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Fermented Pigeon Pea (*Cajanus cajan*) Ingredients in Pasta Products

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Pigeon pea (*Cajanus cajan* var. *aroíto*) seeds were fermented in order to remove antinutritional factors and to obtain functional legume flour to be used as pasta ingredients. Fermentation brought about a drastic reduction of α-galactosides (82%), phytic acid (48%), and trypsin inhibitor activity (39%). Fermented legume flours presented a notable increase of fat and total soluble available carbohydrates, a slight decrease of protein, dietary fiber, calcium, vitamin B_2, vitamin E, and total antioxidant capacity, and a decrease of soluble dietary fiber, Na, K, Mg, and Zn contents. No changes were observed in the level of starch and tannins as a consequence of fermentation. The fermented flour was used as an ingredient to make pasta products in a proportion of 5, 10, and 12%. The supplemented pasta products obtained had longer cooking times, higher cooking water absorptions, higher cooking loss, and higher protein loss in water than control pasta (100% semolina). From sensory evaluations, fortified pasta with 5 and 10% fermented pigeon pea flour had an acceptability score similar to control pasta. Pasta supplemented with 10% fermented pigeon pea flour presented higher levels of protein, fat, dietary fiber, mineral, vitamin E, and Trolox equivalent antioxidant capacity than 100% semolina pasta and similar vitamins B_1 and B_2 contents. Protein efficiency ratios and true protein digestibility improved (73 and 6%, respectively) after supplementation with 10% fermented pigeon pea flour; therefore, the nutritional value was enhanced.

KEYWORDS: Fermentation; pigeon pea; pasta ingredients; nutritional value

INTRODUCTION

Plant protein provides nearly the 80% of the protein intake in developing countries, as compared to 43% in developed ones (*1*). Moreover, in developing countries, malnutrition and deficiency of micronutrients are highly prevalent and even increasing. Factors of direct influence on nutritional disorders are inadequate food consumption, diseases, and poor bioavailability of many nutrients in vegetable diets.

To improve the nutrient intake, food preparation technologies have been advocated to effectively increase the nutrient availability of vegetable diets. These technologies must be simple and easily affordable in terms of economy and labor input. One such household level technology is fermentation (*2*), which has been widely practiced in many developing countries since it is one of the oldest and economical methods of processing and preserving foods. Fermented products are a significant part of the diet of many people in developing countries, and its popularity is increasing in the Western world due to desirable changes in texture, organoleptic characteristics, and elimination of off-flavors (*3*). This process has been suggested as a technological procedure for partial or total removal of α-galactosides, compounds that are closely related with the occurrence of flatulence (*4*, *5*). The fermentation process affects the nutritional quality of food by improving the nutrient density and increasing the bioavailability of nutrients. Microorganisms responsible for this process utilize the biochemical constituents of the food material, changing them from one form to another with the aid of microbial enzyme systems; promote degradation of antinutritional factors, predigestion of certain food components, and synthesis of promoters for absorption; and influence the nutrients uptake by the mucosa (*2*, *6*, *7*).

While population growth has increased the demand for food, rising prosperity has increased the demand for quality food. At the same time, consumers demand convenience foods, since they are becoming increasingly health conscious; therefore, there is a need to diversify food products (*8*).

Plant foods such as cereals and legumes have been considered as the major potential sources of protein for feeding growing populations (*9*). Among food legumes, red gram or pigeon pea (*Cajanus cajan*) is a valuable source of proteins, minerals, and vitamins and occupies a very important place in human nutrition in many developing countries (*10*, *11*). Besides these nutrients, this legume is also rich in non-nutritional compounds as phytic acid, polyphenols, saponins, trypsin inhibitors, and oligosaccharides, which are known to limit the utilization for human nutrition. Fermentation could be applied as an adequate process

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of low cost, low energy requirements, and high yield, in order to enhance organoleptic and nutritional qualities of this legume and to diminish non-nutritional substances.

Pigeon pea proteins are a rich source of lysine but are usually deficient in the sulfur amino acids, methionine and cysteine. Although low in some essential amino acids, pigeon pea could be considered a high protein material to offset the amino acid deficiencies of cereal proteins as wheat. Enrichment of pasta with cereal germ or leguminous material increases the nutritional quality because of a larger amount of protein, vitamins, and minerals. The level of substitution will depend on the formulation, preparation, and processing of pasta products (*12*).

The aim of the present study was to ferment pigeon pea (*C. cajan* var. *aroíto*) seeds to remove non-nutritive compounds and to use the fermented flour to supplement durum semolina flour to make pasta products with higher nutritional value and similar acceptability than the pasta without supplementation that can be included in human diet.

MATERIALS AND METHODS

Preparation of Samples. *Legumes*. Pigeon peas (*C. cajan*, var. *aroíto*) were obtained from The National Agricultural Research Institute (Yaracuy, Venezuela).

Fermented Seeds. The fermentation process was carried out as described by Granito et al. (*5*). Briefly, dry seeds were previously washed with distilled water and suspended in distilled water in a proportion of 1:4 (w/v). Natural fermentation was carried out at 42 °C for 48 h, being stirred at 440 rpm (Fermentor Microferm New Brunswick Scientific Co., Inc. Edison, NY). The fermented seeds were drained, freeze-dried (Labconco freeze dryer, MO), and ground to 0.5 mm for analysis. Fermentation was carried out in triplicate.

Pasta Preparation. Fermented pigeon pea flours were used to supplement whole durum wheat semolina to make pasta products. Pasta products with different levels of fermented pigeon pea flours are described as follows: semolina 100% (control), semolina:fermented pigeon pea flour (95:5), semolina:fermented pigeon pea flour (90:10), and semolina:fermented pigeon pea flour (88:12).

Pasta products were prepared in triplicate, as follows: Homogenized flours were mixed with water to a moisture level of 31.5%, blended (Kitchen Aid classic model) for 2 min, and left to stand for 15 min. The dough obtained was stretched and extruded (single screw pasta machine Columbus, model Marchio Depositati), and spaghetti was formed (at laboratory scale), predried at ambient temperature for 1 h, dried (convection forced air oven) at 50 °C for 2 h, and ground to 0.5 mm particle size.

Chemical Analysis. *Water, Protein, Fat, and Ash Content.* These analyses were carried out according to AOAC (*13*) (methods 960.52, 920.30, and 923.03). The factor of 6.25 was used as a conversion factor to calculate the protein content from nitrogen.

Mineral Determination. Specific minerals were analyzed by inductively coupled plasma atomic emission spectroscopy, according to AOAC (*13*) method 984.27.

Soluble Carbohydrate Content. Monosaccharides, disaccharides, and α-galactosides were determined by high-performance liquid chromatography (HPLC) following the procedure described by Granito et al. (*5*).

Starch Content. The content in starch of raw and fermented pigeon pea flours was determined as in Sotomayor et al. (*14*).

Energy Value. The energy value was calculated by the Atwater system (*15*). Factors applied to nutrients were 4 kcal/g for protein and carbohydrates and 9 kcal/g for fat.

Dietary Fiber Content. The total, insoluble, and soluble dietary fiber amounts were determined according to Prosky et al. (*16*).

Vitamin Analysis. Vitamins B_1 and B_2 were determined by HPLC according to Prodanov et al. (*17*). Vitamin C was determined by capillary electrophoresis following the method described in Thompson and Trenerry (*18*), modified by Frias et al. (*19*). Tocopherol isomers and vitamin E activity were determined by HPLC according to Frias et al. (*19*).

Tannin Content. It was determined by the Folin−Ciocalteu method as in Kuiters (*20*). Galic acid (Sigma Chemicals) was used as the standard.

Total Antioxidant Capacity. The antioxidant capacity was determined as Trolox equivalent antioxidant capacity (TEAC) according to Re et al. (*21*), modified by Frias et al. (*19*).

Inositol Phosphate Content. Inositol phosphates (IP_6, hexainositol phosphate; IP_5, pentainositol phosphate; IP_4, tetrainositol phosphate; and IP_3, tri-inositol phosphate) were extracted according to Kozlowska et al. (*22*) and quantified by HPLC according to Lerhfeld (*23*).

Trypsin Inhibitor Activity (TIA). It was determined as in Vidal-Valverde et al. (*24*).

Pasta Cooking Quality. The pasta cooking quality was determined according to Abecassis et al. (*25*) and Matsuo et al. (*26*), using the following parameters:

Cooking Time. Ten grams of pasta product was dispersed in 100 mL of boiling water. Every minute, a piece of pasta was held between two glass plates and was compressed. The optimum cooking time (min) was established when no white core was observed after the compression.

Cooking Water Absorption. The cooked pasta was drained and weighed to determine the water absorption.

Cooking Loss. Solids extracted from the cooking water were calculated by concentrating the cooking water until dryness in an oven at 100 °C.

Protein Loss in Water. It was determined in the cooking water by the Biuret method, as described in Robinson and Hodgen (*27*).

Sensorial Analysis. Pasta products were cooked in boiling water without the addition of salt, drained, and placed in warm conditions until testing. A 19 member semitrained panel evaluated the pasta products for overall quality. Panelists were asked to evaluate the pasta products and mark the acceptability in a nonstructured scale (15 cm) (left extreme, 1 = extremely unacceptable, and right extreme, 15 = extremely acceptable) (*28*).

Biological Analysis. A biological balance technique was used by recording changes in body weight and food intake and then calculating the nitrogen intake and fecal excretion. True protein digestibility (TD) and protein efficiency ratio (PER) were determined according to Allison (*29*).

Experimental Design and Diets. The experimental diets were prepared by adding the appropriate amount of cooked pasta fortified with fermented pigeon pea flour or cooked 100% semolina pasta to commercial maize starch to provide 10% protein content to the diet. Minerals mix (3.5% from Harland Teklad), maize oil (5.0%), vitamins mix AIN-76A (1.0% from Harland Teklad), and choline bitartrate (0.2%, Sigma) were also added. During a 14 day testing period, experimental diets, a protein free diet, a casein diet, and water were supplied. During the first 3 days of experiments, the rats were allowed to adapt to the diet and experimental conditions. The animals and their feed intake were weighed every second day. In order to calculate protein digestibility, the feces of each animal were collected the last 7 days of the experiment. Nitrogen consumed by each animal for this period was quantified. The feces were oven-dried at 100 °C for 24 h. The dried samples were ground to 0.5 mm. The experimental diet and feces were analyzed for protein (N × 6.25) by micro-Kjeldahl method.

Animals. In each experiment, six week old Sprague−Dawley rats (three males and three females) weighing about 50 g were selected at random and they were housed from day 0 of the experiment in individual stainless steel metabolic cages in an environmentally controlled room.

Statistical Analyses. Data are expressed as means ± standard deviations of three determinations and were subjected to a multifactor analysis of variance using the Statgraphics Statistical Graphics 5.0 System software program (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Table 1 collects the content of non-nutritional compounds in raw and fermented pigeon pea flours. The fermentation

Table 1. Effect of Fermentation on Non-nutritional Compounds Content of Pigeon Pea Seeds (*C. cajan* var. *aroíto*)[a]

composition	pigeon pea	
	raw	fermented
raffinose[b]	1.23 ± 0.08 b	0.13 ± 0.05 a
stachyose[b]	2.35 ± 0.20 b	0.28 ± 0.04 a
verbascose[b]	1.94 ± 0.24 b	0.55 ± 0.14 a
total α-galactosides[b]	5.52 ± 0.22 b	0.97 ± 0.21 a
IP_6 (phytic acid)[b]	0.46 ± 0.02 b	0.24 ± 0.01 a
IP_5[b]	0.17 ± 0.01 b	0.06 ± 0.01 a
IP_4[b]	0.12 ± 0.01 b	0.03 ± 0.01 a
IP_3[b]	ND a	ND a
total inositol phosphates[b]	0.72 ± 0.05 b	0.34 ± 0.01 a
TIA (TIU/mg)	24.72 ± 0.45 b	15.11 ± 0.08 a
tannins (galic acid)[b]	0.39 ± 0.02 a	0.39 ± 0.02 a

[a] Values are the mean ± standard deviation of three determinations. The same superscript in the same row means no significant difference ($P \leq 0.05$). [b] Values in g/100 dry matter; ND, nondetected.

process induced a drastic reduction in total α-galactosides due to the decrease of raffinose, stachyose, and verbascose (89, 88, and 72%, respectively). Fermentation is a catabolic process where these oligosaccharides are hydrolyzed by α-galactosidase and invertase either as endogenous enzyme or from microorganisms involved (*30*−*34*). Odunfa (*35*) studied the activity of the α-galactosidase and *β*-fructosidase enzymes during fermentation and found that the maximum activity occurred after 24 h at temperatures between 40 and 60 °C.

Modification of inositol phosphate content during the pigeon pea fermentation is also shown in **Table 1**. Fermentation brought about a sharp decrease (48%) on phytic acid (IP_6). IP_5 and IP_4 also suffered a noticeable decrease (65 and 75%, respectively), and IP_3 was not detected. Phytate hydrolysis, due to phytases enzymes that are activated during the fermentation process, improves bioavailability of minerals (as calcium, magnesium, copper, zinc, and iron) (*2*). Similar results have been shown by different authors. Sudarmaji and Markakis (*36*) observed that during tempeh preparation, phytic acid decreased by one-third in comparison with raw soybean. Chitra et al. (*37*) found a phytic acid reduction of 53% after pigeon pea-induced fermentation with *Lactobacillus*. Granito et al. (*5*) reported a drastic reduction of IP_6 after fermentation of *Phaseolus vulgaris* seeds. A similar response was found by Egounlety and Aworth (*33*) in fermented soybean, cowpeas, and ground bean. Doblado et al. (*34*) showed that natural and controlled fermentation with *Lactobacillus plantarum* caused an 85% reduction on phytic acid of *Vigna sinensis*. Furthermore, Sathe and Venkatachalam (*38*) pointed out that the extent of phytate removal is dependent on the type of microorganism, the fermentation conditions, the removal of fermentation solution, and the initial phytate amount present in the raw material.

Fermentation of pigeon pea seeds affected also TIA and caused a reduction of 39% (**Table 1**). Results obtained agree with those reported during natural fermentation of lentils (*30*) and during natural and controlled fermentation of *V. sinensis* with *L. plantarum* (*34*).

The tannin content of pigeon pea remained unchanged after the natural fermentation process (**Table 1**). Nevertheless, Bartolome et al. (*39*) found that natural fermentation of lentils led to a general increase of low molecular weight phenolic compounds.

The effect of fermentation on the nutrient content of pigeon pea (*C. cajan* var. *aroíto*) is summarized in **Table 2**. Protein, soluble dietary fiber, and ash suffered significant ($P \leq 0.05$) reductions (5, 46, and 41%, respectively). The contents of starch (total, available, and resistant), insoluble, and total dietary fiber were not modified significantly ($P \leq 0.05$). However, fermentation caused negative changes in nutritionally important minerals such as calcium, sodium, potassium, magnesium, and zinc (losses from 6% for calcium to 53% for sodium) (**Table 2**).

Table 2. Effect of Fermentation on Nutrient Content of Pigeon Pea Seeds (*C. cajan* var. *aroíto*)[a]

composition	pigeon pea	
	raw	fermented
protein[b]	29.26 ± 0.24 b	27.72 ± 0.58 a
fat[b]	2.36 ± 0.38 a	2.89 ± 0.10 b
fructose[b]	0.29 ± 0.04 a	1.84 ± 0.24 b
glucose[b]	ND a	2.25 ± 0.30 b
galactose[b]	ND a	4.52 ± 0.89 b
sucrose[b]	3.87 ± 0.01 b	0.33 ± 0.02 a
total available carbohydrates[b]	4.10 ± 0.14 a	8.94 ± 1.44 b
total starch[b]	41.27 ± 1.12 a	40.74± 0.79 a
available starch[b]	39.47 ± 1.31 a	38.58 ± 1.21 a
resistant starch[b]	2.80 ± 0.60 a	2.43 ± 0.65 a
insoluble dietary fiber[b]	34.88 ± 1.02 a	35.16 ± 0.24 a
soluble dietary fiber[b]	4.23 ± 0.69 b	2.28 ± 0.78 a
total dietary fiber[b]	39.12 ± 0.32 b	37.38 ± 1.03 a
energy (kcal/100 g)	320.00	372.01
ash[b]	3.99 ± 0.07 b	2.37 ± 0.03 a
calcium[c]	200.93 ± 1.52 b	188.60 ± 1.62 a
sodium[c]	89.70 ± 1.55 b	42.17 ± 5.79 a
potassium[c]	1290.37 ± 40.97 b	760.54 ± 3.15 a
magnesium[c]	110.49 ± 2.64 b	78.37± 3.09 a
zinc[c]	7.85 ± 0.05 b	4.66 ± 0.50 a
vitamin B_1[c]	0.31 ± 0.02 a	0.37 ± 0.01 b
vitamin B_2[c]	0.39 ± 0.02 a	0.35 ± 0.02 a
α-tocopherol[c]	1.06 ± 0.03 a	1.07 ± 0.10 a
β-tocopherol[c]	0.06 ± 0.01 a	0.06 ± 0.01 a
γ-tocopherol[c]	9.31 ± 0.34 b	6.64 ± 0.17 a
δ-tocopherol[c]	0.27 ± 0.01 a	0.26 ± 0.02 a
vitamin E (α-TE/100 g dm)	2.02 ± 0.03 b	1.77 ± 0.10 a
vitamin C[c]	ND a	ND a
TEAC (*μ*mol Trolox/g)	33.21 ± 0.67 b	31.88 ± 1.56 a

[a] Values are the mean ± standard deviation of three determinations. ND, nondetected. The same superscript in the same row means no significant difference ($P \leq 0.05$). [b] Values in g/100 dry matter. [c] Values in mg/100 dry matter.

Some authors have reported a reduction on protein content during legume fermentation. Granito et al. (*5*) found a decrease in total protein content of *P. vulgaris* flours, and they showed a relationship between the protein reduction and the water volume used during fermentation. In the present study, once the fermentation process was finished, supernatant was discarded and soluble proteins could leach to this solution. Different results have been presented by Martín-Cabrejas et al. (*40*) who did not show protein reduction during natural fermentation of *P. vulgaris*.

The results obtained about the starch content are similar with the ones reported by Urooj and Puttaraj (*41*) in bengal gram and by Veena et al. (*42*) in bengal gram, cowpea, and green gram who did not find a significant effect in starch content after the fermentation process as compared with the raw seeds. However, Granito et al. (*5*) and Doblado et al. (*34*) reported a slight decrease on starch content during natural fermentation of kidney beans and cowpeas.

The reduction of soluble dietary fiber found as consequence of pigeon pea fermentation agrees with that reported by different authors after natural fermentation of bengal gram, cowpea, green gram, and kidney beans (*5*, *40*, *42*).

With regard to ash and mineral content, Akinyele and Akinlosotu (*6*) found similar results in fermented cowpeas. Mineral losses could be due to their utilization by the micro-

Table 3. Cooking Quality Parameters of Pasta Products with 100% Semolina and Supplemented Semolina with Fermented Pigeon Pea (*C. cajan* var. *aroíto*) Flours[a]

pasta	cooking time (min)	cooking water absorption (%)	cooking loss (%)	protein loss in water (mg/100 g)
semolina 100% (control)	15.00 ± 1.00 a	152.00 ± 2.58 a	3.00 ± 0.02 a	1.80 ± 0.10 a
supplemented pastas with fermented pigeon pea flour				
semolina:fermented pigeon pea (95:5)	17.67 ± 0.58 c	224.00 ± 0.56 d	6.00 ± 0.02 b	2.80 ± 0.57 b
semolina: fermented pigeon pea (90:10)	16.67 ± 0.58 b	226.00 ± 2.60 c	7.00 ± 0.12 b	2.90 ± 0.26 b
semolina: fermented pigeon pea (88:12)	14.67 ± 0.58 a	217.00 ± 1.35 b	8.00 ± 0.00 c	2.90 ± 0.57 b

[a] Values are the mean ± standard deviation of three determinations. The same superscript in the same column means no significant difference ($P \leq 0.05$).

Table 4. General Acceptability of Cooked Pasta Products Made with 100% Semolina or Supplemented Semolina with Fermented Pigeon Pea (*C. cajan* var. *aroíto*) Flours[a]

pastas	general acceptability score
semolina 100% (control)	9.65 ± 3.18 b
supplemented pastas with fermented pigeon pea flour	
semolina:fermented pigeon pea (95:5)	8.68 ± 0.06 b
semolina:fermented pigeon pea (90:10)	7.15 ± 0.32 b
semolina:fermented pigeon pea (88:12)	5.48 ± 0.06 a

[a] Hedonic scale (nonstructured) test. Scale, 1–15 points; 1, extremely unacceptable; 15, extremely acceptable. Values are the mean ± standard deviations. The same superscript in the same column means no significant difference ($P \leq 0.05$) between the pasta products.

organisms involved in the fermentation process; furthermore, some minerals could leach to the processing liquid and, therefore, be removed.

The fat content increased 23% during pigeon pea fermentation (**Table 2**). Granito et al. (*5*), however, did not show significant fat changes during fermentation of *P. vulgaris*.

The sucrose content suffered a large decrease (91%) during fermentation while fructose increased sharply (534%). However, glucose and galactose, monosaccharides that were not detected in the raw seeds, were present in a reasonable amount (2.2 and 4.5 g/100 g dry matter) in fermented pigeon pea flour (**Table 2**). As a result, the content of total available carbohydrates of fermented flours increased twice the value of raw pigeon pea. Similar results have been published previously during natural fermentation of *P. vulgaris* (*5*) and lentils (*4*, *43*). The increase suffered by monosaccharides can be due to the enzymatic activity exerted by the microorganisms and/or the endogenous seed enzymes action on sucrose and polysaccharides during fermentation.

The effect of fermentation of pigeon pea on vitamin content is also shown in **Table 2**. Vitamin B_1 increased significantly (19%) in fermented pigeon pea flours, but vitamin B_2 did not show significant ($P \leq 0.05$) reduction as compared with unfermented seeds (**Table 1**). In tofu preparations, Fernando and Murphy (*44*) reported riboflavin losses due possibly to leaching effect and light exposition. Kazanas and Fields (*45*) pointed out that the riboflavin content in fermented legume flours depends on the experimental conditions and natural flora involved. The vitamin E content decreased 12% after pigeon pea fermentation, mainly due to a noticeable reduction in γ-tocopherol content. Vitamin E is also susceptible to processing conditions such as light, oxygen, or traces of transition metal ions (*46*); hence, these factors could cause its removal after fermentation. Vitamin C was not detected neither in raw nor in fermented seeds, and similar results have been published by Doblado et al. (*34*) in fermented *V. sinensis* flours.

Table 5. Chemical Composition of Cooked Pasta Products Made with 100% Semolina Or Supplemented Semolina with Fermented Pigeon Pea (*C. cajan* var. *aroíto*) Flours[a]

composition	semolina 100% (control)	semolina:fermented pigeon pea flour (92:8) pasta
protein[b]	14.81 ± 0.37 a	17.79 ± 0.22 b
fat[b]	0.07 ± 0.01 a	0.18 ± 0.06 b
fructose[b]	0.27 ± 0.01 a	1.06 ± 0.06 b
glucose[b]	0.73 ± 0.01 a	1.13 ± 0.05 b
galactose[b]	nda	0.49 ± 0.03 b
sucrose[b]	0.86 ± 0.02 a	0.82 ± 0.04 a
total available carbohydrates[b]	1.86 ± 0.03 a	3.50 ± 0.06 b
total starch[b]	73.85 ± 1.09 a	78.68 ± 1.75 b
available starch[b]	71.15 ± 1.99 a	74.74 ± 0.05 b
resistant starch[b]	3.85 ± 0.81 a	3.94 ± 1.69 a
energy value (kcal/100 g)	362.71	401.50
insoluble dietary fiber[b]	3.96 ± 0.03 a	5.13 ± 0.18 b
soluble dietary fiber[b]	0.74 ± 0.08 a	1.89 ± 0.25 b
total dietary fiber[b]	4.73 ± 0.05 a	7.02 ± 0.43 b
ash[b]	0.56 ± 0.03 a	1.29 ± 0.01 b
calcium[c]	35.51 ± 1.84 a	85.85 ± 0.49 b
sodium[c]	23.95 ± 0.18 a	66.47 ± 1.51 b
magnesium[c]	46.43 ± 2.13 a	60.85 ± 1.00 b
zinc[c]	1.89 ± 0.03 a	3.57 ± 0.18 b
vitamin B_1[c]	0.12 ± 0.00 a	0.11 ± 0.00 a
vitamin B_2[c]	0.10 ± 0.01 a	0.11 ± 0.01 a
α-tocopherol[c]	ND a	0.53 ± 0.01 b
β-tocopherol[c]	ND a	ND a
γ-tocopherol[c]	1.30 ± 0.02 a	2.39 ± 0.15 b
δ-tocopherol[c]	ND a	ND a
α-TE vitamin E (units/100 g)	0.13 ± 0.01 a	0.77 ± 0.02 b
TEAC (μmol Trolox/g)	2.24 ± 0.33 a	3.35 ± 0.07 b

[a] Values are the mean ± standard deviation of three determinations. The same superscript in the same row means no significant difference ($P \leq 0.05$). [b] Values in g/100 dry matter. [c] Values in mg/100 dry matter; ND, nondetected.

The total antioxidant capacity, measured as TEAC, decreased slightly, but significantly ($P \leq 0.05$), after pigeon pea fermentation, but only a 4% reduction was observed (**Table 2**). Doblado et al. (*34*), however, reported an increment of TEAC after the fermentation of *V. sinensis* for 48 h.

Fermented pigeon pea seeds presented a better nutritional value than raw seeds since some non-nutritive compounds as α-galactosides, phytic acid, and TIA decreased, while some compounds such as available sugars increased. These obtained fermented pigeon pea flour provide a reasonable level of protein, starch, dietary fiber, minerals, and vitamins, nutritional qualities that make it a convenient ingredient to be incorporated in the formulation for semolina pasta enrichment.

Table 6. Composition in Non-nutritive Compounds of Cooked Pasta Products Made with 100% Semolina or Supplemented Semolina with Fermented Pigeon Pea (*C. cajan* var. *aroíto*) Flours[a]

composition	semolina 100% (control)	semolina:fermented pigeon pea flour (90:10) (92:8) pasta
raffinose[b]	ND a	ND a
stachyose[b]	ND a	ND a
verbascose[b]	ND a	ND a
total α-galactosides[b]	ND a	ND a
IP_6 (phytic acid)[b]	0.10 ± 0.01 a	0.18 ± 0.01 b
IP_5[b]	0.03 ± 0.00 a	0.04 ± 0.00 a
IP_4[b]	ND a	0.02 ± 0.00 b
IP_3[b]	ND a	ND a
total inositol phosphates[b]	0.13 ± 0.01 a	0.24 ± 0.00 b
TIA (TIU/mg)	ND a	ND a
tannins (galic acid)[b]	0.37 ± 0.01 b	0.28 ± 0.01 a

[a] Values are the mean ± standard deviation of three determinations. The same superscript in the same row means no significant difference ($P \leq 0.05$). [b] Values in g/100 dry matter.

Table 3 shows the cooking quality parameters of pasta products prepared with 100% semolina (control) and supplemented semolina with different proportions of fermented pigeon pea flour (5, 10, and 12%). The cooking time of pasta products supplemented with fermented flour at 5 and 10% was slightly ($P \leq 0.05$) higher (18 and 11%) than control pasta while spaghetti fortified with 12% fermented pigeon pea flour showed similar cooking times. These results do not agree with those obtained by Ferreira et al. (*47*) in pasta products prepared with wheat and soybean blends where the cooking times in fortified pasta were smaller than in 100% semolina product. Cooking water absorption values were significantly higher ($P \leq 0.05$) than those for control pasta. According to Casangrandi et al. (*48*), an acceptable weight increment for pasta products will be equivalent to twice the original weight (≥200%). Results obtained in this paper are slightly higher, and it has been considered that fortified spaghetti with fermented pigeon pea flour has an acceptable quality.

The supplementation of semolina with fermented pigeon pea flours caused an increment in cooking loss (**Table 3**). Different authors have observed that when the cooking time increases, the cooking losses increase (*49*−*51*). Higher cooking losses in noodles from pea and wheat blends and in noodles from defatted soy-supplemented whole durum wheat as compared with 100% wheat noodles have been previously reported (*52*, *53*). Cooking loss could be attributable to the structural changes in the protein network due to the substitution of wheat protein by legume protein. According to Ferreira et al. (*47*), solid losses could be a consequence of protein and starch degradation due to the temperature employed during pasta cooking. In the present paper, cooking loss, although higher than control pasta, still satisfies values ≤9% as it has been suggested as acceptable in pasta making (*54*). Results obtained are in accordance with those found by Granito et al. (*55*) in spaghetti supplemented with defatted corn, cassava, cowpea, and gluten. Similar results have been reported by Bergman et al. (*56*) in cooking parameters of pasta products fortified with cowpea meal in comparison to 100% semolina pasta.

Small substitution (5, 10, and 12%) of semolina by fermented pigeon pea flour induced higher protein loss in water than those observed in control pasta (**Table 3**). Dexter and Matsuo (*49*) reported a larger protein loss in water for spaghetti made from poor quality wheat than those made from better quality wheat. This was attributable to a greater proportion of extractable gluten protein for the poorer quality wheat. They also found an increase in insoluble protein content in cooked spaghetti when the cooking time was close to 12 min.

Table 4 shows the general acceptability of cooked control and fortified pasta products. Spaghetti supplemented with 5 and 10% fermented pigeon pea flour showed a similar acceptability score ($P \leq 0.05$) than control pasta. When the substitution level was increased to 12%, the score was significantly ($P \leq 0.05$) lower and this product was not well-accepted. Raggaee et al. (*57*) reported a good consumer acceptability for products formulated with fermented lentils. Frías et al. (*58*) pointed out that changes occurring during legume fermentation impart modifications of texture and organoleptic characteristics such as flavor, aroma, taste, and appearance, especially the elimination of beany flavor. From the acceptability results collected in **Table 4**, it was decided to make spaghetti with a 10% substitution and to carry out chemical, nutritional, and biological studies.

Table 5 reports the chemical composition of cooked pasta products made with 100% semolina or supplemented with 10% fermented pigeon pea flour. Fortified pasta showed a higher content of protein (20%), fat (157%), total available soluble sugars (88%), total and available starch (7 and 5%, respectively), insoluble, soluble, and total dietary fiber (30, 155, and 48%, respectively), ash (130%), and minerals (142% for calcium, 178% for sodium, 31% for magnesium, and 89% for zinc) than the control pasta (100% semolina). Furthermore, cooked fortified pasta led to an increase in energy value (11%). The chemical composition of cooked pasta fortified with 10% fermented pigeon pea flour is in accordance with results reported by Endres (*59*) for pasta products fortified with 15% soy protein isolates and by Goñi and Valentin-Gamazo (*60*) for spaghetti supplemented with 25% chickpea flour. The ratio between soluble and insoluble dietary fiber obtained was 1/2.7, which was quite similar with those found by Wittig et al. (*61*) (1/4.5) in enriched spaghetti with dietary fiber extracts. Mineral values in supplemented pasta with fermented pigeon pea flour were higher than those reported for spaghetti (*62*, *63*).

Table 7. Biological Values of Casein and Cooked Pasta Products Made with 100% Semolina or Supplemented Semolina with Fermented Pigeon Pea Flour (*C. cajan* var. *aroíto*)[a]

diets	food intake (g/rat/day)	protein intake (g/rat/day)	weight gained (g/rat/day)	true digestibility (TD)	protein efficiency ratio (PER)
casein	7.81 ± 0.51 b	0.87 ± 0.06 c	2.40 ± 0.30 c	93.13 ± 2.19 c	2.77 ± 0.34 c
semolina 100% (control) pasta	6.36 ± 1.07 a	0.64 ± 0.11 a	0.69 ± 0.15 a	84.51 ± 3.88 a	1.11 ± 0.30 a
semolina:fermented pigeon pea flour (90:10) pasta	6.14 ± 0.34 a	0.79 ± 0.04 b	1.52 ± 0.15 b	89.62 ± 0.69 b	1.92 ± 0.16 b

[a] Values are the mean ± standard deviations of six determinations. The same superscript in the same column means no significant difference ($P \leq 0.05$).

Table 5 also collects the vitamin content of cooked pasta products. Substitution of semolina with 10% fermented pigeon pea flour had no significant effect on the content of B vitamin. However, vitamin E presented a sharp increase (from 0.13 to 0.77 vitamin E units) mainly due to the presence of α-tocopherol in fortified pasta, which was not detected in control pasta. According with the RDA, spaghetti fortified with 10% fermented flour provides 9−10% of the recommended intake of vitamin B_1, 8−9% of the RDA of vitamin B_2, and 5% of the RDA of vitamin E (*64*). The total antioxidant capacity (TEAC) increased as a consequence of fortification (80%).

Pasta products enriched with 10% fermented pigeon pea flour did not contain α-galactoside and provide larger ($P \leq 0.05$) phytic acid contents than 100% semolina pasta, while the tannin content was reduced significantly ($P \leq 0.05$) (24%) (**Table 6**).

Table 7 collects biological parameters for cooked pasta products studied and the reference casein diet. Fortified cooked pasta showed significantly ($P \leq 0.05$) higher protein intake and weight gained than 100% semolina pasta. TD and PER were notably improved as a consequence of fortification (6 and 73%, respectively) as compared with those values for control pasta. The improvement obtained in PER was possibly due to supplementation of amino acids between legumes and cereal, since fortified pasta products lacked in TIA. In cooked spaghetti supplemented with free α-galactosides flour from *L. angustifolius* varieties, an increase in PER of 73−86% was obtained in comparison with 100% semolina pastas (*65*). However, results obtained in the present paper are higher than values presented by Casagrandi et al. (*48*) in macaroni made with wheat flour supplemented with raw pigeon pea flour in 5, 10, and 15% (PER values ranging from 0.97 to 1.09 and TD from 76.8 to 84.5%) and those reported by Canniati-Brazaca et al. (*66*) for pasta elaborated with a mixture of 30% pigeon pea protein and 70% rice protein where the PER value was 1.7 and the TD value was 73.4%.

It can be concluded that pasta products prepared by fortifying semolina flour with 10% fermented pigeon pea flours did not contain non-nutritive compounds and provided higher protein contents and PERs than 100% semolina pasta. These enriched pasta products presented similar sensorial acceptability than semolina products; therefore, they can be included in the human diet.

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