Heliyon 7 (2021) e07824

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Influence of pH and temperature on the physicochemical and functional properties of Bambara bean protein isolate

Simon Pierre Ngui [a], Carine Emilienne Nyobe [a,c], Christian Bernard Bakwo Bassogog [a,c], Erasmus Nchuaji Tang [d], Samuel René Minka [a], Martin Alain Mune Mune [b,*]

[a] Department of Biochemistry, University of Yaoundé I, PO Box 812, Yaoundé, Cameroon
[b] Faculty of Science, The University of Maroua, PO Box 814, Maroua, Cameroon
[c] Centre for Food and Nutrition Research, PO Box 6163, Yaoundé, Cameroon
[d] Institute of Agricultural Research for Development, Njombe, Cameroon

ARTICLE INFO

Keywords:
Bambara bean
Protein isolate
Heat treatment
Secondary structure
Functionality

ABSTRACT

Bambara bean is a rich low-cost protein source and a functional ingredient in the food industry. We investigated the effects of temperature and different pH on the physicochemical and functional properties of Bambara bean protein isolate. Vicilin was the major protein of Bambara bean as revealed by SDS PAGE analysis. The emulsifying capacity of protein isolate was highest at 80 °C, pH 9 while emulsion stability was highest at pH 4. Generally, increase in temperature decreased protein solubility at pH 4 and 7, while increase was observed at pH 9 and 100 °C. The hydrophobicity of isolate was highest at pH 4 and lowest at pH 9, regardless of temperature. Protein isolate possessed highly compact β-sheet and α-helix secondary structures in proportions greater than 75% (at pH 9 and 50 °C). Increase in temperature generally promoted protein rearrangement and partial unfolding. Protein secondary structure and surface hydrophobicity can predict food functionality, directly affecting protein behavior during formulation and long-term storage. This study clearly demonstrated the potential of exploiting pulse protein isolates as nutritional and functional ingredients through temperature and pH control.

1. Introduction

Bambara groundnut (*Vigna subterranea*), extensively cultivated in low altitudes of sub-Saharan Africa is a legume crop of little relevance on a global scale (Eltayeb et al., 2011; Mazahib et al., 2013). However, in Africa, it compares to groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in terms of productivity (Adebowale et al., 2011; Hillocks et al., 2012). Bambara groundnut is also under considerable production in America, India, Sri Lanka, and Indonesia (Mcwatters et al., 2003; Goli et al., 1997). In Cameroon, it is common in all the ten regions with the exception of a few production basinsin the South West and South East plains (Pasquet and Fosto, 1991). Bambara groundnut is drought tolerant with good yield performance in unfertilized soils, exhibiting remarkable resistance to pests and diseases (Thammarat et al., 2015). Besides this, it is a potential replacement of animal protein in local households, often formulated in sauces and consumed with roots, tubers and cereals (Mune Mune and Sogi, 2015). Despite its ability to withstand harsh climate and contribute to household protein requirement, Bambara groundnut is under little exploitation at both the indigenous and development research communities (Yao et al., 2015).

At the development research level, Bambara groundnut is an exemplar pulse for climate change resilience (Mayes et al., 2019). In addition to climate change adaptation, Bambara groundnut flour perfectly incorporates as a protein ingredient in cookie and vegetable diary formulations (Okafor et al., 2015; Falade et al., 2014; Murevanhema & Jideani, 2013). The successful incorporation of Bambara flour in industrial food formulation depends on its physicochemical and functional properties (Mune et al., 2018). However, limited scientific evidence elucidates the major factors coordinating the successful exploitation of Bambara groundnut flour in the food industry. Bambara protein is rich in essentially amino acids with substantial levels of lysine and methionine that greatly influence structure and function primordial in food formulation (Mune Mune and Sogi, 2015; Mune et al., 2018). The application of protein rich sources in food engineering make use of processing parameters sometimes involving pH and temperature adjustments for stability. Temperature and pH are parameters that differentially affect protein

* Corresponding author.
E-mail address: alainmune@gmail.com (M.A. Mune Mune).

https://doi.org/10.1016/j.heliyon.2021.e07824
Received 5 May 2021; Received in revised form 6 July 2021; Accepted 16 August 2021
2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

structure and function (Barba et al., 2012; Trujillo et al., 2002). Under well-defined temperature and pH regimes, the use of Bambara flour as an essential protein ingredient in food formulation is justified. With this justification coordinated by intrinsic properties such as surface hydrophobicity, Bambara protein could improve novel food functional properties such emulsion capacity and stability (Cui et al., 2013; Nishinari et al., 2014). With this conception, this study aimed to determine the effects of different temperature and pH treatments on the structure and function of Bambara protein isolate.

2. Materials and methods

2.1. Material

We purchased dried seeds of the Bambara bean [*Vigna suhterrenea (L) verde*] red cultivar at the Mfoundi market (Yaounde, Cameroon). They were then sorted and cleaned and the good quality seeds kept at 4 °C in a polyethylene bag until used. Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO) supplied the chemical reagents (acrylamide and potassium iodide).

2.2. Methods

2.2.1. Preparation of Bambara bean flour

The clean Bambara bean sample was ground in a milling machine (Semap) fitted with a fine sieve of 200 um mesh size. Thereafter, flour was defatted in hexane at a 1:5 (w/v) ratio (Maguire et al., 2004). All defatted samples were oven-dried at 45 °C, packed in paper bags and cold-stored at 4 °C in a refrigerator.

2.2.2. Extraction of the Bambara bean protein isolate

The extraction of the Bambara bean protein isolate followed the modified isoelectric precipitation method of Mune Mune and Sogi (2016). A slurry of the defatted flour was prepared by washing 100 g of flour in 1000 ml of distilled water in duplicate after adjusting the pH to 4.5 using 1 M HC1. After washing for 15 min, the slurry was centrifuged at 3800 x g for 20 min and 4 °C and the precipitated protein was re-suspended in distilled water and the pH adjusted to 7 with continuous stirring. The resulting protein suspension was freeze-dried.

2.2.3. Proximate composition of protein isolate

Moisture, ash, total lipid and crude protein (*N* x 6.25) contents were determined according to AOAC (1990). Crude fibre content of the protein isolate was determined according to Goering and Van Soest (1970).

2.2.4. Heat treatment

Exactly 0.45mg of the Bambara bean protein isolate was suspended in a phosphate buffer at pH 4, 7 and 9 (0.01 M, 45 ml) and stirred for 1 h at 25 °C. Thereafter, 9 ml of the buffer suspension was dispensed in test tubes and heated at 50 °C, 70 °C, 80 °C and 100 °C for 10 min. The heated tubes were cooled to ambient conditions in a water bath and the protein isolate in the tubes used for the determination of hydrophobicity, secondary structure, solubility and emulsifying capacity. Values were compared with the unheated (control) sample.

2.2.5. Hydrophobicity

The bromophenol blue fixation method was used to determine surface hydrophobicity of heat treated samples and the control (Chelh et al., 2006). Aliquots of 500µl of the heat treated samples were mixed with 200µl of BPB (1 mg/ml) and vigorously stirred. The following steps of the procedure were described by Mune et al. (2017) and Mune Mune and Sogi (2016).

2.2.6. Secondary structure

The secondary structure of proteins in the heat-treated samples and the control was characterized by Fourier Transform Infrared (FTIR) spectroscopy (Mune Mune and Sogi, 2016; Mune et al., 2017). Briefly, 0.05 mL of the samples was put between two aluminum foils. FTIR spectra were obtained in the wavenumber range of 400–4000 cm^{-1} during 32 scans with 4 cm^{-1} resolution using a FTIR spectrometer (IRAffinity-1 Shimadzu, Japan). Data were analyzed as described by Mune et al. (2017) and the secondary structural features were calculated from the amide I envelope by non-linear regression fitting of Gaussian peaks of the original spectra. Peaks assignments were generated using the results of Farrell et al. (2001).

2.2.7. Solubility

Aliquots of 1 mL of the protein suspension from the heat treatment and the control were stirred for 30 min and then centrifuged at 4000 rpm for 20 min (Mune Mune and Sogi, 2016). Protein concentration in each supernatant was determined by the Kjeldahl method (AOAC, 1990). Protein solubility was calculated as indicated in Eq. (1):

$$\text{Solubility } (\%) = \frac{w_1}{w_0} \text{x } 100 \quad (1)$$

where W_1 was the weight of protein in the supernatant (g), W_0 was the weight of protein in the sample (g).

2.2.8. Emulsifying properties

Emulsifying capacity (EC) and emulsion stability (ES) at different temperatures were measured according to Lawal (2004). The heat-treated protein solution (1 ml) was mixed with 1 ml of soybean oil to obtain an oil to water ratio (OWR) of 1:1 (v/v). The dispersion then mixed at high speed for 1 min at room temperature, using a magnetic stirrer (Illkirch, France), centrifuged at 1,100 rpm for 10 min in an Eppendorf AG (Hamburg, Germany) centrifuge. The emulsifying capacity was derived following Eq. (2):

$$\text{Emulsifying capacity(EC)} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total content in the tube}} \text{x100} \quad (2)$$

ES was determined by heating the emulsion at 80 °C for 30 min before centrifuging at 1100 rpm for 10 min and derived as shown in Eq. (3):

$$\text{Emulsion stability(ES)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \text{x100} \quad (3)$$

2.2.9. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedures of Laemmli (1970). The gel system consisted of 12% (w/v) polyacrylamide for the resolving gel (pH 8.8) and 4.5% (w/v) for the stacking gel (pH 6.8). Five milligrams of both flour and the protein isolate of Bambara beans were suspended in 1 mL of distilled water and diluted 25 times before loading. Samples were prepared under reducing conditions using β-mercapto ethanol, heated at 95 °C in a water bath for 5 min, and then centrifuged for 5 min at 5000 rpm. Fifteen microliters of each sample were loaded into the wells. Electrophoretic separation was carried out at 70 V for the stacking gel and at 100 V for the resolving gel. Protein bands were fixed by immersion of the gel in a 10% (v/v) acetic acid solution for 30 min, stained in a 0.1% (w/v) Coomassie brilliant blue R-250 solution (Bio-Rad) for 30 min and then distained in 10% (v/v) acetic acid and 10% (v/v) methanol for 40 min.

2.3. Statistical analysis

Data analysis and visualization were done using STATISTICA™ (version 5.5, 2002; Statsoft Inc., USA) and SPSS (16.0 version 10.1, 2000, SPSS Inc., USA). Means were compared by the one-way ANOVA applying the Tukey post hoc test for mean separation at $p < 0.05$ level of significance. Correlation was estimated by the Pearson method. Results are expressed as mean ± standard deviation of triplicate values.

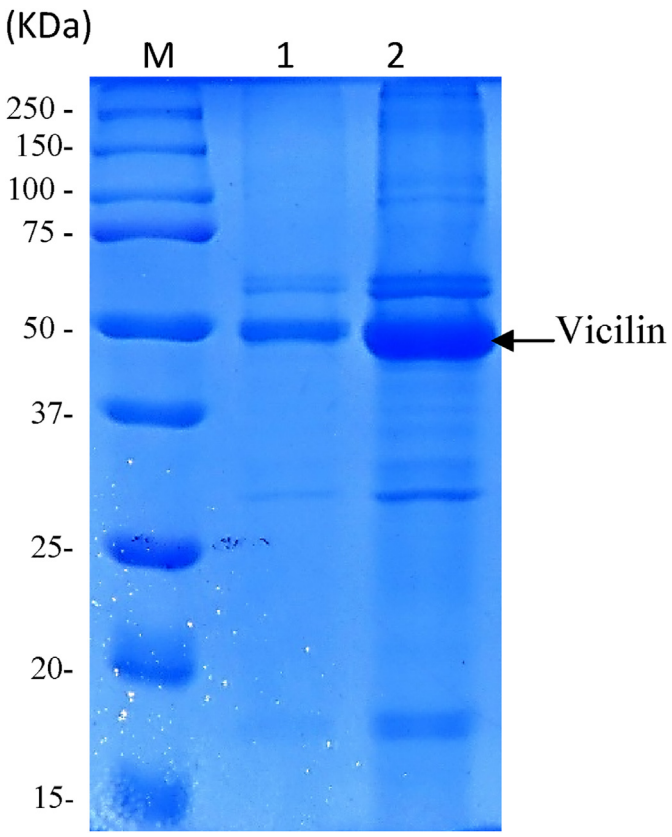

Figure 1. SDS-PAGE patterns of Bambara bean protein. Lane M– Molecular weight standards - Precision Plus Protein™ Dual Color Standards (Bio-Rad); Lane 1 –flour of Bambara bean; Lane 2–Bambara bean protein isolate.

3. Results and discussion

Protein (81.28%, dwb) was the main component in the protein isolate, which also contained ash (3.71%, dwb), fat (6.17%, dwb) and crude fibre (4.07%, dwb). Moisture content was 3.45%. Similar observations have been reported in previous studies (Mune Mune and Sogi, 2015; Mune Mune et al., 2020).

3.1. Structural characterization

3.1.1. Electrophoretic analysis

The SDS-PAGE pattern of Bambara bean proteins showed specific bands with molecular weight ranging from ~ 17 kDa to ~ 118 kDa (Figure 1; Figure S1 Supplementary material). Generally, protein isolate contained mainly globulins (vicilin) and low amount of albumins. Vicilin is a trimeric or hexameric protein devoid of disulphide bonds that play an important role as seed storage protein. Its molecular weight is reported at 200.0 ± 50.0 kDa (Adebowale et al., 2011; Pernollet and Mosse, 1983). Albumins play metabolic functions in pea such as protease and lectin inhibitors (Lam et al., 2018). Three main bands of vicilin subunits were observed under reducing conditions, with the major band expressing a molecular weight of ~ 50 kDa. The other two medium bands weighed ~ 60 kDa and ~ 66 kDa. Albumins were observed at the top of the gel with molecular weight ~100–118 kDa and, then at the bottom with low molecular weight ~ 17 kDa and ~27 kDa.

3.1.2. Secondary structure of protein

Fourier Transform Infrared Spectroscopy (FTIR) is a valuable method for studying conformational changes of proteins in the solid, crystalline and liquid states. Mune Mune et al. (2016; 2018) successfully applied this method and elucidated the secondary structure of legume proteins. The influence of temperature on protein secondary structure at different pH in the amide I region is shown in Table 1 and Figure 2(a,b). The amide I band was further resolved by Fourier self-deconvolution (Figure 2b). Then, the bands were easily assigned as follows: α-helix: 1648-1658 cm^{-1}; β-sheet: 1616-1640 cm^{-1}; β-turn: 1660-1700 cm^{-1}; Irregular structure: 1640-1644 cm^{-1} (Farrell et al., 2001; Achouri et al., 2012). In Figure 2a and Table 1, it was observed that β sheet and α helix were the main secondary structures of Bambara bean protein isolate, with proportions ranging from 15.02 to 67.34 % and 15.47–75.69 %, respectively, depending on pH. In addition, [β-sheet + α-helix] proportion was found between 73 % (at pH 9 and 70 °C) and 100 % (at pH 9, 50 °C).

High β-sheet and α-helix proportion is a main characteristic of compacted globulin storage proteins, and it was also noticed that anti-parallel β-sheet could be formed in aggregated protein molecules (Ellepola et al., 2005). However, β-sheet and α-helix proportion behaved differently following heat treatment, depending on pH. At pH 4, heat treatment caused an increase in β-sheet and a decrease in α-helix, while at pH 7, β-sheet decreased and α-helix increased proportionally. This observation probably underlined the co-existence of several conformations of protein

Table 1. Effects of heat and pH on Secondary structure (means and standard deviations) of Bambara bean protein isolate.

pH	Temperature	Secondary Structure composition (%)			
		β-sheets (%)	α-helix (%)	Irregular structure (%)	β-turn (%)
4	25	15.20 ± 0.51^{e2}	75.69 ± 2.52^{a1}	$1.58 \pm 0.05N^{h4}$	7.53 ± 0.25^{f3}
	50	53.57 ± 1.78^{b1}	33.17 ± 1.10^{ef2}	9.54 ± 0.32^{c3}	3.72 ± 0.12^{ij4}
	70	27.80 ± 0.93^{d2}	61.38 ± 2.04^{b1}	2.41 ± 0.08^{h4}	7.24 ± 0.24^{f3}
	80	29.28 ± 0.98^{d2}	63.75 ± 2.12^{b1}	5.59 ± 0.19^{e3}	2.55 ± 0.08^{j3}
	100	71.80 ± 2.4^{a1}	23.04 ± 0.77^{g2}	1.51 ± 0.05^{h4}	3.65 ± 0.12^{ij3}
7	25	67.34 ± 2.24^{a1}	15.47 ± 0.52^{h2}	11.59 ± 0.38^{b3}	5.6 ± 0.19^{gh4}
	50	45.69 ± 1.53^{c1}	37.09 ± 1.24^{ef2}	4.95 ± 0.17^{ef4}	12.27 ± 0.41^{d3}
	70	30.73 ± 1.02^{d2}	46.96 ± 1.57^{c1}	4.56 ± 0.15^{fg4}	17.75 ± 0.60^{b3}
	80	20.85 ± 0.70^{e2}	63.21 ± 2.11^{b1}	5.80 ± 0.20^{e4}	10.14 ± 0.34^{e3}
	100	43.39 ± 1.45^{c1}	39.64 ± 1.32^{de2}	12.72 ± 0.42^{a3}	4.25 ± 0.14^{hi4}
9	25	40.74 ± 1.36^{c1}	35.00 ± 1.17^{ef2}	$12.570.27^{a4}$	16.03 ± 0.53^{c3}
	50	55.57 ± 1.85^{b1}	44.43 ± 1.49^{cd2}	0.00 ± 0.00^{i3}	0.00 ± 0.00^{k3}
	70	41.76 ± 1.40^{c1}	31.18 ± 1.04^{f2}	5.32 ± 0.18^{ef4}	21.74 ± 0.72^{a3}
	80	29.89 ± 1.00^{d2}	61.51 ± 2.05^{b1}	1.70 ± 0.06^{h4}	6.90 ± 0.23^{fg3}
	100	44.89 ± 1.50^{c1}	46.91 ± 1.53^{c1}	3.96 ± 1.29^{g3}	4.24 ± 0.14^{hi3}

Means followed by different letter (a, b, c, d, e, f, g, h, i) in the same column are significantly ($p < 0.05$) different. Means Followed by different number (1, 2, 3, 4, 5) in the same line are significantly ($p < 0.05$) different.

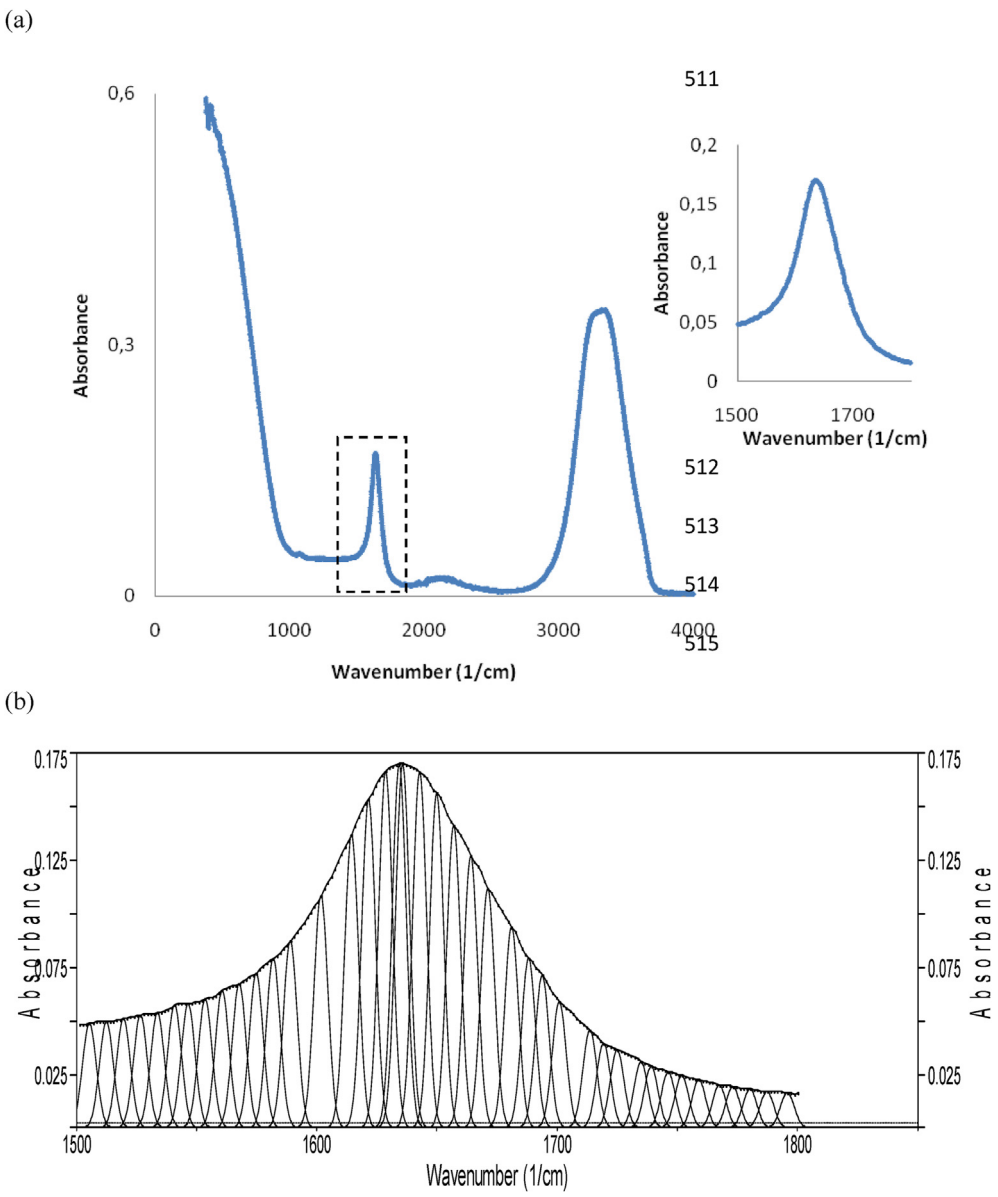

Figure 2. (a–b). Typical FTIR spectra of Bambara bean protein isolate (here at pH 7and 25 °C) (a) and deconvoluted amide I band (b).

during heating. Furthermore, there was probably inter-conversion between β-sheet and α-helix, since correlation between both proportions was significant ($R^2 = 0.98$; $p < 0.01$) during heating at pH 4 and 7. At pH 9, significant ($p < 0.05$) increase in β-sheet proportion was observed at 50 °C compared to untreated sample, and a decrease at 80 °C. At the same pH, heat treatment caused an increased in the α-helix proportion of protein isolate (except at 70 °C). Irregular structure probably resulted from protein unfolding and denaturation, and high β-turn proportion is a product of protein unfolding of higher order structures. However, high β-turn proportion was important for protein flexibility, which contributed to the stabilization of the water-oil emulsion, increasing globular protein emulsion capacity (Mune Mune and Sogi, 2015).

3.2. Physicochemical properties

3.2.1. Hydrophobicity

Surface hydrophobicity of proteins is an important property that eases a quick understanding of solubility, protein-protein interaction and functionality allowing the integration of proteins in food formulation. Protein hydrophobicity is a function of either ionization state of the chemical functional groups, which results from intrinsic factors (i.e. constitutive amino acids, size, and protein conformation) or extrinsic factors such as solvent (ionic strength and temperature), stirring time and protein concentration. The effect of temperature on hydrophobicity (mg bound BBP/g protein) of the Bambara bean protein isolate at different pH is shown in Figure 3a. As expected, protein hydrophobicity was significantly higher at pH 4 and lower at pH 9. At pH 4, the net charge of protein was around zero then hydrophobic protein-protein interactions were predominant, while at pH 9 the protein is negatively charged and protein-water interactions were predominant. At pH 4, hydrophobicity decreased with temperature up to 70 °C, then increased thereafter. Decrease in hydrophobicity was probably resulting from protein aggregation by the mean of hydrophobic interaction, and the increase was due to partial unfolding and exposure of regions previously buried by steric hindrance of the bulky protein structure. At pH 9, heat treatment caused a decrease in protein hydrophobicity. Decrease in protein hydrophobicity occurred at temperature 50–80 °C, while non-significant differences in hydrophobicity compared to untreated protein showed at 100 °C. Jiang et al. (2015) reported an increase in hydrophobicity with increasing temperature for soy protein isolate at 2% (w/v). Stanciuc et al. (2015)

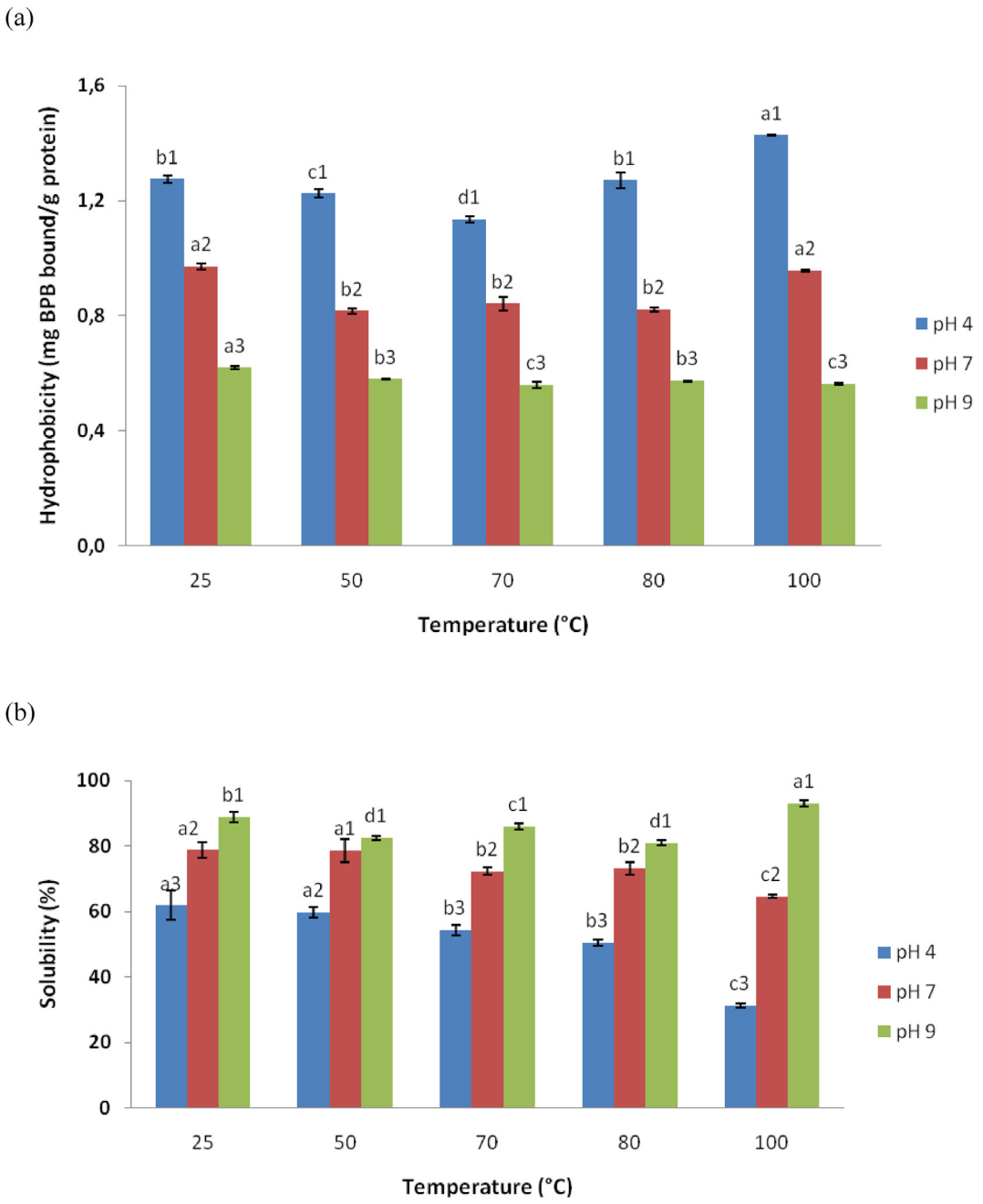

Figure 3. (a–b). Effects of heat and pH on surface hydrophobicity (a) and protein solubility (b) of Bambara bean protein isolate. Means followed by different letters at same pH are significantly ($p < 0.05$) different. Means followed by different numbers (1,2,3) at same temperature are significantly ($p < 0.05$) different.

noted that change in surface hydrophobicity reflected change in the structure of horseradish peroxidase in response to heat treatment.

3.2.2. *Solubility of protein*

Protein solubility correlates to foam formation, emulsification, gelation and as such defines the usability of proteins in liquid foods and beverages (Mune et al., 2017; Zayas, 1997). Effect of temperature on the solubility of the Bambara bean protein isolate at different pH is shown in Figure 3b. The shape of the influence of temperature on protein solubility varies at different pH. The solubility of globular proteins generally correlates negatively with hydrophobicity (Mune Mune and Sogi, 2015). In this regard, the solubility of vicilin protein isolate was significantly lower at pH 4, and higher at pH 9. This low protein solubility at pH 4 is consistent with that of several legumes with isoelectric pH between 4 and 5 (Chavan et al., 2001; Mwasaru et al., 1999; Hermansson, 1979). The negative charge of legume proteins at basic pH explains its high solubility, by maintaining repulsive electrostatic interactions between neighboring side chains, and promoting oligomer dissociation and polypeptide unfolding (Moure et al., 2006). At pH 4 and 7, non-significant difference in solubility was found at 50 °C compared to untreated protein, afterward solubility decreased at temperatures 70–100 °C. The lower protein solubility (31%) occurred at pH 4 and the highest (93%) at pH 9, at 100 °C. The protein solubility behavior upon heat treatment varies with the source of protein. Lee et al. (2019) although working on Yellow Mealworm Larvae proteins also noted that solubility varies not only with temperature but also with the source of the protein.

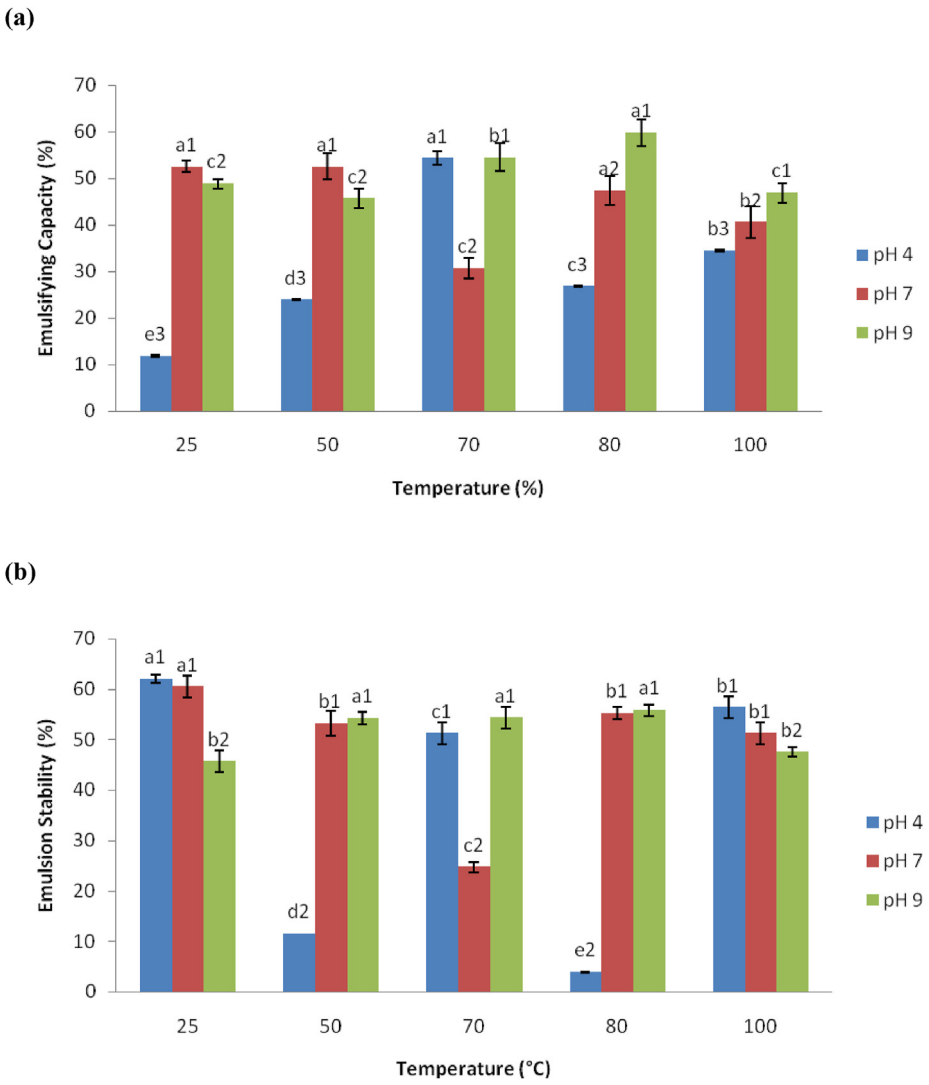

Figure 4. (a–b). Effects of heat and pH on emulsion stability (a) and emulsion capacity (b) of Bambara groundnut isolate protein. Means followed by different letters at same pH are significantly ($p < 0.05$) different. Means followed by different numbers (1,2,3) at same temperature are significantly ($p < 0.05$) different.

3.3. Emulsifying properties

3.3.1. Emulsifying capacity

According to Mune et al. (2017), emulsifying properties are essential in developing novel plant foods. This is justified by the influence of solubility, hydrophobicity and molecular flexibility on the emulsifying properties of globular proteins (Zayas, 1997). The influence of temperature on the emulsifying properties of the Bambara bean protein isolate at different pH is shown in Figure 4(a,b). Emulsifying capacity (EC) and emulsion stability (ES) were determined at pH 4, 7 and 9. For utilization of Bambara bean protein isolate without heat treatment in applications where high EC is required, it could be recommended to manage under neutral or basic pH. Mune Mune and Sogi (2015) and Carvalho et al. (2006) found similar results. It was also found that EC of protein isolate was significantly higher at pH 7 and 9 compared to pH 4 at all temperatures, except for the isolate treated at 70 °C. Generally, adequate combination of solubility, hydrophobicity and molecular flexibility account for high EC of globular proteins. Heat treatment increased EC of vicilin isolate at pH 4. The maximum EC (54.4%) occurred for the isolate at 70 °C. At pH 7, non-significant difference was found between EC of the untreated protein isolate and those treated at 50 and 80 °C (52%), and EC decreased for the isolate treated at 70 and 100 °C. At pH 9, increase in EC was observed for the vicilin isolate at 70 °C (55%) and 80 °C (60%) compared to control, while non-significant difference was found for the isolate treated at 50 and 100 °C. Solubility probably accounted for high EC at pH 7 and 9, since it conferred to the proteins a better ability to diffuse to the oil/water interface then lowering interfacial tension. In another hand, surface hydrophobicity and molecular flexibility of proteins played important role for EC at pH 4.

3.3.2. Emulsion stability

The influence of temperature on the ES of the Bambara bean protein isolate varied according to the pH applied Figure 4(a,b). The untreated protein isolate (at 25 °C) exhibited better ES (62%) at pH 4 and 7. At pH 9, heat treatment at 50–80 °C increased ES of protein isolate (55%), which decreased to 48% when the isolate was heated to 100 °C. Similar results were observed by Mwasaru et al. (1999) who noticed that emulsion stability of the *Cajanus cajan* protein isolate was maximal at pH 4. In addition, Mune et al. (2018) pointed out that High ES required molecular rearrangement of the adsorbed proteins at the oil-water interface to form a thick layer, which prevents coalescence with low repulsive forces between the proteins. In another hand, at pH 4 protein isolate heated at 50 and 80 °C showed low ES (12 and 4%, respectively). At pH 7, non-significant difference ($p > 0.05$) in ES (55%) was observed for isolate heated at 50, 80 and 100 °C, and heat treatment at 70 °C produced protein isolate with lower ES (25%). Molecular flexibility of globular

proteins induced by partial unfolding probably played important role in ES of protein isolate, since it enhanced rearrangement of protein at the oil-water interface.

4. Conclusion

We studied the effect of temperature on secondary structure, physico-chemical and emulsifying properties of Bambara bean protein isolate at different pH conditions. The SDS-PAGE analysis indicated that vicilin (7S globulin) was the main protein in the protein isolate. β-sheet and α-helix were the main secondary structures found in Bambara bean protein isolate, and heat treatment produced partial unfolding and aggregation of proteins. Solubility and hydrophobicity were also affected by heat treatment at different pH, and high hydrophobicity was observed at pH 4, while high solubility was found at pH 9. Emulsifying properties were also significantly affected by changes in pH and temperature. Briefly, adequate control of pH and heat treatment could be important for the application of Bambara bean protein in industrial products where high emulsifying properties are required.

Declarations

Author contribution statement

Simon Pierre NGUI: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Emilienne NYOBE, Christian BakwoBassogog: Performed the experiments; Wrote the paper.

Erasmus NchuajiTang: Analyzed and interpreted the data; Wrote the paper.

Samuel René Minka: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Martin Alain MuneMune: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2021.e07824.

Acknowledgements

This work benefited resource provision from the Food and Nutrition Research Centre (IMPM) of the Ministry of Scientific Research and Innovation, Cameroon.

References

Achouri, A., Nail, V., Boye, J.I., 2012. Sesame protein isolate: fractionation, secondary structure and functional properties. Food Res. Int. 46, 360–369.

Adebowale, Y.A., Schwarzenbolz, U., Henle, T., 2011. Protein isolates from Bambara groundnut (*Voandzeia subterranean L.*): chemical characterization and functional properties. Int. J. Food Prop. 14 (4), 758–775. Association of Official Analytical Chemists. (1990). Official methods ofanalysis (15th ed.). Arlington, TX : Author.

Aoac, 1990. Official Methods of Analysis, fifteenth ed. Association of Official Analytical Chemists. (USA), Arlington.

Barba, F.J., Esteve, M.J., Frígola, A., 2012. High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: a review. Compr. Rev. Food Sci. Food Saf. 11 (3), 307–322.

Carvalho, A.V., Garcia, N.H.P., Amaya-Farfán, J., 2006. Physico-chemical properties of the flour, protein concentrate, and protein isolate of the cupuassu (Theobroma grandiflorum Schum) seed. J. Food Sci. 71 (8), S573–S578.

Chavan, U.D., McKensie, D.B., Shahidi, F., 2001. Functional properties of protein isolates from beach pea (Lathyrusmaritimus L). Food Chem. 74, 177–187.

Chelh, I., Gatellier, P., Sante-Lhoutellier, V., 2006. Technical note: a simplified procedure for myofibril hydrophobicity determination. Meat Sci. 74, 681–683.

Cui, C., Zhao, M., Yuan, B., Zhang, Y., Ren, J., 2013. Effect of pH and pepsin limitedhydrolysis on the structure and functional properties of soybean protein hydrolysates. J. Food Sci. 78 (12), C1871–C1877.

Ellepola, S.W., Choi, S.M., Ma, C.Y., 2005. Conformational study of globulin from rice (*Oryza sativa*) seeds by Fourier- transform infrared spectroscopy. Int. J. Biol. Macromol. 37 (1–2), 12–20.

Eltayeb, A.R.S., Ali, A.O., Abou-Arab, A.A., Abu-Salem, F.M., 2011. Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranean*). Afr. J. Food Sci. 5 (2), 82–90.

Falade, K.O., Ogundele, O.M., Ogunshe, A.O., Fayemi, O.E., Ocloo, F.C., 2014. Physico-chemical, sensory and microbiological characteristics of plain yoghurt from Bambara groundnut (*Vigna subterranea*) and soybeans (*Glycine max*). J. Food Sci. Technol. 1–8.

Farrell Jr., H.M., Wickham, E.D., Unruh, J.J., Qi, P.X., Hoagland, P.D., 2001. Secondary structural studies of bovine caseins: temperature dependence of b-casein structure as analyzed by circular dichroism and FTIR spectroscopy and correlation with micellization. Food Hydrocolloids 15, 341–354.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Procedures and Some Applications). Agriculture handbook, agricultural research service, USA.

Goli, A., Begemann, F., Ng, N., 1997. Characterization and evaluation of IITA's Bambara groundnut collection. In: Proceedings of Proceedings of the Workshop on Conservation and Improvement of Bambara Groundnut, pp. 101–111.

Hermansson, A.M., 1979. Methods of studying functional characteristics of vegetable proteins. J. Am. Oil Chem. Soc. 56, 272–279.

Hillocks, R., Bennett, C., Mponda, O., 2012. Bambara nut: a review of utilization, market potential and crop improvement. Afr. Crop Sci. J. 20 (1), 1–16.

Jiang, L., Wang, Z., Li, Y., Meng, X., Sui, X., Qi, B., Zhou, L., 2015. Relationship between surface hydrophobicity and structure of soy protein isolate subjected to different ionic strength. Int. J. Food Prop. 18 (5), 1059–1074.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259), 680.

Lam, A.C.Y., Can Karaca, A., Tyler, R.T., Nickerson, M.T., 2018. Pea protein isolates: structure, extraction, and functionality. Food Rev. Int. 34 (2), 126–147.

Lawal, O.S., 2004. Functionality of African locust bean (*Parkia biglobossa*) protein isolate: effects of pH, ionic strength and various protein concentrations. Food Chem. 86, 345–355.

Lee, Ha-jung, Kim, Ji-han, Da-somJi, Lee, Chi-ho, 2019. Effects of heating time and temperature on functional properties of proteins of Yellow Mealworm Larvae (*Tenebrio molitor* L.). Food Sci. Anim. Res. 39 (2), 296–308.

Maguire, L.S., O'sullivan, S.M., Galvin, K., O'connor, T.P., O'brien, N.M., 2004. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. Int. J. Food Sci. Nutr. 55 (3), 171–178.

Mayes, S., Ho, W.K., Chai, H.H., Gao, et al., 2019. Bambara groundnut: an exemplar underutilised legume for resilience under climate change. Planta 250, 803–820.

Mazahib, A., Nuha, M., Salawa, I., Babiker, E., 2013. Some nutritional attributes of Bambara groundnut as influenced by domestic processing. Int. Food Res. J. 20 (3), 1165–1171.

Mc Watters, K.H., Ouedraogo, J.B., Ressurection, A.V.A., Hung, Y.-C., Phillips, R.D., 2003. Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. Int. J. Food Sci. Technol. 38, 403–410.

Moure, A., Sineiro, J., Dominguez, H., Parajo, J.C., 2006. Functionality of oilseed protein products. Rev. Food Res. Int. 39 (9), 945–963.

Mune Mune, M.A., Sogi, D.S., 2015. Functional properties of protein concentrates of cowpea and Bambara bean involving different drying techniques. J. Food Process. Preserv. 39 (6), 2304–2313.

Mune Mune, M.A., Sogi, D.S., 2016. Emulsifying and foaming properties of protein concentrates prepared from cowpea and Bambara bean using different drying methods. Int. J. Food Prop. 19 (2), 371–384.

Mune Mune, M.A., Sogi, D.S., Minka, S.R., 2017. Response surface methodology for investigating structure–function relationship of grain legume proteins. J. Food Process. Preserv., e13524

Mune Mune, M.A., Bayiga, A., Nyobe, E.C., Bassogog, C.B., Minka, S.R., 2018. Protein quality, secondary structure and effect of physicochemical factors on emulsifying properties of *Irvingia gabonensis* almonds. Curr. Nutr. Food Sci. 14, 1–19.

Mune Mune, M.A., Stănciuc, N., Grigore-Gurgu, L., Aprodu, I., Borda, D., 2020. Structural changes induced by high pressure processing in Bambara bean proteins at different pH. LWT Food Sci. Technol. 124, 109–187.

Murevanhema, Y.Y., Jideani, V.A., 2013. Potential of Bambara groundnut (*Vigna subterranea L.* Verdc) milk as a probiotic beverage—a review. Crit. Rev. Food Sci. Nutr. 53 (9), 954–967.

Mwasaru, M.A., Muhammad, H., bakar, J., Che Man, Y.B., 1999. Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeon pea (Cajanuscajan) and cowpea (*Vigna unguiculata*) protein isolates. I. physicochemical properties. Food Chem. 67 (4), 435–443.

Nishinari, K., Fang, Y., Guo, S., Phillips, G., 2014. Soy proteins: a review on composition, aggregation and emulsification. Food Hydrocolloids 39, 301–303.

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. Pakistan J. Nutr. 14 (10), 653–657.

Pasquet, R.S., Fosto, M., 1991. Les légumineuses alimentaires du Cameroun, Premiers résultats. In: Boutrais, J. (Ed.), Du politique à l'économique, études historiques dans le bassin du Lac Tchad. ORSTOM, Paris, pp. 317–360.

Pernollet, J.C., Mosse, J., 1983. Structure and location of legume and cereal seed storage. In: Daussant, J., Mosse, J., Vaughan, J. (Eds.), Seed Proteins. Academic Press, New York, pp. 155–192.

Stanciuc, N., Aprodu, J., Ioniţa, E., Bahrim, G., Râpeanu, G., 2015. Exploring the process–structure–function relationship of horseradish peroxidase through investigation of pH- and heat induced conformational changes. Spectrochim. Acta Mol. Biomol. Spectrosc. 147, 43–50.

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 (4), 1584–1595.

Trujillo, A.J., Capellas, M., Saldo, J., Gervilla, R., Guamis, B., 2002. Applications of high-hydrostatic pressure on milk and dairy products: a review. Innovat. Food Sci. Emerg. Technol. 3 (4), 295–307.

Yao, D.N., Kouassi, K.N., Erba, D., Scazzina, F., Pellegrini, N., Casiraghi, M.C., 2015. Nutritive evaluation of the Bambara groundnut Ci12 Landrace [*Vignasubterranea (L.) Verdc.* (Fabaceae)] produced in Côte d'Ivoire. Int. J. Mol. Sci. 16 (9), 21428–21441.

Zayas, J.F., 1997. Functionality of Proteins in Food. Springer, New York, NY, p. 373.