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A Nutritious Powder Mixture Formulation (Multimistura) as Food Supplement Chemical Composition and Analysis of Protein Fractions

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Abstract: The chemical composition of the nutritious powder mixture used here showed that this product had carbohydrate concentration of 65.97 % and protein content, around 15.85%. These results show that the chemical composition is dependent of type of available components when the formulation is prepared. The protein fraction when dialyzed produces a globulin and albumin fraction. The protein content determined by dye binding method was of 14.70 %, where globulin represented 11.2 % and albumin 3.57 %. Effect of protein fraction on α-amylase and bovine trypsin was studied. Human salivar α-amylase was inhibited by protein fraction with salivar amylase inhibitor (α-IA) level of 270 μg of inhibitor to 1.0 mg from nutritious powder mixture. Proteinase inhibitor activity was measured against trypsin and the results showed that protein fraction of nutritious powder mixture had trypsin inhibitor activities level (TIA) of level of 20.2 μg of inhibitor to 1.0 mg of protein fraction from nutritious powder mixture.

Key words: Nutritious powder mixture, Albumins, Globulins, Amylase inhibitor, Trypsin inhibitors

Introduction

The high incidence of malnutrition in many development countries has given cause for the efforts to be focused on the utilization of new sources of foods and their formulations. Example of those formulations are the nutritious powders mixtures that are alimentary complement used on foods and are composed of different dry and raw parts of vegetables, principally. These formulations are used in the combat the malnutrition in the Brazil. However, this current interest in found accessible and inexpensive sources foods calls for critical evaluation of the different sources for relative protein quality, content of anti-nutritional factors, physic-chemical properties and the effects of processing conditions.

Most studies designed to answers questions related to the nutritional quality of foods have focused on proteins in the globulin fractions. However in albumin fractions are present high amounts of antinutritional factors as lectins, a-amylase inhibitors and proteinase inhibitors, witch are identified as components responsible for the poor nutritive value of many foods (Liener, Nitsan, Srisangnam, Rackis and Gumbmann, 1985 and Shewry, 1995). a-Amylase inhibitors and proteinase inhibitors are found in many plants, particularly among legumes. They play a physiologic role in the control of endogen enzymatic activity no necessary in seeds in development and have possible role in the defense of plants against microbial and insect pests too (Xavier-Filho, 1991). The latter group of enzyme inhibitors, especially trypsin inhibitors, found in seeds has received particular attention because their potential deleterious effects in animal and human nutrition causing pancreatic enlargement in diets containing unheated proteins (Liener, 1994).

The purpose of the present study was to determine chemical composition in a formulation of nutritious powder mixture and to analyze the protein fractions with relation the presence of inhibitors of digestive enzymes as bovine pancreatic trypsin and human salivar a-amylase and to relate the findings to the nutritional evaluation these proteins. This alimentary complement is used on foods and is a formulation composed of powder mixture of dry and raw parts of vegetables and others components, principally, not used conventionally in the human alimentation.

Material and Methods

Nutritious Powder Mixture: The formulation of nutritious powder mixture was purchased from local markets and was composed of 30% wheat flour, 30% corn meal, 30% wheat bran, 6% cassava leaves bran dust and 3% dust of eggs shell.

Chemical Composition: 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: Mixture was ground to fine powder. Meal 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 Proteins: Protein concentration was determined by dye binding method developed by Bradford (1976) using bovine serum albumin as standard.

Assay of A-Amylase Inhibitors Activity: Aliquots of 80 ml of human saliva a-amylase (1.0 mg. ml^{-1}) was pre-incubated with 100 ml of nutritious powder mixture protein fractions (2.0mg. 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, reaction was terminated. Aliquots of 100 ml were added in 10 ml of 1 mM iodine, 24 mM potassium iodid solution. Absorbance was read at 565 nm. One unit of inhibitory activity was defined as the amount of inhibitor that decreases an absorbance of 0.01 at 565 nm after correction of enzyme blanks. All the assays were made in triplicate.

Assay of Trypsin Inhibitor Activity : Proteolytic activity was determined with BApNA (Na-benzoil-DL-arginine-*p*-nitro-anilide) as substrate. Aliquots of 100 ml of trypsin (0.3 mg. ml^{-1}) were pre-incubated with 400 ml (2.0mg. ml^{-1}) of appropriated buffer (50 mM Tris-HCl, pH 7.5) containing nutritious powder mixture 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 solution. Absorbance was read at 405 nm. One unit of inhibitory activity was defined as the amount of inhibitor that decreases an absorbance of 0.01 at 405nm after correction of enzyme blanks. All the assays were made in triplicate.

Results and Discussion

Although legume and cereal provide good sources of protein, they contain a wide range of constituents in their protein fractions as proteinase inhibitors, α-amylase inhibitors and lectins that are specially investigated with regard to bioavailability of nutrients to mammalian. In foods these protein components exert a negative impact on the nutritional quality of protein. In this present investigation was shown that antinutritional components were present in proteins fractions of the nutritious powder mixture that was used in this study. Results previously published have demonstrated the adverse effects of these antinutritional protein factors, when foods are consumed in raw forms or not adequately processing (Bressani, 1985; Della-Gatta, Piergiovanni, Ng, Perrino and Carnovale, 1989 and Marconi, Ng and Carnovale, 1993).

The chemical composition of the nutritious powder mixture (Table 1) used here showed that this product had high concentration of carbohydrates (65.97 %), certainly due the presence of wheat flour, corn meal, wheat bran, that are rich in starch, in the formulation. The protein content, around 15.85%, is compared with protein content of the grains from cereal, also present in the formulation. These results show that the chemical composition is variable between "Multimistura" and is dependent certainly of type of available components when the formulation is prepared. The formulation that was studied by Madruga and Camara (2000) had a total protein content around 13.6%, inferior to the analyzed by us, but had a superior concentration of carbohydrate in relation the formulation in this study.

The protein fraction from nutritious powder mixture when dialyzed against water produces a globulin fraction and an albumin fraction. The protein content of crude extract determined by dye binding method was of 14.9 %, where globulin fraction represented around 11.2 % and albumin fraction 3.56 % of the total protein content (Table 2). This result is different those other found because the Kjeldahl method (AOAC, 1984) used to chemical composition studies overestimate protein content in samples. Most of the literature on degradation of proteins concerns the major storage proteins (globulins), which constitute 50-75% of total seed proteins (Khan, Gatehouse and Boulter, 1980 and Shewry, 1995). This fraction of globulin proteins, in their native form, is resistant to mammalian enzymes, but after cooking treatments becomes susceptible to digestive enzymes (Deshpande and Nielsen, 1987; Sales, Macedo and Xavier-Filho, 1992). This treatment becomes available amino acids as proline, glutamine and phenylalanine that are founded in expressive concentrations in globulins from selected crop seeds (Greenfield, *et al.*, 1998).

Effect of protein fraction from nutritious powder mixture on α-amylase and bovine trypsin was studied. Human salivar α-amylase was inhibited by protein fraction with salivar amylase inhibitor (α-IA) level of 270 μg of inhibitor to 1.0 mg of protein fractions from nutritious powder mixture (Table 2).

Table 1: Proximate composition per 100g dry nutritious powder mixture and its constituents.

Component	Nutritious powder mixture
Dry matter (g) (fresh wt)	90.43 ± 0.17
Ash (g)	2.95 ± 0.09
Fat (g)	2.33 ± 0.34
Protein (g)	15.85 ± 1.15
Crude Fiber (g)	3.34 ± 0.13
Carbohydrate (g)	65.97 ± 0.83

Table 2: Protein fractionation (globulin and albumin) per 100g of nutritious powder mixture and analysis of Anti-α-amylase human salivar activity (αIA) and Anti-trypitic activity (TIA) per ìg/mg of protein fractions from nutritious powder mixture.

Components	Nutritious powder mixture
Total protein* (g)	14.9 ± 3.6
Globulins (g)	11.2 ± 1.4
Albumins (g)	3.56 ± 0.09
αIA (ìg)	270.0 ± 0.043
TIA (ìg)	20.2 ± 2.6

* Protein concentration was determined by dye binding method developed by Bradford (1976).

Proteinase inhibitor activity was measured against trypsin at pH 7.5 and the results showed that protein fraction of nutritious powder mixture had trypsin inhibitor activities level (TIA) of level of: 20.2 μg of inhibitor to 1.0g of protein fractions from nutritious powder mixture.

The inhibitory activity that was observed in assays, probably, is present in the complements corn and wheat of the mixture, because cereal seeds and also legume seeds are rich in α-amylase and trypsin inhibitors (Liener, Nitsan, Srisangnam, Rackis and Gumbmann, 1985 and Shewry, 1995). In general, the α-amylase inhibitors show be stable in human gastrointestinal secretions, slows dietary starch digestion *in vitro*, rapidly inactivates amylases in the human intestinal lumen, and, at acceptable oral doses, may decrease intraluminal digestion of starch in human (Layer, Carlson and Dimagno, 1985). Otherwise, trypsin inhibitors considerably inhibit the growth of young animals due to interference with normal gut and systemic metabolism of pancreas, liver and muscle (Liener, 1994). In rat, pancreatic hypertrophy and hyperplasia is primarily a result of interference with CCK (cholescystokinin)-mediated feedback control of exocrine pancreatic secretion, in contrast, with pigs or dogs, in which feedback regulation is mediated via secretin, no increase of pancreas enlargement was observed (Liener, 1994). Antinutritional protein factors are so 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 processing as dehulling, soaking and cooking (Nti and Plahar, 1996). The soaking is a treatment that remove 28% of trypsin inhibitor activity (Roman, Bender and Morton, 1987), cooking eliminates 77% of the trypsin inhibitor activity initially present in the sample and considerable reduction around 91% is reached with cooking (boiling 15 minutes) followed by roller drying or autoclaving (15 min at 15 psi, 121 °C) (Wang, Lewis, Bennan and Westby, 1997). The risks of ingestion of enzyme inhibitors should be reduced by best choice of components of these mixtures and also by inactivation of antinutritional proteins before consummation. As well as the seeds, the formulations of nutritious powder mixture should be well processed for posterior use as food to avoid risks for the long term to human health and especially to children health.

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