Electrophoretic Characterization of Protein Isolates from *Lonchocarpus sericeus* (Sengal lilac)

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Abstract

There is an increasing interest in exploiting new sources of plant proteins as food products to meet the demand for foods, and confront food sustainability challenges particularly in Tropical Africa ravaged by drought, unplanned population growth, and conflicts. These effects have led to adverse effects on livestock production and a serious drop in animal protein supply, increase in food prices and unprecedented high import bills amidst shortage of exchange currency. L. sericeus seed is an under-utilized oil seed legume and a very good source of protein. Therefore, it has great potential as an alternative to other oilseed legumes. To facilitate its use in food system, the knowledge of its protein content of L. sericeus seed using SDS-PAGE and to determine the effect of processing methods on the seed protein isolates. Proteins were isolates from raw and processed L. sericeus seed. Molecular weights of each protein sample were investigated with the highest band at 102kDa and the lowest band 24kDa. This study revealed that the seed has a distinct protein pattern similar to other legumes; therefore, processing method has slight difference in the protein electrophoretic pattern.

Keywords: *Loncocarpus sericeus*, Electrophoresis, Protein, SDS-PAGE

Introduction

Malnutrition is among the greatest global current societal challenges with a basic lack of protein, energy foods and micronutrients (Ritchie & Roser, 2017), resulting in poor diet and malnutrition, causing health, economic and environmental burdens (Behrman, 2020). Countries are also experiencing the double burden of malnutrition, where undernutrition coexists with overweight, obesity and other diet-related non-communicable diseases (NCDs); these phenomena are more severe in Sub-Saharan Africa (SSA) and are becoming an increasingly public health problem (Akombi, Agho, Merom, Renzaho, & Hall, 2017; Behrman, 2020; WHO, 2017). Malnutrition-induced stunting is prevalent in Africa and differs across countries, ranging from 7.9 to 57.7%, with an average of 30% for the region (WHO, 2017). Moreover, malnutrition has been strongly linked with suboptimal brain development, resulting in diminished cognitive capacity, compromised immune systems, reduced educational performance and economic productivity of nations (Mokria et al., 2022).

Also, the United Nations World Food Programme (UN-WFP) has warned of severe famine arising from drought and conflicts. In a recent report by the United Nations Food and Agriculture Organisation(UN-FAO), higher food import bills that would not necessarily translate into more food inflows was predicted for the least developed countries (LDCs), sub-Saharan Africa (SSA) and low-income food deficit countries (LIFDCs) (UN-FAO, 2021)An advanced quantity and enhanced quality of food are required to feed a global population that is growing and becoming wealthier. Combating famine and its attendant consequences, therefore, requires an integrated approach that will include the exploitation of lesser known and underexploited food supplies that abound in the Sub-Sahara Africa (McClements & Grossmann, 2022). Plant proteins, and specifically those from legume crops, are increasingly recognised as sustainable and functional food ingredients (Diedericks et al., 2020). Plant protein including mung bean, chick pea have been studied as functional egg substitutes through various modification (Wang et al., 2022, Lima et al., 2022)

There is an increasing interest in exploiting new sources of plant proteins as food products to increase access to food, confront food sustainability challenges, enhance diet quality and nutritional status particularly in Tropical Africa ravaged by drought, unplanned population growth, and conflicts. Also, the world's population according to the United Nations is expected to increase to 9.8 billion by 2050 and 11.2 billion by 2100 (United Nations, 2017), thus, leading to a rise in the demand for highly nutritious foods and price increases. Plant proteins have been found to be more sustainable and cost-effective compared to animal proteins and as such, there's a growing interest in exploiting new sources of plant proteins as alternative food products. Pulses accumulate large amounts of proteins during their development and are therefore considered as promising protein sources (Duranti & Gius, 1997). Understanding protein microstructure is important as other intrinsic variables like matrix composition, component interactions as

well as the molecular configuration affect the rate and extent of hydrolysis and nutritional value (Mondal et al., 2022). Quantitative data on internal protein structure can be related to the mechanical and rheological properties, and image analysis techniques including electron microscopy, XRD, SEM, XPS have proved to be reliable methods Xiaoyan et al., (2015).

Information on the nature of proteins of underutilised food trees and legumes indigenous to sub-Sahara Africa is often hard to find, hence, food trees and legumes that are rich in protein may therefore be overlooked in agriculture and nutrition development planning, projects and policies. The purpose of this study, therefore, is to investigate and document the properties of proteins of *Lonchocarpus sericeus* thereby understanding their potential contribution to household food diet diversification and socioeconomic improvement, eliciting environmental protection and increasing their abundance on different landscapes.

These effects have led to adverse effects on livestock production and a serious drop in animal protein supply, increase in food prices and unprecedented high import bills amidst shortage of exchange currency.

2.0 Materials and methods

Sample collection and preparation

Lonchocarpus sericeus seeds were obtained from the premises of Federal polytechnics Ilaro, Ogun state, Nigeria. Seeds were divided into two lots. One lot was decorticated manually and as further divided into three portions. One portion was boiled in distilled water on a gas burner for 6 hours after which it was drained using sieve and then sun dried (sample1). The same treatment was given to another portion which was unshelled (sample 2). The second decorticated portion was roasted in fine sand bath until brownish in colour, the roasted sample washed in distilled water, drained and sun-dried (sample 3). The same treatment was also given to another unshelled portion (sample 4). The third portion was left raw, washed and dried (sample 5); the sixth portion was unshelled and was not given any treatment (sample 6). All the above samples were grounded separately in a blender. The blended fine powder kept in an air tight container for further analysis.

Protein isolation

L. sericeus flour was dispersed in distilled water (10% w/v) and the pH was adjusted to 8.0 with 1 N NaOH. The dispersion was stirred using a magnetic stirrer for 1 hour at room temperature (20 ± 2 C). Insoluble non-protein fraction was separated from protein extract by centrifugation at 17,500 g or 15 minutes in an SS34 Sorvall centrifuge (Sorvall Instruments, Wilmington, DE). In order to increase the yields, the extraction and centrifugation process was repeated twice on the solid residue. The protein extract (supernatant) collected was acidified with 1 N HCl to pH 4.5. The protein precipitate obtained in this way was collected by centrifugation at 11,200 g for 3 minutes. To eliminate all soluble components, the pellet was washed with distilled water. The protein was resuspended in distilled water maintaining about 10% (w/v) total solid and the pH was adjusted to 7.0. Clear protein suspension was obtained by decanting and filtering through a double layer of muslin cloth.

Drying of protein isolate solution

For freeze drying, protein solution was first frozen for 24 hours at 18^{o}C. Frozen samples were then freeze-dried (DYNA-VAC Freeze dryer VRI-8 high vacuum pump, DHF 600, Dyna-vac Engineering incorporated, Australia) at 48 C compressor temperature and 0.5 mm vacuum pressure. Freeze-dried lentil protein isolate was ground using a mortar and pestle and sieved through a No. 40 mesh.

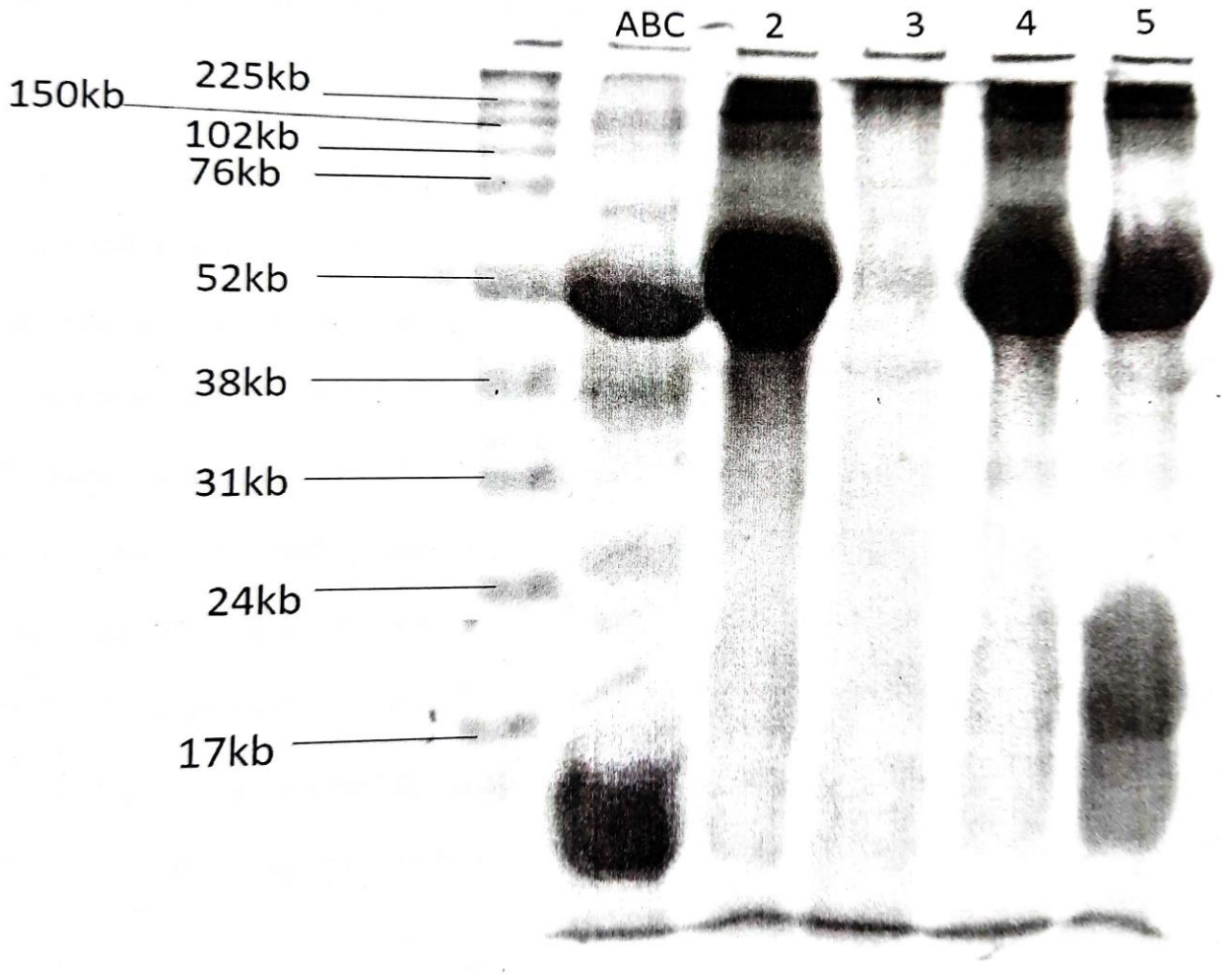
SDS-PAGE Characterisation

Sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDP-PAGE) was done on LKB 2001 vertical electrophoresis unit. The procedure was according to Laemimli methods (1970). The protein samples were dissolved in 1.5 M tris-HCL (pH8.8) buffer containing 1% SDS, 1% 2-mercaptoethanol, 40% sucrose and 0.2% bromophenol blue to provide 1 mg/ml protein solution. The mixture was then heated in boiling water for 5 minutesfollowed by centrifugation at 10000g for 10 minutes. For each sample, 10µL of supernatant were loaded onto each lane.
The electrophoresis was performed at constant current of 15mA in stacking gel (14×16×0.15cm) and 25mA in separating gel (lower 14cm) until the tracking dye reached the bottom of the gel. After electrophoresis, the gel was

stained for 3hours with 0.05% coomassie brilliant blue R in acetic acid solution, and then distained for 6 hours by methanol solution.

Results and discussion

To investigate variations among seed storage proteins from *L. sericeus* SDS-PAGE was performed under reducing and non-reducing conditions. Bands obtained under the different conditions are indicative of the purity of the protein, since it lacks disulphide bonds amongst its subunits. As such the protein would not be dissociated under reducing conditions, thus exhibiting similar electrophoretic patterns in the absence and presence of the reducing agent; contrary to the other major storage proteins legumin and albumin (Mession et al., 2015; Tang, 2008; Tang & Sun, 2011).



Protein bands were observed within 17kDa and 225kDa. The highest protein band was observed at 102kDa while the lowest band was observed within 24kDa range. All the samples tested except sample 3 show that the protein bands observed at higher molecular weight may be classified as globulin, since globulins as earlier reported by Duranti et al (2008) are higher of δ and γ conglutins. The globulin and glutelin protein fraction of the deffated seed of L. sericeus seed as reported my Ezeagu and Gowda (2006) constituted of major seed protein present 28.59% and 52.55%) while prolamin and albumin are least (0.44and 4.44% respectively). This is similar to the glutein and globulin fractions of walnut (Juglans regia L.) as reported by Xiaoying et al, 2014(72.06% and 15.67%).

Samples 1, 2, 4 and 5 showed a major band at 52 kDa, and this is often denoted as the β subunit in legume seed protein and is thus indicative of the major subunit composition of vicilin, this being the largest band present. (Chang, Alli, Molina, Konishi, & Boye, 2012; Maruyama et al., 1998; O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004b; Tzitzikas et al., 2006), this is an indication of presence of vilicin in the sample.

Sample 1 shows a minor band at 38kDa which is denoted to be leucine a protein is identical to the 36-kDa protein which was isolated by Larsson et al (1991). Leucine is a protein found around the cartilage.

Conclusion

SDS-PAGE result of raw and processed deffated *L. sericeus* flour revealed similsr protein bands except for whole roasted *L. sericeus* flour. This may be attributed to heat involced in the roasting process as heat denatures protein content. *L.sericeus* has a potential promising as a low protein when animal protein is scarce. Its protein isolate can be used in food industry as food additives or ingredients.

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