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Original Research Article

Nutritional ranking of 30 Brazilian genotypes of cowpeas including determination of antioxidant capacity and vitamins

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ABSTRACT

This study aimed to establish a nutritional ranking of 30 genotypes of cowpea (*Vigna unguiculata* L. Walp). The results showed the proximate composition varies among genotypes in protein (20–30%) and dietary fiber contents (20–35%), in protease inhibitors (2–4 UI/μg protein), lectin (40,000–640,000 UH/kg meal) and essential amino acid levels, in vitro protein digestibility (30–40%) and in the apparent concentration of expressed proteins. The antioxidant capacity varied from EC_{50} of 9.54–38.7 mg seed extract/mL DPPH and the highest values detected for alpha- and delta-tocopherol were 0.38 mg/g and 1.88 mg/g, respectively. The analysis of a nutritional quality index based on the weighted average content of total protein, dietary fiber, iron, zinc, protease inhibitors and lectins allowed ranking genotypes. In descending order the 5 best genotypes were: BRS-Cauamé > BRS-Tumucumaque > Canapuzinho > BRS-Potengi > BRS-Urubuquara.

1. Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is one of the most important grain legume crops that grow in tropical and subtropical zones of the world, being the major source of dietary protein, calories, dietary fiber, minerals and vitamins for a large segment of world population (Phillips et al., 2003). Particularly, the poor population (ca. 25 million people) from Northeastern Brazil eats cowpeas mainly to obtain protein and minerals such as iron and zinc in order to replace the animal protein sources that are of high cost (Maia et al., 2000). In addition, cowpeas are the most important source of dietary fiber for these people. Nevertheless, cowpeas possess some undesirable properties that are common to other legume seeds, such as methionine and cysteine deficiency as well as considerable contents of antinutritional factors like protease inhibitors, lectins, phytic acid, tannins, among others (Giami, 2005; Duranti, 2006). One way to overcome these undesirable characteristics while increasing the levels of key components, such as proteins, minerals and dietary fiber, is the development of new cultivars with improved nutritional attributes through conventional breeding of plants.

Chemical composition and nutritional properties of cowpeas vary considerably according to cultivar. For effective utilization of newly developed cowpea cultivars for human nutrition, the removal or reduction of antinutrients and evaluation of their nutritional properties are necessary (Giami, 2005). As a matter of fact, the concern with the adverse effects of genetic modification of foods on human health should be directed not only to food produced by rDNA technology, but also to all products including those produced by conventional breeding methods since these also

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carry the potential for introducing unintended compositional changes that may have adverse effects on human health (Atherton, 2002). Recently, Vasconcelos et al. (2010) reported the importance of chemical and nutritional monitoring of new cowpea cultivars. The 3 cowpea cultivars studied in that work showed considerable differences in antinutrient levels, for both the whole seeds and their protein fractions. So it is clear that the conventional plant breeding may also result in new cowpea cultivars with nutritionally undesirable properties, as well as in more nutritious seeds. Thus, this work aimed to establish a nutritional ranking among 30 Brazilian genotypes of cowpeas, including new genotypes. The results obtained in this study may point toward the appropriate selection of parental genotypes to be used in cowpea breeding programs in order to obtain more nutritious cowpea genotypes or to indicate new genotypes for immediate consumption by population.

2. Materials and methods

2.1. Biological samples and chemical reagents

Azocasein (Sigma A2765), bovine serum albumin (96%) (Sigma A2153), chymotrypsin from bovine pancreas (type I-S) (Sigma C7762), coomassie brilliant blue G and R (Sigma B0770 and B0149, respectively), dimethyl sulfoxide (99.9%) (Sigma D8779), Kunitz-type soybean trypsin inhibitor (type I-S) (Sigma T9003), N-a-benzoyl-L-arginine-pnitroanilide (L-BAPNA) (Sigma B4875), total dietary fiber assay kit (Sigma TDF100A), trypsin from bovine pancreas (type I) (Sigma T8003) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Molecular mass markers (K494-500UL) were purchased from Amresco (Solon, OH, USA). All other chemical reagents used in the experiments were of analytical grade. Rabbit blood was obtained by puncturing the marginal ear vein of healthy animals maintained at the Federal University of Ceará (Fortaleza, Brazil) and female mice of Swiss strain were obtained from an outbred colony maintained at the same institution.

2.2. Seed samples and processing

Mature seeds of 30 genotypes of cowpea were obtained from a germplasm bank kept by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Meio-Norte, Teresina, Brazil). Each genotype studied was obtained as a pool of 3 different samples of the same genotype. The *V. unguiculata* (cowpea) genotypes studied were "BR3-Tracuatea (TR)", "BR5-Cauamé (CM)", "BR5-Guariba (GU)", BR5-Marataoa (MA)", "BR5-Milênio (MI)", "BR5-Nova Era (NE)", "BR5-Pajeú (PJ)", BR5-Paraguaçu (PA)", BR5-Potengi (PG)", "BR5-Rouxinol (RO)", "BR5-Tumucumaque (TU)", "BR5-Urubuquara (UR)", "BR5-Xique-xique (XI)", "BR17-Gurguéia (GG)", "Canapuzinho-2 (CP)", "Canapuzinho (CA)", "Inhuma (IN)", "MNC01-611F-11 (MK)", "MNC01-614F-15 (MZ)", "MNC01-631F-11 (NX)", "MNC01-631F-15 (NY)", "MNC01-631F-20-5 (NW)", "MNC01-649F-2 (NK)", "MNC99-510F-16-1 (MX)", "MNC99-510F-16-3 (MY)", "MNC99-537F-14-2 (MW)", "Paulistinha (PL)", "Patativa (PT)", Pingo de Ouro-1-2 (PI)" and Pingo de Ouro-2 (PO)". The seeds were cleaned and stored in screw-top plastic jars at room temperature (25 °C) from where a part was ground into fine flour (mesh size 1.0 mm) by using a blender (Mallory, Maranguape, Brazil) and a coffee mill (Cadence, Caxias do Sul, Brazil) at maximum speed and stored in air-tight plastic containers at 4 °C until analysis.

2.3. Proximate composition

Moisture, ash and crude lipid contents were determined according to AOAC (2000). Nitrogen was determined by the standard macro-Kjeldahl method (AOAC, 2000) using a digestion apparatus combined with the photocolorimetric method described by Baethgen and Alley (1989) to obtain total crude protein content ($N \times 6.25$). Total dietary fiber was determined by Prosky-AOAC method (AOAC, 2000) using the total dietary fiber assay kit (Sigma–Aldrich Co., St. Louis, MO, USA). All determinations were run in triplicates (3 analyses of the same sample). The digestible carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight − (g crude protein + g crude lipid + g ash + g dietary fiber)]/100 g.

2.4. Mineral composition

The determination of minerals (Fe, Zn, Na, K, Ca, Mg, Mn, and Cu) was done by atomic emission spectroscopy (Optima 4300 series, Perkin Elmer, USA). For each test 200 mg of seed meal were treated with 3 mL concentrated HNO_3 (Merck, Uppsala, Sweden) and 2 mL H_2O_2 30% (v/v) (Merck). This mixture was heated in microwave oven (Multiwave, Anton Par) under pressure and maximum power, with heating program set to 20 min and cooling to 15 min. After decomposition the suspension was diluted to 30 mL with deionized water (Milli-Q) and a calibration curve was prepared using multi-elementary solution for quantitation of minerals (Gouveia et al., 2002). All determinations were run in triplicates.

2.5. Preparation of crude extracts and soluble protein determination

The crude extracts of cowpea seeds were prepared by suspending 1 g of seed flour into 10 mL of 0.05 M sodium phosphate buffer, pH 7.0, with 0.15 M NaCl, mixing with a magnetic stirrer at 4 °C, maximum speed for 4 h. The mixture was filtered through nylon cloth and centrifuged at $15{,}000 \times g$, for 15 min. The supernatant was utilized for the assays of lectin activity, acute toxicity against mice, antioxidant activity and for the determination of electrophoretic profiles of the soluble proteins of each genotype. The protein content in the crude extracts and in each protein fraction was determined by the method described by Bradford (1976), using bovine serum albumin as standard.

2.6. Antinutritional and/or toxic factors

Lectin activity was assayed by serial 2-fold dilution of crude extracts (Vasconcelos et al., 2010). The extracts were diluted with 0.15 M NaCl and mixed with rabbit erythrocytes (20 mg/mL suspension prepared in 0.15 M NaCl) treated with trypsin from bovine pancreas in a concentration of 1 mg/mL. The degree of agglutination was monitored visually after the tubes had been left to stand at 37 °C for 30 min and at room temperature (22 ± 3 °C) for an additional 30 min. The results are reported as haemagglutination titre (HU), which is the reciprocal of the highest dilution giving visible agglutination. Trypsin inhibitor activity was determined by a slight modification of the method originally described by Kakade et al. (1974) using trypsin and L-BAPNA (substrate) (Hamerstrand et al., 1981). Activity was expressed as the amount of trypsin inhibited, calculated from a calibration curve using soybean trypsin inhibitor. Chymotrypsin inhibitor activity was determined by the method described by Erlanger et al. (1961), using azocasein as substrate. The activity was expressed as inhibition units/mg of proteins, which is defined as a decrease in 0.01 of absorbance at 440 nm. Acute toxicity to mice ($n = 6$) was verified by intraperitonial injection (30 mL/kg body weight) of diluted and crude water extract of each seed according to Vasconcelos et al. (1994). Procedures involving laboratory animals were approved by the Animal Experimentation Ethics Committee of Universidade Federal do Ceará (CEPA) in

accordance to N.11.794/2008 Act, which governs the creation and use of laboratory animals in teaching and research across the country. All determinations were run in triplicates.

2.7. SDS-electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). Treated samples containing approximately 10 μg of protein content were inoculated in wells and electrophoresis was carried out at 20 mA constant current for 2 h. Protein bands were visualized by staining with 0.05% Coomassie Brilliant Blue R-250. Protein markers employed were β-galactosidase (116 kDa), myosin (212 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumine (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (20.1 kDa) and lysozyme (14.2 kDa) (AMRESCO Inc., OH, USA).

2.8. Amino acid analysis

The genotypes of cowpea that showed high content of total proteins (≥25%) were submitted to analysis of amino acid composition, in vitro protein digestibility, antioxidant activity and the content of some vitamins. For the amino acid analysis, seed flours were hydrolyzed with 6 M HCl containing 10 g/L phenol at 110 °C for 22 h, in sealed glass tubes under N_2 atmosphere. HCl and phenol were removed by evaporation and the amino acid compositions were established after chromatography on a Biochrom 20 system (Pharmacia). Tryptophan was determined according to the method described by Pintér-Szakács and Molnár-Perl (1990). All determinations were run in triplicates.

2.9. Sequential in vitro protein digestibility

Sequential in vitro protein digestion experiments using pepsin and trypsin were carried out according to the method described by Tang et al. (2009). Briefly, seed flours were dispersed in 0.1 N HCl (pH 1.5) (1%, w/v) and incubated in a water bath at 37 °C for 3–5 min. An aliquot of pepsin powder (enzyme:protein = 1:100, w/w) was added and the mixture was incubated at 37 °C for 120 min. The pH of the mixture was adjusted to 7.0 with 1.0 N NaOH to stop the digestion reaction. The neutralized pepsin-digested mixture was then mixed with trypsin powder (enzyme:protein = 1:100, w/w) to initiate further digestion for another 120 min. The digested samples were mixed with an equal volume of 10% TCA to obtain the precipitates. Protein digestibility (%) was defined as $(N_o - N_t)/N_{tot} \times 100$, where N_t represents the TCA-precipitated nitrogen content after pepsin + trypsin digestion (mg), N_o the TCA-precipitated nitrogen content in the protein samples before digestion (mg), and N_{tot} total nitrogen content in the protein samples (mg). All determinations were run in triplicates.

2.10. Antioxidant activity: free radical scavenging capacity

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to the method reported by Yepez et al. (2002). The diluted crude extract (0.1 mL) in different concentrations (10–10,000 μg/mL) was mixed with 3.9 mL of methanolic solution containing DPPH• radicals (6.5×10^{-1} mol/L). The reduction of the DPPH• radical was measured by monitoring continuously the decrease of absorption at 515 nm. The percentage scavenging of DPPH• radical was calculated according to the formula: % scavenging effect = $100 \times [(A_{DPPH^\bullet} - As)/A_{DPPH\cdot}]$, where *As* is the absorbance of the solution when the sample has been added at a particular level and $A_{DPPH^\bullet}$ is the absorbance of the DPPH• solution. The percentage of remaining DPPH• was plotted against the sample/standard concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH• by 50% (EC_{50}). Based on the parameter EC_{50}, the result was expressed in terms of mg of seed extract per mL of DPPH• in the reaction medium. All determinations were run in triplicates.

2.11. Vitamins: beta-carotene and alfa- and delta-tocopherol

The content of beta-carotene and alpha- and delta-tocopherol in 7 cowpea genotypes (total crude protein >25%) followed the methodology described by Sousa et al. (2008). Seed flour (500 mg) of each genotype were weighed in graduated glass tubes with screw cap and 10 mL of methanol–water (90:10, v/v) containing 5% KOH were added. The tubes were placed in a water bath at 70 °C for 30 min. After cooling at room temperature, 5 mL of the saponified extract, 1.5 mL of Milli-Q water and 2.5 mL of n-hexane were mixed in a platform mixer for 10 min. After centrifugation of 15 min in order to allow phase separation, 1 mL was transferred from the upper hexane phase to test tubes and left under a stream of air bath at about 50 °C for total evaporation of the solvent. The residue was then suspended in 1 mL of methanol at the time of chromatographic analysis. The chromatographic system consisted of a Waters Spherisorb S5 ODS2 column (250 mm × 4.6 mm) and a mobile phase consisting of methanol–tetrahydrofuran (95:5, v/v) at a rate of 1.5 mL/min, using a pump (AKTAbasic 10) Model P-900, Amersham. 100 mL aliquots of the residue suspended in methanol were inject manually using a Rheodyne 7210 sample injector (Hamilton Co.). This procedure was performed with the standards and samples. The monitor (AKTAbasic UV-900) was set at 450 nm and 292 nm for simultaneous reading of carotenoids and tocopherols, respectively. The chromatograms were recorded by the control system UnicornTM 5.0 (UNICORN 5.0 software, Amersham Biosciences, Uppsala, Sweden). The concentrations of beta-carotene, alpha- and delta-tocopherol in whole seeds of cowpea were calculated by comparing the peak areas of known concentrations of solutions prepared with commercial standards (beta-carotene, alpha-tocopherol and delta-tocopherol, Sigma) with those produced by extracts of seed flour with the same retention time. All determinations were run in triplicates.

2.12. Statistical analyses

The data of proximate composition (only protein and dietary fiber), mineral composition, antinutritional and/or toxic components (except lectin results), in vitro digestibility, antioxidant activity and vitamins were presented as mean ± standard deviations of 3 determinations. For other analyses, the data were shown as means of 3 determinations and the standard deviation values were omitted since they were less than 5% of the mean. To rank the 30 genotypes according to nutritional quality, an index was constructed as a weighted average of desirable nutritional attributes of each genotype. For each component it was established a minimum (desirable nutritional attributes) or maximum amount (undesirable nutritional attributes) that the component must have. Quantities beyond the limit, in the case of minimal values (proteins and fiber over 25%, iron, over 60 mg/g and zinc, 30 mg/g) count positively. As to maximal values (toxic factors and/or antinutritional factors: protease inhibitors, less than 3 UI/g and lectins, less than 80,000 HU) the reverse is true. The index was calculated by multiplying each value in excess or shortage by its respective arbitrary weight (4 for proteins, 3 to iron and zinc, 2 to dietary fiber and 1 to each toxic and/or antinutritional factors) followed by the algebraic sum of each term. The end result of this sum was then divided by the sum of the weights.

3. Results and discussion

The results on proximate composition of the genotypes (Table 1) showed values compatible to those of literature (Giami, 2005; Vasconcelos et al., 2010). Regarding the content of total proteins, the Brazilian genotypes showed values varying from 20 to 30%, which are frequently reported in literature for cowpea seeds (Onwuliri and Obu, 2002; Giami, 2005; Vasconcelos et al., 2010). The deviations from mean values showed a variation coefficient of 14%The genotypes with the highest protein contents (≥25%) were MNC01-649F-2 (28.3%), BRS-Cauamé (27.8%), BRS-Paraguaçu (27.7%), BRS-Marataoã (27.4%), Canapuzinho (25.0%), BRS-Tumucumaque (24.8%) and MNC01-631F-15 (24.6%). Onwuliri and Obu (2002) have reported values as high as 39% total proteins, but protein contents of over 25%, as were observed in this study, are already considered quite high for pulses, which are staple foods in many regions of the world (Iqbal et al., 2006). Besides total proteins, the total dietary fiber content, which is another important macronutrient present in cowpeas, showed evident variation, between 20 and 35% among the studied genotypes and more than half of these showed high dietary fiber contents (≥25%). This is an interesting finding since the consumption of dietary fiber has been related to prevention of cardiovascular disease, diabetes and digestive tract diseases, considering that it lowers the glycemix index of food as well as serum cholesterol levels (Brownlee, 2011). Thus it becomes clear that the nutritional properties of foods produced by traditional genetic breeding should be monitored since the levels of important components for human nutrition, such as protein and dietary fiber, can show significant variation (Wang et al., 2003).

To assess whether, in addition to protein content, the amino acid composition was different among the genotypes, we selected those that contained ≥25% of total protein to have amino acid composition determined (Table 2). Overall, the amino acid composition of the genotypes studied was comparable to those reported by Vasconcelos et al. (2010). Proteins from cowpea have good levels of some essential amino acids, phenylalanine + tyrosine, leucine and lysine and of all non-essential ones, but their shortage in sulfur amino acids has also been well documented (Maia et al., 2000; Vasconcelos et al., 2010). There were no significant differences in the levels of nonessential amino acids, however, the levels of essential valine, isoleucine and tryptophan showed a variation coefficient of 14% among the genotypes. Nevertheless, the values for the sulfur amino acids, which are the limiting factors in pulses, did not vary much among the genotypes. Vasconcelos et al. (2010) have not reported significant variation in 3 studied genotypes, while Onwuliri and Obu (2002) observed 29% variation among the values obtained for leucine (287.5–643.8 mg/g N) of 6 different genotypes. In such cases the change may not have been intentional, but the change in the amino acid composition of globulins has been sought both through improvement from the natural variation and genetic manipulation, usually to increase the expression of proteins rich in sulfur amino acids (Wang et al., 2003).

The cowpea crude extracts did not show toxicity to mice under the conditions used in this study and this was a good attribute shared by all 30 genotypes. On the other hand, all studied genotypes showed inhibitory activity against both trypsin and chymotrypsin and at the same magnitude, with a variation coefficient of 11% for chymotrypsin inhibitors and 17% for trypsin

Table 1
Proximate composition (% dry weight basis)[a] of 30 newly developed Brazilian cowpea genotypes obtained by conventional plant breeding.

Cowpea genotypes	Proteins[b]	Lipids	Ash	Dietary fiber	Digestible carbohydrates[c]
BR17-Gurguéia	23.9 ± 0.2 abc	1.0 ± 0.1	3.8 ± 0.0	35.6 ± 0.3 ak	35.7
BR3-Tracuateua	24.0 ± 0.7 abc	1.4 ± 0.1	4.2 ± 0.0	28.3 ± 0.9 bck	42.1
BRS-Cauamé	27.8 ± 1.1 d	1.3 ± 0.0	3.3 ± 0.1	29.5 ± 0.9 bdk	38.1
BRS-Guariba	22.7 ± 0.9 abce	1.4 ± 0.0	3.7 ± 0.1	24.9 ± 0.3 eho	47.3
BRS-Marataoã	27.4 ± 1.4 df	1.6 ± 0.1	4.5 ± 0.2	28.8 ± 0.5 bcfk	37.7
BRS-Milênio	17.7 ± 0.7 gh	1.5 ± 0.0	3.6 ± 0.0	19.5 ± 1.3 g	57.8
BRS-Nova Era	17.4 ± 1.0 g	1.3 ± 0.0	4.2 ± 0.0	22.0 ± 0.5 eg	55.0
BRS-Pajeú	21.3 ± 0.4 ai	1.4 ± 0.1	4.4 ± 0.1	22.3 ± 0.3 egi	50.7
BRS-Paraguaçu	27.7 ± 0.9 dj	1.3 ± 0.0	3.9 ± 0.1	31.5 ± 0.2 dfj	35.8
BRS-Potengi	23.5 ± 0.5 abc	1.4 ± 0.0	4.2 ± 0.2	27.2 ± 1.4 bhlo	43.8
BRS-Rouxinol	19.3 ± 0.9 gi	1.2 ± 0.0	4.4 ± 0.1	25.9 ± 0.6 chmo	49.2
BRS-Tumucumaque	24.8 ± 0.5 bf	1.2 ± 0.0	3.6 ± 0.0	33.5 ± 0.3 aj	37.0
BRS-Urubuquara	22.7 ± 1.5 abce	1.4 ± 0.0	3.4 ± 0.0	21.0 ± 0.4 gn	51.5
BRS-Xique-xique	17.7 ± 0.7 gh	1.3 ± 0.0	4.6 ± 0.0	24.4 ± 0.5 gn	52.0
Canapuzinho	25.0 ± 0.8 bfj	1.5 ± 0.0	4.5 ± 0.0	28.0 ± 0.8 bckp	41.0
Canapuzinho-2	17.9 ± 1.7 gk	1.3 ± 0.0	3.4 ± 0.0	27.7 ± 1.2 bko	49.7
Inhuma	19.8 ± 0.8 gil	1.3 ± 0.0	3.9 ± 0.1	30.4 ± 0.6 dkq	44.6
MNC01-611F-11	22.6 ± 0.9 abcl	1.5 ± 0.0	4.1 ± 0.0	23.4 ± 0.4 eimn	48.4
MNC01-614F-15	23.8 ± 0.4 abc	1.4 ± 0.0	3.9 ± 0.1	33.0 ± 0.9 ajq	37.9
MNC01-631F-11	23.2 ± 0.5 abcm	1.4 ± 0.0	4.4 ± 0.0	25.0 ± 0.8 himo	46.0
MNC01-631F-15	24.6 ± 0.8 cf	1.3 ± 0.0	3.6 ± 0.1	26.9 ± 0.0 bhor	43.6
MNC01-631F-20-5	18.7 ± 0.7 gi	1.3 ± 0.0	4.6 ± 0.1	24.2 ± 1.2 emr	51.2
MNC01-649F-2	28.3 ± 0.7 d	1.4 ± 0.0	4.4 ± 0.1	32.2 ± 0.6 dj	33.7
MNC99-510F-16-1	22.5 ± 0.6 abcl	1.2 ± 0.1	4.2 ± 0.1	28.5 ± 0.6 bck	43.6
MNC99-510F-16-3	20.6 ± 0.5 eiklm	1.4 ± 0.0	4.2 ± 0.1	27.2 ± 0.4 bchlo	46.6
MNC99-537F-14-2	23.1 ± 1.0 abce	1.3 ± 0.0	3.9 ± 0.0	23.5 ± 1.0 emn	48.2
Patativa	22.7 ± 1.3 abce	1.4 ± 0.0	4.2 ± 0.1	25.1 ± 0.6 himop	46.6
Paulistinha	23.0 ± 0.5 abce	1.3 ± 0.1	4.2 ± 0.0	24.4 ± 0.7 elmr	47.1
Pingo de Ouro-1-2	22.3 ± 0.3 abcl	1.4 ± 0.0	3.8 ± 0.0	24.9 ± 0.1 eho	47.6
Pingo de Ouro-2	20.4 ± 0.9 chikl	1.4 ± 0.0	4.3 ± 0.0	28.3 ± 0.7 bck	45.6
RDI[d]	50 (g/day)	65 (g/day)	–	25 (g/day)	300 (g/day)

[a] All values in dry basis are means ± SD ($n = 3$). Mean values followed by different letters in the same column represent significantly different ($P < 0.05$). Only the protein and dietary fiber contents were submitted to statistical comparison due to the main focus of this study.
[b] $N \times 6.25$.
[c] The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight − (g crude protein + g crude lipid + g ash + g dietary fiber)]/100 g.
[d] Reference daily intake in 101.9 (c) (8) iv. FDA U.S. Food and Drug Administration (2010).

Table 2
Amino acid composition[a] (mg/g N) of 7 newly developed Brazilian cowpea genotypes with high content of crude protein (>25 g/100 g seed flour) compared to hen egg protein and FAO/WHO/UNU (1985) scoring pattern for different children age groups.

Amino acids	Cowpea genotypes[b]							Hen egg	Children[c]	
	CM	MA	PA	TU	CA	NY	NK		2–5 years	10–12 years
Essential										
Thr	248	257	256	253	248	234	254	302	212	175
Val	303	296	228	278	273	368	285	339	219	156
Leu	522	525	494	482	504	614	508	351	412	275
Ile	257	233	189	216	210	292	244	475	175	175
Lys	520	480	477	500	502	503	501	678	362	275
Phe + Tyr	651	617	629	654	639	646	661	820	394	138
Met + Cys	105	105	111	105	125	100	96	462	156	138
Trp[d]	NA[e]	72	92	95	76	91	67	247	69	6
Non-essential										
Asx	676	719	717	670	682	626	645	376		
Glx	1134	1145	1162	1098	1177	1028	1059	530		
Ser	307	344	366	347	333	280	316	333		
Gly	253	269	257	254	255	254	259	197		
Ala	301	312	305	302	301	281	292	358		
His	239	236	249	224	225	223	240	142	119	119
Arg	464	435	484	478	471	483	529	641		
Pro	510	475	491	549	484	481	555	376		

[a] The standard deviation values were omitted since they were less than 5% of mean ($n = 3$).
[b] CM, BRS-Cauamé; MA, BRS-Marataoã; PA, BRS-Paraguaçu; TU, BRS-Tumucumaque; CA, Canapuzinho; NY, MNC01-631F-15; NK, MNC01-649F-2.
[c] Patterns of amino acid requirements for different age groups (FAO/WHO/UNU, 1985).
[d] Trp was determined according to the method described by Pintér-Szakács and Molnár-Perl (1990).
[e] Not analyzed.

Table 3
Values for trypsin and chymotrypsin inhibitory and lectin activities from 30 newly developed Brazilian cowpea genotypes obtained by conventional plant breeding.[a]

Cowpea genotypes	Trypsin inhibitory[b]	Chymotrypsin inhibitory[b]	Lectin[c]
BR17-Gurguéia	2.9 abc	3.0 ab	160,000 a
BRS-Tracuateua	2.3 acd	2.6 ac	320,000 b
BRS-Cauamé	2.9 ae	2.9 ad	40,000 c
BRS-Guariba	2.6 adf	2.6 abe	80,000 d
BRS-Marataoã	3.2 abcg	3.0 f	320,000 b
BRS-Milênio	2.9 ef	2.5 abg	80,000 d
BRS-Nova Era	3.3 adf	3.2 abgh	320,000 b
BRS-Pajeú	3.1 ac	3.6 cdegi	160,000 a
BRS-Paraguaçu	2.6 abc	2.9 ad	320,000 b
BRS-Potengi	2.6 ef	2.3 abe	320,000 b
BRS-Rouxinol	2.7 h	3.8 cdei	160,000 a
BRS-Tumucumaque	2.4 deg	2.4 aci	160,000 a
BRS-Urubuquara	2.3 ae	2.4 ac	320,000 b
BRS-Xique-xique	3.1 ef	2.8 ac	320,000 b
Canapuzinho	2.8 aci	3.3 cdi	80,000 d
Canapuzinho-2	2.7 adf	2.8 abd	320,000 b
Inhuma	2.7 abcg	3.1 cdei	320,000 b
MNC01-611F-11	3.0 degi	2.9 ac	160,000 a
MNC01-614F-15	3.8 degi	3.9 cdehi	80,000 d
MNC01-631F-11	2.5 ae	2.7 cdeij	160,000 a
MNC01-631F-15	3.0 ae	2.9 abghj	160,000 a
MNC01-631F-20-5	2.7 acd	2.8 ac	320,000b
MNC01-649F-2	2.7 adj	3.1 ci	320,000 b
MNC99-510F-16-1	2.5 bdei	3.1 k	80,000 d
MNC99-510F-16-3	2.4 e	2.2 ac	320,000 b
MNC99-537F-14-2	2.8 deg	2.6 ab	320,000 b
Patativa	2.4 chj	2.8 aci	80,000 d
Paulistinha	3.1 adj	3.0 bf	80,000 d
Pingo de Ouro-1-2	3.0 ch	4.2 i	640,000 e
Pingo de Ouro-2	3.0 de	2.9 aci	80,000 d

[a] Mean values followed by different letters in the same column represent significantly different ($P < 0.05$); the standard deviation values were omitted since they were less than 5% of mean ($n = 3$).
[b] Trypsin and chymotrypsin inhibitory activities were expressed as inhibition units per mg of proteins (U/mg protein), which is defined as the decrease of 0.01 in the absorbance at 440 nm.
[c] Activity is expressed as haemagglutination titre (HU) per kg of flour (HU/kg flour), where HU is the reciprocal of the highest dilution giving visible agglutination.

inhibitors (Table 3). The elimination of protease inhibitors is of great interest, but one must first decide whether the absence of such compounds would cause any loss to the plant (Wang et al., 2003). The presence of lectins was detected in large amounts and although the majority of the genotypes showed hemagglutinating activity in the order of 320,000 UH (13 of 30 genotypes studied) the extreme values were extremely far apart (40,000 UH, in BR5-Cauamé to 640,000 UH in Pingo de Ouro-1-2). Vasconcelos et al. (2010) have commented that even the high levels of lectin activity in some genotypes of cowpeas do not show relevance once this can be abolished after ordinary thermal treatment.

To assess the degree to which variations in proximate and amino acid composition and levels of toxic and/or antinutritional factors affected protein digestibility, seeds of 7 genotypes with protein contents ≥25% were analyzed for in vitro protein digestibility. The results (Table 4) showed that the digestibility ranged from 29.39% (in MNC01-649F-2) to 44.05% (in BRS-Marataoã) but it was not possible to draw any correlation among the observed digestibility and factors such as dietary fiber, lectins and protease inhibitors. However, it must be kept in mind that the presence of phenolic compounds, especially tannins, which is one of the main factors that affect protein digestibility, was not

Table 4
Values for in vitro protein digestibility[a] (%) of seed flour from 7 newly developed Brazilian cowpea genotypes with high content of crude protein (>25 g/100 g seed flour).

Cowpea genotypes	In vitro protein digestibility[b]
BRS-Cauamé	34.98 ± 0.01 a
BRS-Marataoã	44.05 ± 0.01 b
BRS-Paraguaçu	43.02 ± 0.03 bc
BRS-Tumucumaque	38.18 ± 0.00 a
Canapuzinho	38.71 ± 0.00 ac
MNC01-631F-15	37.03 ± 0.01 a
MNC01-649F-2	29.39 ± 0.01 d

[a] Values are means ± S.D. ($n = 3$). Sequential in vitro digestion using pepsin and trypsin were carried out according to the method described by Tang et al. (2009).
[b] Mean values followed by different letters in the same column represent significantly different ($P < 0.05$).

determined in this work. Mesquita et al. (2007), working with 21 lines of *Phaseolus vulgaris*, have also reported no correlation between the digestibility and trypsin inhibitor or phenolic compound levels. In addition, there must also be differences between the results obtained by in vitro determination and those in vivo (Boye et al., 2010). Beans protein digestibility is usually between 40 and 70% (Mendonça et al., 2003) and the values are lower in raw seeds, increasing significantly after heat treatment (Shoshima et al., 2005). Most of cowpea proteins consist of globulins followed by albumins (Vasconcelos et al., 2010; Wang et al., 2003) and it is well known that globulins are resistant to digestion (Phillips et al., 2003). This fact could explain the low digestibility observed and this is somehow evident when the electrophoretic profiles of the genotypes are analyzed (Fig. 1). BRS-Marataoã showed the lowest concentration of globulins (45 kDa) and at the same time the highest digestibility among the 7 genotypes studied. Even more striking differences could have been observed if all the 30 genotypes had been analyzed for protein digestibility. There was a noticeable variation in the intensity of globulin bands (44–63 kDa), which are apparently in greater concentration in the genotype MNC01-631F-11 and in lowest concentration in the genotype BRS-Marataoã. Also, proteins of low molecular mass (below 30 kDa) appeared in higher concentration in the genotypes MNC01-611F-11, MNC01-631F-11, BR5-Guariba and proteins of high molecular mass (about 100 kDa) in genotypes Pingo de Ouro-1-2 and MNC01-631F-11. These results reinforce that the genetic breeding accomplished by conventional techniques can produce important changes in expression patterns of proteins present in seeds.

The mineral contents of the 30 analyzed genotypes were homogeneous (Table 5), with variation coefficient over 15% only for sodium. Concerning the improvement of zinc and iron levels (70 and 60 mg/kg, respectively) which were intended by the breeding program, iron levels reached the intentional amount but zinc levels were still far below the desired amount, with an average of 32.83 mg/kg. This result is noteworthy since over 60% and over 30% of world population are iron and zinc deficient, respectively (White and Broadley, 2009). Thus, more attention should be given to zinc in future breeding programs.

Seeds of 7 genotypes with protein contents over 25% were analyzed for the presence of antioxidant activity by observation of the ability to capture free radicals and content of some vitamins. The results on the ability to capture free radicals (Table 6) showed that the genotype BRS-Paraguaçu has the lowest activity (EC_{50} 38.7 mg seed extract/mL DPPH) while MNC01-631F-15 the highest activity (EC_{50} 9.54 mg seed extract/mL DPPH). These results are better than those reported by Heimler et al. (2005) studying different strains of P. vulgaris, with values ranging from 39 to 2810 mg seed extract/mL DPPH, i.e. the worst performance observed in the present study was equivalent to the best result described for *P. vulgaris*. Even knowing that legume seeds are not traditional sources of carotenoids, the search for this compound was performed in some genotypes, since there are reports in the literature on the presence of carotenoids in legume seeds (Amarowicza et al., 2011). None of the genotypes analyzed had detectable levels of beta-carotene (results not shown). Nevertheless, the presence of delta-tocopherol was detected in all genotypes and alpha-tocopherol was observed in 6 of the 7 genotypes

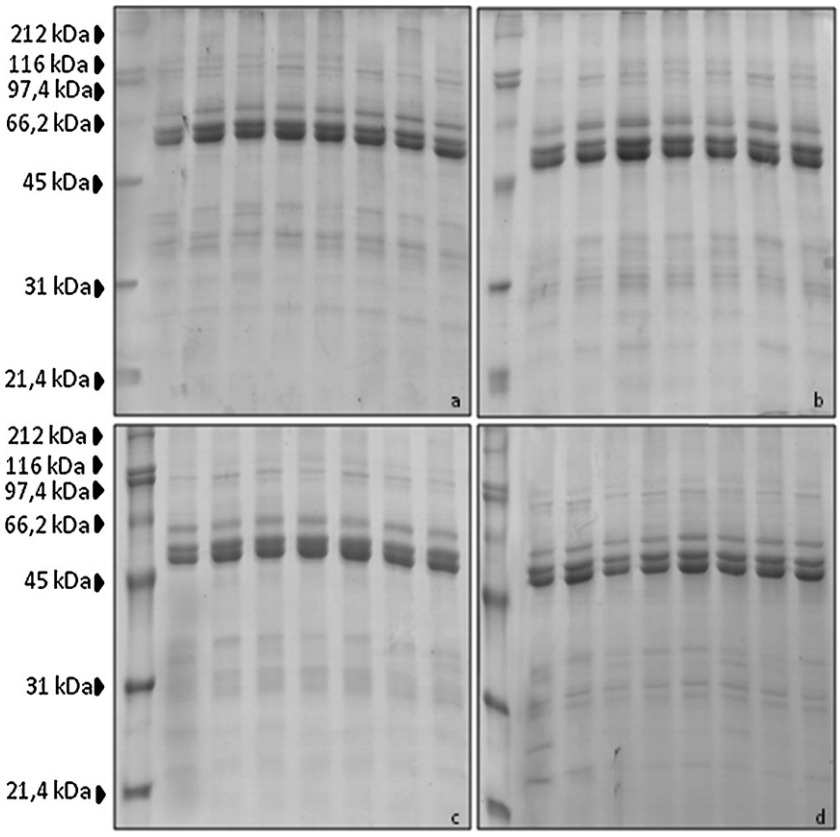

Fig. 1. Profiles of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of crude extracts of 30 newly developed Brazilian cowpea genotypes. From left to right: (a) Molecular markers, BRS-Pajeú, Pingo de Ouro-1-2, MNC99-537F-14-2, BRS-Xique-xique, MNC01-611F-11, BR5-Potengi, Pingo de Ouro-2, BR3-Tracuateua; (b) Molecular markers, MNC99-510F-16-1, BRS-Milênio, MNC01-631F-11, Inhuma, Paulistinha, BRS-Guariba, BR17-Gurguéia; (c) Molecular markers, BRS-Nova Era, BRS-Urubuquara, MNC01-614F-15, Canapuzinho-2, BRS-Rouxinol, MNC99-510F-16-3, Patativa; (d) Molecular markers, MNC01-649F-2, BRS-Tumucumaque, BRS-Marataoã, BRS-Cauamé, BRS-Paraguaçu, MNC01-631F-15, Canapuzinho, MNC99-537F-14-2. Molecular markers employed were myosin (212 kDa), β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumine (45 kDa), carbonic anhydrase (31.0 kDa) and soybean trypsin inhibitor (21.4 kDa).

Table 5
Mineral composition[a] (mg/100 g seed flour) of 30 newly developed Brazilian cowpea genotypes obtained by conventional plant breeding compared with patterns of mineral requirement for different age groups.

Cowpea genotypes	Fe	Zn	Na	K	Ca	Mg	Mn	Cu
BR17-Gurguéia	6.1 ± 0.0 ak	3.2 ± 0.0 ab	9.1 ± 0.5	1169 ± 36	44 ± 1	160 ± 4	2.0 ± 0.0	2.1 ± 0.0
BR3-Tracuateua	6.5 ± 0.2 abk	3.9 ± 0.4 cde	14.7 ± 0.6	998 ± 19	35 ± 1	131 ± 2	2.0 ± 0.0	2.2 ± 0.0
BRS-Cauamé	7.2 ± 0.3 cde	3.7 ± 0.4 ac	9.2 ± 0.5	1049 ± 70	37 ± 4	151.1 ± 9	1.9 ± 0.0	2.1 ± 0.0
BRS-Guariba	6.9 ± 0.1 bcf	3.3 ± 0.1 afg	9.5 ± 0.8	973 ± 9	35 ± 1	145 ± 1	2.9 ± 0.0	2.0 ± 0.1
BRS-Marataoã	6.1 ± 0.1 ak	3.0 ± 0.1 bfhi	10.1 ± 0.2	1225 ± 36	37 ± 0	147 ± 3	2.0 ± 0.0	2.1 ± 0.0
BRS-Milênio	6.8 ± 0.0 bcg	4.1 ± 0.1 ce	17.7 ± 0.7	957 ± 32	39 ± 0	137 ± 4	2.1 ± 0.0	2.2 ± 0.0
BRS-Nova Era	6.5 ± 0.1 abk	3.0 ± 0.0 bfij	15.0 ± 1.3	1004 ± 45	35 ± 2	140 ± 9	1.7 ± 0.0	2.1 ± 0.1
BRS-Pajeú	7.3 ± 0.1 cd	3.3 ± 0.0 afg	13.6 ± 0.8	1164 ± 38	35 ± 1	144 ± 7	2.1 ± 0.1	2.1 ± 0.1
BRS-Paraguaçu	6.3 ± 0.2 afgh	2.9 ± 0.0 bfik	12.0 ± 0.6	1081 ± 18	32 ± 0	151 ± 3	1.8 ± 0.0	2.1 ± 0.0
BRS-Potengi	8.1 ± 0.1 h	3.3 ± 0.0 ahjklm	8.4 ± 0.1	1048 ± 8	39 ± 0	159 ± 0	1.8 ± 0.0	2.1 ± 0.0
BRS-Rouxinol	7.3 ± 0.3 cd	3.4 ± 0.1 ahjg	12.4 ± 0.9	1251 ± 60	39 ± 0	145 ± 2	2.4 ± 0.0	2.1 ± 0.0
BRS-Tumucumaque	7.5 ± 0.2 di	3.6 ± 0.0 admn	10.0 ± 0.7	1106 ± 40	39 ± 4	158 ± 10	2.0 ± 0.0	2.1 ± 0.0
BRS-Urubuquara	7.4 ± 0.1 cd	4.4 ± 0.2 e	11.6 ± 0.2	1095 ± 39	51 ± 3	169 ± 9	1.9 ± 0.0	2.2 ± 0.0
BRS-Xique-xique	7.6 ± 0.2 dh	3.3 ± 0.1 aghjkl	14.1 ± 0.4	1127 ± 50	33 ± 0	142 ± 3	2.0 ± 0.0	2.0 ± 0.0
Canapuzinho	7.5 ± 0.2 dij	3.0 ± 0.1 bfhi	15.0 ± 1.3	1150 ± 91	37 ± 2	155 ± 8	2.0 ± 0.0	2.0 ± 0.0
Canapuzinho-2	6.6 ± 0.2 ab	3.4 ± 0.0 aghjk	9.0 ± 1.0	1098 ± 3	32 ± 1	140 ± 1	1.7 ± 0.0	2.1 ± 0.0
Inhuma	7.2 ± 0.0 cde	3.0 ± 0.0 bfik	13.9 ± 0.2	1117 ± 21	39 ± 0	150 ± 0	1.9 ± 0.0	2.0 ± 0.0
MNC01-611F-11	7.6 ± 0.5 dh	3.3 ± 0.3 aghjkl	14.7 ± 0.1	1238 ± 37	39 ± 1	159 ± 1	2.1 ± 0.1	2.0 ± 0.0
MNC01-614F-15	6.6 ± 0.2 ab	2.9 ± 0.2 bfil	13.6 ± 0.4	1011 ± 32	34 ± 0	130 ± 4	1.8 ± 0.2	2.1 ± 0.0
MNC01-631F-11	6.9 ± 0.5 bcjl	3.1 ± 0.1 bfhin	12.8 ± 0.1	1183 ± 11	37 ± 1	147 ± 2	2.0 ± 0.2	2.0 ± 0.0
MNC01-631F-15	6.2 ± 1.1 afk	2.7 ± 0.4 i	13.3 ± 0.0	1125 ± 25	38 ± 3	133 ± 0	1.9 ± 0.0	2.1 ± 0.0
MNC01-631F-20-5	7.0 ± 1.2 bcd	3.7 ± 0.8 cgm	15.7 ± 0.4	1189 ± 49	31 ± 1	136 ± 1	2.2 ± 0.0	2.0 ± 0.0
MNC01-649F-2	7.0 ± 1.2 bci	3.1 ± 0.8 bfhio	13.7 ± 0.7	1219 ± 18	36 ± 1	147 ± 3	2.1 ± 0.0	2.1 ± 0.0
MNC99-510F-16-1	6.0 ± 2.0 k	3.5 ± 2.8 agno	10.2 ± 1.7	1116 ± 21	38 ± 4	148 ± 3	2.0 ± 0.0	2.1 ± 0.0
MNC99-510F-16-3	6.9 ± 0.1 bcil	3.1 ± 1.1 bfhin	12.7 ± 0.6	1148 ± 23	41 ± 3	150 ± 3	2.2 ± 0.0	2.0 ± 0.0
MNC99-537F-14-2	6.7 ± 1.3 abe	2.8 ± 0.1 bi	11.5 ± 0.1	1033 ± 55	32 ± 1	148 ± 9	1.8 ± 0.0	2.1 ± 0.0
Patativa	7.4 ± 2.4 cd	3.3 ± 0.4 aghjkl	15.5 ± 1.0	1190 ± 6	30 ± 1	142 ± 1	2.0 ± 0.0	2.1 ± 0.0
Paulistinha	6.6 ± 0.8 ab	2.8 ± 1.4 bfi	11.7 ± 0.3	1045 ± 79	35 ± 1	131 ± 7	1.8 ± 0.0	2.1 ± 0.0
Pingo de Ouro-1-2	6.4 ± 1.1 afgkl	3.3 ± 0.9 aghjkl	9.8 ± 0.7	1034 ± 17	29 ± 1	135 ± 2	1.7 ± 0.0	2.1 ± 0.0
Pingo de Ouro-2	7.2 ± 0.8 cde	3.2 ± 1.1 aghjkl	14.1 ± 1.1	1068 ± 23	44 ± 8	142 ± 2	1.9 ± 0.0	2.0 ± 0.0
RDA/*AI*[b] (mg/day)	*0.3*–**27**	*2*–**13**	*120–1500*	*400–5100*	*21–130*	*30*–**420**	*0.003–2.6*	*0.2*–**1.3**

[a] Values in dry basis are means ± S.D. ($n = 3$). Mean values followed by different letters in the same column represent significantly different ($P < 0.05$).
[b] The mineral requirements for the most and less demanding age group (IOM – Dietary reference intakes: the essential guide to nutrient requirements, 2006). Recommended Dietary Allowance (**RDA**) (bold letters): the average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group. Adequate Intake (*AI*) (italic letters): the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when an RDA cannot be determined.

analyzed (Table 6). Grelaap and Gunterb (1995) have reported 34.1 mg/g of gamma-tocopherol and 6.3 mg/g of delta-tocopherol in seeds of *P. vulgaris*. These values are higher than the highest value reported in the present study (BRS-Marataoã 0.38 mg/g for alpha-tocopherol and 1.88 mg/g for delta-tocopherol). The total content of tocopherols in *V. unguiculata* can be far higher than those observed in the present study since the levels of gamma-tocopherol, not analyzed in this study, were higher than those of alpha-and delta-tocopherol for all seeds analyzed by Grelaap and Gunterb (1995).

In order to rank the 30 genotypes according to nutritional quality, an index was constructed as a weighted average of desirable nutritional attributes of each genotype as was presented previously. The weights assigned to the different components were chosen arbitrarily, but following the relative importance of each, as follows: protein content higher than 25% (weight 4), considering that about 5 genotypes had levels above 25%; fiber content, greater than 25% (weight 2), considering that most genotypes showed contents greater than 25%; iron and zinc, more than 60 mg/kg and 30 mg/kg (weight 3), respectively, based upon the average values

Table 6
Values for free radical scavenging capacity and levels of alpha- and delta-tocopherol from 7 newly developed Brazilian cowpea genotypes with high content of crude protein (>25 g/100 g seed flour).[a]

Cowpea genotypes	DPPH• EC_{50} (mg seed extract/mL DPPH•)[b]	Alpha-tocopherol (g/kg seed flour)	Delta-tocopherol (g/kg seed flour)
BRS-Cauamé	26.10 ± 2.67 a	0.02 ± 0.00 a	1.36 ± 0.04 ab
BRS-Marataoã	12.63 ± 0.24 b	0.38 ± 0.05 b	1.88 ± 0.02 ab
BRS-Paraguaçu	38.7 ± 1.78 a	0.16 ± 0.02 c	1.90 ± 0.05 ab
BRS-Tumucumaque	32.73 ± 1.11 a	0.07 ± 0.04 ac	1.30 ± 0.01 a
Canapuzinho	11.36 ± 0.70 b	0.02 ± 0.01 a	0.79 ± 0.04 b
MNC01-631F-15	9.54 ± 0.98 b	0.02 ± 0.00 a	1.25 ± 0.07 ab
MNC01-649F-2	26.26 ± 5.22 a	ND[c]	1.18 ± 0.13 a
Controls			
Butyl-hydroxy-toluene (BHT)[d]	0.165 ± 0.01 c	–	–
Quercetin[d]	0.081 ± 0.03 d	–	–
Rutin[d]	0.295 ± 0.01 e	–	–

[a] Values are means ± S.D. ($n = 3$). Mean values followed by different letters in the same column represent significantly different ($P < 0.05$).
[b] Efficient concentration (EC), as mg of sample required to decrease one g of the initial 2,2-diphenyl-1-picrylhydrazyl (DPPH•) concentration by 50%.
[c] Not detected; the limit of detection was 0.0024 μg/μL based upon serial dilution of the used standards.
[d] Substances that are frequently used as standard for antioxidant activity studies.

obtained for the studied genotypes. The upper limits for trypsin and chymotrypsin inhibitors were established as smaller than 3 IU/μg protein (weight 1 for each) based on the average of the respective values found for the genotypes studied. The upper limit for hemagglutinating activity was assigned as less than 80,000 UH/kg flour (weight 1) based on the most frequent low value observed for the genotypes studied. Besides, all values of hemagglutinating activity were divided by a factor of 10,000 to avoid very high figures for the index.

The results showed that in the descending order the 5 best genotypes were: BRS-Cauamé > BRS-Tumucumaque > Canapuzinho > BRS-Potengi > BRS-Urubuquara.

4. Conclusions

V. unguiculata has a great genetic potential to be exploited since it shows great variability in nutrient and anti-nutrient contents. Many of these variations (intentional or unintentional) can be generated by conventional genetic breeding to attend the nutritional needs in developing countries. In addition, the utilization of a nutritional quality index will allow pinpointing the genotypes that gather the largest number of desirable nutritional attributes and then assist in the planning of new crosses in the breeding program.

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