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Original article

Influence of extraction methods on functional properties of protein concentrates prepared from South African bambara groundnut landraces

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Summary Functional properties of protein concentrates prepared from three bambara groundnut landraces using acid precipitation and salt solubilisation methods were evaluated. The protein content of bambara grains (26–27%) was similar for the three landraces. The acid precipitation gave a much higher yield of protein concentrates (52%), which were also high in protein (79%) compared to the salt solubilisation method (yield: 25%, protein content: 57%). Functional properties of proteins were more influenced by the methods of preparation rather than the landraces. Protein concentrate prepared by salt solubilisation method showed higher emulsifying (63–66%), foaming (53–57%), water (1.4–2.0 mg mL^{-1}) and oil absorption properties (2.2–2.6 mg mL^{-1}) than the acid-precipitated concentrates (53–57%, 63–66%, 2.0–2.7 mg mL^{-1}, 1.4–1.7 mg mL^{-1}). The foaming capacity and stability of all the protein concentrates decreased with increasing pH from 3 to 8. Salt solubilisation may be the most appropriate method for the enhanced functionality and utilisation of bambara groundnuts' protein concentrates.

Keywords Acid precipitation, bambara groundnut, functional properties, landrace, protein concentrate, salt solubilisation.

Introduction

Bambara groundnut (*Vigna subterranea* L. *Verdc*) is a neglected legume of the African origin (Adegbola & Bamishaiye, 2011). It is the third most important after groundnut (*Arachis hypogeal*) and cowpea (*Vigna unguiculata*) in Africa (Adegbola & Bamishaiye, 2011). Bambara is indigenous to South Africa and is grown mainly in Limpopo, Mpumalanga and KwaZulu-Natal provinces of South Africa (Mabhaudhi & Modi, 2013). The protein content of bambara grain may vary between 15% and 25% (Adegbola & Bamishaiye, 2011; Hillocks *et al*., 2012; Murevanhema & Jideani, 2013). This is similar to cowpea and slightly lower when compared to that of soya bean (Adegbola & Bamishaiye, 2011). Furthermore, bambara groundnut is highly drought tolerant and produces better yield under harsh agronomic condition, which gives it an advantage over other legume grains such as groundnut and soybean (Mazahib *et al*., 2013). Despite these attributes, the agro-ecological, genetic potential as well as the nutritional importance of bambara groundnuts has not been fully researched (Boateng *et al*., 2013). The crop is still cultivated from local landraces in South Africa.

Plant protein concentrates may be utilised in foods for the improvement of both nutritional and functional quality of the food products. The yield, composition and functionality of proteins may vary depending on the grain varieties and method of extraction (Zayas, 1997). The functional properties of soya bean protein and concentrates have been extensively researched (Kwon *et al*., 2010; De la Caba *et al*., 2012; Rebholz *et al*., 2012). For example, the protein yield of soya bean concentrate (16.2%) prepared by micellisation method was found to be substantially low, three to four times that obtained by isoelectric precipitation. However, soya bean protein concentrate obtained by isoelectric precipitation showed lower foaming capacity compared to soya bean concentrate extracted using the micellisation method (Adebowale *et al*., 2011). This suggests that methods of extraction may have influence on the functionality of proteins and, consequently, their application in foods. A comparative study on

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chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut cultivated in Thailand was carried out by Kudre *et al.* (2013). Findings show that all protein isolates contain substantial amount of lysine. However, by DSC, mung bean and bambara isolates were characterised by two endothermic peaks, whereas three peaks were found for black bean isolates, suggesting some differences in thermal stability and structural composition. In a recent study, Kudre & Benjakul (2014) investigated further the effects of heat treatment (50–80 °C) in combination with ethylenediaminetetraacetic acid (EDTA) on functional and sensory properties of bambara protein isolates. Results show that bambara protein isolates prepared in the presence of EDTA exhibited higher emulsion activity and stability indices as well as higher foam expansion and stability than those prepared in the absence of EDTA, regardless of the heating temperature at 95% level of significance. Although diverse studies on bambara landraces have been reported in Africa, particularly in Nigeria (Adebowale *et al.*, 2011; Adegunwa *et al.*, 2013), results have revealed some differences in their functional properties. However, as stated as above, bambara from Southern Africa remain underutilised. Due to the increasing interest in alternative protein sources for human nutrition and functional applications in foods, the knowledge of the physicochemical properties of South African bambara protein may be required to facilitate utilisation and value addition. Therefore, this study aims to investigate the functional properties of protein concentrate extracted from South African bambara groundnut landrace using acid precipitation and salt solubilisation methods.

Materials and methods

Materials

Three cultivated landraces of bambara groundnuts were obtained from Josini, KwaZulu-Natal province of South Africa. These were identified based on the seed coat colour as red, maroon and cream.

Preparation of defatted flours

Bambara groundnuts were dehulled manually using mortar and pestle. Dehulled grains were thereafter ground in a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA) and sieved through a screen mesh of 355 μm to obtain fine flour. Bambara flours was defatted with n-hexane in the ratio 1:5 (flour:solvent) for 3 h on a magnetic stirrer at speed of 198 ***g***. Defatted flours were placed in fume hood overnight to remove the remaining hexane.

Preparation of concentrates

Acid precipitation and salt solubilisation methods were followed for the preparation of bambara groundnut concentrates. For acid precipitation, the method utilised by Adebowale *et al.* (2007) was adopted with some modifications. Briefly, defatted flour was suspended in water at 1:10 (flour to water ratio) and pH was adjusted to 8.0 with 1 M NaOH, to facilitate protein solubilisation. The suspension was stirred for 4 h at 32 °C in a shaking water bath (Scientific, 132A, Pretoria, South Africa). Centrifugation was thereafter carried out (Eppendorf 5810R, Hamburg, Germany). The centrifuge was set at 4000 ***g*** for 30 min at 4 °C. After centrifugation and recovery of the supernatant, the precipitate was resuspended in half the volume of initial water and extraction was carried out as described above. The supernatants were pooled together and pH adjusted to 4.0 with 0.5 M HCL to precipitate the protein concentrates, which were recovered by centrifugation at 5000 ***g*** for 30 min at 4 °C. The protein concentrate was freeze-dried (model 22KBTES_55, ZIRBUS technology, Bad Grund, Germany) and kept at 4 °C until required.

Protein concentrates were prepared using the method of Teixeira *et al.* (2013), with some modifications. Defatted flours were suspended in 0.5 M NaCl (1:10 w/v). The suspension was stirred in a shaking water bath for 4 h at 32 °C. The suspension was centrifuged at 12 000 ***g*** for 20 min at 4 °C. The clear supernatant was dialysed (cut-off 10 kDa) against distilled water for 48 h. The dialysed extract was freeze-dried and stored at 4 °C until required.

Analysis

Grain composition

Moisture, fat and ash contents were determined using AOAC methods (AOAC, 2000) with analytical numbers 950.46, 960.39 and 920.153, respectively. The protein content (N × 6.25) was determined by Kjeldahl method with No. 928.08. Total carbohydrate was calculated by difference.

Protein yield and protein content

Yield of protein concentrate was determined as the dry weight of protein concentrate after precipitation and solubilisation, respectively, per weight of the defatted flour as shown below (Qayyum *et al.*, 2012). The protein content (N × 6.25) of the defatted flour and the protein content of the concentrates were determined by Kjeldahl method (AOAC, 2000)

$$\text{Yield}\,(\%) = \frac{\text{Protein concentrate recovery} \times \text{Protein content of concentrate}\,(\%) \times 100}{\text{Protein content of defatted flour}\,(\%)}$$

Colour
Colour measurement of the concentrates was carried out using colour flex (A60-1014-593; Hunter Associates Laboratory, Reston, VA, USA) on the basis of lightness (L^*), red-green (a^*) and yellow-blue (b^*) values. Soya bean isolate was used as reference. The instrument was calibrated against white and black colour tiles before colour measurement. Total colour difference (ΔE) was calculated as shown below

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}.$$

Water and oil absorption capacity
The water absorption capacity and oil absorption capacity of protein concentrates obtained by acid precipitation and salt solubilisation were determined using the method described by Mundi & Aluko (2012), with some modifications. One gram of each sample was dispersed in 10 mL of distilled water (or sunflower oil) in a 50-mL preweighed centrifuge tube. The dispersion was vortexed for 1 min, allowed to stand for 30 min and then centrifuged at 4000 ***g*** for 30 min at room temperature. The supernatant was decanted, excess water (or oil) in the upper phase was drained for 15 min, and the tube containing the residue was weighed again to determine the amount of water or oil retained per gram of the sample.

Foam capacity
Foams were formed as prescribed by Aluko *et al.* (2009), with some modifications. Suspensions were prepared by dispersing 300 mg in 5 mL of 0.1 M phosphate buffer at pH 7.0. Sample suspensions were homogenised at 3600 ***g*** for 1 min using Polytron homogenizer (PT 210; Fisher Scientific, Water Side, UK). Volumes of the suspension were recorded before and after homogenisation. Foaming capacity was determined as follows using the mean of three measurements.

$$\text{Foam capacity (FC)} = \frac{\text{Volume after homogenisation} - \text{Volume before homogenisation} \times 100}{\text{Volume before homogenisation}}$$

Foam stability was determined as the volume of foam that remained after 8 h at room temperature expressed as percentage of the initial volume.

The effect of pH on foaming properties was carried out by adjusting the suspension to the desired pH 3.0, 6.5 and 8.0 using either 1 M HCl or 1 M NaOH. Homogenisation was thereafter carried out as described above. The final volume after the addition of HCl or NaOH was used as the volume before homogenisation for the calculation of the foaming capacity.

Emulsifying activity and stability
Emulsifying activity and stability were determined using the method described by Lawal *et al.* (2007). Five millilitre (5 mL) portions of protein solution were homogenised with 5 mL of sunflower oil. The emulsions were centrifuged at 1100 ***g*** for 5 min. The height of the emulsified layer and that of the total contents in the tube was measured. The emulsifying activity (EA) was calculated using the expression below.

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

Emulsion stability was determined by heating the emulsion at 80 °C for 30 min after which it was centrifuged at 1100 ***g*** for 5 min.

$$\text{ES}\,(\%) = \frac{\text{Height of emulsified layer after heating} \times 100}{\text{Height of emulsified layer before heating}}$$

Statistical analysis

Experiments were conducted in triplicate. Mean scores of some of the results and their standard deviation were reported. Data were subjected to analysis of variances, and Duncan multiple range (Duncan, 1995) test was used to separate the means.

Results and discussion

Grain composition

The carbohydrate, protein, fat, ash and moisture content of the three landraces of bambara grain were found to be similar (Table S1). The protein contents (26–27%) of bambara landraces observed in this study appeared slightly higher than those previously reported for the previous research (Mahala & Mohammed, 2010; Adegbola & Bamishaiye, 2011). The protein contents of bambara landraces in comparison with other legumes were seemingly higher than those of cowpea and chickpea (Hillocks *et al.*, 2012), but similar to that of kidney bean (Wani *et al.*, 2013). Carbohydrate was the major nutrient in bambara, with approximately 56%, which was within the values reported for bambara groundnut flour (Adegbola & Bamishaiye, 2011; Hillocks *et al.*, 2012; Murevanhema & Jideani, 2013). The carbohydrate contents of the landraces grains appeared slightly lower than those of cowpea, faba bean and chickpea (Hillocks *et al.*, 2012). Bambara landraces contained low fat contents, which are within the range reported by Hillocks *et al.* (2012).

Protein content and protein yield of the concentrate

Protein content and yield varied with the method of extraction rather than the landraces (Table S2).

Concentrates prepared by acid precipitation gave much higher protein contents and yields (79% and 52%), respectively, compared to that obtained by salt solubilisation. The variations in protein contents and yields as a function of the extraction methods seem to be in agreement with literature (Boye *et al*., 2010). Previous studies conducted on soya and other legumes such as chickpea, broad bean and kidney bean have found acid precipitation method with relatively high protein content (Castel *et al*., 2012; Qayyum *et al*., 2012). Therefore, the protein content of the concentrates obtained through acid precipitation in this research is in agreement with the result reported for pea, chickpea, lentil, broad bean and kidney bean (Castel *et al*., 2012; Qayyum *et al*., 2012). Similarly, the higher protein yield obtained in this study may be attributed to the adjustment in pH using NaOH (pH 8), which may have enhanced the extractability of the protein. Proteins have been reported to exhibit higher solubility at pH above their isoelectric point (Adebowale *et al*., 2011). Okezie & Bello (1988) found that a change in pH of the extracting medium resulted in higher protein extractability of winged bean flour. Castel *et al*. (2012) reported high protein yield for *Amaranth mantegazzianus* protein concentrate when acid precipitation method was used.

Similar studies have found salt solubilisation method with lower protein yield and content. Adebowale *et al*. (2007) reported lower yield for mucuna bean protein concentrate prepared by salt solubilisation when compared to the acid precipitation method. The protein content of the concentrates extracted through salt solubilisation in this study is similar to those previously reported for bambara protein concentrates and *Bauhinia cheilantha* seeds (Boateng *et al*., 2013; Teixeira *et al*., 2013).

Colour

The colours of bambara protein concentrates were significantly influenced by the method of extraction (Table S3). The protein concentrates prepared by acid precipitation appeared slightly brownish in colour with low lightness (L^*) and high redness (a^*) and yellowness (b^*) values compared to the concentrates prepared by salt solubilisation. The variation in colour indices was reflected in the total colour difference (ΔE). The colours of the salt solubilisation protein concentrates were much similar to that of the reference soya protein. The use of acid produced slightly brownish concentrates. The browning of the acid-precipitated isolate may due to changes in pH during the preparation. Similar findings have been reported for kidney bean when the same method was used for the preparation of its isolates (Wani *et al*., 2014) and lentil protein isolates (Joshi *et al*., 2011). Unlike the method of extraction, the colour of the grain coats did not seem to have any major effect on the colour of protein concentrates after preparation.

Water and oil absorption capacity

Water absorption capacity (WAC) was influenced by the landrace and extraction methods (Fig. S1). Protein concentrate prepared through salt solubilisation absorbed slightly more water (2.4 mL g^{-1}) than those prepared through acid precipitation. For the acid precipitation method, the cream landraces showed a slightly high water absorption capacity (2.0 mL g^{-1}) than the red and maroon landraces. On the other hand, the red landraces showed higher values of water absorption capacity than other landraces in the concentrates obtained through the salt solubilisation concentrate. The higher water absorption capacity value for concentrates obtained through salt solubilisation is in agreement with the result reported for chickpea protein when similar method of extraction is used (Boye *et al*., 2010). Albumin and globulin are the major storage proteins in the concentrate obtained by salt solubilisation. These proteins may have been responsible for the high WAC. Bambara concentrates obtained by salt solubilisation method recorded a low protein compared to that obtained by acid precipitation (Table S2). Possibly, the presence of nonprotein contents in salt-solubilised concentrates may have contributed to its high WAC when compared to the acid-precipitated concentrates. Water absorption capacity of protein concentrates in this study is similar to isolates from kidney bean and Ginkgo biloba seeds (Deng *et al*., 2011; Shevkani *et al*., 2014).

Previous reports have recommended water absorption capacity ranging from 1.49 to 4.72 mL g^{-1} for use in viscous food (Mundi & Aluko, 2012). The high water absorption capacity reported in this study, especially for the concentrates prepared by salt solubilisation, falls within the stated range. Therefore, this suggests that bambara concentrates may be used in the formulation of some foods such as dough, soups, processed cheese and baked product.

Oil absorption capacity (OAC) was influenced by both landrace and extraction methods (Fig. S2). OAC of the protein concentrates prepared by salt solubilisation for the three landraces was higher (red: 2.2 mg mL^{-1}; maroon: 2.6 mL g^{-1}; and cream: 2.2 mL g^{-1}). These values were almost twice the values of acid precipitation concentrate (red: 1.7 mg mL^{-1}; maroon: 1.4 mL g^{-1}; and cream: 1.5 mL g^{-1}). This result is similar to findings earlier reported for chickpea protein isolate. Boye *et al*. (2010) reported a higher oil absorption capacity for chickpea protein isolate extracted using salt when compared to isoelectric precipitation. Similar result

was reported by Mwasaru *et al.* (1999) for cowpea and pigeon pea concentrate in which both salt solubilisation and acid precipitation method were used for extraction. The oil absorption capacity is crucial and of great importance from an industrial point of view. This is due to its influence on the emulsifying capacity, a highly desirable characteristic in products such as mayonnaise (Mundi & Aluko, 2012). The oil absorption capacity of the concentrates obtained by salt solubilisation in this study is in agreement with that reported for chickpea protein isolate (Boye *et al.*, 2010), but higher than that of soya bean protein isolates (Shevkani *et al.*,2014). Findings from this study suggest that bambara protein concentrates prepared through salt solubilisation could be used in flavour retention, improvement of palatability and extension of shelf life.

Foaming capacity

The foaming capacity of the three landraces decreased with increased pH (Fig. S3). All the three landraces showed similar foaming capacity at pH 6.5 and 8.0. However, at pH 3.0, the maroon landrace showed a slightly higher foaming capacity compared to other landraces. Furthermore, the foaming capacity of the concentrates extracted by salt solubilisation was higher (approximately 1.3 times) than the concentrates obtained by acid precipitation method.

Decrease in foaming capacity with increase in pH has been reported for both mucuna bean and cowpea (Aluko & Yada, 1995; Adebowale *et al.*, 2007). Foaming indicates the ability of protein to form films around gas bubbles in water. The solubility of protein in aqueous and its ability to diffuse at the air–water interface, unfold, form a cohesive and strong film to prevent rupture and coalescence are important factors in foaming capacity (Damodaran, 1996). Grahams & Phillips (1976) linked good foaming capacity with flexible protein molecules that can reduce surface tension, while highly ordered globular proteins, which are relatively difficult to surface-denature, gave low foaming capacity. Hence, one may suggest that bambara groundnut proteins may be high in flexible protein at acidic pH. The highest foaming capacity at the acidic pH 3 could be due to the decrease in attractive hydrophobic forces among the protein molecules, which occur at acidic regions. This development leads to repulsion, which facilitates the flexibility of the protein molecules, making them to diffuse more rapidly in the air–water interface to encapsulate air particles, leading to high foaming capacity (Adebowale & Lawal, 2003). The results of the foaming capacity obtained in this study for both methods of extraction are in agreement with values reported for pigeon pea and mucuna bean concentrate when similar extraction methods were used (Adebowale & Lawal, 2003). The higher foaming capacity of the salt solubilisation may be due to the presence of albumin, which has weakened the hydrophobic interaction. This may result in an increase in solubility and flexibility that allows for a wider spread on the air—water interface, better encapsulation of air particles, and consequently, an increase in foam formation (Shevkani *et al.*, 2014). Furthermore, the salt used in the preparation of the salt solubilisation concentrate may be responsible for its higher foaming capacity. This is because there is higher solubility of vegetable protein in salt solutions, coupled with the ability of the salt to aid diffusion and spread at the interface (Akintayo *et al.*, 1999).

Foaming stability

The foaming stability decreased as pH increased for all the landraces. Also, the same trend was observed for the extraction methods (Table S4). After 8 h, the highest foaming stability was observed at pH 3 for all the landraces. However, concentrates obtained by salt solubilisation had better foaming stability than the concentrates obtained through acid precipitation at all pH. Similar results were reported for soybean protein extracted by salt solubilisation and acid precipitation (Adebowale *et al.*, 2011). Boye *et al.* (2010) also reported lower foaming stability for pea proteins obtained through acid precipitation. The maroon landrace concentrate obtained by acid precipitation showed the highest (83%) foaming stability compared to other landrace concentrates. The highest foaming stability observed at pH 3 could be due to the formation of stable molecular layers in the air–water interface of the foams. Protein adsorption and viscoelasticity at an air–water interface are maximal near or at isoelectric pH because protein is not strongly repelled. Higher foaming stability at low pH 4 has been previously reported for mucuna protein concentrate (Adebowale & Lawal, 2003). The results obtained for the foaming activity and stability in this study indicates that the concentrates prepared through salt solubilisation could serve as replacements of better-known proteins in food applications such as whipping, toppings and ice cream.

Emulsion properties

The emulsifying activity and stability of the cream landrace were higher compared to the red and maroon landraces (Figs S4 and S5). The maroon landrace concentrates obtained through acid precipitation had the lowest emulsion stability in comparison with other landraces.

The methods of extraction adopted had an influence on the emulsifying activity and stability of the

concentrates. The emulsifying activity of concentrates obtained through salt solubilisation (approximately 64%) was slightly higher than the concentrate obtained through acid precipitation method (approximately 55%). The higher emulsifying activity and stability observed for the salt solubilisation concentrates may be attributed to its lower protein content (Table S2) compared to the acid precipitation concentrate. This may be due to the fact that the emulsifying capacity of proteins tends to decrease as protein concentration increase (Mao & Hua, 2012). Boye *et al*. (2010) reported higher emulsifying properties for micellised pea, chickpea and lentil protein concentrate compared to the isoelectric concentrate. Generally, the high emulsifying activity and stability of bambara concentrate obtained in this study, especially concentrates prepared through salt solubilisation, could serve as a potential ingredient in food formulations such as sausages, ice creams and mayonnaises**.**

Conclusions

Bambara groundnut landraces are good sources of proteins and carbohydrates. Acid precipitation produces bambara concentrates with high protein content and yield compared to concentrates prepared by salt solubilisation. Protein concentrates prepared through salt solubilisation method exhibited better functional properties in terms of water absorption capacity, oil absorption capacity, foaming capacity, foaming stability and emulsion activities when compared to concentrates obtained through acid precipitation. This study suggests that salt solubilisation may be the most appropriate method for the enhanced functionality and utilisation of bambara groundnuts' protein concentrates.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Water absorption capacity of acid precipitation and salt solubilisation concentrates of three bambara groundnut landraces.

Figure S2. Oil absorption capacity of the acid precipitation and salt solubilisation concentrates from three landraces of bambara groundnut.

Figure S3. Effect of pH on foaming capacity of on the landrace acid precipitation and salt solubilisation concentrate.

Figure S4. Emulsifying activity of acid-precipitated and salt-solubilised concentrate of bambara landraces.

Figure S5. Emulsifying stability of acid-precipitated and salt-solubilised concentrate from landrace bambara groundnut.

Table S1. Proximate composition of the three landraces of bambara groundnut grains.

Table S2. Protein yield and content of bambara groundnut concentrates obtained by acid precipitation and salt solubilisation.

Table S3. Colour values of concentrates prepared by acid precipitation and salt solubilisation of bambara landraces.

Table S4. Effect of pH and extraction methods on foaming stability (%) of bambara landrace protein concentrates.