus vulgaris* L.)

JESUS M. PORRES, PILAR ARANDA, MARÍA LÓPEZ-JURADO, AND GLORIA URBANO*

Departamento de Fisiología, Instituto de Nutrición y Tecnología de Alimentos, Campus Universitario de Cartuja s/n, Universidad de Granada, Granada 18071, Spain

The effect of natural and controlled fermentation with an inoculum of *Lactobacillus plantarum* and additional thermal treatment (dry heat at 120 °C for 20 min) on the availability of N, P, Fe, Cu, Zn, Ca, and Mg from *Phaseolus vulgaris* L. var. *carrilla* was estimated using an in vitro method based on equilibrium dialysis. Natural and controlled fermentations caused significant reductions in the pH and phytate content (36%) of the bean flours, with a concomitant increase in the titratable acidity and free phosphorus content, and had no effect on the other nutrients studied. The percentage of dialyzable N, P, Cu, and Mg was significantly improved by both types of fermentation, whereas Zn dialyzability was significantly reduced. The greatest reduction was observed for the bean flour fermented with an inoculum of *L. plantarum*. The percentage of dialyzable Fe improved significantly as a result of natural fermentation but was not affected by controlled fermentation. The application of dry heat at 120 °C for 20 min caused a significant increase in Fe dialyzability and a further reduction in the percentage of dialyzable Zn in fermented bean flours but did not affect the dialyzability of the other nutrients studied.

KEYWORDS: ***Phaseolus vulgaris*; natural fermentation; controlled fermentation; thermal treatment; phytate; nutrient dialyzability**

INTRODUCTION

Legumes are important sources of macronutrients, micronutrients, and antioxidant compounds and have a great potential for human and animal nutrition. The bean is the most common legume in human consumption (*1*), and the variety of bean used in the present study is widely consumed in Spain (*2*). Several culinary and technological processes have been developed to improve the nutritive value of legumes (*3*, *4*). Fermentation is one of the oldest and most economical methods of food production and preservation known (*5*). Fermentation can be spontaneously initiated with the microbiota naturally present in the legume (*6*, *7*) or controlled by the use of specific cultures or starters from a batch of previously fermented product (*8*, *9*). Legumes are fermented to improve their sensory characteristics, such as flavor and taste, and to enhance their nutritive value by improving the density and availability of nutrients (*10*, *11*). This can be achieved by degradation of antinutritional factors, by the predigestion of certain food components, and by the synthesis of promoters for absorption (*12*, *13*). In addition, by increasing the titratable acidity and reducing the pH of the food to levels below 4.5, fermentation precludes the proliferation of contaminating acid-intolerant species of bacteria and fungi.

Changes occurring during the fermentation process are mainly due to endogenous enzymes of the seed and the enzymatic activity of the microflora present in the legume. Differences in the final nutritional value of the fermented product depend on whether the whole seed or flour suspensions with different ratios of flour to water (*7*, *14*) are used. Fermentation may be combined with other culinary (soaking and cooking) and technological (germination prior to the fermentation process) treatments to further improve the nutritional value of the fermented product.

Several authors have pointed out the importance of thermal treatment for the reduction of antinutritional factors and in improving the nutritional quality and organoleptic properties of food products (*15*, *16*). Free amino acids, peptides, and reducing sugars formed during the fermentation process may act as precursors of the Maillard reaction products that develop during the thermal treatment and influence the flavor and aroma of the food.

In vitro techniques based on the diffusibility of nutrients through a dialysis membrane under conditions that resemble those found in the gastrointestinal tract can be a reliable indicator of the potential availability of these nutrients from different foods (*17*−*19*). Therefore, our objectives for the present study were (i) to determine the effect of natural and controlled fermentation with an inoculum of *Lactobacillus plantarum* and

* Corresponding author (telephone 34-958-243885; fax 34-958-248959; e-mail paranda@ugr.es).

10.1021/jf0300083 CCC: $25.00
Published on Web 07/12/2003

Table 1. Chemical Composition of Raw and Fermented Bean Flours in Dry Matter[a]

	pH	titratable acidity (mequiv of NaOH/100 g)	total N (g/100 g)	insoluble N (g/100 g)	soluble protein N (g/100 g)	soluble nonprotein N (g/100 g)
RB	6.4 ± 0.01c	3.2 ± 0.2a	3.86 ± 0.05a	0.36 ± 0.02a	3.07 ± 0.03c	0.45 ± 0.02a
NT48	4.5 ± 0.02b	51.6 ± 0.76b	3.93 ± 0.04a	0.79 ± 0.02c	1.94 ± 0.02ab	1.20 ± 0.02b
NT48A	4.5 ± 0.01b	53.2 ± 1.10b	3.98 ± 0.06a	1.01 ± 0.05d	1.82 ± 0.07a	1.18 ± 0.01b
PL48	3.7 ± 0.02a	108.2 ± 2.27c	3.85 ± 0.07a	0.64 ± 0.03bc	1.86 ± 0.02ab	1.34 ± 0.01c
PL48A	3.7 ± 0.03a	105.1 ± 1.56c	3.90 ± 0.03a	0.55 ± 0.04b	2.03 ± 0.05b	1.32 ± 0.02c

	ash (%)	phytate (mg/g)	free P (mg/100 g)	total P (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	Cu (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)
RB	4.03 ± 0.08a	11.76 ± 0.28b	28.8 ± 0.81a	451.5 ± 4.20a	8.66 ± 0.16b	4.76 ± 0.07a	1.21 ± 0.06ab	70.3 ± 1.43a	181.7 ± 2.15a
NT48	3.88 ± 0.01a	7.93 ± 0.35a	196.1 ± 9.38bc	468.06 ± 2.41ab	8.30 ± 0.10ab	5.13 ± 0.02b	1.13 ± 0.08ab	74.2 ± 0.29a	191.4 ± 1.28ab
NT48A	3.91 ± 0.01a	8.21 ± 0.08a	206.5 ± 4.25c	486.2 ± 6.71b	8.10 ± 0.11a	5.19 ± 0.06b	1.08 ± 0.04a	73.2 ± 0.41a	197.8 ± 3.17b
PL48	3.84 ± 0.03a	7.25 ± 0.27a	169.3 ± 10.01b	469.6 ± 1.78ab	8.41 ± 0.05ab	5.01 ± 0.06ab	1.32 ± 0.04b	72.7 ± 0.23a	185.2 ± 2.47a
PL48A	3.81 ± 0.08a	7.32 ± 0.31a	177.0 ± 8.56bc	465.1 ± 16.16ab	8.40 ± 0.05ab	4.91 ± 0.12ab	1.16 ± 0.02ab	71.1 ± 1.43a	183.9 ± 1.78a

[a] Values are means ± SEM from four replications. The same letter in the same column indicates no significant differences ($p < 0.05$). RB, raw bean flour; NT48, naturally fermented bean flour; NT48A, naturally fermented bean flour plus dry-heating at 120 °C for 20 min; PL48, controlled fermentation with an inoculum of *L. plantarum*; PL48A, controlled fermentation plus dry-heating at 120 °C for 20 min.

fermentation combined with a thermal treatment of dry heat at 120 °C for 20 min on the pH, titratable acidity, and nutrient and phytate compositions of *Phaseolus vulgaris* L. var. *carrilla*; and (ii) to study the influence of the two types of fermentation and the additional dry-heating treatment on the dialyzability of N, Fe, P, Cu, Zn, Ca, and Mg.

MATERIALS AND METHODS

Beans. *Raw Bean.* Raw bean flour (RB) was from *P. vulgaris* L. var. *carrilla*.

Fermentation. Raw beans were washed with distilled water and dried on a stove at 55 °C for 24 h. After drying, bean samples were ground in a ball mill and sieved, and the 0.050−0.250 mm fraction was collected. The bean flour was aseptically suspended in sterilized distilled water at 300 g/L concentration. The suspension was allowed to ferment naturally with the microorganisms present in the seed (*20*) (NT48) or was inoculated with 10% inoculum (v/v) of *L. plantarum* CECT 748 (PL48) at 37 °C for 48 h without aeration in a 5 L stirred fermentor (Infors ISF-100, Infors AG) at 150 rpm. After fermentation, the samples were collected and freeze-dried.

Thermal Treatment of the Fermented Bean Flours. NT48 and PL48 bean flours were dry-heated for 20 min at 121 °C (NT48A and PL48A).

Analyses. *pH and titratable acidity* were determined as described by Barampama and Simard (*8*) and Frias et al. (*12*). Titratable acidity was expressed as milliequivalents of NaOH per 100 g of dry matter (DM).

Chemical Analysis. The moisture content of the different bean flours was determined by drying to constant weight in an oven at 105 ± 1 °C. Total nitrogen was determined according to Kjeldahl's method. Crude protein was calculated as N × 6.25. Soluble protein and nonprotein nitrogen were measured using the methodology described by Periago et al. (*21*). The ash content of the different diets was measured by calcination at 500 °C to a constant weight. Samples of ashed material were dissolved in 6 N HCl, filtered, and diluted to 25 mL before analysis. Iron, zinc, copper, calcium, and magnesium contents were determined by atomic absorption spectrophotometry using a Perkin-Elmer AAnalyst 300 spectrophotometer. Lanthanum chloride was added to calcium and magnesium samples to prevent interferences caused by phosphate ions. Phosphorus was measured spectrophotometrically using the technique described by Chen et al. (*22*). Analytical results for Fe, Cu, Zn, P, Ca, and Mg were validated by a standard reference whole-meal flour (no. CRM-189) (Community Bureau of Reference, Commission of the European Communities). Phytic acid and free phosphorus were determined using the methodology described by Latta and Eskin (*23*) and Chen et al. (*22*). Phytase activity was determined after extraction of the bean flours in 0.2 M citrate buffer (pH 5.0) during 60 min at 4 °C with a flour-to-buffer ratio of 1:8 (w/v). The extract was spun down at 3000*g* for 10 min and the supernatant desalted using Sephadex G-25. The enzyme activity was assayed by measuring the free phosphorus released after incubation of the desalted fractions with sodium phytate during 30 min at 50 °C in 0.2 M citrate buffer (pH 5.0). One unit of phytase activity was defined as the amount of phytase activity that liberates 1 μmol of inorganic phosphorus from sodium phytate per minute at pH 5.0 and 50 °C.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). Proteins were extracted from the bean flours with 50 mM Tris-HCl (pH 7.8) containing 1% SDS. SDS-PAGE was done according to the method of Laemmli (*24*). The final concentration of acrylamide in the running gel was 15%. The gels were fixed and stained with 0.2% Coomassie brilliant blue R-250 in methanol/acetic acid/water (5:4:1 v/v/v). The mixture of molecular weight markers (Merck) consisted of cytochrome *c* (12.3 kDa), myoglobin (16.9 kDa), carboanhydrase (30 kDa), ovalbumin (42.7 kDa), albumin (66.25 kDa), and ovotransferrin (78 kDa).

Dialyzability. The in vitro method of Miller et al. (*25*) was adapted to assess iron, zinc, phosphorus, calcium, magnesium, copper, and nitrogen dialyzability of bean samples. Dialyzable nitrogen and minerals were expressed as a percentage of the total present in each digestion vial, assuming that the dialyzable component had equilibrated across the dialysis membrane by the time the dialysis bag was removed at the end of the digestion period.

Statistics. Data were analyzed using one-way ANOVA (Statgraphics Statistical Graphics). Tukey's HSD was applied to determine significance of differences in chemical composition and nutrient dialyzability. The level of significance was set at 0.05.

RESULTS

Natural and controlled fermentations reduced the pH of bean flours by 30 and 42%, respectively (**Table 1**). The pH reduction was not further affected by an additional dry-heat treatment at 120 °C and 1 atm for 20 m applied to flours NT48A and PL48A. The fall in pH was followed by a concomitant increase in the titratable acidity of the fermented beans; the bean flour fermented with an inoculum of *L. plantarum* (PL48 and PL48A) had the lowest pH and the highest titratable acidity.

Average total nitrogen content of the bean flours used in the present study was 3.90 ± 0.06 g/100 g of DM. In raw bean flour, 79.3% of the total nitrogen content corresponded to soluble protein nitrogen, whereas 11.5% corresponded to soluble nonprotein nitrogen (NPN) and the remaining 9.2% was not soluble at the basic pH used for nitrogen extraction. Natural and controlled fermentation of bean flours significantly increased the levels of insoluble and soluble nonprotein nitrogen and

Table 2. Percentage of Dialyzable Nitrogen, Phosphorus, Iron, Zinc, Copper, Calcium, and Magnesium from Raw and Fermented Bean Flours[a]

	N	P	Fe	Zn	Cu	Ca	Mg
RB	38.3 ± 1.77a	11.6 ± 0.30a	3.47 ± 0.06a	61.1 ± 0.49d	21.1 ± 1.90a	25.5 ± 0.69c	67.3 ± 0.54a
NT48	54.2 ± 2.53b	52.9 ± 1.39c	7.51 ± 0.09b	53.8 ± 0.76c	50.9 ± 1.45b	18.8 ± 0.72a	74.9 ± 1.28b
NT48A	54.9 ± 2.71b	50.8 ± 0.61bc	13.51 ± 0.5d	49.1 ± 1.01b	55.3 ± 1.46b	19.8 ± 0.90ab	74.8 ± 1.28b
PL48	53.7 ± 2.11b	48.8 ± 0.56bc	3.72 ± 0.05a	51.9 ± 0.79bc	52.1 ± 1.06b	22.3 ± 0.72bc	78.4 ± 0.49b
PL48A	52.8 ± 1.52b	47.6 ± 1.80b	10.74 ± 0.15c	45.4 ± 0.55a	56.8 ± 1.40b	23.1 ± 1.19bc	75.7 ± 0.49b

[a] Values are means ± SEM from four replications. The same letter in the same column indicates no significant differences ($p < 0.05$). RB, raw bean flour; NT48, naturally fermented bean flour; NT48A, naturally fermented bean flour plus dry-heating at 120 °C for 20 min; PL48, controlled fermentation with an inoculum of *L. plantarum*; PL48A, controlled fermentation plus dry-heating at 120 °C for 20 min.

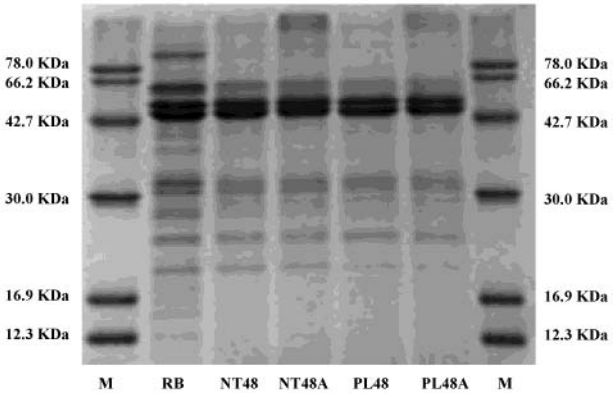

Figure 1. SDS-PAGE of the proteins extracted from raw and fermented bean flours. An equal amount of legume nitrogen (1.45 μg) was loaded in each lane. M, molecular weight markers; RB, raw bean flour; NT48, naturally fermented bean flour; NT48A, naturally fermented bean flour plus dry-heating at 120 °C for 20 min; PL48, controlled fermentation with an inoculum of *L. plantarum*; PL48A, controlled fermentation plus dry-heating at 120 °C for 20 min.

significantly reduced the content of soluble protein nitrogen. The highest amount of insoluble nitrogen was found in the flour of naturally fermented beans dry heated at 120 °C for 20 min (NT48A), whereas the highest proportion of soluble nonprotein nitrogen was found in the bean flours fermented with an inoculum of *L. plantarum* (PL48 and PL48A). No differences related to the thermal treatment were observed in the content of soluble nonprotein nitrogen among the fermented bean flours.

Figure 1 shows the changes in the SDS-PAGE pattern of proteins from the bean flours used in the present study. Raw bean flour exhibited the typical band pattern generally ascribed to phaseolin subunits (43−51 kDa) and two bands of high density at a molecular weight of ~32 kDa generally ascribed to *Phaseolus* phytolectins. The processes of natural and controlled fermentation caused the disappearance of several protein bands after 48 h of fermentation, whereas the 32 kDa bands diminished to a lesser extent and the β'' (46 kDa) and β' (43 kDa) subunits of phaseolin were hardly affected. Fermentation of the beans led to the appearance of high molecular weight protein aggregates that remained at the top of the resolving gel and were especially evident in the flours of beans that were fermented and then dry heated (NT48A and PL48A).

Raw bean flour had average ash, total P, Ca, and Mg contents of 4.03, 0.451, 0.07, and 0.18%, respectively, and Fe, Cu, and Zn contents of 8.66, 1.21, and 4.76 mg/100 g of DM, respectively (**Table 1**). In general, ash or mineral content was not greatly modified by the different treatments applied. Raw bean flour had a phytate content of 11.76 mg/g of DM and a phytase activity measured at pH 5 and 50 °C of 79.8 ± 4.15 units/kg of DM. Natural and controlled fermentations of beans produced an average 36% reduction of phytate content. No significant differences were found among the phytate contents of the different fermented bean flours. Phytase activity was lost as a result of the fermentative process. Dry-heating treatment applied to the fermented bean flours (NT48A and PL48A) did not produce any additional decrease in the levels of phytate. In all of the fermented bean flours, the reduction of phytate content originated a concomitant increase (5.8−7.1-fold) of free P that was highly correlated to the reduction of phytate ($r = 0.85$). No significant differences were found in the free P contents of the different fermented bean flours. The percentages of dialyzable N, P, Cu, and Mg from beans was significantly increased by natural and controlled fermentations compared to those of raw bean flour (RB), but no differences were observed between the different fermentation types or when thermal processing was applied after fermentation (**Table 2**). No significant differences in the percentage of dialyzable Fe were found between RB and beans fermented with an inoculum of *L. plantarum* (PL48). Fe dialyzability was significantly enhanced by natural fermentation of beans (NT48) and by the thermal treatment of dry heat applied to the flours of fermented beans (PL48A and NT48A). The naturally fermented bean flour plus thermal treatment at 120 °C for 20 min (NT48A) had the highest Fe dialyzability of all the treatments studied.

The percentage of dialyzable Zn was significantly reduced by natural and controlled fermentations of bean flour compared to raw beans. The dry-heat treatment applied to flours NT48A and PL48A caused an additional drop in Zn dialyzability, and the lowest value was found for the flour PL48A (45.4% versus 61.1% in RB).

Calcium dialyzability was not significantly affected by fermentation with an inoculum of *L. plantarum* (PL48), whereas natural fermentation of beans (NT48) caused a slight but significant reduction in the percentage of dialyzable Ca compared to raw bean flour. No significant differences related to the thermal treatment applied to the flours of fermented beans were found.

DISCUSSION

The process of fermentation is usually associated with a fall in pH and increased titratable acidity of foods due to the production of organic acids that is inherent to this treatment (*8*, *26*, *27*). Under our experimental conditions, the lower pH and higher titratable acidity of beans fermented with an inoculum of *L. plantarum* compared to the beans fermented with the endogenous microflora present in the seed may be related to differences in the type of bacterial population and growth or to the pattern of organic acid production by the two types of fermentation (*20*, *28*, *29*).

The content of total and soluble nonprotein nitrogen found in the beans used for the present study was within the range of values found in the literature for different varieties of beans (*21*, *30*). The increase in the insoluble nitrogen content during

the natural or controlled fermentation of beans was related to the appearance of high molecular weight protein aggregates in the SDS-PAGE and may have been caused by the mild hydrothermal treatment associated with the fermentative process (48 h at 37 °C) and subsequent dry-heat treatment at 120 °C for 20 min; it has been reported that similar thermal treatments reduce the protein solubility (*31*, *32*).

Nonprotein nitrogen (NPN) is usually constituted of free amino acids, polyamines, low molecular weight peptides, puric or pyrimidinic bases, and alkaloids. The increase in NPN observed in the flours of fermented beans compared to RB is similar to that described by other authors during cocoa or sorghum fermentation (*27*, *33*) and can be attributed to the digestion of legume storage proteins by bacterial or legume proteinases, which originate smaller polypeptides that are not precipitated at the acidic conditions used for soluble protein and NPN separation. This is confirmed by the disappearance or reduction of band intensity of polypeptides observed in the SDS-PAGE of raw and fermented bean flours. The absence of any increase in polypeptides of molecular weight >12.3 kDa over the fermentation period suggests that the proteins break down mainly into components with masses lower than this. The smaller polypeptides would be able to diffuse through the 12 kDa membrane used in our experiments, causing the increase in dialyzable nitrogen observed for all of the fermented bean flours. This is supported by the high correlation ($r = 0.82$) found between the amount of NPN and the percentage of dialyzable nitrogen.

These results suggest that the different fermentation processes applied under our experimental conditions have predigested the protein of bean flour and probably improved its potential digestive utilization.

The phytate content and mineral composition of the beans used for the present study were within the range of values found in the literature (*19*, *34*−*36*). Of the total phosphorus present in the variety of beans studied, 74% was in the form of phytic acid, which has been described as poorly available for monogastrics. The reductive effect of natural and controlled fermentation on the amount of phytic acid present in beans (36%) can be attributed to the action of endogenous phytase from the seed (*37*), phytase activity of the microorganisms present in the fermentation process (*38*), or a combination of the two. The phytate reduction achieved by the fermentation processes used in the present study was low compared to what has been reported for different varieties of beans (*19*, *39*) and cereals such as millet (*29*). This may have been caused by pH and temperature conditions during the fermentative process that were not optimal for an efficient degradation of phytic acid by phytase. The thermal treatment of dry heat at 120 °C for 20 min applied to flours NT48A and PL48A did not cause any additional decrease of the phytate content, as has also been found for chickpea or lentil flour subjected to a similar thermal treatment (*40*, *41*). In contrast, Fernandez et al. (*42*) found a 40% reduction of phytate content after dry-heating faba bean flour at 120 °C for 15 min. This seems to indicate that the physical breakdown of phytate caused by heat is influenced by the particular type of legume seed, given that enzymatic hydrolysis is not to be expected.

The decrease in phytate content caused by controlled and natural fermentations was highly correlated to an increment in the levels of free P ($r = 0.85$), whereas the increased levels of free P were highly correlated ($r = 0.98$) to a significant improvement in the percentage of dialyzable phosphorus from the different fermented bean flours compared to the raw bean control group. These results suggest that fermentation is an optimal process to improve the bioavailability of the latter mineral.

The percentage of dialyzable magnesium was high in the RB, which denotes a high solubility and thus potential availability of this mineral. The decrease in phytate levels (36%), in lignin content of naturally fermented beans (50%) (*43*), and in the cellulose fraction of insoluble fiber (31−59%) (*43*) could be responsible for the significant improvement in Mg dialyzability observed in the flours of fermented beans compared to the raw bean control.

The low percentage of dialyzable Ca obtained from RB was in agreement with the in vitro results obtained by Lombardi-Boccia et al. (*44*) in white and mottled bean seeds. The low amount of diffusible calcium from the RB found under our experimental conditions could be due to the presence of dietary fiber or phytate or to the fact that most of the calcium in beans is present in the seed hull (70−80%) complexed by oxalate (*45*, *46*). Neither natural nor controlled fermentation of bean flour improved the percentage of dialyzable Ca despite the reduction in the phytate and dietary fiber content caused by the fermentation process. It is possible that the reduction in these compounds was not sufficient for the improvement to become apparent. Furthermore, the percentage of dialyzable Ca might be affected by the presence of other factors with a greater influence on calcium solubility—factors that are resistant to hydrolysis or that might be synthesized during the fermentation process.

The digestive utilization of calcium from beans and lentils studied in vivo using the rat as an experimental model was high, in contrast to that observed in the present experiment (*40*, *47*). These differences could be attributed to the hydrolysis of undialyzable calcium complexes by the microflora of the large intestine, which would release calcium and make it available for absorption (*44*, *48*).

The zinc dialyzability from raw beans used in the present study was high, similar to that described by Wolters et al. (*49*) for French beans. Natural and controlled fermentations of bean flours decreased zinc solubility despite the phytate reduction and the improvement in phytate/Zn and Ca × phytate/Zn molar ratios (25 and 0.436, respectively, in raw beans versus 15 and 0.277, respectively, in fermented beans) caused by the different fermentation processes. The inhibitory effect of fermentation on zinc dialyzability could be attributed to the organic acids produced by the fermentation process that decrease zinc solubility at the pH conditions (6.7) used to resemble digestion in the small intestine. Zinc dialyzability was not correlated to the pH or titratable acidity of the fermented samples under our experimental conditions; no significant differences were found between the percentages of dialyzable zinc from the bean flours fermented naturally or with an inoculum of *L. plantarum*.

The further reduction in the zinc dialyzability observed in heat-treated bean flours (PL48A and NT48A) compared to their unheated controls (PL48 and NT48) can be attributed to Maillard reaction products formed during the thermal treatment, as has been suggested by Whitelaw and Weaver (*50*) and Navarro et al. (*51*).

Natural fermentation of bean flour (NT48) caused a significant increase in the percentage of dialyzable Fe, which may be related to the reduction in the levels of phytate (33%), lignin (50%) (*43*), and polyphenolic compounds (*52*) and to the production of small peptides and organic acids by the endogenous microbiota present in the seed (*29*) that have an enhancing effect on Fe solubility (*35*). Under the present experimental conditions, the only differences between the bean flour fer-

mented with an inoculum of *L. plantarum* and the naturally fermented bean flour were in lignin content, which was not decreased by the controlled fermentation (PL48) (*43*), and in the significantly higher titratable acidity of the flour fermented with the inoculum of *L. plantarum*, which is probably associated with a predominance of lactic acid over other different organic acids that are present in the naturally fermented bean flour. The lower Fe dialyzability found in flour PL48 when compared to flour NT48 could be attributed to these differences that would counteract the positive effect of the different factors previously mentioned for the naturally fermented bean flour. Ekholm et al. (*53*) reported that the enhancing effect of lactic acid on mineral solubility was small. Furthermore, lactate may facilitate the oxidation of ferrous to ferric iron at pH 6.7 of the dialysis step (*54*) and thus affect its solubility.

The increase in iron dialyzability found in flours NT48A and PL48A, compared to flours NT48 and PL48 (1.8−2.8-fold), could be attributed to the effect of the soluble melanoidins formed during the course of Maillard reactions, given that the rest of the components that could affect Fe dialyzability were not altered. These compounds are able to complex Fe (*55*), rendering it more soluble and capable of diffusing through the dialysis membrane.

The increment in Cu dialyzability from fermented beans observed in the present experiment is in agreement with the findings of Sripriya et al. (*29*) and of Usha Antony and Chandra (*6*) for fermented pearl millet. The 2.5-fold increase in the Cu dialyzability of fermented bean flours was independent of the type of fermentation process and could be due to the combination of several factors, such as small peptides that are able to complex Cu and enhance its diffusion through the dialysis membrane, the observed reduction in phytate content, and the appearance of organic acids with an enhancing effect on Cu solubility (*56*). The improvement on Cu dialyzability was independent of the pH and titratable acidity of the samples.

Under our experimental conditions, the thermal treatment applied after natural and controlled fermentation (NT48A and PL48A) did not result in a decrease of Cu dialyzability, in contrast to the observations of other authors working with pure Maillard reaction products, who have reported a negative effect on Cu solubility (*57*, *58*).

In conclusion, natural fermentation and the combination of natural fermentation and dry-heating at 120 °C for 20 min were found to be the most efficient and least expensive processes to develop a food product that reduces the risk of energy and micronutrient deficiencies in children and the elderly. Our results indicate that the process of natural fermentation enhances the dialyzability of N, P, Fe, Cu, and Mg, thus favoring a food product with high nutrient availability.

ACKNOWLEDGMENT

We thank Rosa Jiménez and Dr. Carmen Lluch Plá for skillful technical assistance and literature support.

LITERATURE CITED

(1) Hedley, C. Introduction. In *Carbohydrates in Grain Legume Seeds. Improving Nutritional Quality and Agronomic Characteristics*; Hedley, C. L., Ed.; CABI Publishing: Wallingford, U.K., 2001; pp 1−13.

(2) Varela, G.; Moreiras, O.; Carvajal, A.; Campo, M. *Estudio Nacional de Nutrición y Alimentación 1990/91*; Publicaciones del Instituto Nacional de Estadística: Madrid, Spain, 1995.

(3) Fernandez, M.; Lopez-Jurado, M.; Aranda, P.; Urbano, G. Nutritional assessment of raw and processed faba bean (*Vicia faba* L.) cultivar major in growing rats. *J. Agric. Food Chem*. **1996**, *44*, 2766−2772.

(4) Porres, J. M.; Urbano, G.; Fernández-Fígares, I.; Prieto, C.; Pérez, L.; Aguilera, J. F. Digestive utilization of protein and amino acids from raw and heated lentils by growing rats. *J. Sci. Food Agric*. **2002**, *82*, 1740−1747.

(5) Reddy, N. R.; Salunkhe, D. K. Fermentation. In *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 3, pp 177−217.

(6) Usha Anthony; Chandra, T. S. Antinutrient reduction and enhancement in protein, starch, and mineral availability in fermented flour of finger millet (*Eleusine coracana*). *J. Agric. Food Chem*. **1998**, *46*, 2578−2582.

(7) Granito, M.; Frias, J.; Doblado, R.; Guerra, M.; Champ, M.; Vidal-Valverde, C. Nutritional improvement of beans (*Phaseolus vulgaris*) by natural fermentation. *Eur. Food Res. Technol*. **2002**, *214*, 226−231.

(8) Barampama, Z.; Simard, R. E. Oligosaccharides, antinutritional factors, and protein digestibility of dry beans as affected by processing. *J. Food Sci*. **1994**, *59*, 833−838.

(9) Ibrahim, S. S.; Habiba, R. A.; Shatta, A. A.; Embaby, H. E. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Nahrung* **2002**, *46*, 92−95.

(10) Vidal-Valverde, C.; Frias, J.; Prodanov, M.; Tabera, J.; Ruiz, R.; Bacon, J. Effect of natural fermentation on carbohydrates, riboflavin and trypsin inhibitor activity of lentils. *Z. Lebensm. Unters. Forsch*. **1993**, *197*, 449−452.

(11) Svanberg, U.; Lorri, W. Fermentation and nutrient availability. *Food Control* **1997**, *8*, 319−327.

(12) Frias, J.; Vidal-Valverde, C.; Kozlowska, H.; Tabera, J.; Honke, J.; Hedley, C. L. Natural fermentation of lentils. Influence of time, flour concentration, and temperature on the kinetics of monosaccharides, disaccharides and α-galactosides. *J. Agric. Food Chem*. **1996**, *44*, 579−584.

(13) Valencia, S.; Svanberg, U.; Sandberg, A. S.; Ruales, J. Processing of quinoa (*Chenopodium quinoa*, Willd): effects on *in vitro* iron availability and phytate hydrolysis. *Int. J. Food Sci. Nutr*. **1999**, *50*, 203−211.

(14) Kozlowska, H.; Honke, J.; Sadowaska, J.; Frias, J.; Vidal-Valverde, C. Natural fermentation of lentils. Influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. *J. Sci. Food Agric*. **1996**, *71*, 367−375.

(15) Vidal-Valverde, C.; Frias, J.; Sotomayor, C.; Diaz-Pollan, C.; Fernandez, M.; Urbano, G. Nutrients and antinutritional factors in faba beans as affected by processing. *Z. Lebensm. Unters. Forsch. A* **1998**, *207*, 140−145.

(16) Hashim, P.; Selamat, J.; Kharidah, S.; Muhammad, S.; Ali, A. Changes in free amino acid, peptide-N, sugar and pyrazine concentration during cocoa fermentation. *J. Sci. Food Agric*. **1998**, *78*, 535−542.

(17) Keane, L. A.; Potter, N. N.; Sherbon, J. W. Estimation of calcium status in selected food systems. *J. Food Sci*. **1988**, *53*, 1111−1112.

(18) Miller, D. D.; Berner, L. A. Is solubility in vitro a reliable predictor of iron bioavailability? *Biol. Trace Elem. Res*. **1989**, *19*, 11−24.

(19) Mamiro, P. R. S.; Van Camp, J.; Mwikya, S. M.; Huyghebaert, A. *In vitro* extractability of calcium, iron, and zinc in finger millet and kidney beans during processing. *J. Food Chem*. **2001**, *66*, 1271−1275.

(20) Doblado, R.; Frias, J.; Muñoz, R.; Vidal-Valverde, C. Antinutritional factor content of dry beans (*Phaseolus vulgaris*) as affected by fermentation. *Pol. J. Food Nutr. Sci*. **2002**, *11/52*, 73−75.

(21) Periago, M.; Ros, G.; Martínez, C.; Rincón, F. Variations of nonprotein nitrogen in six Spanish legumes according to the extraction method used. *Food Res. Int*. **1996**, *29*, 489−494.

(22) Chen, P. S.; Toribara, T. Y.; Warner, H. Microdetermination of phosphorus. *Anal. Chem.* **1956**, *28*, 1756−1758.
(23) Latta, M.; Eskin, M. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* **1980**, *28*, 1313−1315.
(24) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.
(25) Miller, D. D.; Schricker, B. R.; Rasmussen, R. R.; Van Campen, D. An in vitro method for estimation of iron availability from meals. *Am. J. Clin. Nutr.* **1981**, *34*, 2248−2256.
(26) Czarnecka, M.; Czarnecki, Z.; Nowak, J.; Roszyk, H. Effect of lactic acid fermentation and extrusion of bean and pea seeds on nutritional and functional properties. *Nahrung* **1998**, *42*, 7−11.
(27) Yousif, N. E.; El Tinay, A. H. Effect of fermentation on sorghum protein fractions and in vitro protein digestibility. *Plant Foods Hum. Nutr.* **2001**, *56*, 175−182.
(28) Svanberg, U.; Lorri, W.; Sandberg, A. S. Lactic fermentation of non-tannin and high tannin cereals: Effects on *in vitro* estimation of iron availability and phytate hydrolysis. *J. Food Sci.* **1993**, *58*, 408−412.
(29) Sripriya, G.; Antony, U.; Chandra, T. S. Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food Chem.* **1997**, *58*, 345−350.
(30) Nikokyris, P. N.; Kandylis, K. Feed protein fractions in various solvents of ruminant feedstuffs. *J. Sci. Food Agric.* **1997**, *75*, 198−204.
(31) Zheng, G. H.; Fasina, O.; Sosulski, F. W.; Tyler, R. T. Nitrogen solubility of cereals and legumes subjected to micronization. *J. Agric. Food Chem.* **1998**, *46*, 4150−4157.
(32) Carbonaro, M.; Cappelloni, M.; Nicoli, S.; Lucarini, M.; Carnovale, E. Solubility-digestibility relationship of legume proteins. *J. Agric. Food Chem.* **1997**, *45*, 3387−3394.
(33) Lerceteau, E.; Rogers, J.; Pétiard, V.; Crouzillat, D. Evolution of cacao bean proteins during fermentation: a study by two-dimensional electrophoresis. *J. Sci. Food Agric.* **1999**, *79*, 619−625.
(34) Donangelo, C. M.; Trugo, L. C.; Trugo, N. M. F.; Eggum, B. O. Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. *Food Chem.* **1995**, *53*, 23−27.
(35) Lombardi-Boccia, G.; Carbonaro, C.; Cappelloni, M.; Carnovale, E. Relationship between *in vitro* Fe and Zn dialysability and peptide composition of albumin and globulins extracted from cooked bean (*Phaseolus vulgaris* L.). *Int. J. Food Sci. Nutr.* **1996**, *47*, 485−492.
(36) Nwokolo, E. Common bean (*Phaseolus vulgaris* L.). In *Food and Feed from Legumes and Oilseeds*, 1st ed.; Nwokolo, E., Smartt, J., Eds.; Chapman and Hall: London, U.K., 1996; pp 159−172.
(37) Lolas, G. M.; Markakis, P. The phytase of navy beans (*Phaseolus vulgaris*). *J. Food Sci.* **1977**, *42*, 1094−1106.
(38) Lopez, H. W.; Ouvry, A.; Bervas, E.; Guy, C.; Messager, A.; Demigné, C.; Rémésy, C. Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. *J. Agric. Food Chem.* **2000**, *48*, 2281−2285.
(39) Gustafsson, E. L.; Sandberg, A. S. Phytate reduction in brown beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **1995**, *60*, 149−152, 156.
(40) Nestares, T.; Barrionuevo, M.; Urbano, G.; López-Frías, M. Effect of processing methods on the calcium, phosphorus, and phytic acid contents and nutritive utilization of chickpea (*Cicer arietinum* L.). *J. Agric. Food Chem.* **1999**, *47*, 2807−2812.
(41) Urbano, G.; López-Jurado, M.; Fernández, M.; Moreu, M. C.; Porres-Foulquie, J.; Frias, J.; Vidal-Valverde, C. Ca and P bioavailability of processed lentils as affected by dietary fiber and phytic acid content. *Nutr. Res.* **1999**, *19*, 49−64.
(42) Fernández, M.; Aranda, P.; López-Jurado, M.; García-Fuentes, M.; Urbano, G. Bioavailability of phytic acid phosphorus in processed *Vicia faba* L. var. Major. *J. Agric. Food Chem.* **1997**, *45*, 4367−4371.
(43) Sanfiz, B. Efectos del proceso de fermentación y autoclave en la fibra alimentaria de *Phaseolus vulgaris*. M.D. Dissertation, Universidad Autónoma de Madrid, 2002.
(44) Lombardi-Boccia, G.; Lucarini, M.; Di Lullo, G.; Del Puppo, E.; Ferrari, A.; Carnovale, E. Dialysable, soluble and fermentable calcium from beans (*Phaseolus vulgaris* L.) as a model for *in vitro* assessment of the potential calcium availability. *Food Chem.* **1998**, *61*, 167−172.
(45) Barnabas, A. D.; Arnott, H. J. Calcium-oxalate crystal-formation in the bean (*Phaseolus vulgaris* L) seed coat. *Bot. Gaz.* **1990**, *151*, 331−341.
(46) Weaver, C. M.; Heanet, R. P.; Proulx, W. R.; Hinders, S. M.; Packard, P. T. Absorbability of calcium from common beans. *J. Food Sci.* **1993**, *58*, 1401−1403.
(47) Nestares, T.; Barrionuevo, M.; López-Frías, M.; Vidal, C.; Urbano, G. Effect of different soaking solutions on nutritive utilization of minerals (calcium, phosphorus, and magnesium) from cooked beans (*Phaseolus vulgaris* L.) in growing rats. *J. Agric. Food Chem.* **2003**, *51*, 515−520.
(48) Younes, H.; Demigné, C.; Rémésy, C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. *Br. J. Nutr.* **1999**, *75*, 301−314.
(49) Wolters, M. G. E.; Diepenmaat, H. B.; Hermus, R. J. J.; Voragen, A. G. J. Relation between *in vitro* availability of minerals and food composition: A mathematical model. *J. Food Sci.* **1993**, *58*, 1349−1355.
(50) Whitelaw, M. L.; Weaver, C. M. Maillard Browning effects on *in vitro* availability of zinc. *J. Food Sci.* **1988**, *53*, 1508−1510.
(51) Navarro, P.; Aspe, T.; Seiquer, I. Zinc transport in Caco-2 cells and zinc balance in rats: Influence of the heat treatment of a casein-glucose-fructose mixture. *J. Agric. Food Chem.* **2000**, *48*, 3589−3596.
(52) Dueñas, M.; Fernández, D.; Hernández, T.; Estrella, I.; Muñoz, R. Compuestos Bioactivos de Judías (*Phaseolus vulgaris*). Cambios originados por la fermentación. In *Proceedings of the II Congreso Nacional de Ciencia y Tecnología de Alimentos*; Orihuela, Spain, 2003.
(53) Ekholm, P.; Virkki, L.; Ylinen, M.; Johansson, L.; Varo, P. Effects of natural chelating agents on the solubility of some physiologically important mineral elements in oat bran and oat flakes. *Cereal Chem.* **2000**, *77*, 562−566.
(54) Kot, E.; Furmanov, S.; Bezkorovainy, A. Ferrous iron oxidation by *Lactobacillus acidophilus* and its metabolic products. *J. Agric. Food Chem.* **1995**, *43*, 1276−1282.
(55) Homma, S.; Aida, K.; Fujimaki, M. Chelation of metal with brown pigments in coffe. In *Amino−Carbonyl Reactions in Food and Biology Systems*; Fujimaki, M., Nakmiki, M. Y., Kato, H., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1986; pp 165−172.
(56) Wapnir, R. A. Copper absorption and bioavailability. *Am. J. Clin. Nutr.* **1998**, *67*, 1054S−1760S.
(57) O'Brien, J.; Morrissey, P. A. Nutritional and toxicological aspects of the maillard browning reaction in foods. *CRC Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 211−248.
(58) Wijewickreme, A. N.; Kitts, D. D.; Durance, T. D. Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model maillard reaction products. *J. Agric. Food Chem.* **1997**, *45*, 4577−4583.

Received for review January 6, 2003. Accepted May 14, 2003. This research was funded by Projects AGL 2000-1609-C02-02 and AGL2002-02905 ALI from the Spanish CICYT.

JF0300083