Effect of Heat Treatment on Nutritional Quality of Germinated Legume Seeds

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The effect of heat treatment of germinated soybean, lupin, and black bean on chemical composition and protein utilization in rats was evaluated. Heat treatment caused complete inactivation of trypsin inhibitors whereas it did not affect phytic acid levels. Proximate components, minerals, and amino acids did not change, but low molecular weight sugars were affected by heat treatment differently for each germinated legume. The sugar digestibility ratio (total digestible sugars/total nondigestible sugars) in germinated black beans doubled after heat treatment. True protein digestibility (TD) increased with heat treatment only in germinated soybean. Net protein utilization was markedly improved (20%) with heat treatment in germinated soybean and lupin. Utilizable protein of heat-treated germinated legumes was 289, 236, and 132 g/kg of legume dry weight for soybean, lupin, and black bean, respectively. Supplementation with methionine did not alter TD but improved all other indices of protein utilization in the germinated legumes, particularly in black bean. All three germinated legumes become equivalent in protein quality when heating and supplementation with methionine are combined with germination. The use of germinated heat-treated soybean, lupin, and black bean on their own and/or as food ingredients is nutritionally advantageous due to the low content of nondigestible oligosaccharides and the high protein utilization.

Keywords: *Legumes; germination; boiling; nutrient and nonnutrient compounds; protein quality*

INTRODUCTION

Legume seeds are important staple foods, particularly in developing countries, due to their relatively low cost, long conservation time, and high nutritional value. However, their wider use is somehow limited by the presence of nonnutrient compounds in the seeds which may have adverse effects for human or animal nutrition. Some examples of these compounds are protease inhibitors, lectins, phenolics, phytates, and α-galactosides (Deshpande et al., 1984; Garcia et al., 1997; Trugo and von Baer, 1998). Consequently, it is desirable to develop transformation processes that could improve the nutritional quality of legumes and also provide new derived products for the consumers.

Germination is considered a potentially beneficial process for legume seed transformation which may decrease undesirable components such as alkaloids and phytates (Muquiz et al., 1998; Oboh et al., 1998; Orúe et al., 1998), increase nutrients such as vitamin C (Riddoch et al., 1998), and increase protein digestibility (Schulze et al., 1997), consequently improving nutritional quality. Additional advantages of germination are reduction in cooking time and improvement of sensorial attributes of the product (Vanderstoep, 1981; Deshpande et al., 1984).

Germination has been shown to decrease the level of α-galactosides of different legume seeds including soybean, black bean, and lupin seed, with the corresponding decrease in carbohydrates available for fermentation in the large intestine of humans. The content of trypsin inhibitors and phytates is also decreased, but considerable amounts of these factors are still present after germination (Souza et al., 1988; Trugo et al., 1990, 1993; Donangelo et al., 1995; Silva and Trugo, 1996).

Information on the biological quality of germinated legume seeds is not readily available particularly when related to in vivo studies of protein and energy utilization. In a previous study (Donangelo et al., 1995), we compared protein utilization and energy digestibility of soybean, black bean, and lupin seed before and after 48 h germination. Germination produced only a discrete increase in protein utilization. Heating can be a complementary treatment to improve protein utilization and to further decrease antinutritional factors. In the present study, we evaluated the effect of heat treatment of the germinated legume seeds on their chemical composition and the effects of heat treatment and supplementation with methionine on protein utilization in rats.

MATERIALS AND METHODS

Samples and Processing. Seeds of a sweet variety of lupin (*Lupinus albus* cv. *multolupa*) were supplied by the Instituto Agronômico do Paraná (Ponta Grossa, Brazil), and black beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) were from EMBRAPA (Rio de Janeiro, Brazil). Germination of the seeds was performed by a modification of the method of Khaleque et al. (1985) as previously described (Donangelo et al., 1995). The seeds were germinated in the dark at 28 °C for 48 h and finally divided in two batches; one was freeze-dried without further treatment and the other was freeze-dried after heat treatment by boiling for 20 min with enough distilled water to cover the seeds.

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Analytical Methods. Proximate analyses were performed by standardized AOAC (1984) methods. Low molecular weight (LMW) sugars were extracted with 50% (v/v) ethanol in a water bath for 60 min at 65 °C and quantified by gas−liquid chromatography according to Bach Knudsen and Li (1991). The sugar digestibility ratio (SDR) was calculated according to Trugo et al. (1995) as (fructose + glucose + sucrose)/(raffinose + stachyose + verbascose).

Starch was analyzed by the enzymatic−colorimetric method of Bach Knudsen (1997). Total nonstarch polysaccharides (NSP) and their constituent sugars were determined as alditol acetates by gas−liquid chromatography for neutral sugars and by a colorimetric method for uronic acids using a modification of the Uppsala procedure (Theander et al., 1994) and the procedure of Englyst et al. (1982) as described by Bach Knudsen (1997). Klason lignin was measured gravimetrically as the residue resistant to sulfuric acid (Theander et al., 1994). Zinc and iron were measured by flame atomic spectrophotometry, and calcium was measured by a colorimetric method, as previously described (Trugo et al., 1993). Phytic acid was determined by the method of Harland and Oberleas (1977). Trypsin inhibitor activity was measured according to Kakade et al. (1974), and results were expressed as trypsin inhibitory units per milligram of dried sample. Amino acid analyses were performed according to Mason et al. (1980). All results are given as means of duplicate analysis.

Rat Bioassay. The experimental procedures for measurements of digestibility and nitrogen balance in rats used in this work have been described by Eggum (1973). The experimental design was a 3 × 3 factorial, with three types of germinated legume seeds (lupin, soybean, and black bean) and three treatments (no heat treatment, heat treatment, and heat treatment plus supplementation with methionine) as factors. However, as the rats refused to eat diets containing non-heat-treated black beans probably due to the high content of protease inhibitors and lectins (Deshpande et al., 1984), they were fed only with the heat-treated black beans, with and without methionine supplementation.

The diets were composed of a N-free mixture (starch, 80.7%; sucrose, 8.9%; cellulose, 5.2%; and peanut oil, 5.2%), vitamins (1.6%), minerals (4%), and the germinated legume seed providing 15 g of N/kg dry weight, either untreated, heat-treated, or heat-treated and supplemented with DL-methionine (0.15 g % diet dry weight).

Groups of five rats were used per treatment (initial weight about 70 g). Each animal received 10 g dry matter (150 mg of N) of diet daily, with a preliminary feeding period of 4 days and a balance period of 5 days. True protein digestibility (TD), biological value (BV), net protein utilization (NPU), and utilizable protein (UP) were determined as already described, including appropriate correction factors (Eggum, 1973).

Statistical Analyses. Evaluation of the effects of heat treatment and of supplementation with methionine on the biological indices for each type of germinated legume, and of legume type for each treatment, was done by analysis of variance. Statistical differences ($p < 0.05$) between groups were determined by Tukey's test.

RESULTS AND DISCUSSION

The legume seeds selected for this study present particular characteristics. Black bean is mainly used for human nutrition as a staple food especially in Latin American countries; soybean is a staple food in Asia, and it is used worldwide for animal nutrition; lupin is a crop not widely used but with potential for both animal and human nutrition. All three legumes are important sources of protein, carbohydrates, and minerals, and additionally, both soybean and lupin are good sources of energy due to their high contents of oil. Germination and heating as separated processes have proved partially beneficial for the nutritional quality of seeds. The study of the effect of combined germination and heating may provide useful information for optimization of use of legume seeds as food products.

Table 1. Chemical Composition of Germinated Legume Seeds with and without Heat Treatment[a]

	lupin		soybean		black bean	
composition	NT	HT	NT	HT	NT	HT
proximate components (g %)						
protein (N × 6.25)	34.9	35.6	41.5	43.2	23.2	24.3
fat	10.1	9.7	19.0	21.8	1.8	1.3
carbohydrates	47.3	48.3	24.1	23.7	56.0	59.2
lignin	2.4	2.3	2.2	1.9	3.2	3.0
ash	3.2	2.9	5.3	4.7	4.6	4.6
minerals (mg %)						
calcium	260	280	300	280	280	300
zinc	4.9	4.5	4.6	4.6	3.4	3.6
ron	8.6	8.9	10.2	8.9	8.6	9.3
phytic acid (g %)	0.6	0.6	1.6	1.4	1.4	1.2
trypsin inhibitors (TIU/mg)	2	nd	46	nd	98	nd

[a] Results are means of duplicate determinations, expressed on dry matter basis. Abbreviations: NT, no treatment; HT, heat treatment; nd, nondetected; TIU, trypsin inhibitor units.

Effect of Heat Treatment on the Chemical Composition of Germinated Legume Seeds. Table 1 shows the results of proximate analyses, as well as levels of minerals, phytic acid, and trypsin inhibitors of the samples. The heat treatment did not have a considerable effect on the proximate and mineral composition of the germinated legume seeds. The small variations observed were considered to be within the range of the method error.

Regarding the nonnutrient components that could have adverse effects on nutrient bioavailability, heating caused a complete inactivation of the trypsin inhibitor in all germinated legumes whereas phytic acid levels were similar in the same germinated legume with and without heat treatment. Therefore, mineral bioavailability in the germinated legumes studied can be similarly adversely affected by phytic acid both before and after heat treatment. However, the lower levels of phytic acid in germinated lupin seeds, either heated or nonheated, in comparison to soybean and black bean should probably contribute toward a better mineral bioavailability in lupin. As for the trypsin inhibitor activities of the germinated seeds, heating was very effective for their inactivation in soybean and black beans, since germination alone (Donangelo et al., 1995) caused a decrease of only 36% and 20%, respectively, in trypsin inhibitor activity.

The germinated legume seeds studied presented marked differences in the carbohydrate composition on a dry basis (Table 2). Soybean and lupin had a very low starch content, since their carbohydrate fraction consisted mainly of LMW sugars and cell wall polysaccharides, whereas black bean had about 30% starch. The distribution of nonstarch polysaccharides also varied between seeds. While noncellulosic polysaccharides were major contributors to this fraction for all seeds, there was a large variation in the proportion of soluble to insoluble noncellulosic polysaccharides, such that the ratio was high in lupin, intermediate in black bean, and low in soybean. Germinated soybean and black bean showed similar cellulose contents (around 3.5%), but lupin had much higher content (around 9%). The polysaccharide fraction was not affected by the heat treatment in all germinated seeds studied.

Germinated lupin seed had the highest content of both soluble and insoluble dietary fiber components (8.1 and 28.1 g %, respectively), whereas the concentration

Table 2. Carbohydrate Composition of Germinated Legume Seeds with and without Heat Treatment[a]

	lupin		soybean		black bean	
component (g %)	NT	HT	NT	HT	NT	HT
LMW sugars						
frutose	0.4	0.4	0.3	0.2	nd	0.2
glucose	0.4	0.4	0.3	0.2	0.1	0.3
sucrose	6.6	6.6	5.3	4.5	5.1	5.5
raffinose	0.5	0.3	0.1	0.1	0.3	0.1
stachyose	2.6	3.8	1.0	1.0	0.6	0.4
verbascose	0.6	0.6	0.1	0.0	nd	0.1
total	11.0	12.1	7.1	6.0	6.1	6.5
SDR	2.0	1.6	4.9	4.5	5.8	10.0
starch	2.4	2.1	1.8	1.7	30.5	31.3
NSP						
S-NCP	8.1	7.5	2.9	3.1	5.3	5.7
I-NCP	16.9	17.8	8.7	9.5	10.8	11.9
cellulose	8.9	8.8	3.6	3.6	3.3	3.8
total NSP	33.9	34.1	15.2	16.0	19.4	21.4
lignin	2.4	2.3	2.2	1.9	3.2	3.0
dietary fiber	36.2	36.4	17.4	17.9	22.6	24.4
soluble/total dietary fiber	0.22	0.21	0.16	0.17	0.22	0.23

[a] Results are means of duplicate determinations, expressed on dry matter basis. Abbreviations: NT, no treatment; HT, heat treatment; nd, nondetected; NSP, nonstarch polysaccharides; S-NCP, noncellulosic polysaccharides; I-NCP, insoluble noncellulosic polysaccharides; LMW, low molecular weight; SDR, sugar digestibility ratio (total digestible sugars/total nondigestible sugars).

in soybean was about half of these values and intermediate in black bean. The ratio soluble/total dietary fiber was similar for germinated lupin seed and black bean (around 0.22) and slightly lower for germinated soybean (0.16). Neither the ratio between soluble and insoluble dietary fiber components nor the total level of dietary fiber changed after heating of the germinated seeds. Thus, germinated lupin similarly to the nongerminated (Donangelo et al., 1995) is a good source of dietary fiber, and it has a level of soluble components that is substantially higher than those usually found in cereals (Nyman et al., 1984).

The LMW sugar content and distribution also showed differences between the germinated legumes (Table 2). The highest total sugar content was found in lupin seeds (11−12 g %), with sucrose being the major contributor to this fraction. A high amount of sucrose is generated during germination in lupin but not in the other legumes as has been previously reported (Donangelo et al., 1995). α-Galactosides were also present in larger amounts in the germinated lupin seed when compared with the other legumes, both before and after the heat treatment. While total α-galactosides were unchanged after heating in germinated soybean (1.2−1.1 g %), they decreased in germinated black bean (0.9−0.6 g %) and increased in germinated lupin (3.7−4.7 g %). This increase may result from hydrolysis of a carbohydrate fraction of higher molecular weight, sensitive to heating, which is not detected under the chromatographic conditions normally used for galactoside analysis. The beneficial effect of germination in decreasing substantially α-galactosides in lupin, black bean, and soybean (Donangelo et al., 1995; Trugo et al., 1993) is therefore partially overcome by heating in the case of lupin.

Relatively high amounts of digestible sugars, mainly sucrose, were found in the germinated seeds, and some decrease after the heat treatment was observed only in the soybean (Table 2). The considerable increase in SDR in germinated black bean after heat treatment is due to both a general increase in digestible sugars and a decrease of raffinose and stachyose. Therefore, heat treatment of germinated black bean appears beneficial in terms of higher energy bioavailability from low molecular weight sugars and lower undesirable intestinal fermentation.

Table 3. Amino Acid Composition in Germinated Legume Seeds with and without Heat Treatment[a]

amino acid (g/16 g of N)	lupin		soybean		black bean	
	NT	HT	NT	HT	NT	HT
aspartic acid	10.09	10.36	11.76	11.72	11.20	11.85
threonine	3.74	3.67	3.80	3.75	4.30	4.43
serine	5.36	5.37	5.43	5.41	6.08	6.40
glutamic acid	18.66	19.59	17.51	17.55	14.48	14.80
proline	4.19	4.44	5.33	5.34	3.89	4.20
glycine	3.93	3.94	4.12	4.19	3.94	3.78
alanine	3.21	3.42	4.21	4.37	4.12	4.36
valine	4.41	4.44	5.18	5.25	5.27	5.65
isoleucine	4.64	4.66	5.03	5.10	4.43	4.88
leucine	7.15	7.17	7.68	7.79	7.61	7.84
tyrosine	4.83	4.70	3.86	3.82	3.39	3.22
phenylalanine	3.94	3.93	5.34	5.41	5.62	5.54
histidine	2.47	2.45	2.81	2.79	2.71	3.06
lysine	4.82	4.78	6.24	6.27	6.29	6.52
arginine	9.90	10.03	7.33	7.39	5.73	6.05
cystine	1.43	1.44	1.58	1.53	0.97	1.13
methionine	0.67	0.75	1.34	1.53	1.11	1.20
tryptophan	0.80	0.82	1.31	1.29	1.15	1.22

[a] Results are means of duplicate determinations, expressed as g/16 g of N. Abbreviations: NT, no treatment; HT, heat treatment.

The amino acid composition (Table 3) varied very little when the germinated seeds were compared before and after heat treatment. A comparison of the amino acid composition of the samples with the FAO standard for infants (OMS, 1985) showed, as expected, that methionine plus cystine were the first limiting amino acids in the germinated seeds, either heated or nonheated. Chemical scores were around 50% for lupin and black bean and 70% for soybean. In the case of germinated lupin, either heated or nonheated, tryptophan also presented a score around 50%. According to these results germinated lupin presents a less adequate amino acid profile for infant consumption when compared to soybean and black bean.

Effect of Heat Treatment and Supplementation with Methionine of Germinated Legume Seeds on Protein Utilization in Rats. Results of the rat bioassay are shown in Table 4. Values for untreated germinated black beans are not given as explained in Materials andMethods. Therefore, for black beans only the results for heat-treated samples and the effect of supplementation with methionine are presented.

Comparing the germinated lupin and soybean, non heat treated, lupin had higher TD, but all other indices (BV, NPU, and UP) were similar between these two legumes. The very low trypsin inhibitor activity in lupin may contribute to a better TD. Slight differences in the essential amino acid profile of the germinated seeds, less favorable for lupin, did not affect significantly retention of the absorbed amino acids, and overall protein utilization was similar in both germinated legumes. Therefore, germinated lupin and soybean have very similar protein values as expressed by UP, which is important when comparing protein-rich materials for animal feed. This is in contrast with observations in nongerminated seeds (Donangelo et al., 1996) that showed a better protein utilization of lupin compared to soybean.

Heat treatment improved TD of germinated soybean (11.3%) probably due in part to suppression of trypsin

Table 4. Protein Utilization in Rats of Germinated Legume Seeds with and without Heat Treatment[a,b]

	true digestibility (%)	biological value (%)	net protein utilization (%)	utilizable protein (g/kg of legume)
soybean				
NT	85.0 ± 1.6	64.7 ± 6.6	54.9 ± 5.5	228 ± 23
HT	94.6 ± 1.6^{c}	70.6 ± 3.7	66.8 ± 4.5^{b}	289 ± 20^{b}
HT + Met	94.0 ± 2.0	82.4 ± 2.0^{e}	77.5 ± 3.3^{e}	335 ± 14^{f}
lupin				
NT	95.0 ± 1.2^{i}	60.6 ± 3.9	57.6 ± 4.0	201 ± 14
HT	$90.7 \pm 3.0^{a,g}$	73.0 ± 3.3^{c}	66.3 ± 4.8^{a}	$236 \pm 17^{b,h}$
HT + Met	93.5 ± 1.0	$78.8 \pm 2.5^{d,g}$	73.6 ± 2.7^{d}	$262 \pm 10^{f,i}$
black bean				
HT	78.5 ± 2.7^{i}	68.9 ± 5.5	54.1 ± 4.9^{i}	132 ± 12^{i}
HT + Met	75.9 ± 2.5^{i}	$97.1 \pm 3.6^{f,i}$	73.7 ± 4.9^{f}	$179 \pm 12^{f,i}$

[a] Abbreviations: NT, no treatment; HT, heat treatment; Met, methionine; utilizable protein, protein (g/kg dry weight of legume) × NPU. [b] Comparison in the same legume between nonheated and heated samples: significantly different from the corresponding NT (a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$). Comparison in the same heat-treated legume between methionine supplementation and no supplementation: significantly different from the corresponding HT (d, $p < 0.05$; e, $p < 0.01$; f, $p < 0.001$). Comparison of lupin and black bean with soybean in the same treatment: significantly different from the corresponding soybean value (g, $p < 0.05$; h, $p < 0.01$; i, $p < 0.001$).

inhibitor activity. The heat treatment of boiling for 20 min that was used in this study probably contributed to exposure of peptide bonds to digestive enzymes without causing substantial oxidative losses of amino acids or extensive Maillard reaction. Mild moist heat treatment has been found to improve TD and particularly BV in some legume seeds (Deshpande et al., 1984) and in cereals (Eggum et al., 1983). However, heat treatment reduced TD in germinated lupin (4.5%) but improved substantially BV in this legume (20%). Although the reason for the lower TD for heat-treated germinated lupin is not evident from our results, chemical reactions between protein and nonprotein components with heating may contribute to this fact as has been observed for cereals (Eggum et al., 1983). The increase in BV observed after heat treatment of germinated lupin cannot be explained by changes in the amino acid profile that was unaffected by this treatment. However, due to the decrease in TD with heat treatment, the relative proportion of essential to nonessential amino acids in absorbed protein from germinated lupin may have changed, with heating resulting in a better utilization. In sorghum, for instance, cooking has been found to decrease protein digestibility of the prolamine fraction which is rich in proline and low in lysine, by binding to polyphenols, thus resulting in a better BV (Eggum et al., 1983).

In our study, the overall effect of heat treatment on protein utilization of the germinated seeds was markedly positive for soybean and lupin with an increase of over 20% in NPU. Comparing the heat-treated germinated legumes, black bean presented the lowest NPU, which was due mainly to its lower TD. Comparing the potential protein value of the three heat-treated germinated legumes in our study, soybean had the largest UP (289 g/kg) followed closely by lupin (236 g/kg). UP in heat-treated germinated black bean was about half that of soybean (132 g/kg), which was partially due to its lower protein content.

Sulfur-containing amino acids are first limiting for most legumes, and methionine supplementation has usually been found to improve protein utilization in legume seeds (Eggum et al., 1993, 1985). In the present study, supplementation of the heat-treated germinated legume proteins with methionine did not alter TD as expected but significantly improved all indices of protein utilization in the three legumes studied. The highest improvement in NPU with methionine supplementation was for heat-treated germinated black bean (increase of 36%) compared with similarly treated lupin and soybean (increase of 9.9% and 16%, respectively). NPU was similar in all heat-treated germinated legumes after supplementation with methionine. The improvement in protein utilization with methionine supplementation in all three heat-treated germinated legumes is consistent with the fact that methionine plus cystine were the first limitants for the germinated legumes. In the case of heat-treated germinated lupin, the additional limitation of tryptophan could explain why it showed the lowest increases in BV and NPU when the diets were supplemented with methionine, compared to the other legumes.

In conclusion, combining heating with germination in soybean, lupin, and black bean is nutritionally advantageous due to the reduction of nondigestible oligosaccharides attained by germination, as previously observed (Donangelo et al., 1995), and improvement in protein utilization attained by heat treatment, as demonstrated in the present study. All three germinated legumes become equivalent in protein quality when both heating and supplementation with methionine are combined with germination. These advantages should be considered in cost/benefit analyses of the use of processed legumes, on their own or as food ingredients, for animal and human nutrition.

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