Anti-nutritional factors, in-vitro trypsin, chymotrypsin, and peptidase multi enzyme protein digestibility of some melon (egusi) seeds and their protein isolates

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In-vitro multi-enzyme protein digestibility (IVMPD) and some anti-nutritional factors (ANF) of five melon (egusi) seed flours (MSF) and their protein isolates (PI) were carried out. Their PI have potentials comparable to that of soya bean. It is important to know the IVMPD and ANF of these MSF and PI as to ensure their safety when adapted for use as alternate protein sources for cow milk, which is relatively expensive in Nigeria. Standard methods were used to produce PI from the seeds of *Citrullus colocynthis, Citrullus vulgaris*, African Wine Kettle gourd (*Lageneria siceraria* I), Basketball gourd (*Lagenaria siceraria* II) and Bushel Giant Gourd (*Lageneria siceraria* III). Standard methods of analysis were used to determine the ANF and IVMPD of the MSF and PI when unheated and at 37°C. Multi-enzymes used were trypsin, chymotrypsin, and peptidase. IVMPD of MSF when unheated ranged from (70.67±0.70) % (C. vulgaris) to (72.07±1.79) % (*L. siceraria* I) while for PI, it ranged from 74.33% (*C. vulgaris*) to 77.55% (*L. siceraria* III). IVMPD of the PI were higher than those of MSF. Heating increased IVMPD of MSF to give an average value of 79.40% and those of PI to give an average of 84.14%. ANF average in MSF are tannin (0.11%), phytic acid (0.73%), % oxalate (1.08). Differences in IVMPD of MSF and their PI at different temperatures may arise from processing conditions that alter the release of amino acids from proteins by enzymatic processes. ANF in MSF were relatively low but were lower in the PI, therefor making the MSF and PI safe for human consumption as an alternate source of protein.

Keywords: Protein Isolates; Multi enzyme protein digestibility; Anti-nutrients; Melon (egusi)

1. INTRODUCTION

Low-cost plant protein is being promoted as a substitute of costlier animal proteins and to reduce malnutrition, mostly in children and in the growing world population. It is known that protein quality depends on both amino acid composition and protein digestibility [1]. Many studies reported that plant proteins tend to have lower digestibility compared to animal protein, presumably due to the presence of anti-nutritional compounds [2, 3]. Some of the wild and underutilised legumes such as Canavalia, Mucuna, and Sesbania, for example, have been investigated and found to possess rich nutraceutical values. However, the greatest impediment to utilising these legumes is the presence of anti-nutrients, which could be successfully removed or deactivated by employing certain processing methods (cooking, dry heat treatments, germination, irradiation, soaking, heating, steaming, fermentation among others) [4-7]. Six underutilised legume seeds grown in Nigeria namely, red, and white lima beans, brown and cream pigeon pea, African yam bean and jack bean were analysed for different anti-nutritional factors [8]. *Sojasapogenol B* was identified as the predominant sapogenol in lima beans and jackbeans by capillary gas chromatography. The content of total inositol

phosphates and individual inositol phosphates (IP6, IP5, IP4 and IP3) were analysed by ion-pair HPLC, being in the range of other legumes. Trace quantities of lupanine were identified as the alkaloid in jackbean, alpha-Galactosides were present in all the legume seeds, stachyose being the predominant galactoside in lima beans, African yam bean and jack bean, and verbascose in pigeon pea. The haemagglutinating activity was estimated as a measure of the lectin content of the samples. African yam bean was found to have the highest haemagglutinating activity. Tannins were found to be in low quantities [8]. It has been reported that protein and thiamine, mineral bioavailability [9, 10] protein and starch digestibility [11, 12] increased, whereas phytic acid [13] and tannin [13, 14] decreased during germination of legumes [15]. Phytate is not only a very stable and potent chelating food component that is considered to be an anti-nutrient by virtue of its ability to chelate divalent minerals and prevent their absorption [16], but it has also been shown to have anticancer and antioxidant activity. It forms an iron chelate that suppresses lipid peroxidation by blocking iron-driven hydroxyl radical generation [17]. Oxalate is a concern because of its negative effect on mineral availability. High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones [18]. Processing was reported to improve the digestibility of the protein by destroying the protease inhibitor and opening the protein structure through denaturation and can also cause a decrease in protein digestibility via the non-enzymatic browning reaction and thermal cross-linking [2]. Heat treatment was reported to have improved in vitro-digestibility of protein [19]. The development of useful in-vitro methods for the determination of protein digestibility has been reported [20], in particular the multi-enzyme in-vitro procedure has shown good correlations with in-vivo methods [20]. In these procedures digestibility is estimated by measuring the fall in pH of the protein suspension caused by enzymatic digestion [20]. The aim of this work is therefore to determine some anti-nutritional factors, the in-vitro-multi-enzyme protein digestibility, and the effects of heating on the in-vitro multi-enzyme protein digestibility of five varieties of melon (egusi) seeds and their protein isolates.

2. MATERIALS AND METHOD

2.1 PREPARATION OF RAW SAMPLES FLOUR

Melon seeds used for this research work are *Citrullus colocynthis, Citrullus vulgaris*, *Lageneria siceraria* I (African wine kettle), *Lageneria siceraria II* (Basketball gourd) and *Lageneria siceraria III* (Bushel giant gourd). The seeds were brought from Ilora in Oyo State, Nigeria and identified at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria [21]. They were dehusked, sundried, picked and milled in a blender. The flour samples were kept in polythene bags, then in plastic containers and stored at a low temperature, prior to analysis.

2.2 PREPARATION OF PROTEIN ISOLATES.

The protein isolates of the above-mentioned seeds were prepared according to the method described by Adebowale, [22]. The melon seed flour that has been defatted in a soxhlet's apparatus with n-hexane for about 9 hours was used as the starting material. The slurry 1:10 of the defatted flour to distilled water was stirred continuously with a magnetic stirrer (Gulfex Medical and Scientific England) for 2 hours with the pH adjusted with 1% sodium hydroxide to alkaline pH for maximum solubilisation of the protein into the solution. The slurry was centrifuged at 3500 rpm for 15 minutes or at 1800 rpm for 30 minutes. Decanting the supernatant into a clean plastic container, the residue protein was further extracted with half the volume of distilled water used initially, repeatedly for two times more. The supernatant was pulled together, and the pH adjusted with 1 M HCl to the pre-determined isoelectric points of the protein samples in other to precipitate the soluble protein in the solution. The extract was further centrifuged at 6000 rpm in a refrigerated centrifuge (Centurion Scientific Limited) at 4°C for 15 minutes. The supernatant was decanted, and the protein concentrate dialysed against distilled water for 24 hours, using dialysis tubes and then freeze dried in a lyophiliser. The Protein Isolates were kept in an airtight polythene bag and labelled appropriately.

2.3 DETERMINATION OF IN-VITRO MULTI-ENZYMES PROTEIN DIGESTIBILITY (IVPD)

The in-vitro multi-enzyme protein digestibility of the samples was determined according to [19]. The enzymes used for IVPD are porcine pancreatic trypsin, bovine pancreatic chymotrypsin and porcine protease type xxiv peptidase all purchased from Sigma USA. The in-vitro method used was a multi-enzyme system consisting of trypsin, chemo-trypsin and peptidase as reported by [19]. The protein isolates were dissolved in distilled water to give a sample suspension of 6.25 mg protein per ml respectively. The pH of each protein- suspension was adjusted to pH 8.0, with 0.1 M HCl and 0.1 M NaOH. The suspensions were kept in water bath at about 37°C with constant stirring. Fresh multi-enzyme solution was prepared, 200 ml of which contained 320mg trypsin, 620 mg chemo-trypsin and 260 mg peptidase. The multi-enzyme solution was maintained in the ice bath and adjusted to pH 8.0 with 0.1 M HCl and 0.1 M NaOH. Five millilitres of the multi-enzyme solution were added to each sample suspension with constant stirring and heated at 37°C. The pH of each of the sample suspension was recorded after adding the multi-enzyme solution and 10 minutes after heating the suspension with the multi enzymes in them. The IVPD was calculated using the equation [19]:

$$y = 210.464 – 18.103x$$

Where:
y = invitro digestibility (%)
x = pH of sample suspension after 10 minutes.

2.4 DETERMINATION OF ANTI-NUTRITIONAL FACTORS

2.4.1 Determination of Tannin

Tannins were determined by spectrophotometry at 725 nm as described in [23]. Total tannins were calculated as the difference of total phenols prior to and after tannin removal from the sample extract using polyvinylpyrrolidone.

2.4.2 Determination of Phytic acid

Phytic acid was determined by spectrophotometry according to [24-26]. Samples were extracted with HNO_3 and reacted with $FeNH_4(SO4)_2$. Following centrifugation, the filtrate was reacted with NH_4CNS and its absorbance was measured at 465 nm.

2.4.3 Determination of Oxalate

Oxalate was determined according to [27].

2.5 STATISTICAL ANALYSIS

Statistical analysis of data was done to determine the mean, standard deviation and coefficient of variation using SPSS. A two-factor analysis of variance (ANOVA) method at significance level $P < 0.05$ was done. The results are expressed as mean ± standard deviation [28].

3. RESULTS AND DISCUSSION

3.1 IN-VITRO MULTI ENZYME PROTEIN DIGESTIBILITY (IVMPD) OF SAMPLES

In-vitro multi-enzyme protein digestibility of the gourd melon (egusi) seed flours is seen in Table I. The IVMPD of the raw samples ranges from (70.67±0.70) % (*C. vulgaris*) to (72.07±1.79) % (*L. siceraria I*) with an average IVMPD of (71.33±1.15) %, Table I. The values are closely related with coefficient of variation 1.15, showing that the values are not significantly different since P≤0.05. The IVMPD of the gourd melon seeds in this study are close to that of dehulled African yam bean flour (72.30-74.10) % as reported by [29], but a little lower than the values of the five varieties of African yam beans when not heated, which ranges from (76.96-82.30) % with an average value of 79.27% as reported by [30]. However, after heating, the IVMPD increased appreciably for all the five gourd seeds with values ranging from (77.50±1.92) % (*C.colocynthis*) to (80.85±0.77) % (*L.siceraria II*), with an average of 79.40% Table I. The percentage difference between the raw and heated gourd melon (egusi) seeds ranging from (8.99 to 13.53) % with an average value of 11.33%. The differences in digestibility values of protein may arise from inherent differences in the nature of protein in food such as amino acid bonding, protein conuration from the presence of non-protein constituents which may modify digestion (dietary fibre, tannins and phytates) to the presence of anti-physiological factor or from processing conditions that alter the release of amino acids from proteins by enzymatic processes [20]. Table II shows the IVMPD of the pro-

Table I - In-vitro multi enzyme protein digestibility of varieties of gourd seeds at room temperature and when heated

Sample	Digestibility (%)			
	Normal	Heated	Difference	% Difference
C.colocynthis	71.11±0.31	77.50±1.92	6.39	8.99
C.vulgaris	70.67±0.70	78.22±2.17	7.55	10.68
L.siceraria I	72.07±0.13	79.76±0.00	7.69	10.67
L.siceraria II	71.20±0.18	80.85±0.77	9.65	13.53
L.siceraria III	71.62±1.79	80.76±0.80	9.14	12.76
Mean	71.33	79.40	8.08	11.33
S.D.	0.82	1.76	1.31	1.82
% C.V	1.15	2.22	16.21	16.06

Table II - In-vitro multi-enzyme protein digestibility of protein isolates (PI) of some gourd seeds at Room temperature and when heated

Sample	Digestibility (%)			
	Normal	Heated	Difference	% Difference
C.colocynthis PI	74.42±0.13	86.19±0.64	11.77	15.82
C.vulgaris PI	74.33±0.51	83.25±0.77	8.92	12.00
L.siceraria I PI	74.69±0.51	86.37±0.38	11.68	15.64
L.siceraria II PI	76.41±0.70	83.02±0.00	6.61	8.65
L.siceraria III PI	77.55±0.19	81.40±0.32	3.85	4.96
Mean	75.50	84.14	8.79	11.41
S.D.	1.37	2.04	3.03	4.66
% C.V	1.81	2.42	34.47	40.84

Table III - Comparison between the in-vitro multi-enzyme protein digestibility of raw gourd seed flours and their protein isolates at room temperature

Sample	Digestibility (%)			
	Raw	Protein isolate	Difference	% Difference
C.colocynthis	71.11±0.31	74.42±0.13	3.31	4.65
C.vulgaris	70.67±0.70	74.33±0.51	3.66	5.18
L.siceraria I	72.07±0.13	74.69±0.51	2.62	3.64
L.siceraria II	71.20±0.18	76.41±0.13	5.21	7.32
L.siceraria III	71.62±1.79	77.55±0.19	5.93	8.28
Mean	71.33	75.50	4.15	5.81
S.D.	0.81	1.37	1.38	1.93
% C.V.	1.15	1.81	33.25	33.22

Table IV - Comparison of in-vitro multi-enzyme digestibility of varieties of gourd seed and their protein isolates when heated (37°C)

Sample	Digestibility (%)			
	Raw	Protein isolate	Difference	% Difference
C.colocynthis	77.50±1.92	86.19±0.64	8.69	11.21
C.vulgaris	78.22±2.17	83.25±0.77	5.05	6.43
L.siceraria I	79.76±0.00	86.37±0.38	6.61	8.29
L.siceraria II	80.85±0.80	83.02±0.00	2.17	2.68
L.siceraria III	80.76±0.64	81.40±0.32	0.64	0.70
Mean	79.42	84.14	4.63	5.86
S.D.	1.76	2.04	3.26	4.23
% C.V.	2.22	2.42	70.41	72.18

tein isolates of the gourd seeds under normal condition and when heated. The IVMPD of the PI are than those of the MSF raw gourd melon (egusi) seed samples in Table I. Under normal condition, the IVMPD of the PI ranges from 74.33% (*C.vulgaris*) to 77.55% (*L.siceraria* III). Heating the protein isolates, further increased the digestibility to values ranging from (81.40 to 86.37) %. These values compare favourably with the IVMPD reported for soy concentrates (87.70%), wheat concentrates (89.90%), cotton seeds (85.30%) and soy isolates (89.60%) [19]. Hence, the protein isolates of the varieties of gourd seeds flours have higher multi enzyme protein digestibility when heated. Processing can improve the digestibility of protein by destroying protease inhibitor and opening of the protein structure through denaturation and can also cause decrease in protein digestibility via the non-enzymatic browning reaction and thermal cross-linking [31, 32].

Under normal room temperature conditions, the digestibility of the protein isolates are far higher than those of the raw flour samples with % difference ranging from 3.64% (*L.siceraria* I) to 8.28% (*L.siceraria* III) with a mean of 5.81%, Table III. Similarly, when heated, the IVMPD of the gourd melon seed protein isolates is equally higher than those of the corresponding gourd seed flours when heated. The difference in the heated gourd seeds flour and the heated gourd seed protein isolates follow similar trend with an average of 5.86%, Table IV.

3.2 RESULT OF ANTI-NUTRITIONAL FACTORS

Table V shows the result of the anti-nutritional factors (ANF) determined in the MSF. Tannin ranged from 0.0927% (*L.siceraria* II) to 0.1283% (*C. Vulgaris*), % Phytic acid ranged from 0.4737 (*L. siceraria* III) to 1.11 (*C. vulgaris*). These values are little lower than 0.60% tannins, 2.23% phytic acid reported for raw moringer leaves [31]. % Oxalate in the MSF ranged from 0.90 (*L. siceraria* II) to 1.35 (*C. vulgaris*). ANF in the PI of the gourde melon seeds are seen in Table VI. Comparing the anti-nutritional factors of the PI with the values in the raw MSF, % Tannin in the PI of the gourd melon (egusi seeds) reduced from 0.1057 to 0.0230 (*C. colocynthis*), 0.1283 to 0.0290

Table V - Anti-nutritional Factors of varieties of gourd melon seeds flours (MSF)

Sample	% Tannin	% Phytic acid	% Oxalate
C.colocynthis	0.1057±0.49	0.6320±1.21	1.08±0.00
C.vulgaris	0.1283±0.29	1.1190±1.21	1.35±0.00
L.siceraria I	0.1003±0.12	0.7487±1.15	0.95±0.06
L.siceraria II	0.0927±0.11	0.6600±0.00	0.90±0.00
L.siceraria III	0.1137±0.06	0.4737±3.52	1.13±0.00
Average	0.10814	0.72668	1.082

Table VI - Anti-nutritional Factors of varieties of gourd seeds protein isolates

Sample	% Tannin	% Phytic acid	% Oxalate
C.colocynthis PI	0.0230±0.00	0.3777±1.15	0.36±0.00
C.vulgaris PI	0.0290±0.00	0.2470±0.00	0.27±0.00
L.siceraria I PI	0.0220±0.44	0.2060±0.00	ND
L.siceraria II PI	0.0407±0.58	0.5833±2.36	ND
L.siceraria III PI	0.0337±0.06	0.2880±0.00	ND

ND = Not Determined

(*C. vulgaris*), 0.1003 to 0.0220 (*L. siceraria* I), 0.0927 to 0.0407 (*L.siceraria II*) and from 0.1137 to 0.0338 (*L. siceraria* III). Tannin therefor ranged from 0.0230 (*C. colocynthis*) to 0.0407 (*L.siceraria* II) for the PI. % Phytic acid in the PI also reduced from 0.6320 to 0.3777 (*C. colocynthis*), from 1.1190 to 0.2470 in (C. vulgaris), 0.7487 to 0.2060 (*L. siceraria* I), from 0.6600 to 0.5833 (*L. siceraria* II) and from 0.4737 to 0.2880 (*L. siceraria* III). % phytic acid in the PI therefore ranged from 0.2060 (*L. siceraria* I) to 0.5833 (*L. siceraria* II). Reduction in the % Tannin and % Phytic acid observed in the PI than in the raw MSF is consistent with the observations made by researchers that anti-nutritional factors, like Tannin and Phytic acid are generally reduced after processing [31]. % Oxalate also reduced in the PI than the few traces noticed in the MSF from 1.08 to 0.36 (*C. colocynthis*) and from 1.35 to 0.27 *(C. vulgaris*). Oxalate in the PI therefor ranged from 0.27 (*C. vulgaris*) to 0.36 (*C. colocynthis*) and oxalate was not determined in the remaining three varieties of *L. siceraria* PI. ANF in the PI of the gourde melon seeds are, generally, much lower than those of the gourd melon (egusi) seed flours.

CONCLUSION

The IVMPD of the gourd melon seed protein isolates PI are higher than those of the corresponding gourd seed MSF samples. Heating significantly increased the IVMPD of both the raw MSF samples and the PI. The anti-nutritional contents of the melon seed flour samples and their corresponding PI were very small and hence, make the samples suitable for human consumption and the application of their protein isolates as protein supplements and as a good substitute for animal proteins which are relatively costly in Nigeria.

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