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Structure, composition and functional properties of storage proteins extracted from bambara groundnut (*Vigna subterranea*) landraces

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Abstract Bambara groundnut is a protein-rich traditional legume. In this study, storage proteins were isolated from three bambara landraces. Bambara protein revealed four major protein bands: one broad band at 55 kDa, two medium bands at 62 kDa and 80 kDa and a high molecular weight (HMW) protein at 141 kDa. The vicilin (7S) subunits with molecular weight of 55 kDa and 62 kDa were major fractions in bambara storage proteins. Bambara proteins showed two endothermic peaks ranging from 64 to 69 °C and 76 to 90 °C, respectively. Bambara protein isolates had well-defined tertiary and secondary structures, respectively, at pH 3.0, and this well-defined structure decreased slightly at higher pH values. The isolates revealed a strong secondary structure dominated by α-helical conformation. Foaming capacities of bambara proteins were dependent on pH with maximum percentage FC observed at pH 3.0, while the emulsion activity increased with increasing pH for all the isolates. Vicilin (7S) fraction seems to be the major storage protein fraction of bambara. Bambara proteins could serve as excellent ingredients for the formulation of food foams and emulsions.

Keywords Bambara groundnut, circular dichroism, landrace, protein isolate, solubility, thermal properties.

Introduction

Bambara groundnut (*Vigna subterranean L. verdc*) is an indigenous African legume. It is a good source of protein (15–27%), similar to cowpea and peanuts (Adegbola & Bamishaiye, 2011; Kudre *et al*., 2013; Arise *et al*., 2015). In Southern Africa, bambara is cultivated mainly in Limpopo, Mpumalanga and KwaZulu-Natal provinces of South Africa (Arise *et al*., 2015). It is a drought-tolerant legume and as a result has great potentials as an alternative legume to peanut and soya bean, which cannot withstand harsh agronomic conditions (Mazahib *et al*., 2013). A recent effort to develop improved genotypes has been initiated by Shegro *et al*. (2013). These authors, using morphological quantitative markers, reported a wide genetic variability among 20 bambara groundnut accessions in South Africa. However, extensive breeding research is required before the new bambara varieties can be released to farmers. Currently, cultivated bambara varieties are predominantly the landrace types, which are under-researched and underutilised (Adegbola & Bamishaiye, 2011). Traditionally, bambara is processed and consumed as snacks and porridges by the people living in growing areas.

Knowledge of protein composition and structure may be important to explain functionality and application of legumes. Structure and composition may vary among legumes of the same genotypes which will affect their functionality. The major storage proteins in legume seeds are the globulins. However, some storage proteins, especially among the dry beans (e.g. mucuna and faba bean), have been found to contain more protein in the albumin fraction content than the globulins (Adebowale *et al*., 2007; Rui *et al*., 2011). The relative amount of 7S and 11S protein fractions have been found to vary in soya bean, and this variation may have significant effects on protein functionality (Barać & Stanojević, 2005). For instance, high gelation has been reported for pea vicilin, whereas legumin did not gel under the same condition (Bora *et al*., 1994). Also, the vicilin in cowpea protein displayed better emulsifying properties than did its legumin (Rangel *et al*., 2003). Environmental

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factors such as pH, ionic strength and temperature are the determinant factors upon which structure–function relationship of protein depends. These dependent factors are often encountered in food systems at various stages of food preservation, processing and storage. Other factors related to growth environment and grain variety can also introduce significant changes in composition, structure and functionality of these proteins.

Physicochemical characteristics of plant proteins such as hemp seed (Malomo *et al.*, 2014), pinto bean and soya bean (Tan *et al.*, 2014) have been well studied. So far, very limited studies have been conducted on bambara protein composition and structural characterisation, especially those grown in Southern Africa. Our recent study evaluated the functional properties of protein concentrates prepared from bambara groundnut (Arise *et al.*, 2015). According to our findings, the concentrate prepared by salt (NaCl) solubilisation followed by dialysis to remove the salt and precipitate the proteins showed better foaming and emulsifying properties compared with the concentrates obtained by isoelectric precipitation. However, the isoelectric precipitation, which was produced by alkali solubilisation followed by acid-induced protein precipitation, produced a higher yield of concentrates

The knowledge of protein composition may be important to better understand the functionality of bambara protein and facilitate its utilisation. Hence, the objective of this study was to determine the physicochemical properties of protein isolates from different bambara landraces.

Materials and methods

Materials

Three bambara groundnut landraces grown in Jozini, KwaZulu-Natal Province of South Africa, were used in this study. These were differentiated based on the seed coat colour as red, maroon and cream. The bambara seeds were stored at 4 °C until use.

Low molecular mass protein calibration kits were from Thermo Scientific laboratories (EU, Lithuania). Precast (4–15%) gradient, polyacrylamide Tris–HCl gels and Coomassie brilliant blue R-250 were from Bio-Rad laboratories (Hercules, CA). All other analytical-grade chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Sample preparation

Preparation of defatted flours

Defatted bambara flours were prepared as previously described (Arise *et al.*, 2015). The fat contents of flours after defatting using soxhlet extractor were less than 0.01%. Protein content (N × 6.25) of the defatted flours was determined by Kjeldahl method (AOAC, 2000).

Storage protein extraction

Protein was extracted from defatted bambara seed flour using a modified isoelectric precipitation procedure (Boye *et al.*, 2010b). Protein concentration was determined using Bradford method (Bradford, 1976). Protein isolates were stored in sealed tube at 4 °C until analysed.

Vicilin extraction

The vicilin extraction procedure was carried out according to the method of Rubio *et al.* (2014) with some modifications. Briefly, defatted flour was extracted (1:10 w/v) with 0.2 M borate buffer (pH 9) containing 0.5 M NaCl and centrifuged (20 100 ***g***, 30 min, 4 °C). The supernatant was retained (supernatant A) and the borate insoluble sediment was re-extracted as described above and centrifuged to obtain supernatant B. Supernatants A and B were combined and adjusted to pH 4.6 with glacial acetic acid in the cold, stirred for 30 min and centrifuged (20 100 ***g***, 30 min, 4 °C). The supernatant was dialysed for 4 days against distilled water and centrifuged under same condition. The sediment obtained was freeze-dried as the 7S vicilin.

Analysis

Protein content and yield

The protein content of isolates was determined using the Bradford method (Bradford, 1976). The protein yield was determined as the dry weight of protein isolate per weight of the defatted flour (Arise *et al.*, 2015) as shown in Table 1.

Amino acid composition

The amino acid contents of the protein isolates were determined using Pico-Tag method (Bidlingmeyer *et al.*, 1984).

Table 1 Protein content and yield of bambara protein isolates

Seed type	Protein content (%)	Protein yield (%)
Red	$91.70^{a} \pm 0.05$	$49.60^{a} \pm 0.03$
Maroon	$89.80^{a} \pm 0.03$	$51.50^{a} \pm 0.04$
Cream	$90.80^{a} \pm 0.04$	$52.50^{a} \pm 0.02$

[a]Mean ± SD. Mean values followed by different superscripts in each column are significantly different at $P \leq 0.05$.

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SDS-page

SDS-PAGE of bambara seed proteins was done as described previously (Mundi & Aluko, 2012). This was carried out under both reducing (with mercaptoethanol) and nonreducing conditions. Precast (4–12%) gradient polyacrylamide Tris–HCl gels were used with a Bio-Rad Criterion Cell under a constant voltage of 200 V. A mixture of standard proteins (10–250 kDa) was used as the molecular weight markers. Gels were stained with Coomassie Brilliant R-250.

Intrinsic fluorescence emission

The method described by Li & Aluko (2006) was used to record intrinsic fluorescence spectra on a Jasco FP-6300 spectrofluorimeter (Jasco, Tokyo, Japan) at 25 °C using a 1-cm path length cuvette. Protein stock solutions were prepared in 0.1 M sodium phosphate buffer, at pH 3.0, 5.0 or 9.0; each buffer was then used to dilute the respective stock solution to 0.002% (w/v) and fluorescence spectra recorded at excitation wavelengths of 275 nm (tyrosine and tryptophan) with emission recorded from 280 to 450 nm. Emissions of the buffer blanks were subtracted from those of the respective samples to obtain fluorescence spectra of the sample.

Differential scanning calorimetry

Thermal properties of all protein isolates were studied using a differential scanning calorimeter (SDT Q600, USA) using the method of Kudre *et al.* (2013), with the exemption that the samples were dispersed in 0.05 M sodium phosphate buffer (pH 7).

Circular dichroism (CD) spectra measurement

CD spectra of samples were measured at 25 °C in a J-810 spectropolarimeter (Jasco, Tokyo, Japan) using the spectral range of 190–240 nm (far-UV) for secondary structure determinations and 250–320 nm (near-UV) for tertiary structure according to the method described by Omoni & Aluko (2006).

Protein solubility profile as a function of pH

The pH solubility profiles of protein isolates were determined according to the method of Kudre *et al.* (2013).

Foaming capacity (FC) and foam stability (FS)

FC and FS were carried out using the method of Adebiyi & Aluko (2011), except that 0.1 M phosphate buffer at pH 3.0, 7.0 and 9.0 was used.

Emulsifying properties

Emulsifying activity and stability were determined using the method described by Arise *et al.* (2015) with some modifications. Protein samples (300 mg) were prepared in 5 mL of 0.1 M phosphate buffer at pH 3.0, 7.0 and 9.0. The resulting suspensions were homogenised with 5 mL of sunflower oil using polytron homogeniser (PT 210: Fisher Scientific, Water side UK) at 10 000 rpm for 1 min. The emulsions were centrifuged at 1100 ***g*** for 5 min at 4 °C; height of the emulsified layer and that of the total contents in the tube were measured. The emulsifying activity (EA) was calculated using the expression below

$$\text{EA}\ (\%) = \frac{\text{Height of emulsified layer in the tube} \times 100}{\text{Height of total content in the tube}}$$

Statistical analysis

All experiments were conducted in triplicate. Data were analysed using one-way or two-way analysis of variance (ANOVA) where necessary and the mean values were compared using Tukey's multiple comparison test with significant differences at $P < 0.05$.

Results and discussion

Protein content and yield

The protein content (approx. 90.7%) and protein yield (51.2%) of the bambara isolate were similar across landraces (Table 1). The protein contents of the isolates in this study were higher than those previously reported for pigeon (82.4%) (Mwasaru *et al.*, 1999) and hemp seed (84.2%) (Malomo *et al.*, 2014) obtained by an isoelectric precipitation method. The protein content of bambara isolate was similar to that of cowpea (89.5%) protein and the value reported for bambara protein isolate (90%) (Mwasaru *et al.*, 1999; Adebowale *et al.*, 2011). Therefore, the results suggest that the bambara protein isolates are of relatively high purity. Also, the protein yield obtained for the isolates (51.2%) is similar to 51% reported for cowpea protein isolates.

Amino acid composition

Glutamic acid and aspartic acid, which may include glutamine and asparagine, respectively, were the major amino acids in bambara protein (Table 2). These amino acids are also the major amino acids found in other legume seeds such as soya bean (Adebowale *et al.*, 2011), fenugreek (Feyzi *et al.*, 2015), mung bean and black bean (Kudre *et al.*, 2013). Bambara landrace

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Table 2 Amino acid (g/100 g protein) composition of bambara seed protein isolates

Amino acid	Red	Maroon	Cream isolate
Isoleucine[a]	2.97	3.16	2.83
Leucine[a]	6.71	6.96	6.69
Lysine[a]	5.95	6.03	5.81
Methionine[a]	1.26	1.26	1.1
Phenylalanine[a]	5.35	5.97	4.83
Threonine[a]	5.11	5.13	4.61
Valine[a]	4.09	4.45	3.82
Histidine	2.41	2.43	2.39
Arginine[a]	6.61	7.31	5.9
Tyrosine	2.73	2.99	2.56
Alanine	3.57	3.92	3.23
Aspartic acid	9.32	10.72	8.49
Glutamic acid	15.66	15.91	14.93
Glycine	2.63	2.81	1.87
Serine	3.99	4.48	3.17
Proline	3.08	3.41	2.42

[a]Essential amino acids; FAO/WHO (2007) for lysine 5.8.

also contained reasonable amount of arginine (approx. 7 g/100 g protein), a conditionally essential amino acid. Arginine is involved in the synthesis of protein and nitric oxide (NO) as well as amino acids such as proline and glutamate in human (Kudre *et al.*, 2013). The lysine content of bambara protein is high (6.0 g/100 g protein) and similar among landraces. Bambara isolate is a good source of lysine which compares favourably to the lysine content in soya bean protein (Adebowale *et al.*, 2011; Amonsou *et al.*, 2012). Based on FAO/WHO (2007) recommended pattern, bambara proteins may be considered good sources of lysine for preschool children and adults. Therefore, bambara protein could offer some potential in food formulation where it could be used to complement cereal proteins which generally lack lysine. Amino acid data from our previous study on bioactivity of bambara protein isolate also showed that bambara was a good source of lysine (Arise *et al.*, 2016). However, bambara protein had limited content of cysteine and methionine in comparison with WHO standard (Arise *et al.*, 2016). But the bambara proteins were limited in methionine, which is consistent with the amino acid pattern of legumes.

SDS-page

Bambara proteins showed four major polypeptide bands: a broad band at 55 kDa, two medium bands at 62 kDa and 80 kDa and a high molecular weight (HMW) protein at 141 kDa. The HMW protein was reduced by mercaptoethanol, suggesting that this was stabilised by disulphide bond (Fig. 1a). A HMW proteins have been reported for pulses such

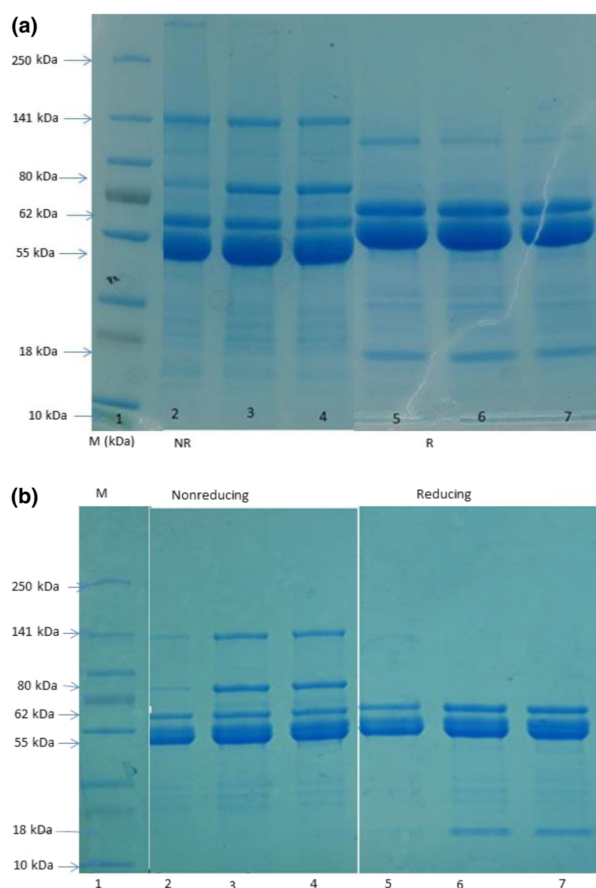

Figure 1 (a) SDS-PAGE patterns of bambara groundnut protein isolates. Lane 1 – standard marker. Lanes 2, 3 and 4 carried out without β-mercaptoethanol. Lane 2, red isolate; lane 3, maroon isolate; lane 4, cream isolate. Lanes 5, 6 and 7 carried out with β-mercaptoethanol. Lane 5, red isolate; lane 6, 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 β-mercaptoethanol. Lane 2, red isolate; lane 3, maroon isolate; lane 4, cream isolate. Lanes 5, 6 and 7 carried out with β-mercaptoethanol. Lane 5, red isolate; lane 6, cream isolate; and lane 7, maroon isolate.

as kidney bean (150 kDa) and field pea (155 kDa) (Shevkani *et al.*, 2015). The protein bands reported at 55 kDa and 62 kDa may correspond to 7S vicilin subunits (Boye *et al.*, 2010b; Rui *et al.*, 2011; Kudre *et al.*, 2013; Shevkani *et al.*, 2015). Similar protein pattern observed in this research has been reported for pea (Park *et al.*, 2010), mung bean and black bean isolate (Kudre *et al.*, 2013) with major broad band at 54, 51, 54 and 52 kDa, respectively. A medium intensity protein band of approx. 62 kDa has also been reported for cowpea (Rangel *et al.*, 2003).

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To confirm the major protein fraction in bambara, the vicilin was extracted from bambara grains and analysed by electrophoresis. Interestingly, SDS-PAGE of vicilin under nonreducing and reducing conditions was not significantly different from those obtained for the protein isolate (Fig. 1b). The two vicilin subunits (55 kDa and 62 kDa) were the main polypeptides and appeared in the gels under reducing and nonreducing conditions. These results further confirm that vicilin may be the major polypeptides of bambara storage proteins.

Intrinsic florescence emission

Intrinsic emission fluorescence spectroscopy reveals the conformational changes of bambara proteins at different pH values (Fig. 2). Fluorescence intensity is a reflection of the overall tertiary conformation of proteins, especially the interactions of aromatic amino acids with the hydrophilic environment (Schmid, 1989). Thus, the information provided enables estimation of the degree of protein folding, which has relationships with solubility and functional performance in food systems like foams and emulsions (Malomo *et al*., 2014).

At pH 9.0, bambara proteins showed a maximum fluorescence wavelength (λ_{max}) of 338 nm, which revealed a characteristic fluorescence profile of tryptophan residues mostly contained within a hydrophobic environment (Mundi & Aluko, 2013). However, at pH 3.0 and 7.0, the λ_{max} (332 nm) showed a blue shift that suggests relocation of tryptophan residues to a more hydrophobic pocket. Moreover, at pH 9.0 the F_{max} values for tryptophan were slightly higher than at pH 3.0 and 7.0. The observed λ $_{max}$ at high pH value in the present study is similar to previous reports on quinoa protein isolates (Abugoch *et al*., 2008) and hemp seed isolates (Malomo *et al*., 2014).

Differential scanning calorimetry (DSC)

The structure of bambara protein was further characterised by DSC (Table 3). The protein isolates showed two endothermic peaks at 68–71 °C and 76–90 °C. These two peaks most likely represent the denaturation of the 7S protein at lower temperature (68–71 °C) and HMW protein at higher temperature (78–90 °C) as indicated by SDS-PAGE (Fig. 1). A similar trend of two endothermic peaks has been reported for cowpea isolates with denaturing temperatures (T_d) of 83–84 °C and 91–93 °C, respectively (Avanza *et al*., 2013). As the high thermal denaturation temperature of the cowpea seed proteins was linked to a high proportion of β-sheet structure (Hirano *et al*., 1992), the structure of bambara protein isolates was further analysed by CD.

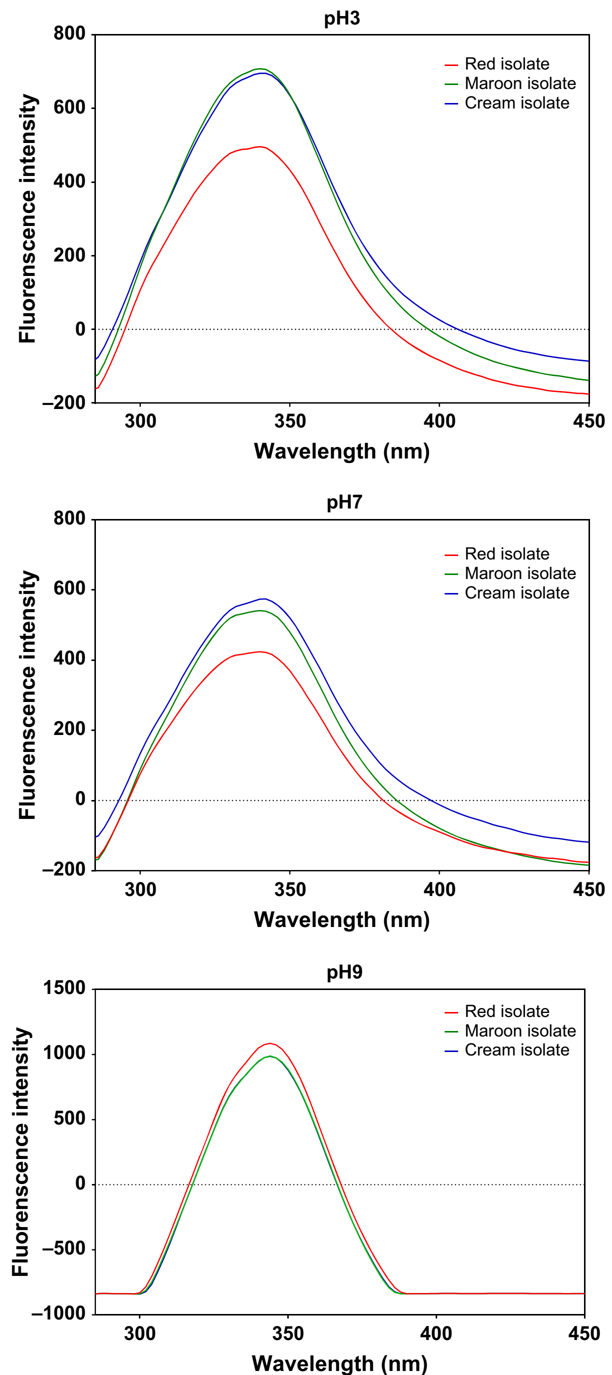

Figure 2 Intrinsic Fluorescence intensity (arbitrary units) of bambara seed protein at different pH values.

Circular Dichroism spectra

The far-UV CD data reflect the secondary structure conformation and degree of ordered arrangement

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Table 3 Thermal properties of bambara seed proteins from different landraces

Samples	Peak	T_o (°C)	T_d (°C)	ΔH (J g^{-1})
Red isolate	I	$64.2^b \pm 0.1$	$71.0^b \pm 0.6$	$6.6^a \pm 0.1$
	II	$78.^{5b} \pm 0.5$	$84.3^b \pm 0.4$	$6.8^a \pm 0.4$
Maroon isolate	I	$62.1^a \pm 0.1$	$68.7^a \pm 0.2$	$6.4^a \pm 0.3$
	II	$78.1^b \pm 0.2$	$90.4^c \pm 0.1$	$11.7^b \pm 0.5$
Cream isolate	I	$66.9^c \pm 0.7$	$69.9^a \pm 0.5$	$6.5^a \pm 0.1$
	II	$76.0^a \pm 0.1$	$78.9^a \pm 0.7$	$6.7^a \pm 0.7$

T_o: Onset temperature; T_d: peak denaturation temperature; ΔH: enthalpy of denaturation Mean ± SD. Mean values followed by different superscripts in each column are significantly different at $P \leq 0.05$.

(Schmid, 1989). Secondary structure fractions such as the α-helix and β-sheet reflect a more ordered conformation, which could determine protein functionality such as solubility Shevkani *et al.* (2015). The CD spectrum of the isolates at pH 3.0 in the far-UV region showed the dominance of a negative peak at about 210 and 220 nm, a weak positive peak in the vicinity of 195 nm and a broad shoulder that extended from 220 to 240 nm (Fig. 3). These features of the spectrum are sufficient indicators of highly ordered structure, including α-helix and β-types (Yang *et al.*, 1986; Yin *et al.*, 2011; Anwer *et al.*, 2016). The isolates revealed a strong secondary structure dominated mostly by α-helix conformation. This is based on intense ellipticity between 210 and 220 nm. All the isolates showed similar ellipticity pattern, but the cream isolate had less intensity in comparison with red and maroon isolates. In addition, the ellipticity at 195 nm was stronger for red and maroon isolates, which indicates the presence of more β-sheet structure when compared to the cream isolate. The presence of a higher level of β sheet structure indicates a more open conformation.

At pH 9.0, the ellipticity at 195 nm for the cream isolate became more intense when compared to that obtained at pH 3.0 and 7.0. For the red and maroon isolate, there is no difference in the ellipticity at pH 9.0. However, there was a reduction in intensity of the α-helix for studied isolates This suggests that increased charge at alkaline pH 9.0 may have produced greater protein–protein electrostatic repulsion resulting in more disorganised structure when compared to that at pH 7.0. This result differs from what was reported for kidney bean vicilin which showed no defined structure at pH 3.0 (Sule Mundi & Aluko, 2013).

The near-UV CD spectra show that the structural conformation of bambara seed proteins was affected by varying the pH conditions (Fig. 4). For example, at pH 3.0, the proteins had peak ellipticity at 276 nm, which is consistent with tyrosine residues within a hydrophobic environment. The results are consistent with the fluorescence intensity data, which also showed

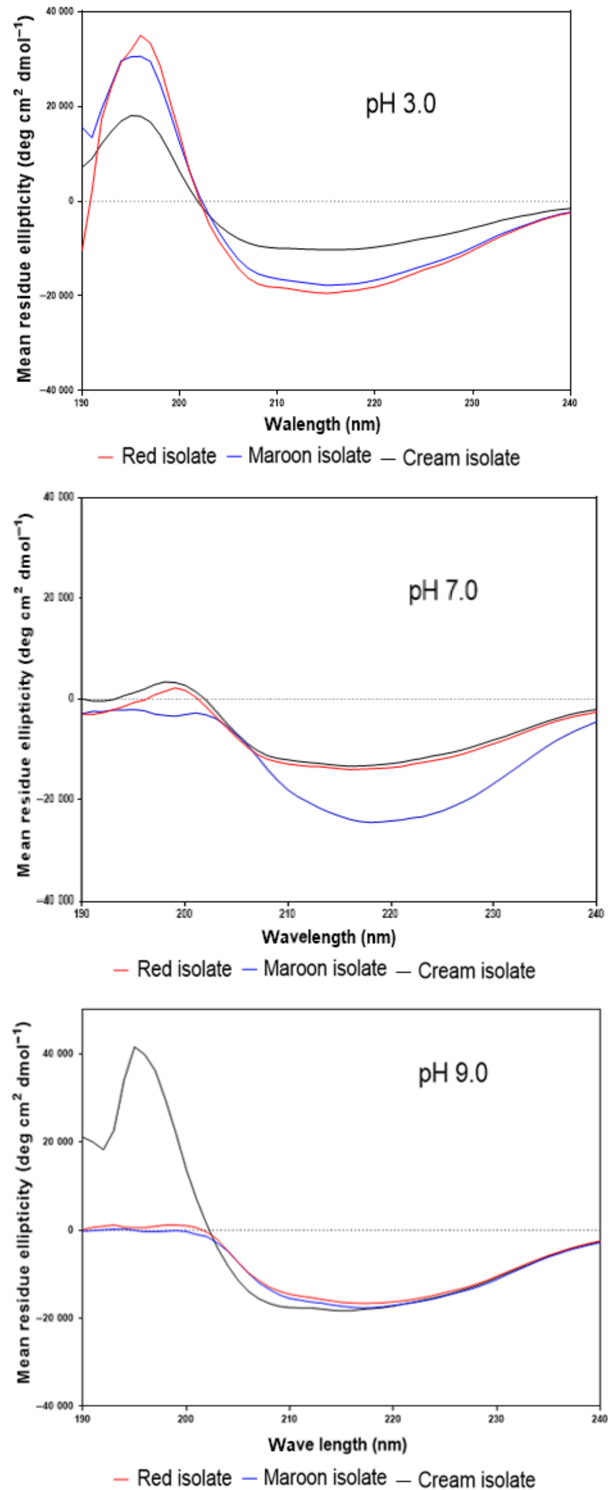

Figure 3 Far-UV circular dichroism spectra of bambara protein isolates at different pH values.

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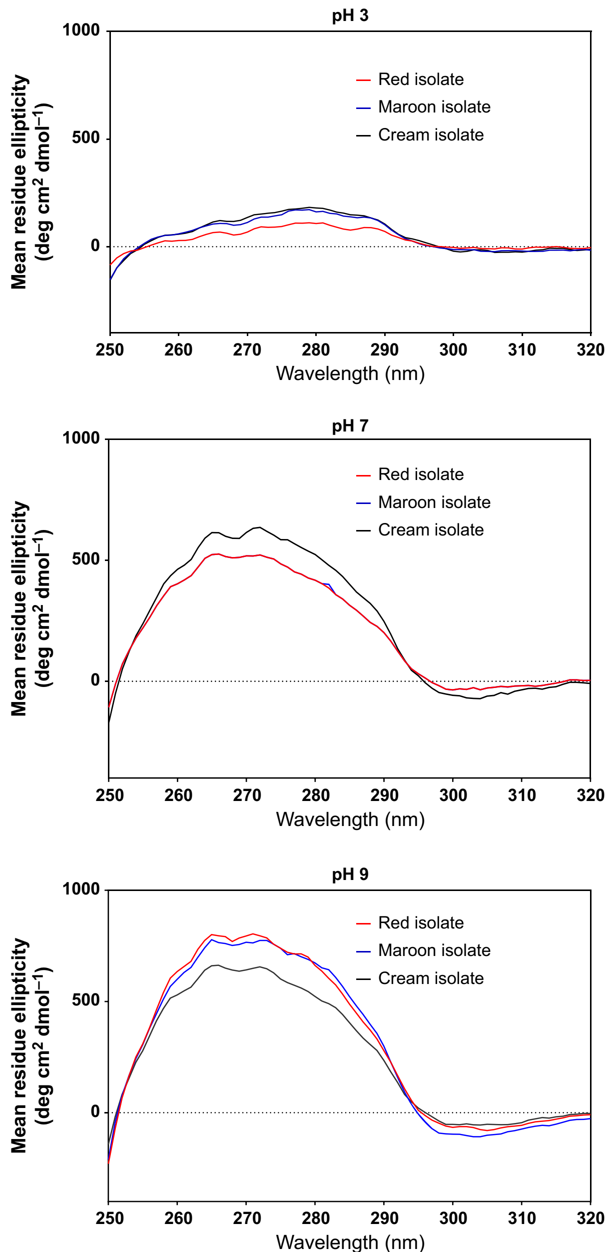

Figure 4 Near-UV circular dichroism spectra of bambara seed protein at different pH values.

the red isolate with less structural compactness at pH 3.0 than the cream and maroon isolates

At pH 7.0, there was an increased ellipticity in comparison with pH 3.0. This may be due to shift in the aromatic amino acid residues into the more hydrophobic interior as the exterior became more hydrophilic. This is revealed in a greater ellipticity values at pH 9.0 for all the proteins which is also similar to the fluorescence intensity data. The result is in line with earlier findings (Kelly *et al.*, 2005; Malomo & Aluko, 2015).

Solubility

Bambara proteins exhibited U-shaped solubility curves in the pH range of 2–10 (Fig. 5). Minimum solubility (approx. 12%) was observed at pH around 4–5. This could be attributed to the intermolecular attraction of protein molecules in the isoelectric zone (Ali *et al.*, 2010). Protein solubility increased when the pH values increased gradually from 4 to 10 or reduced from 4 to 2 reaching a maximum of approximately 79%. This is because at high acidic or high alkaline pH values, proteins carry net positive or negative charges, respectively, and thus, electrostatic repulsion and ionic hydration could have promoted protein solubilisation (El Nasri & El Tinay, 2007). The results obtained in this study are similar to that reported for kidney bean protein isolate, chickpea and cowpea which showed an increase in protein solubility at pH values above and below their isoelectric point (Sanjeewa *et al.*, 2010; Mundi & Aluko, 2012).

Foaming capacity and foam stability

The foaming capacities of bambara proteins were dependent on pH with maximum percentage FC value observed at pH 3.0 (Fig. 6). FC profiles were generally similar among landraces. pH-dependent foaming capacity of globular storage protein has previously been reported (Malomo *et al.*, 2014). At pH 3, the unfolded state of protein enables it to diffuse and adsorb at air–liquid interface. The degree of protein solubility significantly affects foaming capacity of proteins (Malomo *et al.*, 2014). The high FC observed at

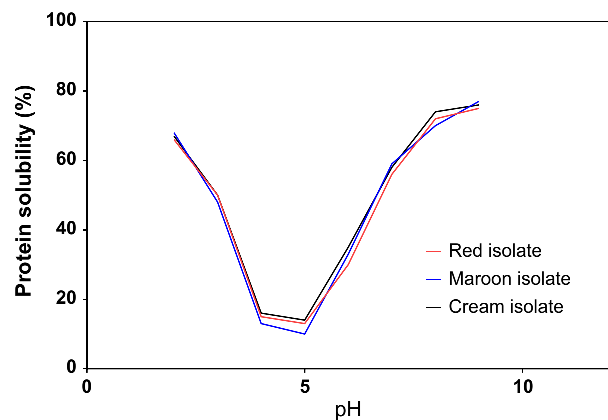

Figure 5 Effects of pH on solubility of bambara groundnut protein isolates.

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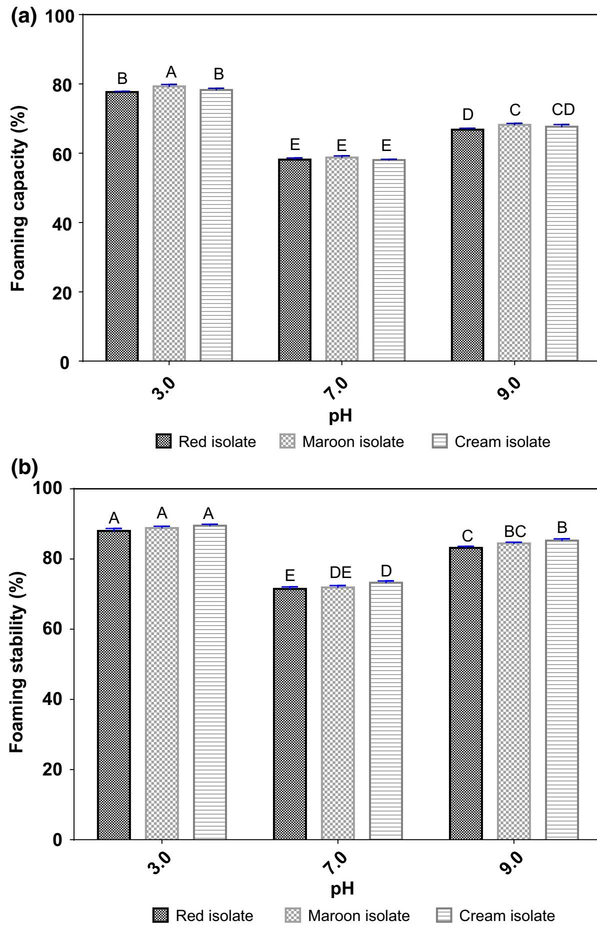

Figure 6 (a) Foaming capacity of bambara protein isolates at different pH values. (b) Emulsion activity of bambara protein isolates at different pH values.

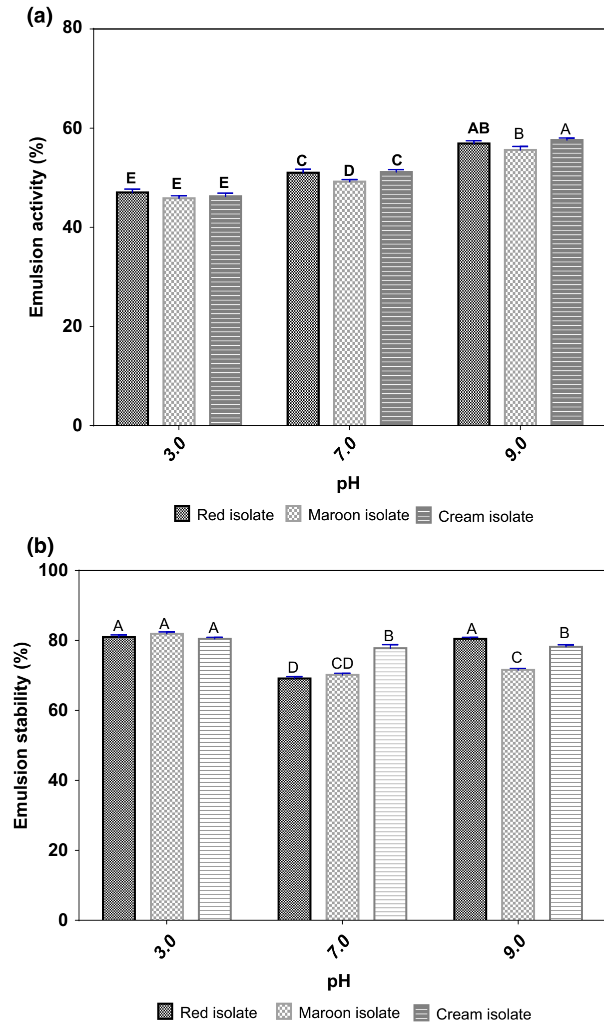

Figure 7 (a) Foaming stability of bambara protein isolates at different pH values. (b) Emulsion stability of bambara protein isolates at different pH values.

pH 3.0 for all bambara protein isolates may be due to increased protein flexibility in more acidic region (Fig. 5). Further, higher FC suggests greater interactions with the aqueous phase which enhance the ability of the protein molecules to encapsulate air particles. Increased interaction with water and a more flexible protein structure at pH 3.0 are consistent with the near-UV CD data, which showed minimal protein structure when compared to pH 7.0 and 9.0.

Minimum FC was observed at pH 7.0 for all the isolates. This could be due to the fact that the balance of protein conformation and net charge probably limits air encapsulation ability. The results obtained in this study are in agreement with the report for hemp seed (Malomo *et al.*, 2014), kidney bean (Mundi & Aluko, 2012) and cowpea protein isolates (Ragab *et al.*, 2004).

Bambara isolates had similar foaming stability. Unlike the landrace, the stability was significantly influenced ($P < 0.05$) by pH. The foaming stability revealed higher FS at pH 3.0 for all the isolates (Fig. 7). This may occur as a result of formation of stable molecular layers in the air–water interface of the foams (Adebowale *et al.*, 2011). Higher FS at low pH has been previously reported for mucuna protein (Adebowale & Lawal, 2003) and hemp seed protein (Malomo & Aluko, 2015).

Emulsion activity (EA) and stability (ES)

The emulsion activity increased with increasing pH for all the isolates (Fig. 6b). Possibly, as the pH increases, the increased net charge enhanced protein unfolding that produced greater exposure of hydrophobic

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groups. The increased number of exposed hydrophobic groups would enhance greater interaction with oil droplets, thus resulting in higher EA. This same trend was observed for *Ginko biloba* seeds (Deng *et al.*, 2011) and kidney bean protein isolates (Mundi & Aluko, 2012).

The emulsion stability result revealed significant difference ($P < 0.05$) for all the isolates at different pH values (Fig. 7b). ES is higher at acidic and alkaline pH in comparison with neutral pH. The lower value of ES observed at pH 7.0 suggests a weak interfacial membrane formation as protein–protein interaction is reduced at this pH for red isolate. In general, the isolates were able to stabilise emulsion very well (>70%) at all pH values which could be attributed to the high protein content of isolates (approx. 90.7%). The results obtained in this study are comparable to that reported for pea, chickpea and lentil protein isolates (Boye *et al.*, 2010a). However, there was no significant interaction between the varieties and pH values.

Conclusion

Bambara landrace storage proteins are good sources of lysine. The vicilin-like protein with two subunits seems to be the major storage protein fraction of bambara seed. The three bambara protein isolates revealed a strong secondary structure dominated mostly by α-helix conformation. Bambara storage proteins are thermally stable and seem to display similar and good foaming and emulsion activities. Therefore, bambara proteins could be employed as ingredients for the production of food foam and emulsion. The high thermal stability of bambara proteins suggests that the protein could be used as ingredient in food systems where moderate heat treatment is required and still prevents its denaturation.

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