

Effect of pH on Emulsifying Activity of Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (*Vigna Unguiculata* L. Walp)

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ABSTRACT

The increasing world population has led to shortage in protein supply which necessitated the quest for new protein sources, particularly from legume. Legumes, naturally do not possess suitable functionality for application in food industry. To improve functionality, different methods of modification (physical, chemical, enzymatic) have been utilized by researchers though with some limitations. However, the use of germination has not been fully explored especially for underutilized Solojo cowpea. Therefore, the aim of this study was to evaluate the potential of germination in improving the nutritional and functional properties of flours and proteins isolated from Solojo cowpea and the corresponding effect of pH on its Emulsifying Activity as well as the stability of the isolates. The protein isolates were obtained as whole cowpea flour (WCF), full fat dark ash solojo (FFDAS) and defatted dark ash solojo (DFDAS) flours from dehulled defatted cowpea flour (DDCF) by isoelectric (CPIA) and micellization (CPIB) precipitation were studied. The emulsifying activity as well as stability were affected by pH as shown in Figures [1-12]. All the flours of DAS and BS showed lowest EA between pH 4 – pH 6. The EA decreased as the H⁺ increased up to the IEP increased with pH to the alkaline region. The DAS isolates had its minimum EA at pH 2- pH 4, while the BS isolate had its minimum EA at pH 2 for all treatment except 6h and 36h, which both had their minimum EA at pH 8. The value ranged between 43.34% and 50.00%; 44.44% and 51.52%; 43.28% and 49.37%; 43.99% and 49.34%; 43.80% and 52.55%; 44.81 and 60.18% for FFDAS, DFDAS, FFBS, DFBS, DAS and BS respectively. ES followed an arrangement comparable to the EA. The lowest ES was reported at pH 4 for most of the flour samples, except for, FFDAS Raw at pH 8 and DFDAS Raw at pH 6. The protein isolates of DAS and BS also had most of the ES minimum at pH 4, except DAS 72h and BS Raw with minimum at pH 2; DAS 36 h, 48 h, BS 6 h and 36 h had their minimum at pH 6; only DAS Raw had minimum ES at pH 8. The ES values ranged between 43.33% and 51.65%; 44.44% and 51.52%; 44.80% and 50.01%; 43.52% and 50.21%. 80.72% and 110.70% 80.66% and 108.82%, for FFDAS, DFDAS, FFBS, DFBS, DAS and BS respectively. There was no marked difference between the ES of the two varieties.

Keywords: Solojo Cowpea, Under-utilized legumes, BS DA, Un-germinated, Protein isolate, Emulsifying Activity

Introduction

The problems of malnutrition and food in-security existing together with fast population growth, and prohibitive nature of animal-based food have necessitated the need to identify and integrate unorthodox protein sources to augment the conventional formulation.

The demand for protein has become so great and is still rapidly increasing due to world population explosion and growth of

the food industry, cumulating in increasing demand for animal protein thereby putting pressure on the conventional animal sources. In the developing countries, animal protein such as meat, egg, fish and milk are out of the reach of many because of shortage in supply which has eventually led to increase in cost which makes adequate quantity consumption impossible [2-4]. Consequently, it is undeniable that conventional animal protein sources are not adequate to meet with the demand, and on the long run unsustainable. It has therefore become imperative to look into new sources of protein to reduce the pressure on the existing sources [3-12].

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Plant proteins play significant role in human diet especially in developing countries where average protein intake is less than required [6]. The proteins in cereals and millets which form the major component of the poor man's diet are deficient in lysine and that they can be supplemented by legumes which are rich not only in lysine but also in threonine [11-14]. In Nigeria, as in most tropical countries little work has been carried out either on composition or cultivation of legume crops.

Cowpea (*Vigna unguiculata* L. Walp) is widely cultivated and consumed in Africa and South America. It provides more than half the plant protein in human diets for the poorest sector of many developing countries. It is an important source of protein in developing countries, especially in West Africa where it is eaten in a variety of ways [8]. The contribution of vegetable protein as functional ingredient is playing an increasing role in food formulation as the food industries move towards creating more market-driven consumer product. Both economy and consumer attitudes have compelled manufacturers to replace milk, egg and meat of animal proteins, with acceptable product from plant sources [16]. The production of plant protein isolates is of growing interest to industry because of the increasing application of plant proteins in food and non-food markets. The use of plant isolates in foods as functional ingredients, to improve the nutritional quality of the product or for economic reasons is much extended. Collaborative efforts towards exploiting the capacity of legumes to curtail the complication of malnutrition (protein) in Africa and to reduce the pressure on the commonly consumed legumes is on-going [1,2].

Emulsification abilities is improved by exposing buried oil-loving groups, improving its surface hydrophobicity and decreasing its molecular mass; these improvements allow for better imbibition along water-oil border. The reason being that such enzymatic polymerization of proteins could be regulated to boost the functionality to the requested level for desired time [17]. Partial enzymatic hydrolysis has also been identified as somewhat easy and beneficial procedure for better palatability and nutritious value of plant proteins [15].

The increasing world population has led to shortage in protein supply which necessitated the quest for new protein sources, particularly from legume. Legumes, naturally do not possess suitable functionality for application in food industry. To improve functionality, different methods of modification (physical, chemical, enzymatic) have been utilized by researchers though with some limitations. However, the use of germination has not been fully explored especially for underutilized Solojo cowpea. Biochemical modification (Germination) as a means of improving functionality has not yet been fully exploited. This work therefore is designed to evaluate the ability of biochemical modification in enhancing the functional properties, and nutritive quality of Solojo protein. Solojo an underutilized legume commonly grown in the South-West region of Nigeria, will be biochemically modified for its possible industrial application through its functional properties.

Materials and Methods

Raw Materials

Two varieties of the underutilized cowpea (*V. unguiculata*) found in South west region of Nigeria where it is called 'solojo' were

used (Figures 1 and 2). Seeds obtained from Bodija market in Ibadan, Western Nigeria, were screened to get rid of every irrelevant materials and unwholesome seeds. The beans were then portioned into six (6). The solojo seeds for germination were sterilized by soaking in 0.07% sodium hypochlorite for 30 min, then rinsed thoroughly. The solojo seeds were then immersed for 6 h in distilled water at ambient temperature (1:10 w/v) (~25°C), then placed in a colander and germinated under subdued light in an open laboratory for, 24 h, 36 h, 48 h and 72 h (Figure 3).



Figure 1: Brown Solojo Cowpea



Figure 2: Dark-Ash Solojo Cowpea

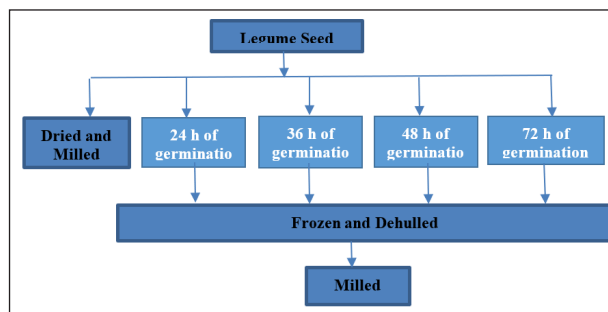


Figure 3: Preparation of Beans Flour/ Schematic representation

Preparation of Flours

Raw Flour

The grains were segregated to remove the spoilt ones; then dry dehulled with a mechanical dry dehuller (fabricated in FIIRO), dried at 40°C and later milled dry to powder then sifted using 80 µm mesh. The flour was stored in flexible bags and preserved at 4°C preceding utilization in a refrigerator freezer.

6 h Soaked Flour

The seeds were segregated to remove the unwholesome ones, then immersed for 6 h in the ratio (1:10 w/v) (seed/water). The grains were then frozen to prevent germination from setting in, then the hull was removed manually, dried for 48 h at 40°C later milled dry to smooth powder prior to sieving using 80 µm mesh screen. The resulting flour was packaged in plastic pack and preserved in a fridge freezer at 4°C pending utilization.

Results and Discussion

The result obtained for the soaked and germinated samples agreed with that of Adebowale and Lawal. The significance of pH on emulsifying activity is more obvious because, emulsifying

activities of dissolvable protein is dependent on the hydrophilic and lipophilic equilibrium which depends on pH. The protein at the oil- water surface, orientate the non-polar residue to the lipid layer and the polar residue to the water layer, hence minimizing strain on the surface.

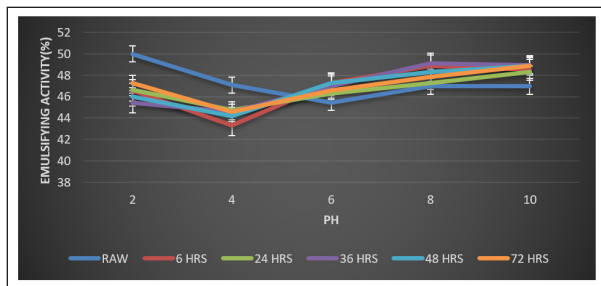


Figure 4: Effect of pH on Emulsifying Activity of FFDAS FFDAS – Full Fat Dark- Ash solojo cowpea of germinated and non- germinated flour

EA for the germinated samples of DAS and BS were found to be generally higher than those of the control. This could be because germination had brought about uncoiling of the protein matrix and thereby allowing for the buried functional groups to come to the fore, which now increases the number of the hydrophilic and lipophilic residues.

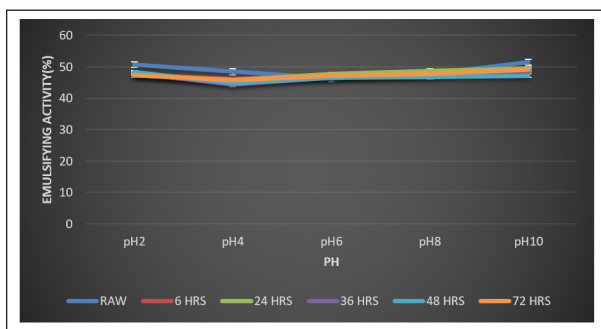


Figure 5: Effect of pH on Emulsifying Activity of DFDAS DFDAS – Defatted dark -ash solojo cowpea of germinated and non- germinated flour

The high ES at extreme acid and alkaline range (pH 2 and pH 10) can be as a result of protein unfolding that results in greater solubility of protein, which thereby brought about an equilibrium of the van der Waals forces of attraction and electrostatic forces of repulsion which are the intermolecular forces holding the lipid phase and protein together, therefore encouraging balance at the interface.

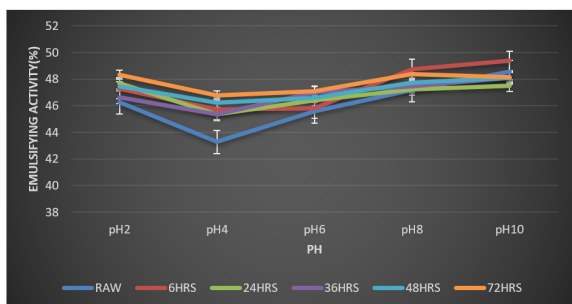


Figure 6: Effect of pH on Emulsifying Activity of FFBS FFBS- Full fat brown solojo cowpea of germinated and non-germinated flour

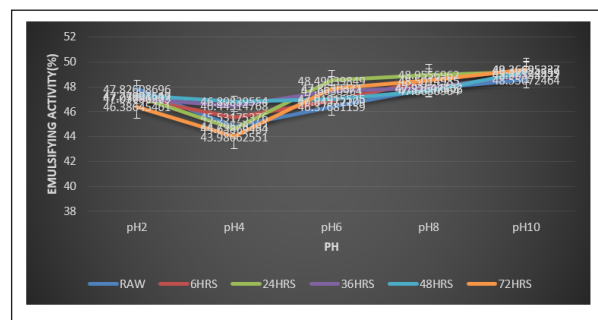


Figure 7: Effect of pH on Emulsifying Activity of DFBS DFBS- Defatted brown solojo cowpea of germinated and non-germinated flour

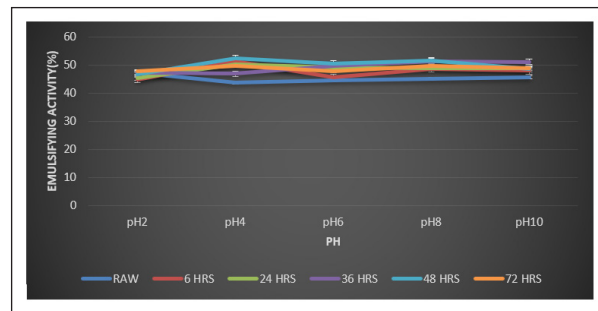


Figure 8: Effect of pH on Emulsifying Activity of DAS DAS- Dark- ash solojo cowpea of germinated and non-germinated protein isolate

As expected, protein close to the isoelectric point ought to perform well when it comes to emulsifying properties, the reason being that, adsorption of protein and visco-elasticity at the oil –water interface are greatest and repulsive forces between proteins are lowest.

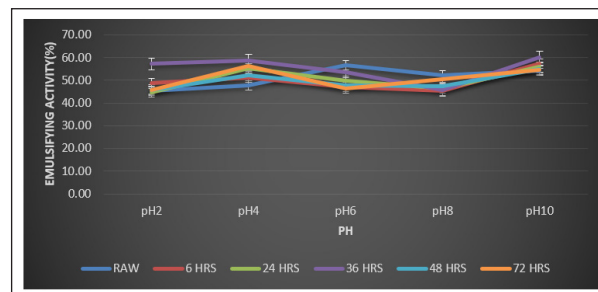


Figure 9: Effect of pH on Emulsifying Activity of BS BS- Brown solojo cowpea of germinated and non- germinated protein isolate

A value range of 44.74% to 60.88% for the two isolates produced by the two different isolation techniques were recorded.

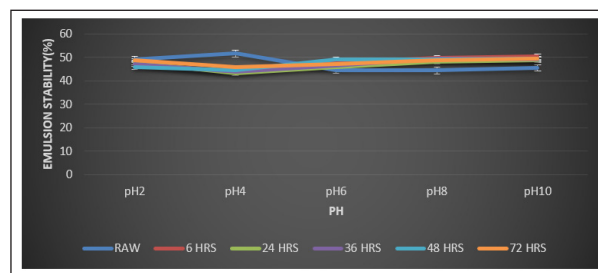


Figure 10: Effect of pH on Emulsion Stability of FFDAS FFDAS – Full fat dark- ash solojo cowpea of germinated and non- germinated flour

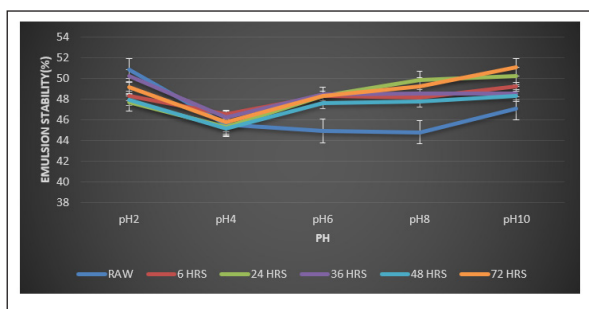


Figure 11: Effect of pH on Emulsion Stability of DFDAS
DFDAS – Defatted dark -ash solojo cowpea of germinated and non-germinated flour

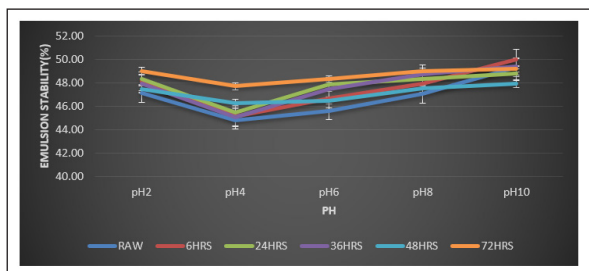


Figure 12: Effect of pH on Emulsion Stability of FFBS
FFBS- Full fat brown solojo cowpea of germinated and non-germinated flour

Discrepancy in Emulsifying Activity of the flours trans-versing the pH spectrum is likely because of the interplay of various component of the flours that affect their characteristics. Our observation agreed with the overall interrelationship amid Emulsifying Activity and protein solubility as recorded in earlier works.

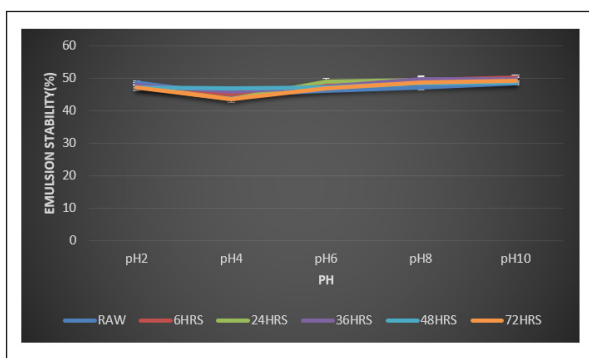


Figure 13: Effect of pH on Emulsion Stability of DFBS
DFBS- Defatted brown solojo cowpea of germinated and non-germinated flour

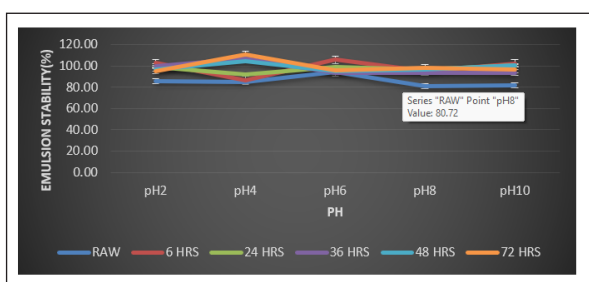


Figure 14: Effect of pH on Emulsion Stability of DAS
DAS- Dark- ash solojo cowpea of germinated and non-germinated protein isolate

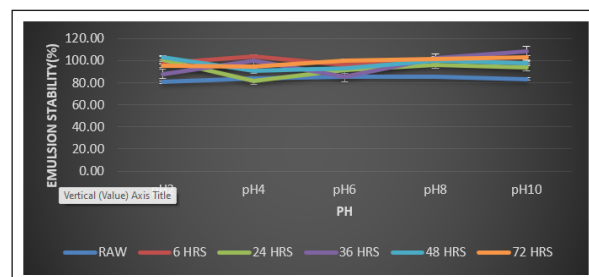


Figure 15: Effect of pH on Emulsion Stability of BS
BS- Brown solojo cowpea of germinate and non-germinated protein isolate

Emulsifying Stability (ES) was near lowest in isoelectric pH region because of clustering and destabilization of the interfacial film. Favorable emulsifying character of the flours make them possibly suitable in food products such as beverages that are milk-like and meat analogs.

Conclusion and Recommendation

According to modern nutrition recommendations, human beings ought to depend majorly on proteins of vegetable and legume origin for their dietary protein needs. Pulses have been found to play very essential role in achieving the required nutritional recommendations, particularly in emerging and third world countries where the consumption of mammalian protein is low because of the high cost. Apart from the high cost, large amounts of saturated fat and cholesterol are other problems associated with animal protein sources.

Concerns about high-cholesterol, has necessitated the recommendation of regular consumption of vegetable protein as opposed to animal protein by nutritionists. This has led to a renewed interest in legume protein because of their high level of protein which ranged between 20 and 60%. They also have good protein quality in respect to their digestible and nutritional characters. Apart from this, the level of fibre in the body also increases with increased consumption of more plant food that helps in reducing the danger of bowel diseases, as well as cancer of the colon and prevalence of osteoporosis. Compared to cereal grains, legume grains are also very excellent source of weight reduction fibers.

Legumes not only possess significant protein content but also essential protein character, research has also shown their capacity to oppose the action of malnutrition especially in emerging nations by including them in the everyday regime. Seed proteins in addition to providing essential amino acid have the useful properties required by industries producing food.

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