

Chapter 7

Bambara Groundnut Protein



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7.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) verdc) is an easy-to-grow legume seed classified in the fabaceae family, the faboidea subfamily and the genus *Vigna*. The nuts were made up of wild (*V. subterranea* var. *spontanea*) and cultivated (*V. subterranea* var. *subterranea*) varieties (Murevanhema and Jideani 2013). The groundnut originated from the African continent (Massawe et al. 2003; Murevanhema and Jideani 2013; Arise et al. 2015; Arise 2016) and found underutilised despite being the third most important legume after common groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Adebowale et al. 2011; Hillocks et al. 2012; Arise 2016). Bambara protein content is similar to those of other pulses, such as cowpea, soybean and groundnut. For instance, Bambara groundnuts had a very high protein content (20.5–32.4%) with an appreciable level of essential sulphur-containing amino acids higher than those found in most legumes (Ijarotimi and Esho 2009; Murevanhema and Jideani 2013; Arise et al. 2015). Recent studies have established the possibility of using Bambara groundnut in various food products such as biscuit, maize snacks, maize pudding, croissant snacks, vegetable milk and yoghurt, and cake production (Falade et al. 2015; Okafor et al. 2015; Arise et al. 2017b; 2018). The chemical compositions of the different varieties of Bambara groundnut and other legume seeds are presented in Table 7.1. The health applications of these protein-rich seeds might be related to the bioactive compounds derived from their proteins.

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Table 7.1 Chemical composition (%) of legumes

Legume type	Species	Protein	Carbohydrate	Fat	Ash	Fibre
Bambara groundnut	<i>Voadnzeia subterranea</i>	32.40	51.79	7.35	5.78	2.68
African yam bean	<i>Sphenogtylis stenocarpa</i>	37.21	44.40	9.49	5.35	3.55
Bambara groundnut	<i>Vigna subterranea</i>	20.60	56.51	6.60	3.25	6.34
Jack bean	<i>Canavalis ensiformes</i>	26.20	57.83	1.95	6.51	1.07
Pigeon pea	<i>Cajanus cajan</i>	24.46	56.83	4.78	4.58	1.10
Cowpea	<i>Vigna unguiculata</i>	24.13	56.60	4.37	4.73	0.97
Soya bean	<i>Glycine max</i>	42.80	19.80	22.80	5.20	2.30
Bauhinia seed	<i>B. Galipinii</i>	38.50	23.30	24.20	4.50	1.40
Chickpea	<i>Acarari etinum</i>	22.83	57.19	5.43	3.04	3.50
Lentil	<i>Lensculinaris medicus</i>	31.12	52.63	0.81	2.62	3.68
Kidney bean	<i>Phaseolus vulgaris</i>	20.09	57.67	2.46	3.85	6.78
Mung bean	<i>Vigna radiate</i>	22.70	58.99	1.36	3.35	4.70

Source: Arise et al. (2016)

7.2 Extraction and Isolation of Bambara Groundnut Proteins

The extraction methods used for most plant protein isolates are assumed to contribute to their physicochemical, digestible, functional and nutritional properties. Therefore, the selection of the appropriate technology and conditions for the extraction of proteins is essential in the processing of food, since these can influence the functional and nutritional properties of the finished product (Boye et al. 2010). Four different methods that could be used to extract protein from protein-rich seeds, which are found applicable to Bambara groundnuts, are isoelectric (acid-alkaline) precipitation (Eltayeb et al. 2011; Kudre et al. 2013; Arise et al. 2015, 2020), micellisation (Adebowale et al. 2011), membrane ultrafiltration coupled with enzymatic digestion (Malomo and Aluko 2015a) and salt solubilisation (Malomo and Aluko 2015b; Arise et al. 2017c).

7.2.1 Isoelectric Precipitation Method

This method is the most common method used for protein extraction in legumes. The method involved the use of aqueous alkali followed by isoelectric precipitation. The technique exploited the advantage of the differential solubility of legume proteins, which is high at alkaline pH, thus requiring the solubilisation of proteins and low at their isoelectric point generally at pH 4–5 (Adebowale et al. 2011; Arise et al. 2020). Despite its limitations, which include low functionality, the isoelectric precipitation has been reported to produce isolates of high protein contents compared to other methods (Adebowale et al. 2011; Arise et al. 2020). For instance, protein contents of 90 and 88% were previously obtained (Adebowale et al. 2011; Kudre

et al. 2013) for isoelectric precipitated Bambara groundnut protein isolates, respectively. However, incomplete protein recovery was experienced, which may be due to inadequate solubilisation or loss in the supernatant during centrifugation of the precipitated protein (Malomo and Aluko 2015b). Besides, poor functional properties such as low foaming, emulsifying and water holding capacity were reported for isoelectric precipitated Bambara protein isolates (Adebowale et al. 2011). However, generation/composition of high amounts of ash during the acid-base neutralization procedure was observed for isoelectric precipitated hemp seed protein isolates (Malomo and Aluko 2015b).

The acid extraction principle was similar to that of alkaline extraction except that the initial protein extraction was conducted under acidic conditions. Since the legume protein solubility was also high at very high acid conditions (pH < 4.0), low pH was used to solubilise the protein then followed by isoelectric precipitation (Boye et al. 2010). A previous finding obtained a Bambara groundnut protein isolate of 79% protein content with low functional properties from the acid precipitation method (Arise et al. 2015).

7.2.2 *Micellisation Method*

This method involved the use of salt (NaCl) for protein extraction or isolation. Although the protein obtained by this method has been reported to have good functional properties, such as high foaming capacity, emulsifying, oil and water holding capacities but with low protein content and yield. For instance, a Bambara groundnut protein isolate with protein content and yield of 75% and 14%, respectively were previously obtained through micellisation method (Adebowale et al. 2011).

7.2.3 *Membrane Ultrafiltration Coupled with Enzymatic Digestion*

The harsh conditions resulting from the common use of acid-alkaline solubility in the traditional isoelectric precipitation procedure undoubtedly have negative effects on protein functionalities, especially protein solubility and foaming properties (Malomo and Aluko 2015a, b). Besides, the composition of high phytate content (which caused protein cross-linking and reduction of protein solubility) with high fibre content had been implicated in the low functionality of isoelectric precipitated protein isolates. Therefore, pre-digestion of protein meal with carbohydrases and phytases (for fibre and phytate digestion) coupled to membrane ultrafiltration (to remove the non-protein materials) is expected to produce protein isolates of high functional properties. However, this novel method has not been previously reported for the production of Bambara groundnut protein isolate, but it has been developed

and proven to be an effective method for production of hemp seed protein isolates with improved food functionalities (Malomo and Aluko 2015a).

7.2.4 Salt Solubilisation

The salt solubilisation method was based on the principle that protein globulin fraction could only be soluble and isolated in the salt solution, thereby producing albumin (filtrate) and globulin (residue). This method involved the use of salt (NaCl) after which the clear supernatant was dialysed using membrane weight cut-off (MWCO) of 10 kDa against distilled water for 48–78 h (Malomo and Aluko 2015a). The dialysed extract was thereafter centrifuged and freeze-dried. The method also reported high functional properties for the resultant protein obtained but with low protein content and protein yield as in the case of low 55% protein content previously obtained for salt-solubilised produced Bambara groundnut isolates (Arise et al. 2015). The salt solubilisation method revealed four major protein bands from Bambara groundnut landraces: one broadband at 55 kDa, two medium bands at 62 and 80 kDa and a high molecular weight (HMW) protein at 141 kDa (Figs. 7.1 and 7.2). The vicilin (7S) subunits with molecular weights of 55 and 62 kDa were major fractions in Bambara storage proteins (Adebawale et al. 2011; Arise et al. 2017c).

7.3 Production of Bambara Protein Isolates, Hydrolysates and Bioactive Peptides

The production of Bambara protein isolates through different methods had been earlier discussed (Sects. 7.2.1–7.2.4) and the resultant isolates could be found applicable as functional ingredients in most food processing industries. Furthermore, Bambara groundnut hydrolysates have been generated using different types of digestive proteases (enzymes) to mimic the actual digestion of protein in the GIT. The digestive proteases, such as alcalase, pepsin, papain, pancreatin, thermolase, trypsin, and others have been used in the past by acting on the protein structures based on their enzyme specificity (point of bond breaking) and optimum conditions (pH, temperature and time). For instance, Bambara groundnut protein hydrolysates and later separated into different molecular fractions of different sizes (<1, 1–3, 3–5 and 5–10 kDa) were obtained from alcalase, trypsin and pepsin digestions (Arise et al. 2016). The resultant hydrolysates and membrane fractions had improved bioactivities such as significant surface hydrophobicity, better antioxidant mechanisms (ferric reducing power, metal chelating, diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activities) when compared with glutathione (Figs. 7.3 and 7.4).

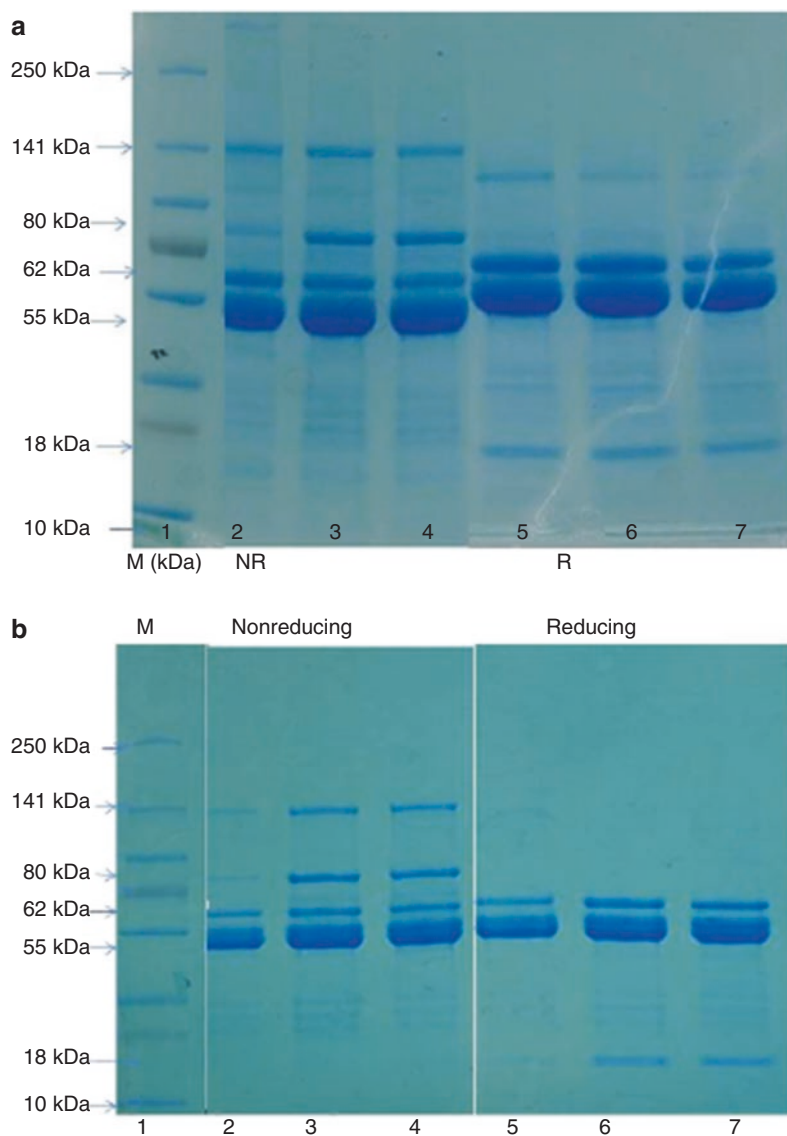


Fig. 7.1 (a) SDS-PAGE patterns of Bambara groundnut protein isolates. Lane 1 – standard marker. Lanes 2, 3 and 4 carried out without b-mercaptoethanol. Lane 2, red isolate; lane 3, maroon isolate; lane 4, cream isolate. Lanes 5, 6 and 7 carried out with b-mercaptoethanol. Lane 5, red isolate; lane 6, cream isolate; and lane 7, maroon isolate. NR nonreducing and R reducing conditions. (b) SDS-PAGE patterns of Bambara groundnut protein Vicilin isolates. Lane 1 – standard marker. Lanes 2, 3 and 4 carried out without bmercaptoethanol. Lane 2, red isolate; lane 3, maroon isolate; lane 4, cream isolate. Lanes 5, 6 and 7 carried out with b-mercaptoethanol. Lane 5, red isolate; lane 6, cream isolate; and lane 7, maroon isolate. **Source:** (Arise et al. 2017c)

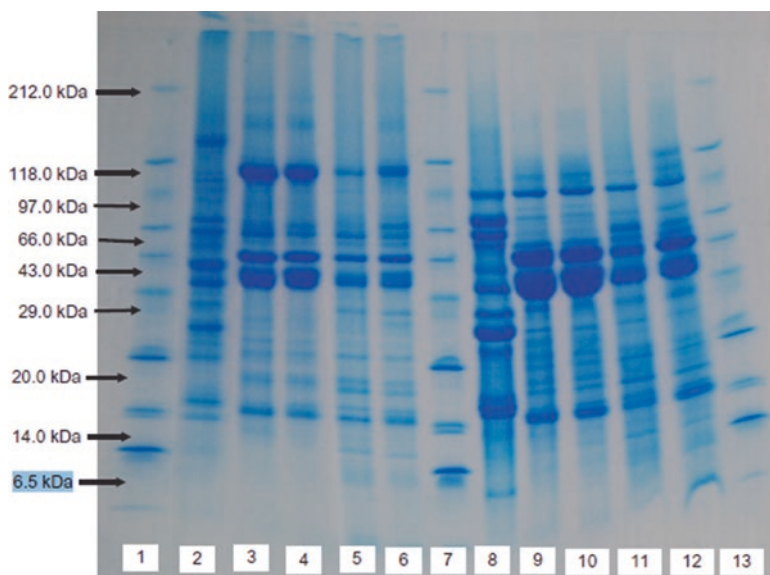


Fig. 7.2 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of Bambara groundnut and soybean protein isolates: Lanes 2, 3, 4, 5, and 6 carried out without Dithiothreitol (DTT). Lanes 1, 7, and 13—Standard reference protein; Lane 2—Soy protein isolate (IEP); Lane 3—Bambara isolate (IEP), white variety; Lane 4—Bambara isolate (IEP), brown diversity; Lane 5—Bambara isolate (MP), white variety; Lane 6—Bambara isolate (MP), brown variety. Lanes 8, 9, 10, 11, and 12 with Dithiothreitol (DTT): Lane 8—Soy protein isolate (IEP); Lane 9—Bambara isolate (IEP), white variety; Lane 10—Bambara isolate (IEP), brown diversity; Lane 11—Bambara isolate (MP), white variety; Lane 12—Bambara isolate (MP), brown variety. **Source:** (Adebowale et al. 2011)

Furthermore, a combination of alcalase, thermolysin and trypsin enzymes produced antioxidant-rich Bambara protein hydrolysates (Mune et al. 2018) and the resultant hydrolysates showed a protective effect against oxidative stress. Likewise, a study showed better and increased DPPH radical scavenging and metal chelating activities (Thammarat et al. 2015) from the Bambara protein hydrolysates obtained through the alcalase digestion (hydrolysis).

Besides the use of Bambara protein hydrolysate as good antioxidant agents, it has also been reported that Bambara protein hydrolysate could be used as antihypertensive agents. A good example is when the Bambara protein hydrolysates obtained from alcalase, trypsin and pepsin hydrolysis exhibited effective inhibitory activities against angiotensin-converting enzyme (ACE) and renin (the dual enzymes implicated in the pathogenesis of hypertension) as shown in Figs. 7.5 and 7.6 (Arise et al. 2017a). However, Bambara groundnut hydrolysates from alcalase, thermolysin and trypsin digestion showed improved ACE (IC_{50} : 52 μ g/mL). and dipeptidyl peptidase-IV (DPP-IV) (IC_{50} : 1.73 mg/mL) inhibitory activities as presented in Table 7.2 (Mune et al. 2018). These findings showcased the Bambara protein hydrolysates as potential ingredients in the formulation of functional foods and nutraceuticals in the management of high blood pressure-related hypertension.

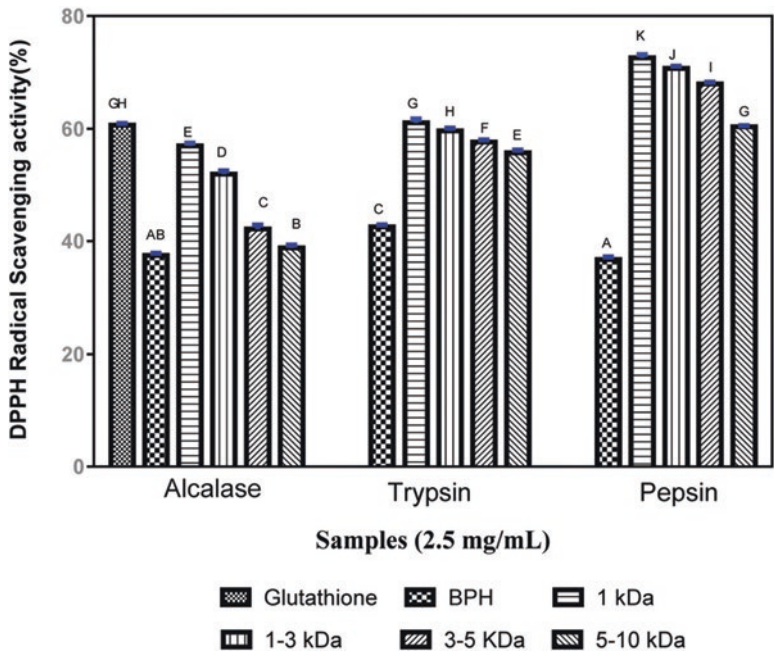


Fig. 7.3 DPPH radical scavenging activities of Bambara protein hydrolysates and membrane fractions (mean $\pm$ standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)). **Source:** (Arise et al. 2016)

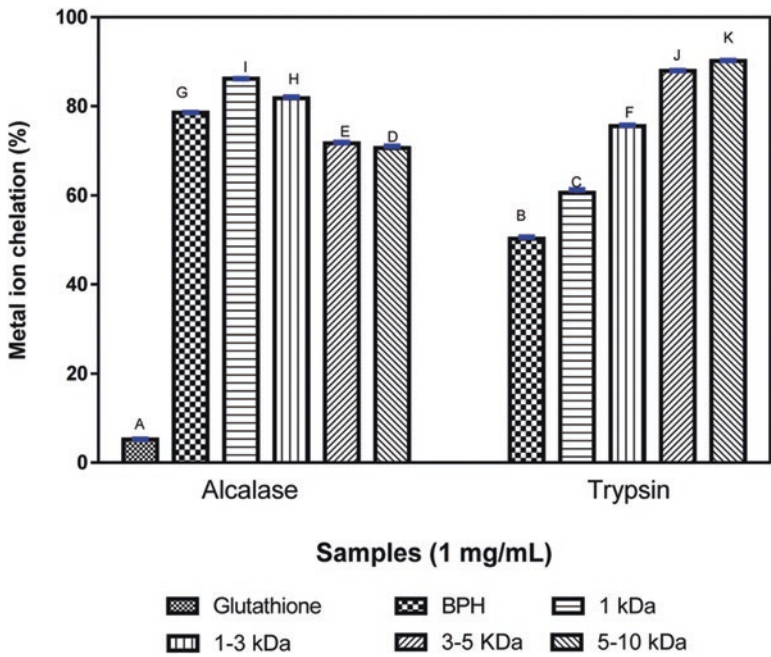


Fig. 7.4 Metal chelating effects of Bambara protein hydrolysates and membrane fractions (mean $\pm$ standard deviation, $n = 3$ with different alphabets having mean values that are significantly different ($p < 0.05$)). **Source:** (Arise et al. 2016)

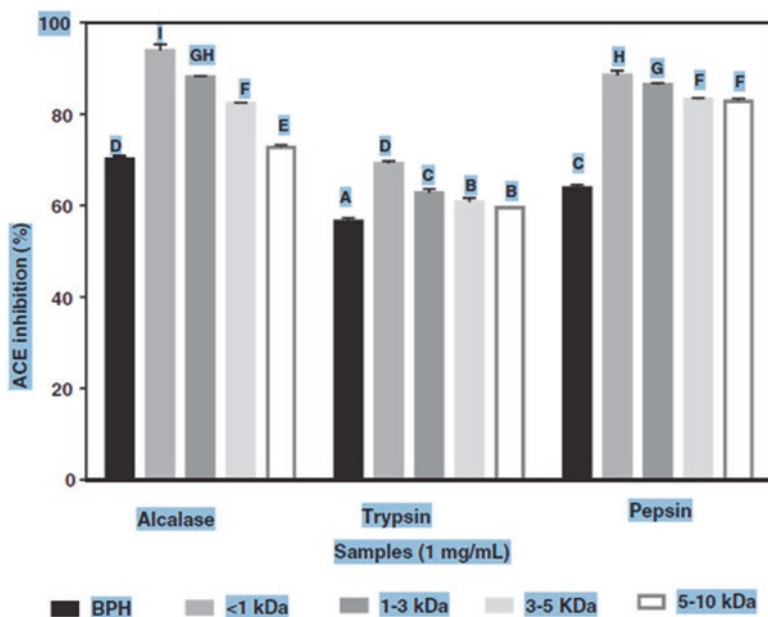


Fig. 7.5 Inhibition of ACE by enzymatic Bambara protein hydrolysate and membrane ultrafiltration fractions at a concentration of 1 mg/mL. Error bars (mean $\pm$ SD, $n = 3$) with different alpha-bets have mean values that are significantly different ($p < 0.05$). **Source:** (Arise et al. 2017a)

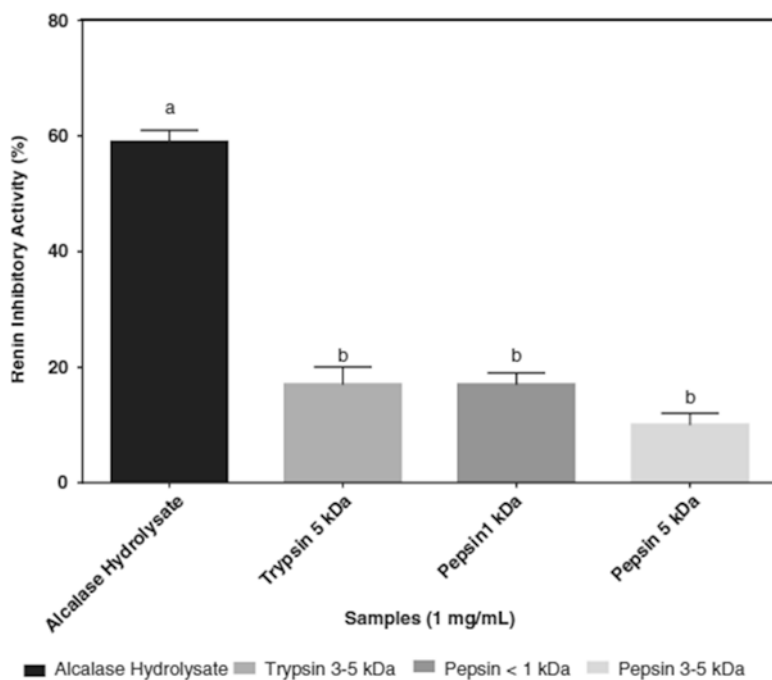


Fig. 7.6 Inhibition of renin by enzymatic Bambara protein hydrolysate and membrane ultrafiltration fractions at a concentration of 1 mg/mL. Error bars (mean $\pm$ SD, $n = 3$) with different alpha-bets have mean values that are significantly different ($p < 0.05$). **Source:** Arise et al. (2017a)

Table 7.2 Antioxidant, ACE and DPPIV inhibitory properties of BBH hydrolysed by Alcalase (BBH-Alc.), Themolysin (BBH-Ther.) and trypsin (BBH-Tryp.) and effects of simulated gastrointestinal digestion on bioactive properties

Samples	Hyrolysates				Stimulated gastro-intestinal digestion			
	ACE (% At100 µg/mL) (IC ₅₀ µg/mL)	DPP-IV (% At 1 mg/mL) (IC ₅₀ in mg/ml)	DPPH (µg trolox eq/mg)	Ferrous chelating (mg trolox eq/mg)	ACE (% At 100µg/mL)	DPP-IV (% At 1 mg/mL)	DPPH (µg trolox eq/mg)	Ferrous chelating (mg trolox eq/mg)
BBH-Alc.	58.301 ± 1.749c (51.812)	44.253 ± 1.327b (1.733)	0.78 1 ± 0.023a	0.498 ± 0.015a	33.843 ± 0.117a	8.996 ± 0.270a	1.420 ± 0.043a	0.25 ± 0.070b
BBH-Ther.	50.597 ± 1.518b (96.579)	44.199 ± 1.326b (1.733)	1.323 ± 0.040b	0.702 ± 0.021b	46.561 ± 2.560b	27.825 ± 0.835b	4.391 ± 0.131b	0.301 ± 0.090c
BBH-Tryp.	28.989 ± 0.870a (536.498)	7.981 ± 0.240a (≥2.5)	5.522 ± 0.166c	1.016 ± 0.030	32.148 ± 1.709a	29.276 ± 0.878b	4.620 ± 0.139b	0.167 ± 0.005a

Keys: BBH (Bambara hydrolysates), Alcalase (BBH-Alc.), Themolysin (BBH-Ther.) and trypsin (BBH-Tryp.). **Source:** Mune et al. (2018)

The improved bioactivities (antioxidant and antihypertensive) of the Bambara protein hydrolysates could be from its higher functional properties. For instance, pancreatin hydrolysis of Bambara groundnut protein resulted in a significant increase in protein solubility (Fig. 7.7), which has a positive effect on other functional properties, such as surface-active, rheological or hydrodynamic properties (Mune et al. 2014). These characteristic effects found the nutritional application of the protein hydrolysates in the fortification of acid beverages and meat products such as sausages where high oil-absorption capacity is required (Mune 2015).

7.4 Amino Acid Composition of Bambara Groundnut Protein and Protein Products

The prominent amino acids in Bambara groundnut protein were glutamic acid and aspartic acid (Aremu et al. 2006; Ijarotimi and Esho 2009; Yao et al. 2015), however, the protein is rich in essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (Aremu et al. 2006; Yao et al. 2015). Although the methionine content of Bambara groundnut is comparably higher than other legumes like soya bean (Aremu et al. 2006; Ijarotimi and Esho 2009), the groundnut still has low tryptophan content when compared with the common soya bean (Yao et al. 2015). Interestingly, the amino acid profile of Bambara groundnut protein is well composed to meet the nutritional needs of most people in developing countries when compared with the required FAO/WHO recommended levels (Table 7.3).

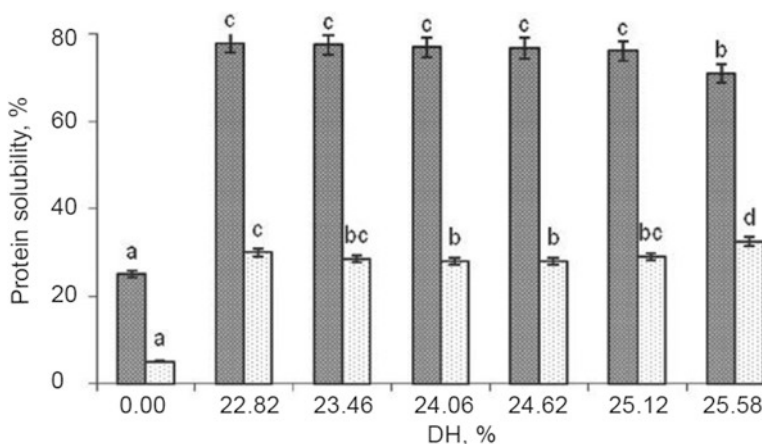


Fig. 7.7 Effect of the degree of hydrolysis (DH) on the solubility of Bambara protein hydrolysates at pH 7 and 4.5. Values followed by different letters are significantly ($p < 0.05$) different. **Source:** Mune et al. (2014)

Table 7.3 Amino acid composition (g/100 g) of Bambara groundnut protein

Essential Amino Acid	FAO*	BBG	Reference
Arginine	2.0	4.0	Hillocks et al. (2012)
Histidine	2.4	2.2	Yao et al. (2015)
Isoleucine	4.2	3.8	Ijarotimi and Esho (2009)
Leucine	4.8	6.8	Eltayeb et al. (2011)
Lysine	4.2	3.0	Mune et al. (2014)
Methionine	2.2	2.0	Aremu et al. (2006)
Phenylalanine	2.8	4.3	Falade et al. (2015)
Threonine	2.6	2.5	Kudre et al. (2013)
Tryptophan	1.4	NR	FAO/WHO (1991)
Valine	4.2	3.8	Thammarat et al. (2015)

NR Not reported; * = Standard values obtained from FAO/WHO (1991)

Notably, the protein extraction, isolation and hydrolysis methods did not have a negative effect on the amino acid composition of Bambara protein products. For instance, the amino acid profile of Bambara protein products (isolates and hydrolysates) similarly showed glutamic acid and aspartic acid as the abundant amino acids present (Table 7.4), while no loss of the profile was recorded during extraction and isolation of the raw protein (Adebowale et al. 2011; Arise et al. 2017a). These acidic amino acids (glutamic and aspartic acids) have been implicated with strong antioxidant effects as a result of the presence of excess electrons that can be donated during interaction with free radicals (Adebowale et al. 2011; Arise et al. 2017a).

7.5 Digestibility of Bambara Groundnut Protein

The peptide bond hydrolysis released H^+ during protein digestion, which caused automatic decreased pH level. The faster decrease, therefore, depicted higher digestion rates and this phenomenon is thus used as an index of protein digestibility (Malomo and Aluko 2015a). One of the major drawbacks limiting the nutritional quality of legume protein generally is their low digestibility. However, pretreatments such as cooking, germination and soaking have led to an increase in the protein digestibility of Bambara groundnut (Yagoub and Abdalla 2007; Adeleke et al. 2017; Oyeyinka et al. 2019). The increased protein digestibility of cooked Bambara groundnut was previously linked to the decrease in the antinutrient contents of the groundnut during thermal processing as well as the structural fragmentation of long polymer chains of its native proteins (Oyeyinka et al. 2019). The application of heat treatment during the pre-processing of Bambara groundnut helped in destroying the heat-labile protease inhibitors, denatured the proteins and opened the structure (especially globulins) for proper digestibility (Adeleke et al. 2017). The faster rate of protein digestion is related with easier protease accessibility to peptide bonds as a result of reduced levels of non-protein materials or sometimes due to the

Table 7.4 Amino acid composition (g/100 g) of Bambara protein isolate and hydrolysates

Amino acid	Isolate	Enzymatic-produced hydrolysates			FAO/WHO (1991)
		Trypsin	Pepsin	Alcalase	
ASP	9.0	8.1	7.4	7.5	
THR	5.2	5.1	4.1	5.1	3.4
SER	4.7	4.2	3.7	4.1	
GLU	14.4	12.4	11.8	12.3	
PRO	4.1	3.7	2.3	4.3	
GLY	2.4	2.2	1.9	2.1	
ALA	2.7	2.4	1.5	2.3	
CYS	0.3	0.3	0.2	0.3	
VAL	4.1	3.9	3.6	3.9	3.5
MET	0.7	0.5	0.3	0.6	
ILE	3.5	3.6	3.3	3.8	2.8
LEU	7.0	6.8	6.7	7.5	6.6
TYR	2.7	2.4	1.8	2.7	1.1
PHE	5.1	4.5	4.1	4.9	6.3
HIS	2.7	2.3	2.1	2.4	1.9
LYS	6.1	5.9	5.8	6.1	5.8
ARG	5.8	5.1	4.6	5.1	
TRP	0.5	0.9	0.5	0.6	
HAA	30.7	29.0	24.3	30.1	
PCAA	14.6	13.3	12.5	13.6	
NCAA	23.4	20.5	19.2	19.9	
AAA	8.3	7.8	6.4	8.2	

Combined total of hydrophobic amino acids-alanine, valine, isoleucine, leucine, tyrosine, phenyl-alanine, tryptophan, proline, methionine, and cysteine (HAA). Positively charged amino acids-arginine, histidine, lysine (PCAA). Negatively charged amino acids-ASX and GLX (NCAA). Aromatic amino acids- phenylalanine, tryptophan, and tyrosine (AAA). **Source:** Arise et al. (2017a, b, c)

pre-digestion reduction in polysaccharides and phytate (as in case of membrane ultrafiltration coupled with enzymatic digestion isolation method). Fibres have been known to have a negative effect on protein digestibility as a result of non-specific interactions between proteins and polysaccharides constituents of foods (Malomo and Aluko 2015a).

The sprouting process on the other hand inherently increased the digestibility of the Bambara groundnut protein by reducing the polyphenols and phytic acid through proteolytic activity of enzymes in the germinated seedling to produce soluble proteins (Yagoub and Abdalla 2007). Moreso, soaking also improved and increased the digestibility of Bambara groundnut protein because the process caused breaking-down and leaching-out of phytic acid-, tannin- and polyphenols-protein complexes (Adeleke et al. 2017).

7.6 Purification and Identification (Sequencing) of Bambara Groundnut Protein

The available literature on the Bambara groundnut has been mainly on the production and health application of the crude protein hydrolysates, hence there existed scanty information on the protein identification (sequencing) of Bambara groundnut proteins. However, protein identification has been done on the dry and malted native (raw) Bambara groundnut proteins (Okpuzor et al. 2010) and presented in Tables 7.3 and 7.4. The procedure involved the drying and malting of the seeds while subsequently extracted in potassium phosphate buffer (pH 7) and precipitated with saturated ammonium sulphate. The multidimensional protein identification technology (MudPit) and liquid chromatography-matrix-assisted laser desorption ionization tandem time-of-flight (LC-MALDI TOF-TOF) mass spectrometry was thereafter used to identify the different protein types. A total of ten (Table 7.5) and twelve (Table 7.6) different types of proteins present in other legume species were identified in the malted and dry seeds, respectively from the 214 peptides isolated after searching 586 proteins of the genus *Vigna* protein databases. Meanwhile, the seed storage protein B and vicilin were observed to be the major proteins (Figs. 7.1 and 7.2) commonly identified in species related to Bambara groundnut protein.

Table 7.5 Proteins identified in malted Bambara groundnut (*Vigna subterranea*)

No	Name	Species	Score	Score	Coverage	Accession
1	Seed storage protein B	<i>Vigna luteola</i>	52.03	52.03	43.04	giL70672852
2	8S globulin alpha subunit	<i>Vigna radiate</i>	15.60	25.16	46.04	giL158251953
3	8S globulin beta isoform precursor	<i>Vigna radiate</i>	9.18	29.23	32.89	giL108743976
4	Chain A, crystal structure of adzuki bean 7S globulin-1	<i>Vigna angularis</i>	4.32	22.37	34.58	giL167013178
5	10 kDa protein precursor (clone PSAS 10)	<i>Vigna radiata</i> var. radiate	4.16	4.16	38.67	giL112671
6	Vicilin protein	<i>Vigna unguiculata</i>	2.00	19.68	37.41	giL160332746
7	BV1 protein	Mungbean yellow mosaic virus Vigna [KA27]	0.32	0.32	7.42	giL8272630
8	Chain 1, crystal structure of the Bowman-Birk inhibitor from <i>V. unguiculata</i> seeds in complex with beta-trypsin at 1.55 angstroms resolution	<i>Vigna unguiculata</i>	0.28	2.77	25.30	giL122920306
9	Heat shock protein 90	<i>Phytophthora melonis</i>	0.20	0.20	5.67	giL156986798
10	8S globulin alpha' isoform precursor	<i>Vigna radiate</i>	0.06	22.71	34.44	giL108743974

Source: Okpuzor et al. (2010)

Table 7.6 Proteins identified in dry Bambara groundnut (*Vigna subterranea*)

No	Protein	Species	Accession	Unused Score	Total Score	% Coverage
1	Seed storage protein B	<i>Vigna luteola</i>	Gil70672852	38.06	38.06	48.74
2	Chain A, crystal structure of adzuki bean 7S globulin-3	<i>Vigna angularis</i>	Gil167013181	20.11	24.27	64.20
3	Dehydrin	<i>Vigna unguiculata</i>	Gil6358640	10.04	10.05	65.64
4	8S globulin alpha subunit	<i>Vigna radiata</i>	Gil158251953	7.46	14.5	51.76
5	Mung bean seed albumin	<i>Vigna radiata</i> var. radiata	Gil1000708	3.70	3.70	13.97
6	Em protein	<i>Vigna radiata</i>	Gil1141784	2.00	2.00	26.26
7	ARG 10	<i>Vigna radiata</i>	Gil2970051	2.00	2.00	18.99
8	PDF 1	<i>Vigna radiata</i>	Gil18146788	1.70	1.70	38.67
9	10 kDa protein precursor (Clone PSAS 10)	<i>Vigna radiata</i> var. radiata	Gil112671	1.52	1.52	30.67
10	Vicillin protein	<i>Vigna unguiculata</i>	Gil160332746	1.50	19.44	64.43
11	Actin	<i>Vigna radiata</i>	Gil6934188	0.96	0.96	8.75
12	7S globulin –2	<i>Vigna angularis</i>	Gil145207915	0.32	14.28	60.60

Source: Okpuzor et al. (2010)

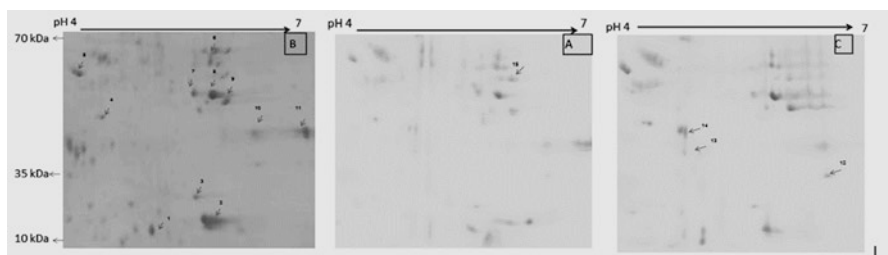


Fig. 7.8 Two-dimensional maps of Bambara (ABC) using immobilized pH gradient (IPG) strips (4–7 non-linear) (a) Red colour Bambara (b) Maroon colour Bambara (c) Cream colour Bambara.
Source: (Arise 2016)

7.7 Proteomic of Bambara Groundnut

The proteomic of Bambara groundnut, which is presented in Fig. 7.8 showed the dominance of acidic polypeptides in three Bambara landraces, which is characteristics of 7S vicilin (Arise 2016). The storage protein (phaseolin), defence-related protein (proteinase inhibitor), stress-related protein (heat shock protein and superoxide dismutase) and proteins involved in metabolism (50S ribosomal protein) were all found in Bambara protein (Okpuzor et al. 2010; Arise 2016). The characteristic

dominance of the acidic polypeptides might have aided the efficacy of the bioactive compounds explored in the bioactivities of Bambara groundnut protein in the food and human health applications.

7.8 Conclusion

The applications of Bambara groundnut protein and protein-derived products in food systems have not been fully explored despite their nutritional values. The isolates and modified products (hydrolysates and bioactive peptides) have improved the possibilities for these proteins to demonstrate their utilisation as functional ingredients in food and human health applications. More so, the advantage (improved nutrition, quality or economy) to facilitate the acceptance of these underutilised proteins as food ingredients to contribute to the structure and nutrition of food products is certainly a step in the right direction. Besides, the use of Bambara groundnut protein and products associated with these proteins is very crucial in the nutraceuticals/functional food production. For instance, peptides from the hydrolysis of these proteins could be bioactive and have a role in disease management/prevention. Although the potentials of Bambara groundnut protein product are at primary experimental level and have yet to receive that commercial recognition seen by the well-known soy protein, nevertheless, these experimental applications must be greatly improved upon for it to become commercially viable. A true understanding of the properties of these protein products would be possible only if their structure, chemical and functional properties have been investigated under conditions relevant to the complex food system and human disease management. The developments, which are likely to have a significant impact on the technological aspects of Bambara groundnut proteins are; improved fractionation techniques, application of molecular biology techniques to modify proteins through enzymatic hydrolysis. It is hoped that databases cataloguing the relevant characteristics of Bambara groundnut proteins will become valuable sources of further information in the not-too-distant future. Lastly, it is likely, that useful studies on the chemistry and technology of Bambara groundnut proteins will continue for a longer period.

References

- Adebowale YA, Schwarzenbolz U, Henle T (2011) Protein isolates from Bambara groundnut (*Voandzeia subterranea* L.): chemical characterization and functional properties. *Int J Food Prop* 14:758–775
- Adeleke O, Adiamo OQ, Fawale OS, Olamiti G (2017) Effect of soaking and boiling on anti-nutritional factors, oligosaccharide contents and protein digestibility of newly developed bambara groundnut cultivars. *Turk J Agric Food Sci Technol* 5:1006–1014
- Aremu MO, Olaofe O, Akintayo TE (2006) A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours. *Pak J Nutr* 5:34–38

- Arise, A.K. (2016). Composition and functional bioactive properties of bambara groundnut protein and hydrolysates. *Thesis submitted to Duran University of Technology, Durban, South Africa*
- Arise AK, Alashi AM, Nwachukwu ID, Ijabadeniyi OA, Aluko RE, Amonsou EO (2016) Antioxidant activities of bambara groundnut (*Vigna subterranea*) protein hydrolysates and their membrane ultrafiltration fractions. *Food Funct* 7:2431–2437
- Arise AK, Alashi AM, Nwachukwu ID, Malomo SA, Aluko RE, Amonsou EO (2017a) Inhibitory properties of bambara groundnut protein hydrolysate and peptide fractions against angiotensin-converting enzymes, renin and free radicals. *J Sci Food Agric* 97:2834–2841
- Arise AK, Aliyu BN, Ajidagba SD (2020) Effect of thermal processing and fermentation on the chemical composition, protein digestibility and functional properties of bambara protein isolate. *Carpathian J Food Sci Technol* 12:148–156
- Arise AK, Amonsou EO, Ijabadeniyi OA (2015) Influence of extraction methods on functional properties of protein concentrates prepared from south African bambara groundnut landraces. *Int J Food Sci Technol* 50:1095–1101
- Arise AK, Dauda AO, Awolola GV, Akinlolu-Ojo TV (2017b) Physico-chemical, functional and pasting properties of composite flour made from wheat, plantain and bambara for biscuit production. *Ann Food Sci Technol* 18:616–624
- Arise AK, Nwachukwu ID, Aluko RE, Amonsou EO (2017c) Structure, composition and functional properties of storage proteins extracted from bambara groundnut (*Vigna subterranea*) landraces. *Int J Food Sci Technol* 52:1211–1220
- Arise AK, Oyeyinka SA, Dauda AO, Malomo SA, Allen BO (2018) Quality evaluation of maize snacks fortified with bambara groundnut flour. *Ann Food Sci Technol* 19:283–291
- Boye J, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, Rajamohamed S (2010) Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Res Int* 43:537–546
- Eltayeb ARS, Ali AO, Abou-Arab AA, Abu-Salem FM (2011) Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranea*). *Afr J Food Sci* 5:82–90
- FAO/WHO. (1991). CODEX CAC/GL 08, 1991. Codex Alimentarius: Guidelines on Formulated Supplementary Foods for Older Infants and Young Children. (4). FAO/WHO Joint Publications: 144.
- Falade KO, Ogundele OM, Ogunshe AO, Fayemi OE, Ocloo FC (2015) Physico-chemical, sensory and microbiological characteristics of plain yoghurt from bambara groundnut (*Vigna subterranea*) and soybeans (*Glycine max*). *J Food Sci Technol* 52:5858–5865
- Hillocks R, Bennett C, Mponda O (2012) Bambara nut: a review of utilisation, market potential and crop improvement. *Afr Crop Sci J* 20:1–16
- Ijarotimi OS, Esho TR (2009) Comparison of nutritional composition and anti-nutrient status of fermented, germinated and roasted bambara groundnut seeds (*Vigna subterranea*). *Br Food J* 11:376–386
- Kudre TG, Benjakul S, Kishimura H (2013) Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *J Sci Food Agric* 93:2429–2436
- Malomo SA, Aluko RE (2015a) Conversion of a hemp seed industrial waste product into a functional protein isolate through membrane ultrafiltration coupled with enzymatic digestion of fibre and phytate. *Innov Food Sci Emerg Technol* 31:151–159
- Malomo SA, Aluko RE (2015b) A comparative study of the structural and functional properties of isolated hemp seed (*Cannabis sativa* L.) albumin and globulin fractions. *Food Hydrocoll* 43:743–752
- Massawe F, Roberts J, Azam-Ali S, Davey M (2003) Genetic diversity in bambara groundnut (*Vigna subterranea* (L.) Verdc) landraces assessed by random amplified polymorphic DNA (RAPD) markers. *Genet Resour Crop Evol* 50:737–741

- Mune M, Minka S, Mbome I (2014) Effects of increasing acylation and enzymatic hydrolysis on functional properties of bambara bean (*Vigna subterranea*) protein concentrate. *Acta Aliment* 43:574–583
- Mune MAM, Minka SR, Henle T (2018) Investigation on antioxidant, angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory activity of Bambara bean protein hydrolysates. *Food Chem* 250:162–169
- Mune MMA (2015) Optimizing functional properties of bambara bean protein concentrate by enzymatic hydrolysis using Pancreatin. *J Food Process Preserv* 39:2572–2580
- Murevanhema YY, Jideani VA (2013) Potential of bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage—a review. *Crit Rev Food Sci Nutr* 53:954–967
- Okafor J, Okafor G, Leelavathi K, Bhagya S, Elemo G (2015) Effect of roasted bambara groundnut (*voandzeia subterranea*) fortification on quality and acceptability of biscuits. *Pak J Nutr* 14:653–657
- Okpuzor J, Ogbunugafor H, Okafor U, Sofidiya M (2010) Identification of protein types in bambara nut seeds: perspectives for dietary protein supply in developing countries. *EXCLI J* 9:17–28
- Oyeyinka AT, Pillay K, Siwela M (2019) Full title-in vitro digestibility, amino acid profile and antioxidant activity of cooked Bambara groundnut grain. *Food Biosci* 31:1–10
- Thammarat K, Leena N, Punnanee S, Soottawat B (2015) Functional and Antioxidative properties of Bambara groundnut (*Voandzeia subterranea*) protein hydrolysates. *Int Food Res J* 22:1584–1595
- Yagoub A, Abdalla AA (2007) Effect of domestic processing methods on chemical composition, in vitro digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranea*) seed. *Res J Agric Biol Sci* 3:24–34
- Yao DND, Kouassi KN, Erba D, Scazzina F, Pellegrini N, Casiraghi AMC (2015) Nutritive evaluation of the Bambara groundnut Ci12 landrace [*Vigna subterranea* (L.) Verdc. (Fabaceae)] produced in Côte d’Ivoire. *Int J Mol Sci* 16:21428–21441