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Analysis of an Alternative Food, Soy "Paçoca", Preparation: Chemical Composition, Anti-nutritional Constituents and Nutritional Evaluation

Ana K. M. Cruz, Luciana G. Alves, Ana H. Araújo, Railene A. Pereira, Creuza B. S. Nascimento, Edda L. Leite, Dilma F. Lima and Maurício P. Sales
Departamento de Bioquímica, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Campus Universitário, Av Salgado Filho s/n, 59072-970 Natal, RN, Brazil

Abstract: The chemical composition of an alternative Brazilian food preparation showed that this product had carbohydrate content 45.90 % and a protein content of 38.92%. Anti-nutritional factors were assayed in soy "paçoca" protein fractions. Human salivary α-amylase was inhibited with salivary amylase inhibitor (α-IA) level of 437.5 μg of inhibitor to 1.0 mg of protein fractions. Trypsin was inhibited with trypsin inhibitor activities level (TIA) of 57μg of inhibitor to 1.0 g of protein fractions. Nutritional evaluation of the soy "paçoca" showed a digestibility of 66.9% compared to 90.0% for casein. The PER of soy "paçoca" was 1.65 and was 4.01 for casein. The NPR of the food preparation was 2.85 and the test diet was 3.02. Indices calculated for the soy "paçoca" food preparation indicated that it was less efficient in supporting growth and maintenance of the weanling rats.

Key words: Soybean, food preparation, anti-nutritional constituents and nutritional evaluation

Introduction

The high prevalence of malnutrition in developing countries, such as Brazil, has directed efforts towards the employment of new sources and new formulations of foods. Soy "paçoca" is an example of a food preparation used as a complement in programs to combat and prevent malnutrition in Natal in the State of Rio Grande do Norte, Brazil. Although, this world wide interest has given rise to accessible and inexpensive sources foods, it calls for a more critical evaluation of protein quality, anti-nutritional factors contents, physical-chemical properties and of the effects of the processing conditions.

Food preparations based predominantly on legumes are characterized by a relatively large amount of proteins and carbohydrates. In general, these preparations also contain significant amounts of fibers, lipids, minerals and vitamins (Alonso *et al.,* 2000). Although legumes are rich protein sources, they also have, in the albumin fraction, high contents of anti-nutritional factors as lectins, a-amylase inhibitors and other proteinase inhibitors, which are responsible for the poor nutritive value of various foods (Liener *et al.,* 1985 and Shewry, 1995). These proteinaceous inhibitors are found in many plants, particularly among legumes. They play a physiological role in endogenous enzymatic activity control, necessary for seed development and in plants defense against microbial and insect pests (Xavier-Filho, 1991). Proteinase inhibitors, especially trypsin inhibitors, found in seeds have received a great deal of attention due to their potential deleterious effects to animal and human nutrition, when these proteins are consumed unheated (Liener, 1994). Attempts to increase the use of legumes in foods have employed a wide range of processing methods and conditions to inactivate or totally eliminate the anti-nutritional protein fractions, as inhibitors and lectins. These methods include soaking, dehulling, heating, germination and fermentation (Nti and Phahar, 1996). This study aims the analysis of an alternative food preparation, the soy "paçoca", through its chemical composition, ingestion effects and digestibility on Winstar rats.

Materials and Methods

Food Preparation: The soy "paçoca" food preparation was composed of: soy protein 27%, cassava meal 16.5%, soy oil 16.5%, banana with peel 17.6%, banana peel meal 12%, onion 2.7%, coriander 2.7%, pumpkin seed powder 1.6%, cassava leaf powder 1%, eggshell powder 1% and salt 1.4%.

Chemical Composition of Food: Dry matter was determined by drying at 105 °C to constant weight (AOAC, 1984). Ash was gravimetrically obtained after combustion of dried sample in a furnace at 500 °C (AOAC, 1984). Total protein was determined by Kjeldahl method (AOAC, 1984). Total fat was quantified by method developed by Bling and Oyer (1959). Total fiber was determined using an acid and alkaline digestion (AOAC, 1984) and total carbohydrate was estimated by subtraction of the chemical components: ash, protein, fat and fiber. Number of composite samples is three with three replicate each.

Meal Fractionation of Food: The food mixture was lyophilized and ground to fine powder. Food powder was extracted with 50 mM Tris-HCl, pH 8.0 (1:10, m/v) for 90 min with constant stirring. The slurry was

centrifuged (10000 x g, 4 °C, 30 min). Supernatant was pooled, dialyzed against water and then centrifuged (10000 x g, 4 °C, 30 min) to separation albumins and globulins. Both protein fractions were freeze-dried.

Determination of Protein: Protein concentration from protein fractions that were utilized in inhibitory enzymatic assays was determined by dye binding method developed by Bradford (1976), using bovine serum albumin (Sigma) as standard.

Assay of α-amylase Inhibitors Activity: The inhibitory assays were performed as to methods described by Desphand *et al.* (1982) with modifications. Aliquots of 40 ml of human saliva a-amylase (1.0 mg. ml^{-1}) were pre-incubated with 100 ml of protein fractions (2.0 mg. ml^{-1}) at 30 °C for 15 min, prior to the addition of 2000 ml of substrate solution (1% soluble starch solution in 0.1 M acetate buffer, pH 5.5 containing 20 mM NaCl and 0.1 mM $CaCl_2$). After 60 min at 30 °C, the reaction was terminated. Aliquots of 100 ml were added to 10 ml of 1 mM iodine, 24 mM potassium iodide solution. Absorbance was read at 565 nm. One unit of inhibitory activity was defined as the amount of inhibitor that decreases the absorbance by 0.01 at 565 nm after correction of enzyme blanks. All assays were performed in triplicate.

Assay of Trypsin Inhibitor Activity: The inhibitory assays were made according methods described by Xavier-Filho (1974) with modifications. Proteolytic activity was determined with Na-benzoil-DL-arginine-*p*-nitro-anilide (Sigma) as substrate. Aliquots of 100 ml of trypsin (0.3 mg. ml^{-1}) were pre-incubated with 400 ml (2.0mg. ml^{-1}) of buffer (50 mM Tris-HCl, pH 7.5) containing protein fractions, at 37 °C for 15 min. The reaction was started by addition of 1000 ml of 0.012 M BApNA solution, at 37 °C, incubated for 10 min and stopped by addition of 1500 ml of 13 % Acetic acid. Absorbance was read at 405 nm. One unit of inhibitory activity was defined as the amount of inhibitor that decreases the absorbance by 0.01 at 405nm after correction of enzyme blanks. All assays were performed in triplicate.

Diets: Iso-nitrogenous diets (Table 1) were prepared from the soy "paçoca" food preparation (Test diet) and from purified high nitrogen casein (Control diet) (Rhoster, Ucrania) to provide 10 % of protein (1.6% nitrogen) on a dry weight basis (Reeves *et al.*, 1993). In addition to protein, the diets contained a 1% vitamin mix (AIN-93-VX) and a 3.5% mineral mix (AIN-93G). Cornstarch was used to make up the remainder of the test preparation. A protein-free diet (Non Protein Control) was also prepared in which the test food preparation was replaced by cornstarch and used to estimate the endogenous nitrogen excretion of the animals.

Table 1: Composition of Casein (control), Soy "paçoca" (test) and Non-protein control (NPC) diets

Diets	Casein (per 100g)	Soy "paçoca" (per 100g)	NPC (per 100g)
Protein	10.3	9.9	-
Lipid	7.0	9.5	7.0
Ash	3.5	1.35	3.5
Crude Fiber	5.0	1.32	5.0
Carbohydrate	62.7	77.9	74.5
Vitamin Mix*	1.0	-	1.0
Salt Mix*	3.5	-	3.5
Choline Bitartarat	2.5	-	2.5
L- Cistein	3.0	-	3.0
Terc butilhydrochinon	0.014	-	0.014

*The casein and NPC diets were prepared as described by Reeves *et al.* (1993) in respect to vitamin and salt mixes concentrations.

Animal and Feed Experiments: This study was conducted according to the AOAC (1995). The weanling male Winstar rats (21 age days, weight ~49g) were housed individually in stainless steel cages with suspended wire bottoms in an animal care room maintained at 22-26 °C and 50-65 % of relative humidity. The animals were distributed into three groups of six animals each. Each group was randomly assigned a diet. Food and water were provided *ad libitum*. Food intake and weight change were monitored and recorded at two days intervals, for a period of 28 days. The protein quality indices as protein efficiency ratio [PER = weight gain of animal on test diet (g) / weight protein consumed by animals on test diet (g)], net protein rate [NPR = (weight gain of test – weight loss by protein-free) animals / weight protein consumed by test animals] and true digestibility [%TD = nitrogen intake of test animals – (fecal nitrogen output by test animals – fecal nitrogen output by protein-free animals) x 100 / nitrogen intake of test animals] were calculated from the data collected.

Statistical Analysis: Comparisons between means were assessed using Student´s *t*-teste at a significance level of $P < 0.05$.

Results and Discussion

Although legume and cereal are good sources of protein, they contain a wide range of other

Table 2: Proximate composition of dry soy "paçoca" and constituents

Component	Soy "paçoca" (per 100g)
Dry matter (g)	90.4 ± 0.3
Ash (g)	3.97 ± 0.2
Fat (g)	16.32 ± 0.5
Protein (g)	31.92 ± 1.3
Crude Fiber (g)	1.80 ± 0.2
Carbohydrate (g)	45.90 ± 0.8

Table 3: Trypitic (TIA) and α-Amilase (αIA) inhibitor activity of protein fractions from Soy "paçoca" food preparation

Anti-Nutritional Components of Soy "paçoca"	
αIA (μg/mg)	TIA (μg/mg)
437.5 ± 0.45	57.0 ± 2.1

constituents in their protein fractions, such as proteinase inhibitors, α-amylase inhibitors and lectins. These compounds have been investigated with regard to their effect on the bioavailability of nutrients to mammalians. These protein components often exert a negative impact on the nutritional quality of protein. In this present investigation, the soy "paçoca" food preparation showed a lower nutritional value compared to the casein control diet. Previously published results have demonstrated the adverse effects of these anti-nutritional protein factors (Layer *et al.*, 1985 and Marconi *et al.*, 1993).

The chemical composition of the soy "paçoca" food preparation is showed in Table 2. The food was composed of 45.90% carbohydrates, probably due to the presence of cassava meal, whole banana with peel, banana peel meal and cassava leaf powder. The protein content was 38.92% and is compared to the protein content of soy present in the preparation. This high content protein content gives this food preparation a excellent nutritional value. But high protein content is not necessarily indicative of high nutritional value. Hence, the effect of the protein fraction, from the soy "paçoca" food preparation on human salivary α-amylase and bovine trypsin was studied.

Human salivary α-amylase was inhibited by soy "paçoca" protein fraction at a concentration of 437.5 μg of inhibitor to 1.0 mg of protein fraction (Table 2). In general, the α-amylase inhibitors are stable in human gastrointestinal secretions, slow down starch digestion *in vitro*, rapidly inactivate amylases in the human intestinal lumen and at acceptable oral doses, may decrease intra-luminal digestion of starch in human (Roman *et al.*, 1987). Proteinase inhibitor activity was measured against trypsin at pH 7.5, revealing that soy "paçoca" protein fraction contained trypsin inhibitor activities (TIA) at a concentration of 57μg inhibitor to 1.0 g protein fraction (Table 3). Trypsin inhibitors negatively affect the growth of young animals as a result of interference with normal gut and systemic metabolism of pancreas, liver and muscle (Liener, 1994). In the rat, pancreatic hypertrophy and hyperplasia, caused by trypsin inhibitor, is primarily due to interference with CCK (cholescystokinin)-mediated feedback control of exocrine pancreatic secretions. In contrast, in pigs or dogs, in which feedback regulation is mediated via secretin, no pancreatic enlargement was observed (Liener, 1994). Antinutritional protein factors are widely distributed in legumes and cereals seeds with significant risk to human health. Studies have demonstrated that high concentrations of inhibitors in raw foods can be completely destroyed by processes such as dehulling, soaking and cooking (Wang *et al.*, 1997). Soaking is a treatment that removes 28% of trypsin inhibitor activity, cooking eliminates 77% and a considerable reduction of 91% is reached with cooking (boiling 15 minutes) followed by roller drying or autoclaving (15 min at 15 psi, 121 °C) (Roman *et al.*, 1987). Risks of ingesting enzyme inhibitors can be reduced by choosing the best components for these food mixtures and also by inactivating anti-nutritional proteins before consumption. Seed mixtures should be appropriately processed before use to avoid long term risks to human health.

The presence of inhibitors and other components in soy "paçoca" food preparation could be responsible for the lower digestibility of this food as determined in the rat bioassay. For example, whole bananas with peel do not affect protein quality but affect the digestibility, as

Table 4: PER, NPR and apparent digestibility (AD) indices determined for Soy "paçoca" test and Casein control diets

Diets	AD (%)	PER	NPR	Relative NPR
Casein control	90.0 ± 1.78	3.02 ± 0.28	4.01 ± 0.72	100
Soy "paçoca" test	66.9 ± 0.12 a	1.65 ± 0.16a	2.85 ± 0.25a	71.07

The results are presented as mean ± SEM. [a] For comparison between diets ($P < 0.05$)

proposed by Ranzani *et al.* (1996). Digestive enzyme inhibitors present in seeds used in the food, principally as the trypsin inhibitors, are responsible for the poor digestibility of dietary protein (Vasconcelos *et al.*, 2001). This is reflected in the results of the apparent digestibility measurement, in which the casein control diet had 90 % digestibility, while the soy "paçoca" food preparation, had 66.9 % digestibility. The PER for soy "paçoca" was 1.65 and for casein control diet was 3.02 as shown in table 3. This soy "paçoca" food preparation did not meet the recommendations of the World Health Organization (WHO, 1974) for formulations prepared from plant sources of protein for nutrition intervention, which must have a corrected PER = 2.1. The relative NPR values were computed from the NPR data by correcting casein to 100. The nutritional evaluation of soy "paçoca" food preparation by NPR was 2.85, showing that protein utilization (relative NPR values) was only 71%. Unlike the PER index, NPR and relative NPR indices take into account the maintenance requirement of the weanling rats (Mensa-Wilmot and Philips, 2001). In conclusion, values for the soy "paçoca" food preparation indicated that it was less efficient than the casein control diet in supporting the growth and maintenance of the weanling rats and that the quality and not the quantity of the protein in the formulation is important in the indication of a new and suitable food.

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