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Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: Principal component analysis

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ABSTRACT

In the Northeast of Argentina, cowpeas are frequently produced by small- and medium-sized farmers for either personal consumption or trade. The objective of this work was to study, using a principal component analysis (PCA), the effect of thermal and non-thermal treatments on the nutritional and anti-nutritional properties of the four different varieties of *Vigna unguiculata*.

Anti-nutrients contents allowed treatment differentiation for each variety because they contributed mainly in the PC1 axis construction (PCA). In contrast, nutrient (proteins and carbohydrates) contribution to treatment differentiation was not significant. Colorado variety presented the highest mineral in vitro availability, with the exception of iron. Thermal treatment increased the protein digestibility of all flours. The typical thermograms of native flours presented a two overlapping endotherm with denaturing temperatures (Td) of 83–84 °C and 91–93 °C respectively and a total enthalpy (ΔH) 7–9 J/g. Heat treatments produced a shift of Td (67–73 °C) with increasing treatment time.

Cowpea seeds varieties, especially Colorado and Cuarenton, because of their nutritional and anti-nutritional components under thermal treatments, could be used as an alternative low cost protein source for food formulas.

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1. Introduction

Cowpea (*Vigna unguiculata* Syn. *Vigna sinensis)* is an annual legume that belongs to the Fabaceae family and is commonly known as southern pea, black-eyed pea, cowpea, alubia, caupi, tape or frijole.

The world production of dried beans was about 23 million tonnes in 2010 (FAO estimations). FAO data from 2010 has the top five producers of dry beans as India, Brazil, Myanmar, China and USA. FAO data from 2009 has the top five exporters of dry beans as China, Myanmar, USA, Canada and Argentina, and the top importers are India and European Union (FAO data from 2010).

In Argentina, total legume production increased up to 406 tonnes in 2008, when 83% corresponded to bean production and the remaining 17% to dry peas, lentils and chickpeas. The province of Salta is the main dry bean producer, closely followed by Jujuy and Tucuman. According to the Argentine Chamber of Legumes (CLERA), in the 2007/08 campaign, 48% of the obtained volume corresponded to alubia bean, 34% to black bean and the remaining 18% to other bean varieties (Lezcano, 2009).

In the Northeast of Argentina, cowpeas are frequently produced by small- and medium-sized farmers for either personal consumption (human or animal) or trade. Cowpeas are also used as green manure, employed in a rotary scheme with other annual crops or in fruit plantations, to increase or sustain soil fertility.

Cowpeas contain significant protein and mineral contents. Chemical and nutritional compositions of cowpea, as well as its cooking properties, vary considerably according to environmental and genetic factors (Giami, 2005).

Cowpeas used as nourishment has been limited due to its beany flavor, its long cooking time, and the presence of certain anti-nutritional factors (polyphenols, tannins and phytic acid). Tannins inhibit the digestibility of protein, whereas phytic acid reduces the bioavailability of some essential minerals (Van der Poel, 1990). Previous studies have shown that different cooking methods improve the nutritional quality of food legumes to variable extents (Chi-Fai, Peter, & Shing, 1997).

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A principal component analysis is used to analyze multivariate data and to generate new sets of variables, these being linear combinations of the original ones (Zagrodzki et al., 1995). The central idea of principal component analysis (PCA) is to reduce the dimensionality of a data set consisting of a large number of interrelated variables. But at the same time it's trying to retain as much as possible of the variation present in the data set. This is achieved by transforming to a new set of variables, the principal components (PC). Which are uncorrelated, and ordered so that the first few retain most of the variation present in all of the original variables (Jolliffe, 2002; chap. 29).

The main aim of this work was to study, using a PCA, the effect of thermal and non-thermal treatments on the nutritional and anti-nutritional properties of the four different varieties of *V. unguiculata* (Cuarenton, Colorado, San Francisco and Z_1) grown in the Northeast of Argentina.

2. Materials and methods

2.1. Material

Four local cowpea varieties grown in the Northeast of Argentina (NEA), named as Cuarenton (CU), Colorado (CO), San Francisco (SF) and Z_1, obtained from Estación Experimental El Sombrero-Corrientes (Instituto Nacional de Tecnología Agropecuaria-INTA) (crops 2009), were used for all the experiments. Cowpea seeds were kept in a hermetic vessel (500 g) and stored at 10 °C until used.

2.2. Processing methods

Soaking: Cowpeas were soaked in solutions of sodium bicarbonate (0.02 g/100 mL; pH 8.3) for 120, 240 and 360 min using a seed-to-liquid ratio of 1:10 (g:mL).

Cooking: Cowpeas were cooked in boiling water temperature for 20, 40 and 60 min in a beaker having a condenser, using a seed-to-distilled water ratio of 1:10 (g:mL).

Autoclaving: Cowpeas were pressure-cooked (2.175 kPa) at 121 °C for 10, 20 and 30 min using a seed-to-distilled water ratio of 1:10 (g:mL).

All the processed samples were washed with distilled water and dried in an oven at 55 °C to a constant weight. Processing methods were performed in triplicate.

2.3. Preparation of seed flour

Treated and non-treated cowpeas (with seed coat) were ground in an electric miller (Braun KSM2 model, coffee grinder, México, 2006) and subsequently sieved through an 80 ASTM (177 μm).

2.4. Chemical analyses

Protein content was determined by the Kjeldahl method (Method 920.87) (N × 6.25). The other constituents, crude fiber (Method 920.86), moisture content (Method 925.10) and ash (Method 923.03), were estimated by the methods of AOAC, 1990. Carbohydrates were determined according to Rose et al. (1991). Potassium, calcium, magnesium, iron and zinc were determined by atomic absorption spectrometry (GBC 932 Plus model, Australia, 2001) using Lanthanum 10,000 ppm (Kalra, 1998). Phosphorus was determined using Murphy and Riley method (Murphy & Riley, 1962) (Metrolab 1700V 2.03 model, Thailand, 1996).

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

All gels were run in minislabs (Bio-Rad Mini Protean II Model). SDS-PAGE was performed according to Laemmli's method (1970) using continuous gels (12%). Protein samples (10 mg/mL) were dissolved in 0.125 mol/L Tris–HCl, pH 6.8, 20 mL/100 mL glycerol, 0.1 g/100 mL SDS, 0.05 g/100 mL bromophenol blue, and centrifuged at 15,800 × g for 5 min at 4 °C. Supernatants were loaded onto the gel (30–40 μg of protein per lane). Samples to be run under reducing conditions were boiled for 1 min in sample buffer containing 5 mL/100 mL 2-mercaptoethanol (2-ME) before centrifugation. Electrophoresis was conducted for 1 h at a constant voltage of 200 V. The following molecular weight standards were used to estimate the molecular masses of polypeptides: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α-lactalbumin (14.4 kDa), (Pharmacia).

2.6. Analysis of anti-nutritional factors

The total content of free phenols in treated and non-treated cowpeas was estimated by the method of Sadasivam and Manickam (1992). Tannin content of both treated and non-treated cowpeas was determined by the vanillin–HCl method of Price, Van Scoyoc, and Butler (1978). The method of Wheeler and Ferrel (1971) was used to determine the phytic acid content.

2.7. In vitro mineral availability (A)

Iron *A*vailability (*A*Fe, g/100 g), zinc (*A*Zn, g/100 g), calcium (*A*Ca, g/100 g) and magnesium (*A*Mg, g/100 g) availability was estimated in pounded samples as described Wolfgor, Drago, Rodriguez, Pellegrino, and Valencia (2002). Iron, zinc, calcium and magnesium availability was calculated as the mineral amount in the dialysate and expressed as a percentage of iron, zinc, calcium and magnesium in the whole sample.

2.8. In vitro protein digestibility (IVPD)

IVPD of raw treated and non-treated cowpeas were determined according to the multienzyme technique modified by Satterlee, Marshall, and Tennyson (1979). In vitro protein digestibility was calculated using the following formula:

$$\%\,IVPD = 234.84 - 22.56x$$

where, x is the pH after 20 min of incubation. The principle involved in this method is that four proteolytic enzymes are used to digest the protein and the pH change is due to the release of amino acids at a fixed time interval.

2.9. Differential scanning calorimetry (DSC)

Thermal analysis was performed in a Q100 V9.8 Build 296 calorimeter (TA Instrument, New Castle, Del., USA). The equipment was calibrated at a heating rate of 10 °C/min by using indium, lauric acid and stearic acid (pro-analysis) as standards. Hermetically sealed aluminum pans were prepared to hold 12–15 mg of freeze-dried flours suspended in water (40 g/100 mL). Samples were scanned at 10 °C/min from 25 to 130 °C. As a reference, a double empty pan sealed with a lead was used. After each run, the pans were punctured and their dry matter content was determined by leaving the pans overnight in an oven at 105 °C. The denaturation temperature, Td (°C), and the enthalpy of transition, ΔH (J/g dry