

Amino Acid Profiles after Sprouting, Autoclaving, and Lactic Acid Fermentation of Finger Millet (*Eleusine Coracan*) and Kidney Beans (*Phaseolus Vulgaris* L.)

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Seeds of finger millet (*Eleusine coracan* (L.) Gaertner) and kidney beans (*Phaseolus vulgaris* L.) were sprouted, autoclaved, and fermented during the processing of a weaning (complementary) food for children. Relative changes in individual amino acids with processing were evaluated. Finger millet and kidney beans both showed a good percentage of essential to total amino acids, with 44.2–44.9% in finger millet and 44.2–45.1% in kidney beans, when compared to 33.9% for the FAO/WHO reference protein for 2–5 year old children. Sprouting resulted in a significant decrease in lysine in kidney beans. Autoclaving caused significant decreases in histidine, while fermentation significantly decreased phenylalanine and increased tryptophan in finger millet. The leucine-to-lysine ratio, which is an indicator of the pellagragenic character of a protein, was significantly improved in finger millet by both sprouting and fermentation.

Keywords: *Finger millet; kidney beans; sprouting; fermentation; amino acids*

INTRODUCTION

Dry kidney beans are widely consumed in Latin America, Africa, and Asia and, for economic reasons, are important sources of protein in the diets of those countries. Being legumes, kidney bean protein is limiting in the sulfur containing amino acids methionine and cysteine. Millets, especially finger millet, constitute a major diet in Africa and Asia where it is extensively used for child feeding. Like all other cereals, finger millet protein is limiting in lysine.

Cereals and legumes also contain significant amounts of antinutrients, which further lower their nutritional quality. Age-old technologies such as sprouting (germination) and lactic acid fermentation have been proposed for processing of more digestible and palatable foodstuffs. When mixed together, the proteins of finger millet and kidney beans complement one another to produce a protein of a better quality by providing to each other significant amounts of the respective limiting amino acids. Such a composite mix can be used as weaning (complementary) food for children.

Sprouting of cereals and legumes involves complex enzymatic reactions, which break down macromolecules such as starch and proteins into smaller units. This makes these foodstuffs more digestible, making sprouting a useful process in the development of weaning foods with improved digestibility. Sprouting has also been found to affect the amounts of various amino acids in cereals and legumes. Chang and Harrold (1988) observed that after 6-days sprouting, tyrosine was signifi-

cantly ($p < 0.05$) increased by 19.9% and 38.4% in navy c-20 beans and pinto beans, respectively.

A mechanism for the increase in lysine and tryptophan during sprouting has been proposed: the degradation of prolamins into lower peptides and free amino acids supplies amino groups, which may be used through transamination to synthesize lysine. Glutamic acid and proline have been implicated in providing nitrogen for synthesis of lysine and other essential amino acids. However, the source of the carbon skeleton for the increased tryptophan synthesis needs to be identified (Chavan and Kadham, 1989).

Heat processing is critical because it may lower the biological value of proteins. Overheating (in terms of temperature and/or exposure time to heat) may adversely affect the availability of lysine, arginine, methionine, and cysteine in beans. The destruction of amino acids, lysine in particular, upon heating can be estimated by measuring lysine availability (Ziena et al., 1991; Friedman, 1996).

Lactic acid fermentation has also been found to affect the amounts of amino acids in cereals and legumes. Hamad and Fields (1979) reported a highly significant ($\alpha < 0.01$) increase in the available lysine content in flour made from 2-day sprouted and 6-day natural lactic acid fermented wheat when compared to fermented oats, rice, millet, and maize. Asiedu et al. (1993) observed that fermentation increased the lysine value by 25% in sorghum and 9% in maize. A similar increase in fermented maize and millet has been observed (Hamad and Fields, 1979). The bacterial fermentation of cereals and cereal plus legume blends produced the highest increase, while yeast fermentation caused a decrease in methionine content. Bacterial fermentation usually involves proteolytic activity, while yeasts mainly degrade carbohydrates. Hence, the changes in amino acid

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composition during bacterial and yeast fermentation may differ. Most of the changes in amino acid amounts reported were obtained during extended periods of fermentation and sprouting. However, these periods are impracticable when using these technologies for processing of human food, as there might be an excessive loss of dry matter through respiration and microbial spoilage. Mbithi-Mwikya et al. (2000) observed that germinating finger millet for longer than 48 h at 30 °C would result in excessive dry matter loss without much improvement in nutritional quality. This time was therefore considered as optimal for sprouting of finger millet. Compared to other cereals, published data on millet proteins, especially amino acid profiles, are scanty. In finger millet, the content of tryptophan has not been determined with certainty (Landry and Delhaye, 1995).

This study was set up to investigate whether technologies such as sprouting and fermentation, when carried out at time/temperature conditions suitable for processing a tropical weaning food, significantly affect the amounts of amino acids and the nutritional quality of protein in finger millet (*Eleusine coracana*) and kidney beans (*Phaseolus vulgaris*).

MATERIALS AND METHODS

Materials. Finger millet (*Eleusine coracana* var. *lima*) was obtained from Kenya (1997 harvest). Kidney beans (*Phaseolus vulgaris* var. *rose coco*) were purchased locally from a shop in Gent. Extraneous materials and broken seeds were removed by sorting. The seeds were washed carefully with distilled water and the floats discarded. They were then drained, spread in trays and left to air-dry overnight (37 °C).

Experimental Procedures. Finger millet and kidney beans were processed separately. They were soaked, sprouted, milled, autoclaved, and fermented.

Raw Sample. Dry grains were milled with a coffee mill and the flour was passed through a 200- μ m sieve. The flour obtained was then packed, double sealed in polyethylene paper (stomacher) bag, and stored in a cold room (4 °C) until analyzed.

Sprouting. About 500 g of dry grains was soaked for 3 h (finger millet) or 8 h (kidney beans) in distilled water (2:1, v/w). The soaking time was optimized to ensure maximum sprouting with minimum loss of dry matter through leaching. The water was drained and the seeds spread on moist blotting paper on wooden trays. They were then covered with perforated aluminum foil to reduce the evaporation rate. The seeds were kept at 30 °C in the dark for 48 h to sprout. At the end of this period, the nonsprouted seeds were discarded and the sprouted ones removed from the moist blotting paper and dried at 37 °C for about 48 h. The sprouted and dried seeds were ground in a coffee mill and passed through a 200- μ m sieve. The flour was then packed, double sealed in a stomacher bag, and stored in a cold room (4 °C) until analysis.

Autoclaving. Prior to fermentation, part of the sprouted flour was mixed with distilled water (10% solids w/v) and then autoclaved at 121 °C for 20 min. The gruel obtained was divided in two parts. One part of the autoclaved slurry was spread on a tray and dried at 37 °C for 36 h. The dry flakes were collected, ground, and passed through a 200- μ m sieve. The flour was then packed, double sealed in a stomacher bag, and stored in a cold room (4 °C) before analysis. The second part of the autoclaved slurry was used for fermentation.

Fermentation. A glass vial of L-(+)-lactic acid producing microorganism *Lactobacillus salivarius* subsp. *salivarius* (LMG 9477^T), obtained in lyophilized form from the BCCN/LMG Bacteria Collection Center of the Ghent University, was used.

The lyophilized microorganisms were incubated for 48 h at 30 °C in MRS broth in order to be activated.

A starter culture was prepared for millet and beans before inoculating the samples. Fifty grams of millet or beans were dispersed in 500 mL of distilled water. The mixture was autoclaved at 121 °C for 20 min, cooled, and inoculated with the activated *Lactobacillus salivarius* at 5% v/v. The starter culture had 10⁵–10⁶ cfu/g (total plate count on MRS broth). Fermentation was carried out for 48 h at 30 °C after which the samples were frozen to –18 °C until further analysis. This fermentation time was optimized to achieve a high nutritional quality by lowering of antinutrients and increase of digestibility with minimum loss in dry matter through microbial respiration.

Amino Acid Analysis. The determination of amino acids was done in triplicate by using the Biotronik LC 3000 amino acid analyzer. The method is based on the separation of the amino acids by cation exchange chromatography (using five lithium acetate buffer solutions of increasing pH and ionic strength), followed by the ninhydrin color reaction and photometric detection at 570 nm for α -amino acids and at 440 nm for the imino acids (Ooghe, 1983). Prior to separation and determination, amino acids were released from the sample protein by acid hydrolysis (Ooghe, 1983).

About 1 g of ground sample (accurately weighed to the nearest 0.1 mg) was boiled in 370 mL of azeotropic (6 M) HCl for 20 h under reflux and under a continuous nitrogen flow. The hydrolysate was made up to 1 L and filtered through a sintered glass filter. An aliquot of the filtrate was evaporated to dryness at 40 °C in a rotavapor system. Twenty-five milliliters of a buffer solution of pH 2.2 was added to redissolve the residue. After filtration (0.22 μ m), 50 μ L of the sample was injected into the analyzer.

Tryptophan Determination. A rapid and simple acid ninhydrin method described by Gaitonde and Dovey (1970) and adapted for the colorimetric determination of tryptophan in protein extracts of beans by Sodek et al. (1975) was used. Kidney beans and finger millet flour samples were partially defatted by suspension in 20 volumes of acetone and stirring occasionally for 0.5 h. After filtration, the powder was left to air-dry. Portions (100 mg) of defatted kidney beans were extracted with 5 mL of 0.2% NaOH in a centrifuge tube for 0.5 h, occasionally stirring and homogenizing with a glass rod. After centrifugation, the residue was re-extracted with a further 5 mL of 0.2 NaOH and the supernatants were combined. Aliquots, 0.5 mL, were taken for tryptophan assay. Determination was carried out in triplicate.

Defatted millet meal (500 mg) was extracted in a centrifuge tube with 2.0 mL of 70% ethanol for 0.5 h at room temperature. The mixture was occasionally stirred and homogenized with a glass rod. Five milliliters of NaOH (0.5%) were then added and extraction continued for another hour. After centrifugation, a clear supernatant was collected and 0.2 mL of it taken for tryptophan assay. Determination was also carried out in triplicate.

Tryptophan values obtained were then corrected for tyrosine interference according to Zahnel and Davies (1973).

Statistical Analysis. Analysis of variance of the results was done at 95% confidence interval ($\alpha < 0.05$) using Tukeys Honestly Significant Difference. This analysis was done using the SPSS 9.0 for Windows (1998) computer software. The means presented are averages of three determinations and the changes discussed are those significant at the 95% level.

RESULTS AND DISCUSSION

Amino acid profiles of finger millet and kidney beans after each processing step are presented in Tables 1 and 2. The protein content of both finger millet and kidney beans, calculated as total amino acids, is also summarized in Tables 1 and 2.

Amino Acid Composition in Raw Finger Millet. Finger millet protein was deficient in lysine but had sufficient amounts of all other amino acids. When compared to the FAO amino acid scoring pattern for children 2–5 years old (FAO, 1991), lysine was limiting,

Table 1. Amino Acid Profiles after Processing of Finger Millet¹

amino acid	raw		sprouting		autoclaving		fermentation	
	mean	sd	mean	sd	mean	sd	mean	sd
essential amino acids								
threonine	4.15 ^a	0.18	4.31 ^a	0.07	4.32 ^a	0.10	4.04 ^a	0.12
valine	6.20 ^a	0.09	5.81 ^a	0.37	6.20 ^a	0.14	6.13 ^a	0.11
cysteine	1.13 ^a	0.09	1.49 ^a	0.41	1.08 ^a	0.09	1.00 ^a	0.07
methionine	2.62 ^{ab}	0.03	2.81 ^a	0.27	2.44 ^{ab}	0.16	2.35 ^b	0.07
isoleucine	4.17 ^a	0.21	3.85 ^a	0.27	4.24 ^a	0.20	4.18 ^a	0.25
leucine	10.24 ^a	0.12	10.05 ^a	0.10	10.48 ^a	0.29	10.14 ^a	0.23
tyrosine	4.28 ^a	0.04	4.29 ^a	0.13	4.36 ^a	0.02	4.33 ^a	0.11
phenylalanine	5.37 ^a	0.01	5.35 ^a	0.03	5.40 ^a	0.04	5.22 ^b	0.06
histidine	2.47 ^a	0.03	2.44 ^a	0.03	2.36 ^b	0.02	2.40 ^{ab}	0.03
lysine	2.58 ^a	0.04	2.75 ^{ab}	0.13	2.57 ^a	0.04	2.75 ^b	0.01
tryptophan	1.45 ^a	0.05	1.44 ^a	0.07	1.41 ^a	0.02	1.66 ^b	0.04
nonessential amino acids								
aspartic acid	5.76 ^a	0.15	6.21 ^c	0.12	5.92 ^{ab}	0.07	6.06 ^{bc}	0.06
serine	5.41 ^a	0.34	5.51 ^a	0.13	5.42 ^a	0.31	5.26 ^a	0.26
glutamic acid	24.11 ^a	0.02	23.75 ^a	0.31	23.94 ^a	0.17	23.90 ^a	0.14
proline	6.26 ^a	0.20	6.30 ^a	0.06	6.24 ^a	0.17	6.38 ^a	0.10
glycine	3.33 ^a	0.05	3.38 ^a	0.04	3.40 ^{ab}	0.05	3.53 ^b	0.05
alanine	6.14 ^a	0.13	6.22 ^a	0.11	6.35 ^{ab}	0.13	6.67 ^b	0.17
arginine	4.33 ^a	0.09	4.04 ^b	0.12	3.88 ^b	0.13	4.01 ^b	0.02
total amino acids*	8.58	0.17	8.56	0.34	8.37	0.06	8.17	0.03

¹ Values are means and one standard deviation of three determinations of amino acid composition expressed in g of AA/100 g of total amino acids. ^{a,b,c} Same superscript within a row means no significant difference ($\alpha < 0.05$). * Total amino acids in g amino acids per 100 g of dry matter.

Table 2. Amino Acid Profiles after Processing of Kidney Beans¹

amino acid	raw		sprouting		autoclaving		fermentation	
	mean	sd	mean	sd	mean	sd	mean	sd
essential amino acids								
threonine	4.74 ^a	0.09	4.63 ^a	0.05	4.72 ^a	0.11	4.60 ^a	0.09
valine	5.04 ^a	0.38	4.98 ^a	0.57	5.06 ^a	0.58	5.07 ^a	0.39
cysteine	0.66 ^a	0.06	0.66 ^a	0.09	0.70 ^a	0.02	0.61 ^a	0.07
methionine	1.22 ^a	0.09	1.21 ^a	0.06	1.31 ^a	0.10	1.25 ^a	0.09
isoleucine	4.35 ^a	0.34	4.13 ^a	0.48	4.31 ^a	0.45	4.37 ^a	0.39
leucine	8.48 ^a	0.24	8.37 ^a	0.34	8.70 ^a	0.23	8.60 ^a	0.35
tyrosine	3.57 ^a	0.04	3.55 ^a	0.05	3.61 ^a	0.06	3.62 ^a	0.02
phenylalanine	5.84 ^a	0.15	5.83 ^a	0.08	5.98 ^a	0.01	5.94 ^a	0.02
histidine	2.94 ^a	0.02	2.93 ^a	0.07	2.90 ^a	0.06	2.92 ^a	0.05
lysine	7.18 ^a	0.06	6.81 ^b	0.12	6.75 ^b	0.15	6.94 ^{ab}	0.12
tryptophan	1.06 ^a	0.03	1.09 ^a	0.03	1.05 ^a	0.03	1.04 ^a	0.03
nonessential amino acids								
aspartic acid	12.70 ^a	0.36	13.02 ^a	0.38	12.21 ^a	0.44	12.61 ^a	0.37
serine	6.58 ^a	0.20	6.82 ^a	0.30	6.49 ^a	0.35	6.51 ^a	0.28
glutamic acid	16.50 ^{ab}	0.14	16.81 ^a	0.23	16.82 ^a	0.29	16.16 ^b	0.13
proline	3.86 ^a	0.14	4.12 ^a	0.33	4.30 ^a	0.23	4.20 ^a	0.37
glycine	4.07 ^{ab}	0.07	3.95 ^b	0.06	4.13 ^a	0.06	4.20 ^a	0.05
alanine	4.45 ^a	0.07	4.60 ^a	0.11	5.03 ^b	0.12	4.93 ^b	0.12
arginine	6.77 ^a	0.11	6.49 ^{ab}	0.12	5.92 ^b	0.31	6.45 ^{ab}	0.25
total amino acids*	22.00	0.45	21.97	0.19	21.43	0.32	21.44	0.38

¹ Values are means and one standard deviation of three determinations of amino acid composition expressed in g of AA/100 g of total amino acids. ^{a,b,c} Same superscript within a row means no significant difference ($\alpha < 0.05$). * Total amino acids in g amino acids per 100 g of dry matter.

with a score of 0.4 in raw finger millet. All other amino acids scored higher than 1 (Table 3). Tryptophan, which is usually the second most deficient amino acid in cereals (Taira, 1968; FAO, 1968), was not deficient in finger millet. Threonine too was not deficient, in contrast to rice, sorghum, and wheat (FAO, 1968). Monteiro et al. (1982) also observed that the contents of lysine, tryptophan, and the sulfur amino acids in Italian millet (*Setaria italica*) were low. Among the millets, finger millet is relatively better balanced in essential to total amino acids because it contains more lysine, threonine, and valine (Ravindran, 1992).

Changes in Amino Acid Composition in Millet during Processing. *Changes during Sprouting.* The sulfur containing amino acids (methionine and cysteine) and lysine increased in finger millet during sprouting

(Table 1). These changes were, however, not significantly different at the 95% confidence interval. One of the sources of the increase in cysteine, which is a common constituent of most enzymes, is the expected turnover in protein during sprouting when a considerable amount of enzyme synthesis occurs (King and Puwastien, 1987). Among the nonessential amino acids, arginine decreased by 6.8% and aspartic acid increased by 7.8% in finger millet during the 48 h sprouting. Other amino acids did not show any significant changes. Among nonessential amino acids, aspartic acid decreased by 4.6% during autoclaving.

Changes during Autoclaving. Among the essential amino acids, a significant decrease was observed only in histidine (3.3%) in finger millet during autoclaving. Other amino acids did not show any significant changes.

Table 3. Amino Acid Chemical Score Profiles after Processing of Finger Millet and Kidney Beans

	essential amino acid scores ^a				
	FAO ref protein ^b	raw	sprouting	autoclaving	fermentation
finger millet					
threonine	3.4	1.2	1.3	1.3	1.2
valine	3.5	1.8	1.7	1.8	1.8
methionine ^c + cysteine	2.5	1.5	1.7	1.4	1.3
isoleucine	2.8	1.5	1.4	1.5	1.5
leucine	6.6	1.6	1.5	1.6	1.5
phenylalanine ^c + tyrosine	6.3	1.5	1.5	1.5	1.5
histidine	1.9	1.3	1.3	1.2	1.3
lysine	5.8	0.4	0.5	0.4	0.5
tryptophan	1.1	1.3	1.3	1.3	1.5
total ^d	33.9	44.7	44.6	44.9	44.2
kidney beans					
threonine	3.4	1.4	1.4	1.4	1.4
valine	3.5	1.4	1.4	1.4	1.4
methionine ^c + cysteine	2.5	0.8	0.7	0.8	0.7
isoleucine	2.8	1.6	1.5	1.5	1.6
leucine	6.6	1.3	1.3	1.3	1.3
phenylalanine ^c + tyrosine	6.3	1.5	1.5	1.5	1.5
histidine	1.9	1.5	1.5	1.5	1.5
lysine	5.8	1.2	1.2	1.2	1.2
tryptophan	1.1	1.0	1.0	1.0	0.9
total ^d	33.9	45.1	45.2	45.1	45.0

^a Amino acid scores, calculated from relative amino acid amounts in sample (Tables 1 and 2)/amino acid content in FAO reference protein. ^b FAO reference protein pattern for preschool-age child (2–5 years) (FAO, 1991). ^c Pairs of amino acids considered physiologically substitutory to one another (FAO, 1991). ^d Total of essential amino acids (g/100 g of total amino acids).

Changes during Fermentation. Fermentation with *Lactobacillus salivarius* caused a 17.8% increase in tryptophan. Other essential amino acids which changed significantly were lysine, which increased by 7.1%, and phenylalanine, which decreased by 3.3%. Amounts of other amino acids were not significantly affected by this lactic acid fermentation.

Mbugua (1987) observed that, after inoculating maize flour with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, tryptophan content increased slightly from 0.53 to 0.70% of crude protein after 24 h of fermentation. During the same period, lysine content decreased by 80%. Chavan and Kadham (1989) reported that among the amino acids released during fermentation, the highest values were 30% for lysine and 20% for methionine. They suggested that a biochemical mechanism, such as transamination, might be taking place during fermentation.

Amino Acid Composition in Raw Kidney Beans.

The combined sulfur containing amino acids methionine and cysteine were limiting in kidney beans. Their combined score (FAO, 1991) was 0.8 in the raw sample. These amino acids are limiting in legumes such as kidney beans but not in cereals such as finger millet (Sgarbieri, 1989). All other essential amino acids, including lysine, scored higher than 1 (Table 3).

Changes in Amino Acid Composition during Processing of Kidney Beans. *Changes during Sprouting.* Lysine content in kidney beans decreased significantly by 5.1% during the 48 h of sprouting. All other amino acids did not show any significant changes.

Hsu et al. (1980) reported small or no changes in the essential amino acid content of yellow peas, lentil, and faba beans after 4 days of sprouting. Lysine decreased by 2.8 and 7% in yellow pea and faba beans, respectively, and increased by 1% in lentil. King and Puwastien (1987) observed a decrease in the total amino acid content, with the exception of cysteine, which increased from 1.4 mg/16 g of N to 1.7 mg/16 g of N after 48 h sprouting of winged beans.

Changes during Autoclaving. Essential amino acids did not change significantly during autoclaving. The observed changes in histidine in finger millet did not occur in kidney beans, suggesting a matrix effect could be in operation during heat treatment.

Among the nonessential amino acids, alanine and glycine increased by 9.4 and 4.6% in kidney beans. Other amino acids in both kidney beans and finger millet did not show any significant changes during this heat treatment. Wu et al. (1996) observed significant changes in amino acid composition after autoclaving (121 °C; 20 min) of red kidney beans: among essential amino acids, lysine, threonine, and tryptophan decreased by 1.2, 3.2, and 4.0%, respectively.

Changes during Fermentation. Of the nonessential amino acids, glutamic acid decreased by 3.9% in kidney beans. Amounts of other amino acids were not significantly affected by lactic acid fermentation.

Significant Overall Amino Acid Composition Changes. In finger millet, tryptophan and lysine increased at the end of the four processing steps by 14.5 and 6.7%, respectively. Phenylalanine decreased by 2.8%. Among the nonessential amino acids, aspartic acid, glycine, and alanine increased by 5.2, 6.1, and 8.6%. Arginine decreased by 7.5%. All other amino acids did not change significantly ($\alpha < 0.05$) during the entire finger millet processing. In kidney beans, alanine increased by 10.8% after the entire processing. All the other amino acids did not change significantly.

Nutritional Changes. Raw finger millet and kidney bean protein contain 44.7 and 45.1% essential amino acids when compared to total amino acids (Table 3). This is higher than the 33.9% essential amino acids in the FAO reference protein (FAO, 1991). The finger millet amino acid profile gives a good ratio of essential to total amino acids, limited only in lysine. Kidney beans on the other hand have a very good ratio of essential to total amino acids, but are limiting in combined methionine and cysteine. When mixed together, these two proteins complement each other, giving a protein of higher

chemical score. This composite protein would be nutritionally beneficial for a child of weaning age. Both foods are suitable for production of composite weaning foods. The percentage of essential amino acids over total amino acids remained fairly constant in both kidney beans and finger millet during the various processing steps. The leucine-to-isoleucine ratio in the millet was between 2.4 and 2.6, while that for kidney beans is 1.9–2.0. Changes in this ratio during processing for both kidney beans and finger millet were statistically insignificant ($\alpha < 0.05$). Excess leucine interferes with the utilization of isoleucine (Harper et al., 1955). Vidyantath-Jha et al. (1991) found that the high leucine-to-isoleucine ratio was responsible for the low biological value of *Euryale ferox*, an aquatic crop. High leucine to lysine levels in proteins are believed to be responsible for the inefficient utilization of lysine. When taken as a major source of protein in the diet, any protein with a leucine-to-lysine ratio less than 4.6 is considered nutritionally safe.

Pellagragenic Character of the Foods. Higher levels of leucine in a diet may induce pellagra, and the leucine-to-lysine ratio is used as an indicator of the pellagragenic character of a food protein (Deosthale et al., 1970). In our study this ratio was between 3.6 and 4.1 for finger millet and 1.2 and 1.3 in kidney beans. This ratio is relatively higher in finger millet since lysine is deficient in this food. It decreased significantly during both sprouting and fermentation but increased significantly during autoclaving in finger millet. However, there were no significant changes in leucine-to-isoleucine ratio in kidney beans during the entire processing.

Overall, it can be concluded that sprouting, autoclaving, and fermentation, under the experimental conditions described under Materials and Methods, significantly changed the content of a few amino acids, which led to slight but significant changes in the overall amino acid profile in both finger millet and kidney beans. Under these conditions, which are suitable for producing a tropical weaning food, the overall nutritional value of their composite protein will hardly be affected.

LITERATURE CITED

- Asiedu, M.; Lied, E.; Nielsen, R.; Sandnes, K. Effect of processing (fermentation and sprouting) on sorghum and maize: II. Vitamins and amino acid composition, biological utilization of maize protein. *Food Chem.* **1993**, *48*, 201–204.
- Chang, K. C.; Harrold, R. L. Changes in selected biochemical components in *in vitro* protein digestibility and amino acid in two bean cultivars during sprouting. *J. Food Sci.* **1988**, *53* (3), 783–787.
- Chavan, J. K.; Kadam, S. S. Nutritional improvements of cereals by sprouting. *Crit. Rev. Food Sci. Nutr.* **1989**, *28* (5), 401–405.
- Deosthale, Y. G.; Mohan, V. S.; Rao, K. V. Varietal differences in protein, lysine and leucine content of grain sorghum. *J. Agric. Food Chem.* **1970**, *18* (4), 644–646.
- Food and Agricultural Organization of The United Nations (FAO). Amino acid scoring pattern. In *Protein Quality Evaluation*; FAO/WHO Food and Nutrition paper; Rome, Italy, 1991; Vol. 51, pp 21–25.
- Food and Agricultural Organization of The United Nations (FAO). Cereals and Grain Products. In *Food Composition Table for use in Africa*. US Department of Health, Education and Welfare, Bethesda, MD 20014, and Food and Agricultural Organization of the United Nations; Rome, Italy, 1968; pp 12–24.
- Friedman, M. Nutritional value of proteins from different food sources. A Review. *J. Agric. Food Chem.* **1996**, *44* (1), 6–29.
- Gaitonde, M. K.; Dovey, K. A rapid and direct method for the quantitative determination of tryptophan on intact protein. *Biochem. J.* **1970**, *117*, 907–911.
- Hamad, A. M.; Fields, M. L. Evaluation of the protein quality and available lysine of sprouted and fermented cereals. *J. Food Sci.* **1979**, *44* (2), 456–459.
- Harper, A. E.; Benton, D. A.; Elvbejhem, C. A. L-Leucine, an isoleucine antagonist in the rat. *Arch. Biochem. Biophys.* **1955**, *57*, 1–12.
- Hsu, D.; Leung, H. K.; Finney, P. L.; Morad, M. M. Effect of sprouting on nutritive value and baking properties of dry peas, lentils and faba beans. *J. Food Sci.* **1980**, *45*, 87–92.
- King, R. D.; Puwatsien, P. Effect of sprouting on the proximate composition and nutritional quality of winged beans (*Psophocarpus tetragonolobus*) seeds. *J. Food Sci.* **1987**, *52* (1), 106–108.
- Landry, J.; Delhay, S. The tryptophan content of pearl millet grains as a function of nitrogen content. *Phytochemistry* **1995**, *38* (1), 5–8.
- Mbithi-Mwikya, S.; Van Camp, J.; Yiru, Y.; Huyghebaert, A. Nutrient and antinutrient changes in finger millet (*Eleusine coracana*) during sprouting. *Lebens. Wiss. Technol.* **2000**, *33*, 9–14.
- Mbugua, S. K. The nutritional and fermentation characteristics of uji produced from dry milled maize flour (unga baridi) and whole wet milled maize. *Chem. Mikrob. Technol. Lebens.* **1987**, *10* (5/6), 154–161.
- Monteiro, P. V.; Virupaksha, T. K.; Rao, D. R. Proteins of Italian millet: Amino acid composition, solubility fractionation and electrophoresis of protein fractions. *J. Sci. Food Agric.* **1982**, *33*, 1072–1079.
- Ooghe, W. On the influence of hydrogen peroxide on the amino acid composition of foodstuffs. Proceedings of the European Conference on Food Chemistry; Rome, Italy, 1983; pp 315–321.
- Ravindran, G. Seed proteins of millets: amino acid composition, proteinase inhibitors and *in-vitro* protein digestibility. *Food Chem.* **1992**, *44*, 13–17.
- Sgarbieri, V. C. Composition and nutritive value of beans (*Phaseolus vulgaris* L.). *World Rev. Nutr. Diet.* **1989**, *60*, 132–198.
- Sodek, L. S.; Vecchia, P. T. D.; Lima, M. L. G. Rapid determination of tryptophan in beans (*Phaseolus vulgaris*) by acid ninhydrin method. *J. Agric. Food Chem.* **1975**, *23*, 1147–1150.
- Taira, H. Amino acid composition of different varieties of foxtail millet (*Setaria italica*). *J. Agric. Food Chem.* **1968**, *16*, 1025–1029.
- Vidyantath-Jha; Barat, G. K.; Jha, U. N. Nutritional evaluation of *Euryale ferox* Salisb. (makhana). *J. Food Sci. Technol.* **1991**, *28* (5), 326–328.
- Wu, W.; Williams, W. P.; Kunkel, M. E.; Acton, J. C.; Huang, Y.; Wardlaw, B.; Grimes, L. W. Amino acid availability and availability corrected amino acid score of red kidney beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **1996**, *44* (5), 1296–1301.
- Zahnely, J. C.; Davies, J. G. Effect of high tyrosine content on the determination of tryptophan in proteins by the acid ninhydrin method. *Biochem. J.* **1973**, *135*, 59–61.
- Ziena, H. M.; Youssef, M. M.; El-Mahdy, A. R. Amino acid composition and some antinutritional factors of cooked faba beans (medammi): Effects of cooking temperature and time. *J. Food Sci.* **1991**, *56*, 1347–1349.

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