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Full Length Research Paper

Amino acid composition and antioxidant properties of African yam bean (*Spenostylis stenocarpa)* protein hydrolysates

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African yam bean (*Spenostylis stenocarpa)* protein isolate was hydrolyzed with pancreatin (was purchased from sigma chemicals, St Loius Missouri, USA) to obtain an hydrolysate that has (42% degree of hydrolysis), which was characterized for its amino acid composition and ability to scavenge free radicals. The African yam bean protein isolate and hydrolysate samples were dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into Technicon Sequential Multi-sample Amino Acid Analyzer. And the major amino acids were found to be glutamate (16.66g and 9.23g), aspartate (11.23g/7.26g), and arginine (6.82g/3.28g) while the contents of sulphur-containing amino acids (methionine and cysteine) were very low (1.33g/0.83g and 1.32g/0.51g). The high degree of hydrolysis helps in breaking down the proteins into lower molecular peptides (albumins) which gives an excellent antioxidant properties with 2,2-diphenyl-1-picryhydrazyl (DPPH).

Keywords: African yam bean protein isolate, hydrolysate, amino acid composition, antioxidant properties, pancreatin, hydrolysis and DPPH.

INTRODUCTION

Legumes are important ingredients of a balanced human diet in many parts of the world due to their high protein and starch contents (Adebowale et al., 2009). They have been consumed traditionally as whole seeds or as ground flour after dehulling. The rapidly growing of food industry, which constantly demands new ingredients, has drawn researchers to legume components i.e. starch and proteins (Adebowale et al., 2009). This legume have been subjected to protein isolation (Adebowale et al., 2009), acetylation and succinylation (Adebowale et al., 2009). The increasing demand for plant protein in lieu of expensive animal protein have been expounded by several researchers (Adebowale et al., 2009). Therefore, the need to intensify research efforts aimed at identifying new legume utilization.

A reappraisal of beneficial effects of legume seed dietary intake is the basis for various health claims. The food components involved in health claims are numerous: lipids, vitamins, oligosaccharides, minerals, fibers, flavonoids, small organic compounds and less frequently, proteins and peptides. Many of these biologically active compounds originate from the plant kingdom, to which a safe and healthy attribute has traditionally been associated. However, in most cases the ability to prevent disease is based on rather generic effects such as anti-oxidant, anti-inflammatory, anti-ageing, detoxification and antigenicity. The African yam Bean (*Sphenostylis stenocarpa*) belonging to this class of legume is one of the under-utilized but widely cultivated in Nigeria which has tendency to broaden the food base for human consumption (Aletor and Aladetimi, 1989). African Yam Bean, like Cowpea (*Virgna unguiculata*) and Pigeon pea (*Cajanus caja*), is rich in both protein and starch (Agunbiade and Longe, 1999). The seeds have protein content which range from 21.0-29.0% with about 50% carbohydrates mainly starch (Eromosele et al., 2008).

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These nutritional components are responsible for the commercial importance of these latter crops. Pulse-derived peptides are generating interest for the production of bioactive peptides because they are more cost-effective in comparison to animal proteins (Aluko, 2008). Bioactive peptides commonly contain 3-20 amino acids per peptide as inactive sequences within large proteins and are released when the parent protein is hydrolyzed by digestive enzymes (in vitro and in vivo), by microbial enzymes, or during food processing (Korhonen and Pihlanto, 2003). Enzymatic hydrolysis of proteins is one approach used to release bioactive peptides, and is widely applied to improve functional and nutritional properties of protein sources (Je et al., 2008). The biological activity of a peptide is widely recognized to be based on amino acid composition (Korhonen and Pihlanto, 2003). Peptides could be used in the formulation of functional foods and nutraceuticals to prevent damage related to oxidative stress in human disease conditions. Moreover, natural antioxidants are desirable because they can be used at higher concentrations without the toxic side effects associated with the use of synthetic equivalents (Aluko, 2008; Li et al., 2008). Nevertheless, to the authors' knowledge, no information is available about antioxidant and amino acid properties of peptides from African yam bean protein hydrolysates, although the antioxidant activities and amino acid profile of enzymatic hydrolysates from other plant proteins, including soy proteins (Chen et al., 1995), wheat protein(Zhu et al., 2006), Buckwheat protein (Chuan et al., 2009) and pea seed (Pownall et al., 2010) have been widely investigated. The antioxidant properties of the hydrolysates, largely depending on protease specificity, degree of hydrolysis (DH) and nature of released peptides (e.g molecular weight and amino acid composition) have been attributed to cooperative or combined effects of a number of properties, including their ability to scavenge free radicals, to act as a chelating agents of metal ions, or act as hydrogen donor. Therefore, the aim of this study was to investigate the enzymatic hydrolysis of AYBPI by pancreatin, determine the amino acid composition of the resulting hydrolysates, and DPPH radical scavenging ability.

MATERIALS AND METHODS

Materials

The African Yam Bean (AYB) sample SSWN90 was obtained from the Institute of Agricultural Research and Training (IAR&T) Ibadan. African Yam Bean Protein Isolate (AYBPI) was prepared from the AYB flour according to the process described, by Aluko and Monu (2003), with slight modifications. The flour was dispersed in a solution of 0.015M NaOH at a ratio of 1:10 (flour: NaOH) and stirred for 2hrs at room temperature (24 ± 2^0C). The resultant slurry was stored at 4^0C for 24hrs before it was centrifuged (3000rpm for 40mins) at 24 ± 2^0C using a magnetic stirrer. Storage of the slurry at 4^0C increased clarity of the supernatant obtained after centrifugation. The supernatant was filtered through whatman no 1 paper and pH of the filtrate adjusted to 4.5 using 10% HCl solution to precipitate the proteins. The precipitated proteins were isolated by centrifugation (3000rpm for 30mins at 24^0C), washed with distilled water. Precipitate was freeze-dried as the protein isolate. Proximate composition of the AYBPI was determined according to the method described by AOAC (2005).

Proteolysis of pancreatin

Four grams of the AYBPI were dispersed in 200ml of distilled water at room temperature. The dispersions were pre-incubated at 37^0c prior to adjusting the pH of the dispersion to 8.5. The mixture of the protein and the enzyme (pancreatin at enzyme – to substrate E/S) ratio of 1:100 was incubated at 37^0C for 1hr 30mins. The pH and the temperature of the mixture were kept constant during the hydrolysis by addition of 2M NaOH for solubilization and 2M HCl for precipitation. After the period of hydrolysis (1hr 30mins) has been completed, the aliquots of the digested mixture were taken out and heated to 70^0C for 15mins, cooled immediately in cold water at room temperature and neutralized by the addition of 2M NaOH. The resulting digests were centrifuged at 1800rpm for 10mins to remove the insoluble residues. The supernatant were then dialysed against 2M HCl and adjusted to pH 7.0 and then freeze-dried to produce the hydrolysate.

The degree of hydrolysis (DH) was determined using Trichloroacetic acid (TCA) assay (Drago and Gonalez, 2001). The degree of hydrolysis was calculated as follows:

$$DH\% = \frac{\text{10\%TCA-Soluble N in hydrolysate} - \text{10\%TCA-Soluble N in sample without hydrolysis} \times 100}{\text{Total N of dispersion}}$$

Chemical analysis

The chemical compositions of AYB flour and its protein products were determined according to AOAC procedures (AOAC, 2005).

Determination of amino acid profile of AYBPI and AYBPH

The determination of the amino acid profile consists of two steps:

i. Hydrolysis of the protein to constituent's amino acids.

ii. The quantitative estimation of the amino acids in the hydrolysate. The hydrolysed amino acids were then

Table 1. Proximate composition (%)of raw and protein isolate of Africa yam bean and the degree of hydrolysis (%) of the hydrolysate.

Proximate composition	Raw AYB	AYB Isolate
Moisture	10±0.1	6.7±0.3
Protein	19±0.3	71.02±0.2
Fat	13±0.3	10±0.2
Ash	9±0.4	3.6±0.2
Fibre	ND	ND
Degree of hydrolysis	None	42±0.1

Table 2. Crude protein (%) of raw, isolate and hydrolysate of AYB

Raw AYB	**Isolate**	**Hydrolysate**
19±0.3	71.02±1.3	52.11±0.2

determined using the Technicon For amino acid analysis, the hydrolysis of the samples was determined using the method described by Speckman et al., 1958

DPPH radical scavenging activity

The DPPH radical scavenging activity of the samples was determined according to the method of Mensor et al., (2001). DPPH methanol solution (1ml, 3mM) was added to 1ml of 300µg/ml methanolic solution of the samples and allowed to react in the dark. The absorbance was taken after 30mins at 517nm with spectrophotometer against methanol as blank and converted into %antioxidant activity. The DPPH radical inhibition as a percentage is calculated by:

(1-(test sample absorbance/blank sample absorbance)×100)

The EC_{50} value (mg/ml; meaning the concentration that causes a decrease in initial DPPH concentration by 50%) was determined from the linear regression equation of the DPPH radical inhibition against the concentration.

RESULTS AND DISCUSSION

Chemical composition

The proximate composition of protein isolate and the degree of hydrolysis of hydrolysate obtained from African yam bean protein (AYB) are as shown in Table 1. The protein content are 19% and 71% for raw AYB and AYB isolate respectively. While the moisture content was 10% and 6.7% for raw AYB and AYB isolate respectively. The increase in the protein level could be due to the removal of soluble non-protein constituents and the slight reduction in that of the hydrolysate could be attributed to the concentration of hydrolysis. These constituents are primarily soluble carbohydrates (mono-, di-, and oligosaccharides), including some low molecular weight nitrogenous substances and minerals. These findings agreed with Deshpande and Campbell (1992) report that grass pea protein isolate contained 83.3-92.1% protein while Adebowale et al., (2009) reported protein content ranging between 91.25-92.50% in different types of isolates prepared from the AYB flour. The %protein obtained depend on the solvent used in their preparation. The degree of hydrolysis (DH) of the hydrolysate was 42%, which compared favourably with report of Aluko and Monu (2003) of 48% obtained from quinoa seed protein hydrolysate. The DH increseased with hydrolysis time, indicating gradual release of peptide fragments hydrolysis. The rate of the hydrolysis or the release of peptide fragments was faster during initial hydrolysis (e.g <30min) and gradually decreased with the hydrolysis time increasing (Chuan et al., 2009).

Amino acid composition

The result of amino acid composition are shown in Table 3. The predominants amino acids in AYBPI and AYBPH

Table 3. Amino acid composition of African yam bean protein isolates and hydrolysates (g/100g), as well as FAO/WHO suggested essential amino acid contents for children and adult.

Amino acids	Raw sample	Protein isolates	Hydrolysates	FAO/WHO SUGGESTED Adult
Threonine	4.58	3.31	2.04	0.9
Tyrosine	3.8	3.33	1.65	
Methionine	5.44	1.33	0.83	
Valine	1.2	4.56	1.85	1.3
Phenylalanine	6.13	5.15	2.53	
Isoleucine	4.89	3.86	1.58	1.3
Leucine	7.9	7.58	3.55	1.9
Lysine	8.8	7.23	3.62	1.6
Cystine	5.44	1.32	0.51	
Histidine	5.51	3	2.02	1.6
Tryptophan	N.D	ND	ND	
Total EAA	53.69	40.67	18.16	8.6
Arginine	6.39	6.82	3.28	
Aspartic acid	10	11.23	7.26	
Glutamic acid	12.1	16.66	9.23	
Serine	5.04	4.50	2.06	
Proline	4.58	3.86	1.32	
Glycine	4.11	3.48	2.26	
Alanine	3.85	4.10	3.04	
Total NEAA	46.07	50.65	30.47	
TEAA/TAA (%)	53.82	44.54	37.34	
TSAA (Meth+Cys)	10.88	2.65	1.34	1.7
AREAA (Phe+Tyr)	9.93	8.48	4.18	1.9
TEAA/TNEAA(%)	116.54	80.30	59.59	
Hydrophobic AA	33.99	30.44	11.66	

Keys:TEAA – Total Essential amino acid TNEAA – Total Non Essential amino acid TAA – Total Amino acid TSAA – Total Sulphur amino acid Ar.EAA – Aromatic Essential amino acid ND – Not determined Hydrophobic Amino acid – Alanine,Proline,Valine,Methionine,Isoleucine,Leucine, and Phenylalanine

are glutamic acid (16.66g/100g and 9.23g/100g), aspartic acid (11.23g/100g and 7.26g/100g), leucine (7.58g/100g and 3.55g/100g), lysine (7.23g/100g and 3.62g/100g), arginine (6.82g/100g and 3.28g/100g), methionine (1.33g/100g and 0.83g/100g) and cystine (1.32g/100g and 0.51g/100g) respectively. The least in both samples are cystine and methionine. The hydrophobic amino acids (alanine, proline, valine, methionine, isoleucine, leucine and phenylalanine) of the AYBPI tends to be higher than the AYBPH which cuold be due to concentration of the acid (precipitation) (2N HCl) and base (solubilisation) (2N NaOH) used in hydrolysing the AYBPI, to give a low molecular weight peptides that will be able to inhibit Angiotensin 1-converting enzymes, produce free radicals and still have a well balanced amino acid composition. The percentage ratio of essential to total amino acid were in the range of 37–45% which were well above 36% considered adequate for an ideal protein (FAO/WHO,1973).

Amino acids are important components for healing and protein synthesis processes; any defficiency in these essential components will hinder the recovery process (Zuraini et al., 2006). According to Wittle *et al*. (2002), glycine together with other essential amino acids such as alanine, proline, arginine, serine, isoleucine and phenylalanine form a polypeptide that promote growth and tissue healing . The amino acid composition of AYBPI is consistent with that previously reported by other researchers (Arogundade et al., 2009). Glutamic and aspartic acid were the major amino acids in all the *S. stenocarpa* protein isolate examined (Chuan-He Tang et al., 2009). A similar report was observed for chickpea, cowpea, lentils, lupin and greenpea (Iqbal et al., 2006; Sijak et al., 2006). AYBPH contains all the essential amino acids in good proportion (Radha et al., 2007), compared with FAO requirement of amino acids for adults, and resembles the protein (sulphur amino acid) found to be limiting in legumes (Oshodi et al., 1995).

With respect to the total EAA in the amino acid FAO/WHO requirement pattern, the AYBPH & AYBPI may contribute adequate levels of total EAA (Table 3). The value obtained is closed to the recommended 39% considered to be adequate for ideal protein food for infants above that recommended (26%), and considered to be adequate for ideal protein for children and 11% for adults (FAO/WHO/UNU,1985). The data suggest that the protein products (hydrolysates) have well-balanced amino acid composition, and are suitable for human consumption as a source of protein nutrition.

DPPH Radical Scavenging Ability

Figure 1 shows the DPPH radical inhibition of the

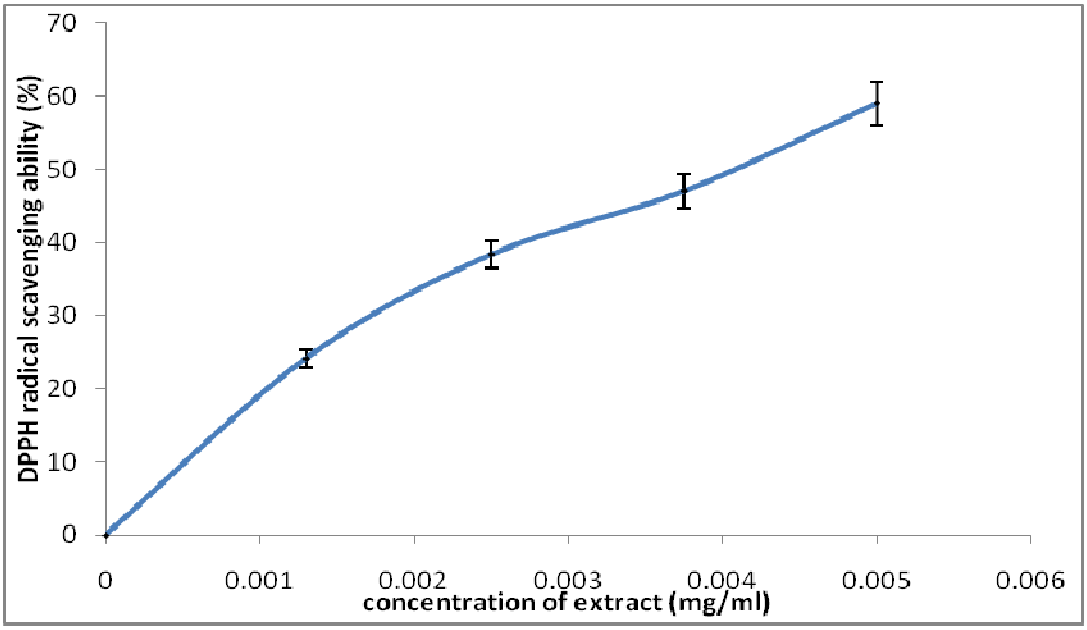

Figure 1. DPPH radical scavenging ability of the African yam bean protein hydrolysates. Each data point is the mean of three determinations, and errors bars represent standard deviation.

hydrolysate of AYBPI , at various concentrations (0-0.005mg/ml). In the present study, the data for AYBPI was not provided, due to its low solubility. DPPH is not a biologically relevant radical; however, it is widely used to evaluate the antioxidant activity of natural compounds (Udenigwe et al., 2009). The relatively stable DPPH radical in ethanol has been widely used to test the ability of some compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity (Jao and Ko, 2002; Shimada et al., 1992). DPPH is a free radical, stable at room temperature, producing a violet solution in ethanol (Xu and Chang, 2007). Reduction of DPPH propable antioxidants resulted in a loss of absorbance, and the degree of discolouration of the solution indicates scavenging efficiency of the added substances (Chen and Ho, 1995). There was increase in absorbance as the concentration increases over the range. This agrees with the work of Chuan *et al.* (2009), who reported that if the concentration is high enough, the hydrolysates might exhibit high antioxidant property. The use of DPPH radical scavenging activity provided an easy and rapid inference to evaluate antioxidant activity. Some previous studies pointed out that high DPPH or other radical scavenging activities for the protein hydrolysates or peptides are usually associated with high hydrophobic amino acid or hydrophobicity (Rajapakse et al., 2005; Li et al., 2008; Chuan *et al.*,2009). This view seems to be consistent with the data obtained for the AYBPH DPPH radical scavenging ability.

During hydrolysis, a wide variety of smaller peptides and free amino acids are generated, depending on enzyme specificity. Changes in size, level and composition of free amino acids and small peptides affect the antioxidative activity (Wu et al., 2003). DPPH is a stable free radical with an absorbance maximum at 517nm in methanol. When DPPH encounters a proton-donating substance such an antioxidant, the radical is scavenged and the absorbance is reduced (Shimada et al., 1992). The result reveals that the AYBPH potentially contained substances which were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

The EC_{50} value is applied as an indication to evaluate the scavenging activity. The lower the EC_{50} value, the higher the free radical scavenging ability. The higher the EC_{50} value obtained for this degree of hydrolysis were calculated from the regression equation to be 0.004mg/ml. The EC_{50} values of the AYBPH are much less than the lowest value (1.3mg/ml) of wheat germ protein hydrolysate prepared with Alcalase (Zhu et al., 2006) and less than the values obtained for Buckwheat protein hydrolysates (0.56-0.94mg/ml) (Chuan et al., 2009).

CONCLUSION

The enzymatic hydrolysis by pancreatin resulted in effective breakdown of the protein aggregates in AYBPI and the resultant hydrolysate as a compound that reduce the amount of free radicals. The amino acid profile of AYBPH was similar to that of AYBPI with the essential amino acids above that which is recommended for adults by FAO/WHO. The results indicate that enzymatic african yam bean protein hydrolysate could be used as potential ingredients to formulate nutraceutical products. Also

based on its amino acid profile it can be used in food product fortification by food industry. The antioxidant from AYBPH can be used as a substitute for the commercial butylated hydroxyltoluene (BHT) and butylated hydroxylanisole (BHA) which were reported to be carcinogenic. More research needs to be done on how to improve on the hygroscopic nature of AYBPH and its production as a dietary supplement or tablet. Improvement of the palatability of protein hydrolysates, while maintaining nutritional value and safety is required.

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