Original Research Article

Chemical and Nutritional Evaluation of Pumpkin (*Cucurbita pepo*) Seed Proteins

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ABSTRACT

Chemical and nutritional properties of pumpkin (*Curcubita pepo*) seed proteins were studied. The seed was processed into defatted flour (CPF) which was further processed into *Curcubita* protein concentrate (CPC) and *Curcubita* protein isolate (CPI) by alkaline water/isoelectric precipitation. Chemical properties of the protein products were determined using standard methods of analysis. Amino acid profile was determined by a fully automated Technicon® liquid chromatography system. Protein digestibility was assessed in-vitro (IVPD) using trypsin-pepsin enzyme method while biological values were determined on the basis of their amino acid profile. Protein efficiency ratio (PER) was estimated according to a standard proposed regression equation. The seed proteins demonstrated high levels of crude protein (CPC=69.98% and CPI=74.15%), vitamin C (CPC=43.46 and CPI=52.36 mg/ml) and vitamin A (CPC=100.56 and CPI= 63.43 I.U/g) with low levels of thiamin and riboflavin. Both proteins showed low and similar ($p>0.05$) levels of sodium (0.14-0.18%), calcium (0.86-1.02%), magnesium (0.53-0.58%) and phosphorus (0.09-0.11%). Percentage ratios of essential to total amino acids obtained for CPC and CPI (44.24% and 45.50% respectively) were greater than 36% which is considered adequate for an ideal protein. Protein biological values obtained for CPC and CPI respectively were: 95% and 53% (chemical score), 2.80 and 1.56 (PER} and 70.10% and 51.28% (essential amino acid index). CPC showed a better digestibility than CPI with IVPD value of 56.88%. Threonine and lysine were the most limiting amino acids in both protein products. All anti-nutrients evaluated were low and below allowable limits. In conclusion pumpkin seed proteins showed good biological values and could be used to improve the quality of other plant proteins or as possible replacement for animal proteins in conventional foods.

Keywords: [pumpkin seed; protein concentrate; protein isolate; amino acid; biological value]

1 INTRODUCTION

The serious consequences of malnutrition particularly among infants and children form a primary roadblock to social and economic development. This condition has engaged the attention of national agencies with numerous new programs to cope with the overall problem. Such programs include the explosive proliferation, distribution and marketing of various protein-rich foods such as legumes and oil seed proteins. Recently more attention has been focused on the use of underutilized agricultural products. This is mostly the case in developing countries like Nigeria where the emphasis has been on improving the use of locally available crops by complementing cereal products with protein sources in order to improve their amino acid profile. Such utilization would also contribute to the production of various new foods. Pumpkin seeds are eaten when roasted or used as baking ingredient for bread and cake mostly in developed countries. The seed and seed oil are rich sources of protein, vitamins, polyunsaturated fatty acids and carotenoids [1]. Pumpkin seed (*Cucurbita pepo*) has received considerable attention in recent years because of its nutritional and health benefits [2]. The extract from *Cucurbita pepo* fruit and seed is known to improve urinary dysfunction and prostatic hyperplasia as well as confers antioxidant, anti-inflammatory and antimicrobial benefits. It has also been used as a hypoglycemic agent [3]. Proteins which are used in food and pharmaceutical industries could be produced as concentrates or isolates and these form important ingredients in many food processes where they exhibit specific functions. This study aims to examine the chemical composition and nutritional potentials of pumpkin seed protein concentrate and isolate.

2. MATERIAL AND METHODS

Pumpkin seeds were extracted from the fruits planted without chemical treatments and harvested from a farm at Umuigu Oboro, Ikwuano Local Government Area in Abia State, Nigeria. All reagents used in this study were of analytical grade.

2.1 Preparation of sample

2.1.1 Preparation of defatted *cucurbita pepo* seed flour

The extracted seeds were washed, sundried and manually decorticated. The seeds were crushed using a household mill (super intermet blender SI-462 model) and defatted to some extent by soaking in n-hexane (Sigma Aldrich) for 36 h with change of solvent every 8 h. The defatted flour was separated from solvent by filtration, dried at room temperature ($27^{o}C \pm 1^{o}C$) and placed in a laboratory fume hood for 24 h to further remove traces of the solvent. The flour was ground to pass through a 355 MIC sieve, packaged in an air tight plastic container and kept in a refrigerator until analyzed.

2.1 2 Preparation of protein concentrate

Cucurbita pepo seed protein concentrate was developed from the flour using isoelectric precipitation and centrifugation [4]. The defatted flour was dispersed in distilled water in the ratio of $^{1}/_{20}$ (w/v) and pH of the mixture was adjusted to 10.0 with 1.0 N NaOH (221465, Sigma Aldrich). The flour suspension was stirred at room temperature ($27 \pm 1^{o}C$) for 1h, and then centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and adjusted to pH 4.5 (Isoelectric point) with 1.0 N HCl. The suspension was centrifuged at 3000 rpm for 15 minutes. The procedure was repeated on the residue to obtain a higher yield. The supernatant was discarded and the precipitate was neutralized with 1.0 N NaOH and oven dried at $45^{o}C$ overnight. The concentrate was packaged in an air-tight container and stored in a refrigerator until analyzed.

2.1.3 Preparation of protein isolate

The defatted flour was dispersed in hot water ($55^{o}C$) at a ratio of 1:15 and the pH was adjusted to 9.0 with 2.0 N NaOH. The slurry was stirred for 45 minutes and allowed to stand for 15 minutes at room temperature ($27 \pm 1^{o}C$). It was then centrifuged at $4^{o}C$ for 30 minutes at 14300 rpm. The supernatant was collected and the pH was adjusted to 4.5 with 2 N HCl followed by stirring for 45 minutes at $25\ ^{o}C$ and centrifugation at 2830 rpm ($4^{o}C$) for 15 minutes. The precipitate obtained was washed twice with distilled water and centrifuged each time at 2830 rpm for 10 minutes. It was then re-suspended in 5 ml of distilled water and neutralized to pH 7.0 with 2 N NaOH. The isolate was oven dried at $45^{o}C$ overnight, packaged in an air-tight container and stored in a refrigerator until analyzed [5].

2.2 Chemical analyses

2.2.1 Chemical composition

Crude protein, fat, ash, moisture, and vitamin C were determined as described by [6] and minerals were determined using the method described by [7]. Carbohydrate was determined by difference and calorific value was obtained using the method of [8]. Thiamin and riboflavin were determined as described by [9]. Vitamin A was determined using the method described by [10]. Tannin, phytic acid and trypsin inhibitor were determined by the methods of [11]; [12] and [13], respectively. Saponin was determined by the method of [14] and cyanogenic glycoside by [9]. Stachyose and raffinose were determined using the method of [15].

2.2.2 Amino acid profile

Amino acids profile was determined using a fully automated liquid chromatography system for amino acid analysis (Technicon sequential multi-sample analyzer; Technicon Industrial systems, New York) according to the method of [16]. The sample was hydrolyzed in 7ml of 6 N HCl at 105 ^{o}C for 22 h under a nitrogen atmosphere. The hydrolyzed sample was mixed with 5ml of acetate buffer (pH 2) and 10 µl of the sample was loaded into the analyzed. The amount of amino acid present in the samples was calculated in g/100g protein.

2.2.3 *In-vitro* protein digestibility

Protein digestibility was determined using the method of [17]. In a centrifuge tube, 1g of sample was suspended in 20 ml of 0.10 M HCl and mixed with 50 mg pepsin from porcine stomach mucosa (Kühl Lagern, Germany) in 1 ml of 0.01 M HCl. The mixture was gently shaken at 37 ^{o}C for 48 h and then centrifuged at 4000 rpm for 10 min. The solid was suspended in the enzyme solution containing 10ml of water and 5 mg trypsin from porcine pancreas (KEM Light Laboratories PVT Ltd, India) in10ml of 0.10 M phosphate buffer (pH 8.0). The mixture was gently shaken for 16 h at 23 ^{o}C in a water bath shaker. The digested mixture was centrifuged and 10ml of 10% trichloroacetic acid (TCA) was added to the supernatant. The supernatant previously obtained from pepsin digestion was also treated in a similar manner. Precipitated proteins were removed by centrifugation at 10,000 rpm for 25 min. The nitrogen content of the TCA-soluble matter of the supernatant was determined by Kjeldahl nitrogen analysis. In-vitro protein digestibility (IVPD) was expressed as percentage enzymatic digestion as shown below;

$$\text{In} - \text{vitro protein digestibility [IVPD (\%)]} = \frac{Nitrogen\ released\ by\ enzyme}{Total\ nitrogen\ content\ of\ undigested\ sample}$$

2.2.4 Protein digestibility corrected amino acid score (PDCAAS)

Protein digestibility was determined using the method of [18] as recommended by [19] using the formula:

$$PDCAAS = uncorrected\ amino\ acid\ score \times protein\ digestibility$$

Where,

$$\textit{Uncorrected amino acid score} = \frac{\textit{mg of EAA in 1g of sample}}{\textit{mg of EAA in reference protein [19]}}$$

2.2.5 Biological values

Biological values of defatted *Cucurbita pepo* seed proteins were determined on the basis of the amino acid profiles. Amino acid score was calculated for each essential amino acid in a given test protein using the FAO/WHO reference pattern and formula [20];

$$Amino\ acid\ score = \frac{mg\ of\ amino\ acid\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ reference\ protein}$$

The method described by [21] was used in calculating the Essential Amino Acid Index (EAAI) of the protein using the amino acid composition of whole egg protein as standard [22].

$$EAAI\ (\%) = 100 \times \sqrt[10]{i = 1 \frac{ai}{ai\ ref}}$$

Where a_i and $a_{i\ ref}$ represent the concentration of essential amino acids in test sample and the reference protein respectively.

Protein efficiency ratio (PER) was estimated according to the regression equation proposed by [23].

$$PER = -0.468 + 0.454\ (Leucine) - 0.105\ (Tyrosine)$$

2.3 Statistical analysis

Two individual determinations of four replicate samples were analysed and the significant difference between chemical compositions of the proteins was tested by ANOVA Duncan's multiple range tests with SPSS statistical software (version 20, IBM SPSS, UK).

3. RESULTS AND DISCUSSION

3.1 Chemical analyses

3.1.1 Chemical composition

Result of the chemical composition of *Cucurbita pepo* seed flour (defatted) and proteins is shown in Table 1. The protein content of *cucurbita pepo* protein isolate was slightly higher (74.15%) than that of the protein concentrate (69.98%). The result from this study is comparable to seed protein isolate of some varieties of watermelon which showed values ranging from 79.05-83.79% protein as reported by [24] and lower than that reported by [25] for different varieties (*Citrullus colocynthis, Citrullus vulgaris* and *Lageneria siceraraia*) of gourd melon seeds which ranged from 88.14-90.91% protein. *Cucurbita pepo* proteins concentrate exhibited a slightly lower protein level than watermelon seed cultivars: *Matera* (72.26%) and sugar baby (71.38%) as reported by [26]. However, the protein content of *Cucurbita pepo* protein concentrate was close to the expected range of 70-85% as reported by [27] while the protein isolate exhibited a lower amount of protein compared to the expected range of 92-94% as reported by [28]. This result may be attributed to incomplete recovery of proteins which may in part be due to losses during the washing process or retention in the residue, due to complexation with other seed components [29]. The ash content of *Cucurbita pepo* seed protein isolate (5.50%) was significantly higher than that of the protein concentrate (1.24%) and slightly higher than the values reported by [25] for *Citrullus colocynthis* (4.70-4.84%) and *Lageneria siceraraia* (4.24-4.54%). *Cucurbita pepo* seed protein concentrate gave higher values for ash content than that reported for watermelon seeds which ranged from 0.4-0.5% [26]. The higher amount of ash in the isolate perhaps may be due to salt formation during protein precipitation at the isoelectric point as reported by [29]. It has also been reported that high ash content in protein isolate could be due to the formation of sodium chloride through the neutralization process during preparation by alkaline water extraction/isoelectric precipitation [30].

Table 1: Chemical composition of *Cucurbita pepo* seed flour and proteins.

Composition	CPF	CPC	CPI
Moisture (%)	3.24^{a} ±0.339	9.36^{b} ± 0.226	7.24^{c} ± 0.0990
Ash (%)	5.38^{a} ±0.311	1.24^{b} ± 0.198	5.50^{a} ± 0.2263
Fat (%)	18.91^{a} ±1.159	12.90^{b} ± 0.283	9.80^{c} ± 0.2828
Crude fibre (%)	1.61^{a} ± 0.042	ND	ND
Crude Protein (%)	57.50^{a} ± 2.969	69.98^{b} ± 1.796	74.15^{b} ± 1.527
Carbohydrate (%)	13.37^{a} ±0.382	6.52^{b} ± 0.113	3.31^{c} ± 0.424
Calorific Value (Kcal/100g)	453.67^{a} ±12.403	422.10^{b} ± 1.273	398.0^{c} ± 2.178
Vitamin C (mg/ml)	16.00^{a} ±0.311	43.46^{b} ± 3.620	52.36^{c} ± 0.976
Vitamin A (I.U /g)	47.31^{a} ±2.305	100.56^{b} ± 1.329	63.43^{c} ± 1.004
Thiamin (%)	0.75^{a} ±0.057	0.74^{a} ± 0.071	0.74^{a} ± 0.099
Riboflavin (%)	0.34^{a} ±0.071	0.26^{a} ± 0.085	0.32^{a} ± 0.028
Na (%)	0.18^{a} ±0.071	0.14^{a} ± 0.085	0.18^{a} ± 0.028
Ca (%)	1.40^{a} ±0.212	0.86^{b} ± 0.156	1.02^{ab} ± 0.028
Mg (%)	0.72^{a} ±0.042	0.53^{a} ± 0.057	0.58^{a} ± 0.085
P (%)	1.09^{a} ±0.113	0.11^{b} ± 0.014	0.09^{b} ± 0.028

Different letters indicate statistically significant differences among samples within the same row ($p<0.05$). Data are means ± standard deviation of duplicate determinations with four replicates samples (n=4). CPF = *Cucurbita pepo* seed flour, CPC = *Cucurbita pepo* seed protein concentrate, CPI = *Cucurbita pepo* seed protein isolate, ND = Not detected.

The fat content of the *Cucurbita pepo* seed protein isolate was significantly ($p<0.05$) lower than the amount detected in the partially defatted flour and protein concentrate respectively. Fat is concentrated with the protein fractions and this could probably have led to its higher content in the seed protein concentrate. Although crude fibre was present in the seed flour, it was not detected in the seed proteins and as such may have been processed out during the sample

digestion. This observation agrees with the previous studies of [31] and [32] for jack bean (*Canavalia ensiformis*), and bambara groundnut protein concentrates respectively and compares to that of [33] who reported <1% crude fibre, for wheat germ protein isolate. The carbohydrate content of the protein concentrate was higher than that of the isolate and this could be as a result of the removal of the insoluble polysaccharides during the preparation of the isolate. The protein concentrate and isolate showed fairly high vitamin C content (43.46 and 52.36%), respectively. The protein samples were low in minerals and showed no significant difference in the levels detected for each mineral in both cases.

The result of antinutritional factors of *Cucurbita pepo* seed products is shown in Table 2. Values obtained for tannin in the protein concentrate (0.76%) and isolate (0.88%) were much higher than the value reported for *Adenopus breviflorus* seed protein isolate (<0.1%) as reported by [34] and some legumes (sweet and bitter lupin seed protein isolates) reported to have 0.32-0.49% [35]. Report has shown that bitterness in plant materials contribute to its high tannin content [36]. Phytic acid content was found to be 0.10% and 0.14% in *Cucurbita* seed protein concentrate and isolate, respectively.

Table 2: Antinutritional factors of *Curcubita pepo* seed products

Antinutrients (%)	**CPF**	**CPC**	**CPI**
Tannin	0.69 ± 0.255	0.76 ±0.127	0.88 ± 0.999
Saponin	0.56 ± 0.170	0.54 ± 0.057	0.51 ± 0.057
Hydrogen cyanide (mg/100g)	4.08 ± 0.113	3.45 ± 0.071	3.98 ± 0.028
Trypsin inhibitor (TIU/g)	2.07 ± 0.382	2.18 ± 0.325	2.06 ± 0.057
Phytate	0.44 ± 0.085	0.10 ± 0.014	0.14 ± 0.064
Stachyose	3.00 ± 0.283	2.80 ± 0.141	0.80 ± 0.078
Raffinose	0.80 ± 0.127	0.70 ± 0.226	0.20 ± 0.028

Data are means ± standard deviation of duplicate determinations. CPF = *Cucurbita pepo* seed flour, **CPC** = *Cucurbita pepo* seed protein concentrate, **CPI** = *Cucurbita pepo* seed protein isolate.

Phytic acid in *Cucurbita* seed proteins was lower than the value (4.67 mg/g) reported by [34] for *Adenopus breviflorus* seed protein isolate. Although limited information is available on the dose of phytate which may have negative effect in humans, the smallest toxic dose of phytates in man is yet to be established. However, it appears that high doses are required for any appreciable effect in man [37] [38]. Hydrogen cyanide (HCN) was found to be 3.45 mg and 3.98 mg in *Cucurbita* seed protein concentrate and isolate, respectively. HCN detected in both proteins in this study were below the safety level for cyanide poisoning in man. The lethal dose range of HCN when ingested by humans is estimated at 50-60 mg/kg body weight per day as reported by [39]. Protein isolate exhibited a lower level of trypsin inhibitor than the protein concentrate. The reduced content of the oligosaccharides in the protein samples may be attributed to processing techniques and solubility during protein precipitation. Values obtained for stachyose and raffinose in both *Cucurbita* proteins ranged between 0.80 - 2.80% and 0.20 - 0.70% respectively. However, the protein isolate showed much lower values than the protein

concentrate and the flour (Table 2). The amount of these oligosaccharides reported by [40] for some legumes such as raw jack bean seed (stachyose 1.80 g/100g and raffinose 1.51 g/100g) is slightly higher than values reported for protein isolate in this study. Result from this study is also partly comparable to the levels of raffinose and stachyose in soaked and cooked dry beans (*Phaseolus vulgaris, L*) as reported by [41] and suggest that protein isolation could also be an effective means of reducing these oligosaccharide in food ingredients. Raffinose, and stachyose have been identified as flatulence inducers and when ingested cause accumulation of gas, discomfort, diarrhea, pain and cramps [42]; a factor which tends to render legumes less acceptable.

3.1.2 Amino acid and protein nutritional quality

The amino acid profiles of *Cucurbita pepo* seed proteins are shown on Table 3. Protein isolate generally exhibited lower amino acid levels compared to the concentrate. This could be attributed to the presence of some antinutrients such as tannin which could affect the nutritional quality of a protein. Rasco [21] reported that some foods contain heat-labile anti-nutritional factors (e.g trypsin inhibitor) and are usually cooked to inactivate the inhibitor while some contain heat stable anti-nutrients (e.g tannins) that can decrease the nutritive value of a protein. The lysine content of *Cucurbita* seed protein concentrate was higher than that of the protein isolate. However, the value reported by [34] for lysine in *Adenopus breviflorus* seed protein isolate (52.40 mg/g equivalent to 5.24g/100g) was higher compared to the result (3.09 g/100g) obtained in this study. The lower content of lysine and the sulphur amino acid in the isolate may also be as a result of the high reduction of albumin (which has been reported to be rich in lysine, cystine and methionine) in the protein products [43]. Lysine is an essential amino acid and a building block of proteins which helps in production of energy in the body from fatty acids. Although high dose of lysine has been found to be toxic in humans, levels up to 800-3000 mg/day has been recommended as safe in adults [44]. Total essential amino acid was found to be highest in *Cucurbita pepo* seed protein concentrate (38.32g/100g protein) and least in the isolate (27.79g/100g protein). Ayodele and Aladesanmi [34] reported a higher value for the total essential amino acid in *Adenopus breviflorus* seed protein isolate, (49.38g/100g). Percentage ratios of essential to total amino acids (E/T, %) for *Cucurbita pepo* seed protein concentrate and isolate were above 36% which is considered adequate for an ideal protein [45]. The present study shows slightly lower level of E/T (42.39%) in *Cucurbita* protein isolate than that reported by [34] for *Adenopus breviflorus* seed protein isolate (50.37%) and whey protein isolate (47.79%) [46]. However, when compared to whey proteins [46] higher levels of phenylalanine (essential amino acid), arginine and glycine were recorded in this study for *Cucurbita* protein concentrate and isolate and the value obtained for histidine in CPC compares favorably with whey protein concentrate (Table 3).

The protein nutritional quality of *Cucurbita pepo* seed protein concentrate and isolate was evaluated (Table 4). The protein concentrate satisfied the FAO/WHO/UNU requirements for the essential amino acids (47). Chemical score is used to assess dietary protein quality. The chemical score based on the content of the sulphur amino acids was above 100% in the protein concentrate. Overall, protein concentrate showed the highest chemical score (95.0%) and protein efficiency ratio of 2.80 while protein isolate had the least (53.0%) and (1.56), respectively, based on the first limiting amino acid and the protein efficiency ratio. The Protein efficiency ratio (PER) is another parameter for protein evaluation with the PER value of < 1.5 indicating low protein quality, 1.5 and 2.0 as intermediate protein quality and >2.0 indicating high protein quality [48]. PER values obtained in this study showed that *Cucurbita* seed protein concentrate is a high quality protein with PER of 2.80 while the value for protein isolate (1.56) indicated an intermediate quality and lower than the PER of 2.67 recorded for *Adenopus breviflorus* seed protein isolate [34]. However the PER obtained in this study for both seed proteins gave values higher than in some legumes as reported for sweet and bitter lupin protein isolates obtained from different isolation techniques [30].

Table 3: Amino acid profile of *Cucurbita pepo* seed proteins (g/100g protein)

Amino acid	Composition (g/100g)		FAO/WHO/UNU (1985) Pre-School Child (2-5yrs) Reference Pattern (g/100g Protein)	Uncorrected Amino Acid Score		PDCAAS	
	CPC	CPI		CPC	CPI	CPC	CPI
Isoleucine	3.91 (6.41)	3.20 (540)	2.80	1.40	1.14	0.80	0.27
Leucine	7.19 (11.60)	5.11 (13.50)	6.60	1.09	0.77	0.62	0.18
Lysine	5.61 (9.83)	3.09 (10.90)	5.80	0.97	0.53	0.55	0.12 [a]
Cysteine [a]	1.19 (2.28)	0.73 (1.90)	-	-	-	-	--
Methionine [a]	1.72 (2.35)	0.68 (3.50)	Methionine + cysteine = 2.50	1.16	0.56	0.66	0.13
†Total sulphur amino acid	2.91 (4.63)	1.41 (5.40)	-	-	-	-	-
Tyrosine	3.22 (3.26)	2.74 (3.90)	-	-	-	-	-
Phenylalanine	**4.39 (3.56)**	**3.72 (3.40)**	Phenylalanine + tyrosine =6.30	1.21	1.03	0.69	0.24
#Total aromatic amino acids	8.78 (8.62)	7.56 (8.80)	-	-	-	-	-
Threonine	3.22 (8.44)	2.30 (5.30)	3.40	0.95	0.68	0.54 [a]	0.16
Tryptophan [a]	1.17 (1.80)	1.10 (1.50)	1.10	1.06	1.00	0.60	0.23
Valine	4.30 (6.09)	3.43 (5.40)	3.50	1.23	0.98	0.70	0.23
Histidine	**2.40 (2.41)**	1.69 (2.00)	1.90	1.26	0.89	0.72	0.21
Arginine	**5.61 (3.18)**	**5.02 (3.00)**	-	-	-	-	-
Aspartic acid	9.29 (12.26)	6.23 (12.30)	-	-	-	-	-
Glutamic acid	12.62 (15.41)	7.10 (17.70)	-	-	-	-	-
Serine	2.49 (6.24)	1.71 (4.50)	-	-	-	-	-
Proline	3.08 (6.28)	2.34 (4.80)	-	-	-	-	-
Glycine	**4.30 (2.00)**	**3.14 (1.90)**	-	-	-	-	-
Alanine	5.49 (4.82)	4.03 (5.60)	-	-	-	-	-
Total amino acids	81.92 [108.22]	57.36 [106.50]	-	-	-	-	-
Essential amino acids	33.91 [52.49]	24.32 [50.90]	-	-	-	-	-
E/T (%)	41.39 [48.50]	42.39 [47.79]	-	-	-	-	-

[a] =Limiting amino acid, †Total sulphur amino acid= Cysteine + Methionine, #Total aromatic amino acids = Tyrosine + Phenylalanine + Tryptophan, values in parenthesis () = equivalent values for whey protein (source: Richard, B.K [46]), E/T [ ] =values calculated from source, E/T= Essential to total amino acid; CPC = *Cucurbita pepo* seed protein concentrate, CPI = *Cucurbita pepo* seed protein isolate; PDCAAS = Protein digestibility corrected amino acid score.

Table 4: In-vitro protein digestibility (IVPD) and protein quality of *Cucurbita pepo* seed proteins.

Sample	Chemical score (%)	Limiting amino acids			EAAI (%)	IVPD (%)	PER
		First	Second	Third			
CPC	95	Threonine	Lysine	Tryptophan	70.10	56.88	2.80
CPI	53	Lysine	Methionine + cystine	Threonine	51.28	23.36	1.56

CPC = *Cucurbita pepo* seed protein concentrate, CPI = *Cucurbita pepo* seed protein isolate, EAAI = Essential Amino Acid Index, PER = Protein Efficiency Ratio.

Cucurbita seed protein concentrate was rich in leucine, total aromatic amino acid (tyrosine and phenylalanine), sulphur amino acid (methionine and cystine), aspartic and glutamic acids but limiting in threonine, lysine and tryptophan while the protein isolate was rich in total aromatic amino acid, aspartic and glutamic acids but limiting in lysine, total sulphur amino acid and threonine. Thus lysine and threonine were the major limiting amino acids noted in *Cucurbita* seed proteins. This result is contrary to the reports on some legume protein isolates obtained under various isolation conditions such as beach pea and pigeon pea seed proteins rich in lysine, leucine, aspartic and glutamic acids but limiting in methionine, and tryptophan [29]; [49]. Protein concentrate had a higher essential amino acid index (EAAI) than the isolate. The EAAI value obtained for *Cucurbita* seed protein isolate is lower than that reported for sweet and bitter lupin isolates prepared by alkaline water extraction/isoelectric precipitation and micellisation. The present results suggest that *Cucurbita pepo* seed protein concentrate could be blended with other oil seed proteins to improve their biological values. *Cucurbita* seed protein concentrate showed a higher digestibility than the isolate. Comparing the digestibility of *Cucurbita* seed proteins from this study with some legumes, *Cucurbita* seed protein isolate exhibited a lower digestibility value (23.36%) compared to those of cowpea meals (73%), and pigeon pea (59%) as reported by [50] and flaxseed protein isolate 90% [51]. Le Guen [52] studied digestibility of protein isolates from two varieties of pea (Finale and Frijaune) in piglets and reported values ranging from 83.7 to 85.4%. The low digestibility values of *Cucurbita* protein concentrate and isolate may be as a result of the globular structure of the proteins and the presence of protease inhibitors (albumins) which hinder the action of digestive enzymes [53].

4. CONCLUSION

In conclusion, the chemical and nutritional properties of *Cucurbita pepo* seed protein concentrate and isolate revealed that the seed has great potentials as food ingredient. The seed is an excellent plant based protein source of phenylalanine, arginine, alanine, leucine and histidine. *Cucurbita* proteins can be used as a possible replacement for animal proteins in conventional foods. Although, threonine and lysine are the first limiting amino acids in the proteins, the level of lysine detected in each case is sufficient to meet the daily recommended dose. However, further supplementation of these two amino acids may be considered in the use of these products for food formulation.

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