Scientific Research Publishing

Agricultural Sciences, 2023, 14, 1268-1276
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Characterization of Seed Storage Proteins in Eight Bambara Groundnut Landraces in Burkina Faso

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How to cite this paper: Traoré-Barro, A., Thiombiano, E.A.M., Gnankambary, K., Rabo, M., Coulibaly, O., Traoré, S. and Traoré, O. (2023) Characterization of Seed Storage Proteins in Eight Bambara Groundnut Landraces in Burkina Faso. *Agricultural Sciences*, **14**, 1268-1276.
https://doi.org/10.4236/as.2023.149085

Received: August 11, 2023
Accepted: September 3, 2023
Published: September 6, 2023

Abstract

The Bambara groundnut *Vigna subterranea* (L.) Verdc. is a drought-resistant indigenous African grain legume with significant nutritional and agronomic potential. This study aimed to characterize the seed storage proteins of eight Bambara groundnut landraces. Seeds of Bambara groundnut landraces were collected from local markets in Burkina Faso, and total soluble protein as well as protein fractions were extracted. Crude protein content was determined by the Kjeldahl method, and soluble proteins were quantified using Bradford dye binding assay. The average crude protein content of the seeds was found to be 18.46%, with variations ranging from 17.69% to 19.17% among the different landraces. Most of the protein content was soluble, constituting approximately 87.04% of the total crude protein. Albumin fraction was the most dominant, representing about 95.42% of the total soluble proteins. The globulin, prolamin and glutelin fractions accounted for 1.82%, 0.13% and 1.17% of the soluble proteins, respectively. The findings provide valuable insights into the protein composition of Bambara groundnut landraces and contribute to our understanding of its nutritional potential, laying the groundwork for further research on crop improvement and sustainable agriculture practices.

Keywords

Bambara Groundnut, Landraces, Protein, Protein Fractions

1. Introduction

The Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous African grain legume that belongs to the *Fabaceae* family. It is grown in many African countries and other regions of the world, including India, Southeast Asia, and South America [1]. It is reported to be drought-resistant and more resilient to climate change than other legume crops, particularly cowpeas and soybeans. Bambara groundnut is used as both food and feed, particularly in poultry, rabbit, and pig farms. The protein content of seeds may vary from one variety to another, and values between 14% and 24% have been reported [2] [3]. The seeds also contain appreciable amounts of carbohydrates (up to 64%), low quantities of lipids (4.2% - 8.8%), 5.5% fiber, and minerals [4] [5].

According to Food and Agriculture Organization (FAO) statistics for 2021, Burkina Faso is the largest producer of Bambara groundnut, with approximately 66,000 tons [6]. However, this production is far below that of cowpea (705,800 tons) and groundnut (264,000 tons). In addition, the yields remain quite low (approximately 0.6 ton/ha) despite the high potential of 3 ton/ha [7]. The low productivity and hard-to-cook features of Bambara groundnut grains underutilized the crop. Several traits, including genetic potential and agronomic and nutritional importance, have not yet been fully investigated. As a result, most germplasms planted by farmers are landraces, where seeds are purchased in the local market at the beginning of the rainy season. These landraces display high genetic variability, reflected in the morphology and color of the seeds and nutritional traits [4].

Nutritional interest in leguminous crops is primarily due to their high protein content. The protein content in Bambara groundnut varies between 9.6% and 40%, a very large variation due to cultivar, growing conditions, and possibly the analytical methods used [4]. This study aimed to characterize seed storage proteins in Bambara groundnut landraces grown in the dry savannahs of Burkina Faso.

2. Materials and Methods

2.1. Plant Material

Bambara groundnut landraces were collected from local markets at two locations. Six originated from Bobo-Dioulasso (11.176634 latitude, −4.283333 longitude) and two from Ouagadougou (12.368430 latitude, −1.470848 longitude). Landraces were distinguished according to seed coat and eye color (**Figure 1**). Landraces L1 and L8 were black and cream, respectively with no pronounced hilum. Landraces L2 and L7 were both cream with black and grey hilum, respectively. Seed coat of landrace L3 was maroon with grey speckles whereas landrace L4 was predominantly cream with maroon speckles. Landraces L5 and L6 seed coat colored in maroon and black, respectively with cream color at the center. Some seed lots consisted of a mixture of two or three landraces. In such cases, landraces were sorted before testing.

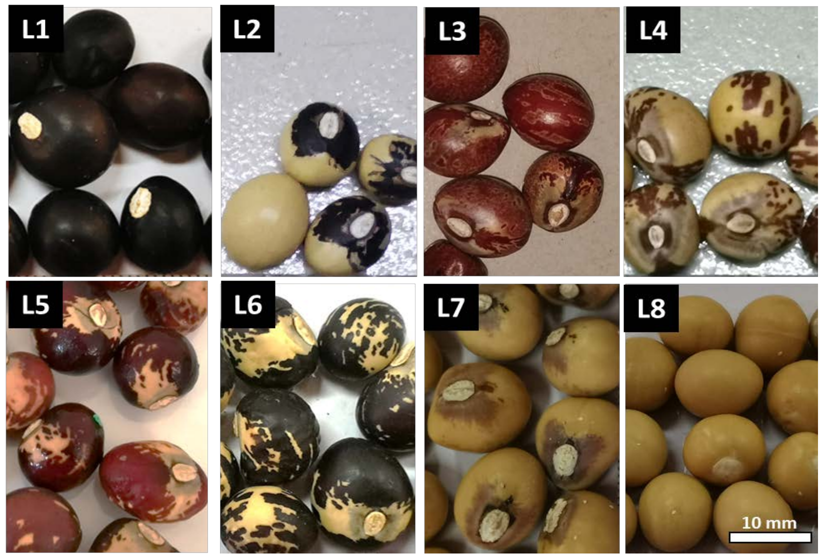

Figure 1. Visual characteristics of Bambara groundnut landraces.

2.2. Total Seed Proteins Extraction and Quantification

Seeds (~10% humidity) were ground in a coffee grinder, and the resulting floor was sieved through a 75 µm sieve. Crude protein content was determined by the Kjeldahl method [8] using an automatic protein analyzer UDK159 (Velp Scientifica, Italy) and a conversion factor of N × 6.25. Total soluble proteins were extracted by dispersing 1 g of the floor in 10 mL of 50 mM sodium phosphate buffer pH 7.8 [9] [10].

The mixture was agitated for 30 min at room temperature and centrifuged at 10,000× g for 10 min. Soluble proteins in the supernatants were quantified using Bradford dye binding assay [11]. Bovine serum albumin was used as a reference protein, and absorbance was measured at 450 nm and 590 nm instead of 595 nm wavelength only for the linearization of the calibration curves [12].

2.3. Fractionation of Seed Proteins

Seed protein fractions were extracted sequentially according to their differential solubilities, using the technique described by Ragab *et al.* [13]. Sequential fractions, including albumins, globulins, prolamins, and glutelins, were extracted using distilled water, 1 M NaCl, 70% (v/v) ethanol, and 0.2% NaOH, respectively. For the albumin fraction, seed flour (3.5 g) was dispersed in 50 mL of distilled water, vortexed, and agitated at room temperature for 30 min, followed by centrifugation at 10,000 ×g for 10 min. The pellet was extracted once more and the supernatants from the two extractions were pooled. The subsequent protein fractions were extracted in the same manner. In an additional experiment, the globulin fraction was extracted in 0.1 M sodium phosphate (Na_2HPO_4) to test the efficacy of this solvent compared to 1 M NaCl [14]. Protein fractions were quantified using Bradford's dye-binding assay, as described for the total soluble protein extract.

2.4. Statistical Analyses

Absorbance readings were recorded, and Bradford assay calibration curves were constructed using Microsoft Excel. All statistical analyses were performed using R software [15]. Assumptions of normality and homogeneity of variance of data on protein content were checked using the Shapiro-Wilk test ($p > 0.05$) and Levene's test ($p > 0.05$), respectively. The mean protein content was compared by analysis of variance (ANOVA, $p < 0.05$), and Fisher's LSD ($p < 0.05$) was used for post-hoc tests.

3. Results

3.1. Total Crude Protein Contents

Figure 2 shows the crude protein content of the seeds of the eight Bambara groundnut landraces. The protein content (%) varied between 17.69 ± 0.27 and 19.17 ± 0.03 with a mean of 18.46 ± 0.13. Despite the narrow range in protein content, statistical analyses showed significant differences among the eight Bambara groundnut landraces ($F7,16 = 31.6$, $p < 0.001$). The highest protein contents were found in landraces LR1 (18.93 ± 0.07) and LR6 (19.17 ± 0.03). Landraces LR2 and LR8 showed the lowest protein contents which were not significantly different ($p = 0.392$).

3.2. Total Soluble Protein Content

Soluble and insoluble protein contents in Bambara groundnut seeds, expressed as percentages of the total crude protein content, are illustrated in Figure 3. The average soluble protein content was 87.04% of the total crude protein, whereas 12.96% accounted for insoluble proteins and non-protein nitrogen. The lowest and highest soluble protein contents were found in landrace L3 (76.51%) and L2 (95.65%), respectively.

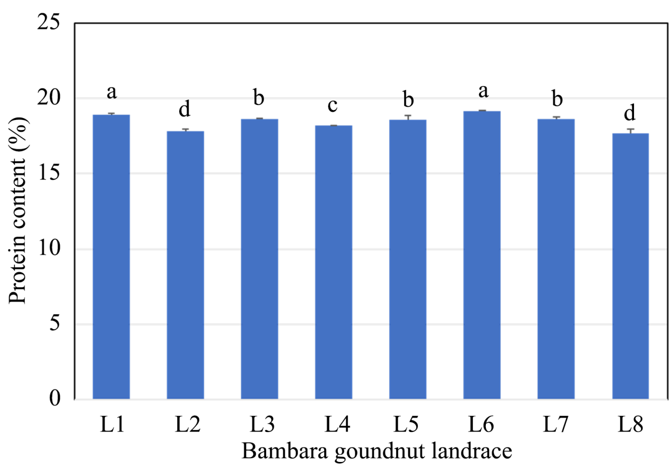

Figure 2. Average crude protein content in Bambara groundnut landraces. Means sharing the same letter are not significantly different. Identical letters on the top of the bars indicate groups with non-significant difference in crude protein content.

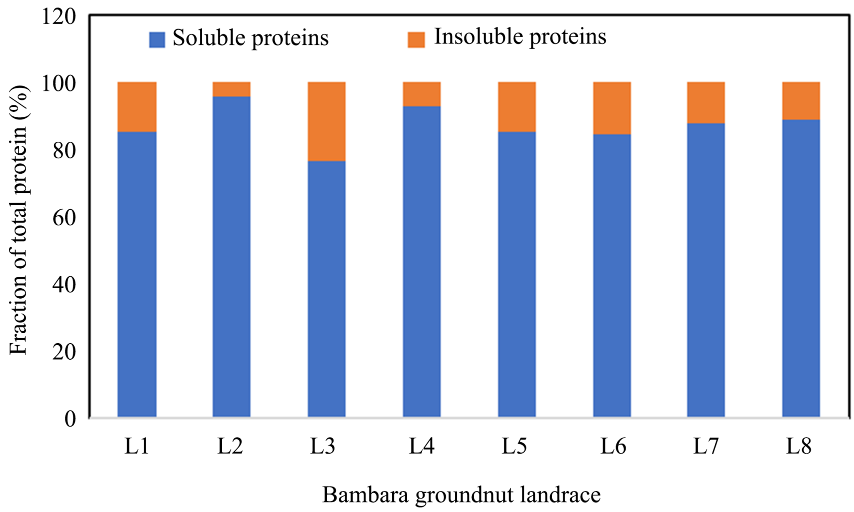

Figure 3. Soluble and insoluble protein content in Bambara groundnut landraces.

3.3. Effect of Globulins Extraction Buffer

Globulin fractions extracted with sodium phosphate and sodium chloride are illustrated in Figure 4. The average protein content (%) in the sodium phosphate fraction was 1.58 ± 0.09 while sodium chloride yielded 0.33 ± 0.05. Two-way ANOVA indicated that extraction with sodium phosphate yielded significantly higher protein content regardless of landrace ($F1,119 = 6833.06$, $p < 0.0001$). However, both extraction solutions showed similar patterns of variation in protein content between the landraces. Statistical analyses also revealed highly significant differences in globulin content between landraces ($F7,119 = 71.53$, $p < 0.0001$). The highest globulin content (1.94%) was recorded in landrace L5 whereas the lowest content was found in landrace L8.

3.4. Protein Fractions

Table 1 summarizes the total soluble protein content and sequentially extracted protein fractions. Total soluble protein extracted in sodium phosphate buffer varied in a narrow range from 14.26% (g of protein/100g of seed floor) to 17.04% with an average protein content of 16.05%. Statistical analyses of protein content showed that only landrace L3 (14.26% protein content) differed significantly from the other landraces. On average, the albumin fraction represented 95.42% of the total soluble protein content. The globulin, prolamin and glutelin fractions represented only 1.82%, 0.13% and 1.17%, respectively. The protein recovery rate was calculated by adding the four fractions. In most landraces, the protein recovery rates were approximately 100%. However, the protein recovery rate was significantly lower for landrace L2 (79.3%).

4. Discussion

Determination of the crude protein content (%) in the seed flour of eight Bambara groundnut landraces revealed that it ranged from 17.69 ± 0.27 to 19.17 ± 0.03).

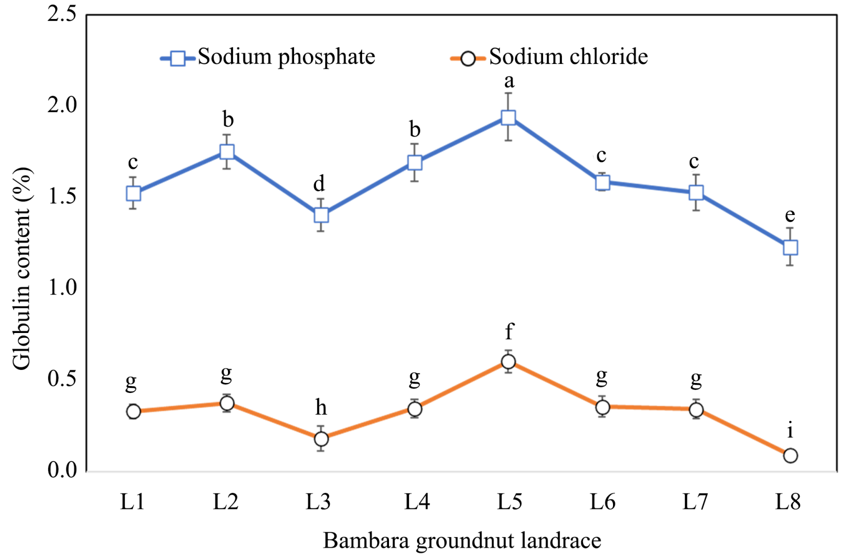

Figure 4. Effect of sodium phosphate and sodium chloride solvents on globulin extraction from seed flour of Bambara groundnut landraces (L1 - L8). Identical letters on the top of the data points indicate groups with non-significant difference.

Table 1. Total soluble protein (% seed flour) and protein fractions (% total soluble protein) in Bambara groundnut landraces.

Landrace	Soluble protein	Albumin	Globulin	Prolamin	Glutelin	% Recovery
L1	16.15 a	98.37 ab	1.79 bc	0.11 c	1.45 a	101.73 a
L2	17.04 a	76.3 c	1.83 bc	0.11 c	1.05 cd	79.29 b
L3	14.26 b	99.32 a	1.84 bc	0.14 a	1.42 a	102.73 a
L4	16.88 a	98.85 a	1.83 bc	0.12 c	0.99 d	101.79 a
L5	15.82 a	99.42 a	2.28 a	0.14 a	1.44 a	103.27 a
L6	16.17 a	93.24 b	1.88 b	0.13 b	1.13 bc	96.39 a
L7	16.35 a	98.89 a	1.74 c	0.13 b	1.19 b	101.95 a
L8	15.74 a	98.96 a	1.38 d	0.13 b	0.71 e	101.19 a

Means followed by the same letter(s) within each column are not significantly different.

Several studies determined the protein content in Bambara groundnut. Doku *et al.* [16] found 17% - 20% crude protein in Bambara groundnut landraces from Ghana. Ranges of 14% - 24% and 15% - 25% were also reported [17] [18]. Slightly higher protein contents of 26% - 27% were found in some South African landraces [19]. Overall, our data are consistent with the results presented in previous studies. The narrow range in protein content suggested that Bambara groundnut landraces may share close genetic backgrounds. It is expected that wider protein content range may be found when extending the study to other landraces in the country. This is exemplified by the 17.4% - 23.5% range found in a set of landraces by [20] as compared to the 17% - 20% range found in previous set of landraces originating from the same country [21].

The total protein content in Bambara groundnut seeds were analyzed regarding both soluble and insoluble components, expressed as percentages of total crude protein content. The total soluble part of the crude protein content was high in most landraces, indicating the efficacy of sodium phosphate buffer to solubilize Bambara groundnut seed proteins [9] [10]. Insoluble protein portions in most landraces were roughly similar to that reported earlier in Bambara groundnut [22] and in cowpea [23].

Several studies made use of the differential solubility of seed proteins to sequentially extract albumin, globulin, prolamin and glutelin fractions [13] [23] [24]. Usually, the globulin fraction was extracted with a solution of sodium chloride. However in a study comparing different solvents, 0.1 M sodium phosphate dibasic was found to be the best solvent for extracting globulins from amaranth seeds [14]. The use of sodium phosphate for Bambara groundnut seeds in this study gave similar results. This solvent clearly outperformed the sodium chloride solution which was no longer used in subsequent extractions.

The distribution of protein fractions within the total soluble protein indicated that the albumin fraction dominated, representing approximately 95.42% of the total soluble proteins. This observation is consistent with previous studies, where albumin has been reported as the major protein component in many plant seeds [13] [14] [20]. Additionally, the pattern of non-albumin soluble protein showed similar levels of globulin and glutelin, and the lowest prolamin content. Such a pattern was also observed in cowpea and Bambara groundnut [13] [22].

5. Conclusion

This study provides valuable insights into the protein composition of Bambara groundnut landraces grown in the dry savannahs of Burkina Faso. The protein content of the seeds ranged from 17.69% to 19.17%, with slight variations among the different landraces. The high protein content and predominance of albumins in the total soluble protein fraction make Bambara groundnut a promising crop for enhancing nutritional security, particularly in regions prone to climate change and food insecurity. Future studies encompassing a broader range of landraces from various regions may reveal wider diversity in protein content. Overall, this study contributes to the knowledge of the nutritional potential of Bambara groundnut as a protein-rich crop and underscores the importance of further exploration of this underutilized crop for food and feed.

Acknowledgements

The authors acknowledge the contribution of the Union Economique et Monétaire Ouest Africaine (UEMOA)/West African Economic and Monetary Union (WAEMU) for providing valuable laboratory equipment necessary for this work.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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