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Nutritional evaluation of cashew (*Anacardium occidentale*, L.) nut protein concentrate and isolate

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This study evaluated the nutritional qualities of a protein concentrate and an isolate produced from cashew nut. The nutritional qualities were evaluated by determining amino-acid composition, *in vitro* digestibility and anti-nutritional factors (tannins, trypsin inhibitor activity-TIA and phytic acid content) in the protein concentrate and isolate using standard analytical methods. The amino-acid with the highest concentration in defatted cashew nut powder (DCNP), cashew nut protein concentrates (CNPC), and cashew nut protein isolate (CNPI) was glutamic acid, which was found to be 22.5, 21.38, and 21.81 g/100 g, respectively. This was followed by leucine, aspartic acid and arginine, in that order. The amino-acid with the lowest concentration in DCNP, CNPC, and CNPI was cysteine. The sulphur-containing amino-acids and some other essential amino-acids (lysine, tryptophan, leucine, isoleucine, and tyrosine) in CNPI were not significantly different ($p>0.05$) from that of DCNP, but were significantly different from that of CNPC ($p<0.05$). CNPC and CNPI were rich in essential amino-acids, and based on the FAO/WHO recommended essential amino-acids pattern requirements for an infant, the limiting amino-acid in CNPC, and CNPI was lysine with chemical scores of 0.68, and 0.63 respectively. However, the anti-nutritional factors (tannins, TIA, and phytic acid contents) of CNPI were found to be lower than those in DCNP and CNPC, while those of CNPI and CNPC were within the range found in the commercial peanut and soy protein concentrates and isolates. The highest *in vitro* digestibility was observed in CNPI (95.30%), while CNPC (87.83%) had a higher value than DCNP (79.93%). The nutritional qualities of the protein concentrate and isolate from cashew nut were found to be comparable to those reported for commercial peanut and soy protein concentrates and isolates. Therefore, the cashew nut products could be suitable as additional source of protein ingredients in food formulations.

Key words: Cashew, protein, nutrition, isolate, concentrate.

INTRODUCTION

Proteins utilised in food processing are of various origins, and can be roughly grouped into animal proteins (gelatin), vegetable proteins (peanut protein), and animal-derivative proteins (milk proteins) (Penny, 1999). Protein concentrates and isolates are used for functional and nutritional food applications in consumer foods (Lin,

1997). Proteins that are essential to growth and health are currently required more in developing countries of the world, because of prevalent outbreak of protein-energy malnutrition in these countries (FAO, 1997). Animal proteins, which are of higher quality and the choice of most individuals, are becoming more expensive to produce.

Supply shortages plus high prices have caused restriction of animal protein consumption in the diets of many families in developing countries of the world (Penny, 1999). However, vegetable proteins which are cheaper and available, offer great potential as a direct food for human consumption.

Cashew is of considerable economic importance because its components have various economic uses. For example, cashew apple is used as an ingredient in the production of cashew beverages and spirits, while cashew kernel is of high food value with about 40-57% oil and 21% protein content (Fetuga et al., 1975). Cashew nut is an important delicacy, which is mainly used in confectionery and as a dessert nut. It was shown that the powdered milk used in the standard milk chocolate recipe can be replaced with 25% roasted cashew kernel (Ogunwolu and Akinwale, 2003).

A recent survey jointly carried out by the Cocoa Research Institute of Nigeria (CRIN) and Bio-hybrids Agriculture Systems Ltd, UK, showed that the number of cashew farmers is increasing yearly while the areas of land cultivated to the crop has increased considerably (Topper et al., 2001). Also, it was reported that the annual raw cashew nut production in Nigeria increased from 727 tons in 1970 to 70,000 tons in the year 2000 (FAO, 2001).

In view of increasing production and limited utilization of cashew globally, and inadequate intake of protein particularly in Africa, where animal proteins are unaffordable by most inhabitants, the cashew kernel is being considered as a suitable raw material to produce protein concentrates and isolates for use in human food products. The work presented here evaluates the nutritional quality of the protein concentrate and isolate produced from cashew nut.

MATERIALS AND METHODS

Source of materials

Cashew nuts were obtained from the cashew plots of Cocoa Research Institute of Nigeria, Ibadan, Nigeria. Chemicals and equipment used were from the Laboratories of Applied Biochemistry group of Institute for plant genetics and crop plant research, Gatersleben, Germany and that of Animal Nutrition Department, Institute of Agricultural Research and Training, Ibadan, Nigeria.

Cashew nut processing, cashew nut powder production and protein extraction

Cashew nut processing, production of cashew nut powder, extraction of protein concentrate and isolate were done as described by an earlier publication (Ogunwolu et al., 2010).

High performance liquid chromatography (HPLC) analysis of cashew nut meal and protein fractions for amino acid composition

Sample hydrolysis

Each sample (0.5 mg) was weighed in triplicates, dissolved in 100 μ l of 6 N HCL and 1 μ l phenol in vials. Each vial was flushed with nitrogen gas for 1 min, and the vials were sealed, vortexed and dried in heating block (BIOBLOCK Scientific, model 92675-Thermolyne corp. USA) at 110°C for 24 h. The samples were allowed to cool to room temperature.

Reconstitution and derivatization of samples

The samples were evaporated to dryness. To each sample, 200 μ l of 20 mM HCL was added, vortexed, centrifuged, and the supernatant removed. A (70 μ l) aliquot of (AccQ. Fluor Borate buffer) was added to 10 μ l of each sample in the sample tube and vortexed. Derivatization buffer (20 μ l) was added to each tube, vortexed, and allowed to stay for 1 min. Samples were transferred to an auto sampler vial (low volume insert) and capped with silicon-lined septum. The vials were heated in a heating block at 550°C for 10 min.

Analysis

Sample diluents were added to each above prepared sample in the tube, mixed and centrifuged (Eppendorf microtube) for 5 min at 5000 rpm. Each sample (30 μ l) was transferred (using pipette) into HPLC vials, closed and loaded into HPLC (Waters with FP1520 intelligent fluorescence detector and water 717 plus-autosampler). Sample injection volumes (of 2 to 8 μ l) were used.

In vitro protein digestibility

This was carried out according to the method described by Hsu et al. (1977). Briefly, each sample (31.25 mg) was dissolved in 5 ml of distilled water and adjusted to pH 8.0 with 0.1 N NaOH while stirring at 37°C. A multi-enzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per 1 ml of distilled water, was maintained in an ice bath and adjusted to pH 8.0 as described above. An aliquot (0.5 ml) of the multi-enzyme solution was added to the protein sample solution and stirred, maintaining the temperature at 37°C. The pH of the solution was recorded 10 min after adding the enzyme solution. The *in vitro* digestibility was calculated using the following equation (Hsu et al., 1977):

$$Y = 210.46 - 18.1x$$

Where, Y = *In vitro* digestibility (%) and x = pH of the sample suspension after 10 min digestion with multi-enzyme solution.

Anti-nutritional factors of cashew nut proteins

Tannins content

This was determined using the vanillin-HCl method as described by Bhagya et al. (2006). Catechin that was used as standard was prepared as follows; catechin (2.5 mg) was dissolved in 1 ml distilled water, and the following concentrations prepared; 2.5 μ g = 1 μ l catechin solution + 999 μ l methanol; 5.0 μ g = 2 μ l catechin

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solution + 998 μ l methanol; 7.5 μ g = 3 μ l catechin solution + 997 μ l methanol; 10.0 μ g = 4 μ l catechin solution + 996 μ l methanol 12.5 μ g = 5 μ l catechin solution + 995 μ l methanol.

Each sample (500 mg) was extracted with 500 μ l methanol at 25°C for 12 h with shaking. Decanted methanol was then made-up to 1.25 ml and filtered using Whatman No. 1 filter paper. Sample extracts (50 μ l) were treated with 250 μ l of a reagent mixture (1:1 4% vanillin in methanol: 8% concentrated HCl in methanol). Reagent mixture (250 μ l) was also added to each standard concentration. These were allowed to incubate at 25°C for 30 min. The colour developed was read with spectrophotometer at 500 nm. Concentration of the standard was then plotted against their measured absorbances, and the regression equation obtained was used to calculate the concentration of tannins as catechin equivalent.

Trypsin inhibition activity (TIA)

This was determined using an enzymatic assay (Bhagya et al, 2006); each sample (250 mg) was extracted with 12.5 ml of 0.01 M NaOH for 3 h with shaking. The sample extracts were diluted 30 times with distilled water. The suspension was maintained between pH 8.4 and 10.0, using 0.1 M NaOH. Trypsin (0.5 ml) solution (1 mg in 50 ml 0.001 M HCl) was added to each sample and incubated at 3°C for 10 min. BAPNA (5 ml) [40 mg of N- α -Benzoyl-DL-Arginine p-nitroanilide hydrochloric acid in 1 ml dimethyl sulfoxide diluted to 100 ml with Tris buffer at 37°C] was added and the reaction was terminated after 10 min by the addition of 0.25 ml of 30% acetic acid. The sample mixtures were then mixed and filtered. The absorbance was measured at 410 nm against the blank. Blank was prepared by mixing 0.25 ml acetic acid with 0.5 ml trypsin 0.5 ml distilled water and 1 ml BAPNA. The absorbance of the pure trypsin solution was also measured under the same conditions. Trypsin inhibitor activity was calculated using the formula:

$$\text{TIA (mg/g)} = \frac{2.632 \times D \times A_1}{S}$$

Where, D = Dilution factor, A_1 = change in absorbance between pure trypsin and sample extracts; S = sample weight (g)

Phytic acid content

This was determined according to the method described by Gullberg et al. (2004); Each sample (100 mg) was extracted using chloroform : methanol : water mixture in the ratio of 20:60:20 ml. The samples were mixed at 4°C and shaken for 20 min. The samples were then centrifuged at 3,000 rpm at 4°C for 10 min. The super-natants were transferred into a new Eppendorf tube, and equal volume (2) of aliquots of each sample were dried in speed vacuum (Refrigerated vapour trap, model; RVT 400 by SAVANT Company, USA) at 35°C for 18 h. Dried pellets were re-suspended in 200 μ l of HPLC-water, and the two aliquots were combined. This sample (200 μ l) was purified using microtiter plate containing a 10 kDa filter (micron, 10 KD, Millipore). HPLC-water (20 μ l) was added to each filter before loading the samples. The filtrates of the samples were then centrifuged at 4°C and 4000 rpm for 60-80 min. The samples were then transferred into HPLC-MS vials. Phytic acid hydrate with calcium (10 mM) from rice was used as a standard: 20 μ l each of the samples and standard were injected into the High performance liquid chromatography-mass spectrometer (HPLC-MS).

Statistical analysis

Determinations were made in triplicates; standard errors of the

mean (SEM) and analysis of variance (ANOVA) in SPSS (version 10.1, 2000, SPSS Inc., USA) were used to analyse the results. Means were separated using Duncan multiple range test. Significance was accepted at 0.05 level of probability.

RESULTS

Amino-acid composition

Amino-acid composition data of DCNP, CNPC, and CNPI are presented in Table 1. The amino-acid contents of the three samples were similar, even though DCNP generally had higher values than CNPI, which were also higher than that of CNPC. The amino-acid with the highest concentration in DCNP, CNPC, and CNPI was glutamic acid with values of 22.5, 21.38, and 21.81 g/100 g, respectively. Leucine was the second most abundant amino-acid followed in decreasing order by aspartic acid and arginine (Table 1). The amino-acid with least content in the DCNP, CNPC, and CNPI samples was cysteine with values of 1.02, 0.97, and 1.01 g/100 g, respectively (Table 3). The contents of sulphur-containing amino-acids and some other essential amino-acids (lysine, tryptophan, leucine, isoleucine, and tyrosine) in CNPI were not significantly different from those of DCNP, but were significantly different from those of CNPC ($p < 0.05$).

Essential amino-acid contents (mg/g protein) and suggested pattern of amino-acid requirements for infants, and pre-school children (2-5 years)

The essential amino-acid contents of DCNP, CNPC, and CNPI were compared with the two suggested patterns of amino-acid requirements for infants, and pre-school children (2-5 years) (Table 2). The calculated amino-acid scores (Tables 3 and 4) show that DCNP, CNPC, and CNPI were rich in essential amino-acids. Based on the FAO/WHO recommended essential amino-acids pattern requirements for infants, the limiting-amino acid in DCNP, CNPC, and CNPI was lysine with chemical scores of 0.69, 0.68, and 0.63, respectively (Table 3).

Taking into consideration the FAO/WHO recommended pattern of essential amino-acid requirements for pre-school children (2-5 years), the limiting amino-acid in DCNP, CNPC, and CNPI was lysine with chemical scores of 0.78, 0.77, and 0.72, respectively (Table 4).

Anti-nutritional factors and *in vitro* digestibility

Factors adversely affecting digestion and nutrient absorption such as Tannin content, TIA, and phytic acid content of DCNP, CNPC, and CNPI are shown in Figure 1. Tannin content of DCNP (1.99%) was found to be higher than that of CNPC (1.82%), which was higher than that of CNPI (0.80%). Trypsin Inhibition activity of the CNPI (0.21 mg/g) was found to be lower than that of

Table 1. Amino-acids composition of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI).

Amino acid	DCNP(g/100 g)	CNPC(g/100 g)	CNPI(g/100 g)
Lysine	4.52±0.11 ^a	4.18±0.18 ^b	4.46±0.12 ^a
Histidine	2.69±0.15 ^a	2.49±0.10 ^b	2.67±0.12 ^a
Arginine	10.18±0.16 ^a	9.83±0.03 ^b	9.95±0.03 ^b
Aspartic acid	10.38±0.10 ^a	10.21±0.04 ^b	10.25±0.05 ^a
Threonine	3.46±0.05 ^a	3.16±0.02 ^b	3.20±0.01 ^b
Serine	5.79±0.02 ^a	5.21±0.03 ^c	5.50±0.02 ^b
Glutamic acid	22.50±0.02 ^a	21.38±0.03 ^c	21.81±0.21 ^b
Proline	5.41±0.01 ^a	5.23±0.03 ^c	5.36±0.01 ^b
Glycine	5.35±0.03 ^a	5.19±0.01 ^c	5.29±0.01 ^b
Alanine	4.39±0.01 ^a	4.04±0.07 ^b	4.15±0.13 ^b
Cysteine	1.02±0.03 ^a	0.97±0.02 ^b	1.01±0.01 ^a
Valine	5.58±0.03 ^a	5.24±0.04 ^c	5.51±0.01 ^b
Methionine	2.28±0.02 ^a	2.21±0.01 ^b	2.27±0.02 ^a
Isoleucine	4.18±0.02 ^a	4.09±0.01 ^b	4.17±0.02 ^a
Leucine	11.48±0.02 ^a	11.32±0.03 ^b	11.47±0.02 ^a
Tyrosine	3.32±0.03 ^a	3.29±0.01 ^a	3.31±0.01 ^a
Phenylalanine	4.53±0.02 ^a	4.49±0.01 ^b	4.51±0.01 ^b
Tryptophan	1.38±0.01 ^a	1.36±0.01 ^b	1.37±0.01 ^a

Means followed by the same alphabetic on the row are not significantly different at $p < 0.05$.

Table 2. Essential amino-acids contents of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI), and suggested pattern of amino-acid requirements for infants and pre-school children (2-5 years).

Amino-acids	DCNP (mg/g protein)	CNPC (mg/g protein)	CNPI (mg/g protein)	*Amino-acid requirements for infants (mg/g protein)	*Amino-acid requirements for children (mg/g protein)
Isoleucine	41.8	41.7	40.9	46.0	28.0
Leucine	114.8	114.7	113.2	93.0	66.0
Lysine	45.2	44.6	41.8	66.0	58.0
Tryptophan	13.8	13.7	13.6	17.0	11.0
Valine	55.8	55.1	52.4	55.0	35.0
Methionine +Cysteine	33.0	32.8	31.8	42.0	25.0
Tyrosine	34.6	32.0	31.6	43.0	34.0

* FAO/WHO/UNU (1991).

CNPC (0.59 mg/g), which was lower than that of DCNP (1.91 mg/g). Phytic acid content of the DCNP (6.44 g/kg) was found to be higher than that of CNPC (4.50 g/kg), which was higher than that of CNPI (3.54 g/kg).

Figure 2 shows the *in vitro* digestibility of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI). The highest digestibility was observed in CNPI (95.30%), while the *in vitro*-digestibility of CNPC (87.83%) was found to be higher than that of DCNP (79.93%).

DISCUSSION

Some of the amino-acid contents of DCNP were signi-

ficantly higher ($p < 0.05$) than those of CNPI and CNPC. This is likely a result of different processing steps involved in the production of CNPI and CNPC, and is in agreement with the findings of Lo and Hill (1971) who reported that the amino-acid content of rapeseed meals were higher than that of rapeseed protein concentrate. Also, similar trends were observed in sunflower flour and its concentrate (Canella et al, 1982).

All the three samples (DCNP, CNPC, and CNPI) were found to be rich in essential amino-acids, and when compared with suggested pattern of amino-acid requirements for infants, and pre-school children (2-5 years); lysine was found to be the limiting essential amino-acid. The use of two amino-acid reference patterns was in

Table 3. The chemical score in defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI) based on amino-acid requirements for infants.

Amino-acids	*DCNP	*CNPC	*CNPI
Isoleucine	0.91	0.91	0.89
Leucine	1.23	1.23	1.22
Lysine	0.69	0.68	0.63
Tryptophan	0.81	0.81	0.80
Valine	1.02	1.00	0.95
Methionine + Cysteine	0.79	0.78	0.78
Phenylalanine + Tyrosine	1.09	1.09	1.08
Threonine	0.81	0.74	0.74
Chemical score	0.69	0.68	0.63
Limiting amino acid	Lysine	Lysine	Lysine

* Calculated based on recommended pattern for infants FAO/WHO/UNU (1991).

Table 4. The chemical score in defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI) based on amino-acid requirements for pre-school children (2-5 year).

Amino-acids	DCNP*	CNPC*	CNPI*
Isoleucine	1.49	1.49	1.46
Leucine	1.74	1.74	1.72
Lysine	0.78	0.77	0.72
Tryptophan	1.26	1.25	1.24
Valine	1.59	1.57	1.50
Methionine + Cysteine	1.32	1.31	1.27
Phenylalanine + Tyrosine	1.25	1.24	1.24
Threonine	1.02	0.94	0.93
Chemical score	0.78	0.77	0.72
Limiting amino acid	Lysine	Lysine	Lysine

*Calculated based on recommended pattern for pre-school children (2-5 yr) FAO/WHO/UNU (1991).

order to include infants in the protein evaluation, which is in accordance with the Food and Agriculture Organisation (FAO) regulation (FAO/WHO/UNU, 1991). The FAO regulation states that amino acid reference pattern for pre-school child (2-5 years) should be used for the evaluation of protein quality for all age categories except infants.

Tannins, TIA, and phytic contents were found to be lowest in CNPI while higher values were recorded for CNPC and CNPI. This may be as a result of processing involved in the production of these products. Reduction in tannin due to processing might have been caused by the activity of polyphenol oxidase or fermenting micro flora on tannins (Reddy and Pierson, 1994). The tannin contents of the samples are within the range of the values reported for commercial peanut protein concentrate and isolate (1.36%) (Fardiaz and Markakis, 1981). This is very important because higher tannin content could make the

proteins unavailable for human nutrition. Previous work suggests, protein-tannin complex appeared to be formed by multiple hydrogen bindings between phenolic hydroxyl groups of tannins and carbonyl groups of protein peptides bonds of digestive enzyme, inhibiting proteolytic enzyme activity in the gastro-intestinal track (Bressani, 1983). Trypsin Inhibition Activity is predominantly proteins and located, for the most part, with the main storage proteins in the protein bodies of the cotyledon. Thus trypsin inhibitors tend to fractionate with the milieu of storage proteins as they are processed (Horisberger et al., 1986). This implies that processing may affect the TIA content of the cashew nut, and would explain the differences in the TIA contents of DCNP, CNPC, and CNPI we observed. The trypsin inhibitors are known to have high amounts of cysteine in their structure (Lawrence and Nielsen, 2001), suggesting that a reduction in the cysteine composition of DCNP, CNPC, and CNPI was associated with an

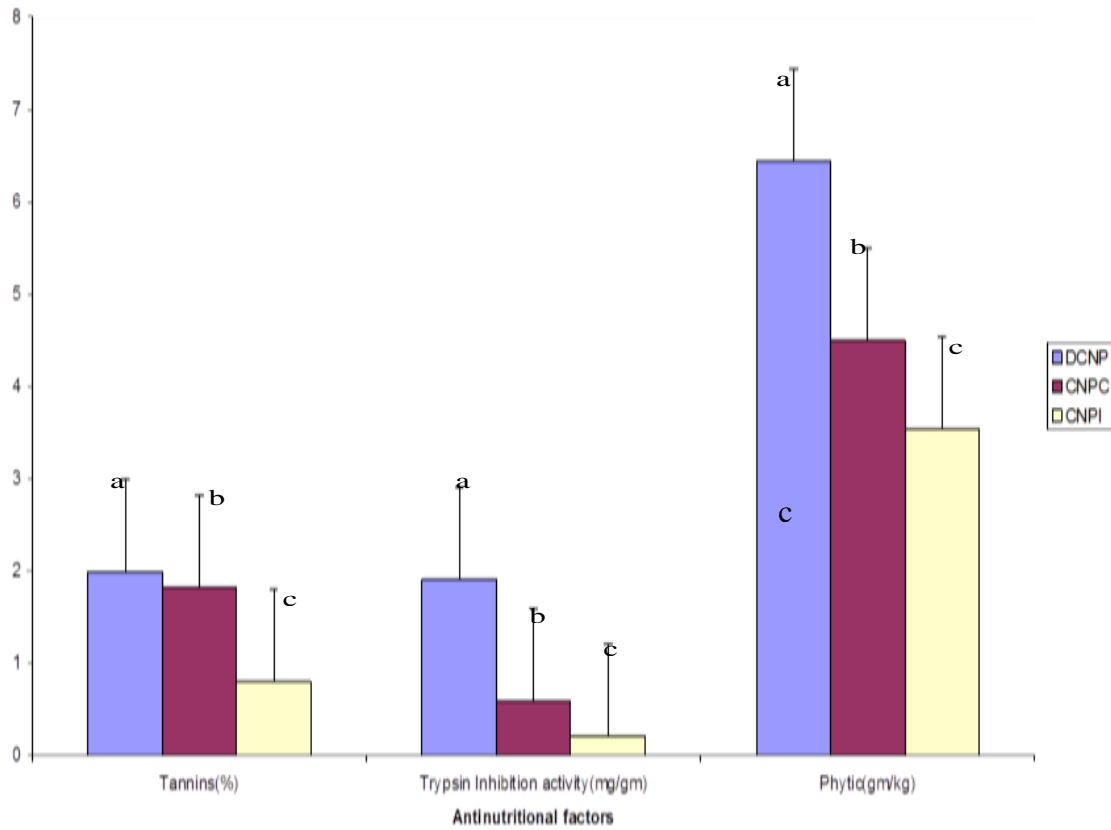


Figure 1. Anti-nutritional factors of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI).

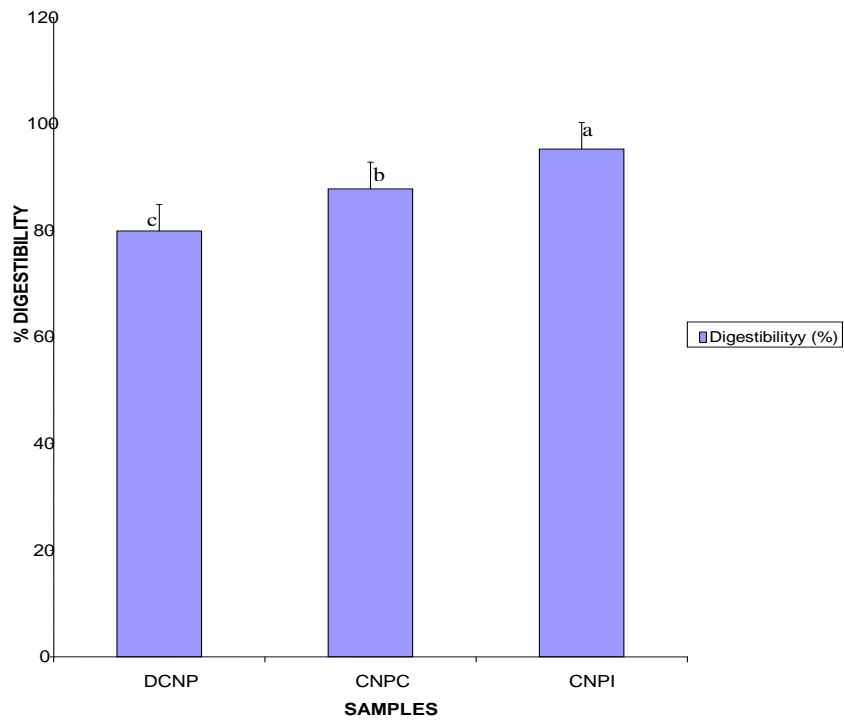


Figure 2. *In vitro* digestibility of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI).

observed reduction in protease inhibitory activity of CNPI and CNPC. The higher TIA content of CNPC (which was methanol precipitated) than CNPI could be explained by the reported findings that TIA is methanol soluble (Idouraine et al, 1991). The value of TIA in DCNP, CNPC, and CNPI was lower than that of defatted soy flour (16 mg/g), commercial soy protein concentrate (8 mg/g of protein), and soy protein isolate (1.4 mg/g of protein), as previously reported (Anderson and Wolf, 1995). This is important because TIA factor was found to form complexes with trypsin enzymes thereby impairing its proteolytic activity, which in turn reduced availability of amino-acids for metabolic processes (Liener, 1989). Phytic acid has long been recognised to interfere with the absorption of minerals, especially calcium, magnesium, iron and zinc; phytic acid is also reported to have anti-carcinogenic properties (Messina and Barnes, 1991). The difference in the phytic acid level of DCNP, CNPC, and CNPI could be as a result of the protein level and method of fractionation. According to a previously reported finding (Chau and Cheung, 1997), protein level, process of fractionation, and protein conformation certainly affected the phytic level in the isolated extracts. Also, at alkaline pH, phytate interaction with proteins diminishes, because the lysine and arginine groups lose their charge and thus the capacity to form complexes; also salts like calcium, magnesium are insoluble under alkaline conditions (Martinez-Dominguez et al., 2002). These may explain the reduction in the phytic acid composition of CNPI and CNPC when compared to DCNP. The phytic acid composition of defatted soybean (13.0 g/kg), soybean protein concentrate (12.5 g/kg), and soybean protein isolate (10.1 g/kg) as previously reported (Honig et al., 1984), are higher than the values obtained in this work for DCNP, CNPC, and CNPI.

The *in vitro* digestibility results indicated that CNPI has highest digestibility, followed by CNPC, and then DCNP. This may be as a result of the processing method used, as isoelectric precipitation denatures proteins extracted from legume flours, making them more susceptible to enzymatic attack (Chau and Cheung, 1997). The digestibility value obtained for CNPI (97%) is similar to what was obtained for soy protein isolate (Gilani and Sepher, 2003). The difference in the digestibility of DCNP, CNPC and CNPI could be as a result of the difference in the level of anti-nutritional factors in these samples (Fagbemi et al., 2005). Different interactions have been described between tannins and dietary protein, and tannins and digestive enzymes (Jansman et al., 1994). Also, tannins have the ability to bind dietary protein into an indigestible form (Glick and Joslyn, 1970). Therefore the reduction achieved for the anti-nutritional factors in the CNPC and CNPI when compared to that of DCNP, could be directly related to this improved digestibility. Digestibility of protein is considered a good approximation of the bioavailability of amino acids of mixed diet and properly processed food products that

contain minimal amounts of residual anti-nutritional factors (FAO/WHO/UNU, 1991).

Conclusion

The high essential amino-acid composition, high digestibility and low anti-nutrients contents of CNPI and CNPC could make them good protein sources for fortification of a variety of food products to combat protein deficiency in many parts of the world, particularly in developing countries.

Conflict of interests

The authors did not declare any conflict of interest.

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