International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 11-05-2021, Accepted: 26-05-2021, Published: 10-06-2021
Volume 6, Issue 3, 2021, Page No. 78-83

Effect of germination on the bulk density of flours and protein isolates from Solojo cowpea (*Vigna Unguiculata* L. Walp)

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Abstract

Effect of germination on nutritional and functional properties of flours derived from two (2) varieties of Solojo Cowpea (DAS-Dark-Ash Solojo and BS-Brown Solojo) were studied before and after dehulling of the germinated seeds while the ungerminated portion of the seeds served as the control. Both varieties (DAS and BS) investigated were soaked in distilled water and germinated at varying periods i.e. 0, 6, 24, 36, 48 and 72hrs. Protein isolates were then obtained from the treated and processed samples by isoelectric precipitation method which was subsequently followed by proximate and anti-nutritional analyses. Functional properties were also analysed. Amino acids and molecular weight of the protein isolates were determined by amino acid analyser and sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis. Surface morphology, functional group and thermal properties were determined for protein isolates by scanning electron microscopy, Fourier Transform Infrared (FTIR) spectrometry and differential scanning calorimeter, respectively. Data were analysed by descriptive statistics and ANOVA at $\alpha0.05$. Germination was observed to generally increase the OAC when compared with the control for full fat, defatted flours and protein isolates. The OAC values of the DAS and BS cowpea at 36 h, which is 2.26 g/g and 2.64 g/g respectively, were higher than those of the mung bean at 36 h which is 0.75 mL/g. Likewise at 24 h treatment DAS and BS cowpea sprouted gave a value of 1.89 g/g and 2.08 g/g respectively contrary to 1.25 mL/g for Mung bean. The bulk densities of germinated and dehulled legume flours were lower compared to control. The isolates had the bulk density varying between 0.36 to 0.50 g/cm^3 for the LBD of DAS and 0.47 to 0.59 g/cm^3 for PBD; while the BS isolate had for LBD 0.35 to 0.47 g/cm^3 and 0.45 to 0.59 g/cm^3 for PBD. Data showed that flours (full fat and defatted) had their WAC increasing with time of germination, the same was observed for the isolates of DAS and BS, with the germinated flour having better WAC than the non-germinated flour (0.95 to 1.94 g/g for FFDAS and 0.94 to 2.10 g/g for DFDAS; 1.41±0.02 to 2.14±0.02 g/g for FFBS; 1.46±0.02 to 2.33±0.01 g/g for DFBS; 2.27±0.06 to 3.41±0.03 g/g for DAS isolate and 2.21±0.09 to 4.15±4.15 g/g for BS isolate). The emulsification capacities of control samples ranged from 55 to 193 ml oil emulsified per gram of sample. On germination and dehulling, the emulsification capacities, activities and stabilities of samples increased significantly. There were increases in foaming capacities and reduction in foam stabilities of all the samples investigated on germination and dehulling. Thus, the study indicated that germination and dehulling improved the functional properties of legumes.

Keywords: dehulled legume, solojo cowpea, emulsification capacities underutilised legumes, ANOVA

Introduction

Legumes represent one of the main plant source of proteins in human diet. They are also generally rich in dietary fibre (Rochfort and Panozzo 2007) [22]. Minor compounds of legumes are lipids, polyphenols, and bioactive peptides (Pastor-Cavada *et al*., 2009) [18]. Legumes provide a good source of protein (18-35%). Plant food diets increase the level of fibre intake which reduces the risk of bowel diseases, including cancer and also reduction in osteoporosis incidence. High protein (18-35%) and carbohydrates (50-60%) contents together with amino acid pattern complementary to that of cereal grains; however make cowpea a potentially important nutritional component in the human diet. Cowpea (Vignaungiculata L.) provides more than half the plant protein in human diets. (Prinyawiwatkul *et al*., 1996).

Most folks in the developing countries rely upon grain legumes as major sources of dietary protein, because, animal proteins are expensive (Shimelis *et al*., 2006) [21]. Various research efforts are now on going on the application of non-animal proteins for the evolution of innovative nutritional produce or substitute against high- priced animal proteins. (Khalid *et al*., 2003) [12]. 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 (Adebowale and Lawal, 2003; Ahmed *et al*., 2011) [1]. 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 (Butt and Batoola 2010) [14].

Functional properties are the physical and chemical characteristics of the specific protein influencing its behavior in food system during processing, storage, cooking and consumption. Examples of functional properties include bulk density, protein solubility, water and oil absorption capacity, emulsifying and foaming properties. The factors that affect the functional behavior of proteins in foods are their size, shape, amino acid composition and sequence, net charge, hydrophobicity, structure, molecular rigidity in response to external environment (pH, temperature, salt concentration) or interaction with other food constituents (Aluko and Yada, 1997) [4]. Formation of an emulsion and its stability is very important for any food systems (Tounkara *et al*., 2013) [8]. Protein has been found to possess the ability to form and stabilize emulsion.

According to modern nutrition recommendations, human beings ought to depend majorly on proteins of vegetable and legume origin for their dietary protein needs (Oreopoulou and Tzia, 2007; Sibt-e-Abbas *et al*., 2015) [23, 16]. 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 (Klupšaitė, and Juodeikienė, 2015) [13]. Legumes will therefore continue to play important part in diets in the foreseeable future.

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.

Fig 1: Some common bean legumes.

Experimental

Materials

The raw material investigated in this research study is Solojo Cowpea (Vigna unguiculata L.) which occur in two varieties i.e. Dark-ash solojo (DAS) and Brown Solojo (BS). These two underutilized varieties found in South-West region of Nigeria where they are called 'Solojo' were obtained from Bodija market in Ibadan, Western Nigeria. They were stored in polyethylene bags at room temperature (25-26ºC).

Methods

The dehulled cowpea seeds were cleaned and screened to get rid of every irrelevant materials and unwholesome seeds. The Solojo seeds (DAS and BS) for germination were sterilized by soaking in 0.07% Sodium hypochlorite (Rumiyati 2012) [20] 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) (~25oC), then placed in a colander and germinated under subdued light in an open laboratory (Rusydi *et al*., 2011) [15] for 0, 6, 24, 36, 48 and 72hrs. Other treated portions of the Solojo seeds (DAS and BS) were dehusked, dried, milled into flour and defatted. Protein was isolated by isoelectric precipitation method. Proximate, antinutritional analysis and functional properties [Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC)] of the flours and protein isolates were determined by standard methods. Amino acids and molecular weight of the protein isolates were determined by amino acid analyzer and sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis. Surface morphology, functional group and thermal properties were determined for protein isolates by scanning electron microscopy, Fourier Transform Infrared (FTIR) spectrometry and differential scanning calorimetry, respectively. Data were analysed by descriptive statistics and ANOVA at α0.05

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 40ºC and later milled dry to powder then sifted using 80 µm mesh. The flour was stored in flexible bags and preserved at 4ºC preceding utilization in a refrigerator freezer.

6hour 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 40oC 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 4oC pending utilization.

Germination of seed

This was implemented by the method of Mubarak (2005) with minor adjustment. The seeds for germination were disinfected by soaking in 0.07 % Sodium hypochlorite (Rumiyati 2012) [20] for 30 mins, then, it was rinsed painstakingly. The Solojo seeds were then immersed for 6 h at ambient temperature in water in the ratio (1:10 w/v) (seed/water) (~25oC), then placed in a colander and germinated under subdued light in an open laboratory (Rusydi, 2011) [15] for various h, 24, 36, 48 and 72 h. The process of germination was terminated by freezing, the seeds were manually dehulled, dried in a draught oven (Schutzart DIN EN 60529-IP 20. Memmert, Germany) at 40oC for 48 h, cooled, milled and packaged in an air tight plastic bag in the refrigerator pending analysis.

Fig 2: Dark- ash Solojo Cowpea

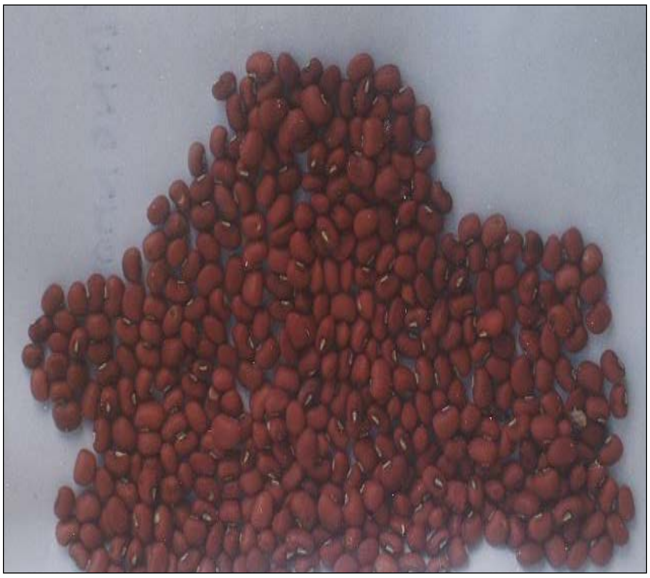

Fig 3: Brown Solojo Cowpea

Fig 4: Germinated Dark- ash Solojo Cowpea

Fig 5: Germinated Brown Solojo Cowpea

Packed and Loose Bulk Density

The bulk density measurement was carried out by weighing 50 g of the sample into a hundred (mL) graduated cylinder. The cylinder was lightly hit against a soft surface about 100 times till no more change in volume occurred. The bulk density was determined as weight/ volume of the test material (Kaur *et al*., 2005).

Result and Discussion

The loose bulk density (LBD) gives an indication of the lowest reachable density without compacting, while, the packed bulk density (PBD) gives the highest reachable density with compaction (Ojo *et al*., 2015). The results of the bulk density of the flours (full fat, defatted) and protein isolates are shown in Tables 4.7-4.9. The result showed there was a rise in BD after flour was defatted, the obtained values ranged between 0.55 to 0.65 g/cm^3 for loose bulk density for the FFDAS variety while that of DFDAS ranged between 0.59 to 0.67 g/cm^3. The PBD for FFDAS was between 0.76 to 0.83 g/cm^3, whilst that of DFDAS ranged between 0.74 to 0.87g/cm^3. The FFBS variety had the LBD ranging between 0.44 - 0.64 g/cm^3; and that of DFBS was 0.54 to 0.67 g/cm^3. The packed density for FFBS was 0.59 to 0.88 g/cm^3 and that of DFBS was between 0.69 and 0.91 g/cm^3.There was a general initial increase in BD with germination for the samples, both full fat flours, defatted flours and the protein isolates. Except for FFDAS samples, which showed decrease at 24hrs and then an increase occurred from 36 h. 24 h, 48 h LBD of FFDAS; 24 hBD of both the DAS and BS protein isolates all showed lower or same values as the raw. The reduction observed goes along with previous literature report by Chinma *et al*. (2009) for two varieties of Tigernut flour; and Desalegn, (2015) for chickpea flour. This could be as a result of decrease in heaviness.

Table 1: Bulk Density Full fat (FFDAS) and Defatted Dark-ash (DFDAS) Solojo Cowpea Flour

FFDAS	Raw	6 h	24 h	36 h	48 h	72 h
LBD (g/cm^3)	0.60±0.02^{bc}	0.60±0.02^{bc}	0.55±0.03^{d}	0.63±0.02^{ab}	0.57±0.03^{cd}	0.65±0.03^{a}
PBD (g/cm^3)	0.76±0.01^{a}	0.81±0.03^{a}	0.83±0.02^{a}	0.79±0.02^{a}	0.79±0.04^{a}	0.79±0.03^{a}
LBD (g/cm^3)	0.59±0.03^{ab}	0.63±0.03^{ab}	0.59±0.02^{b}	0.67±0.04^{a}	0.63±0.03^{ab}	0.59±0.04^{b}
PBD (g/cm^3)	0.76±0.04^{c}	0.86±0.02^{a}	0.87±0.03^{a}	0.83±0.03^{ab}	0.80±0.02^{b}	0.74±0.03^{c}

Loose bulk density - LBD
Packed bulk density - PBD
Full fat dark ash Solojo Cowpea - FFDAS
Defatted dark ash Solojo Cowpea - DFDAS
Means in rows not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

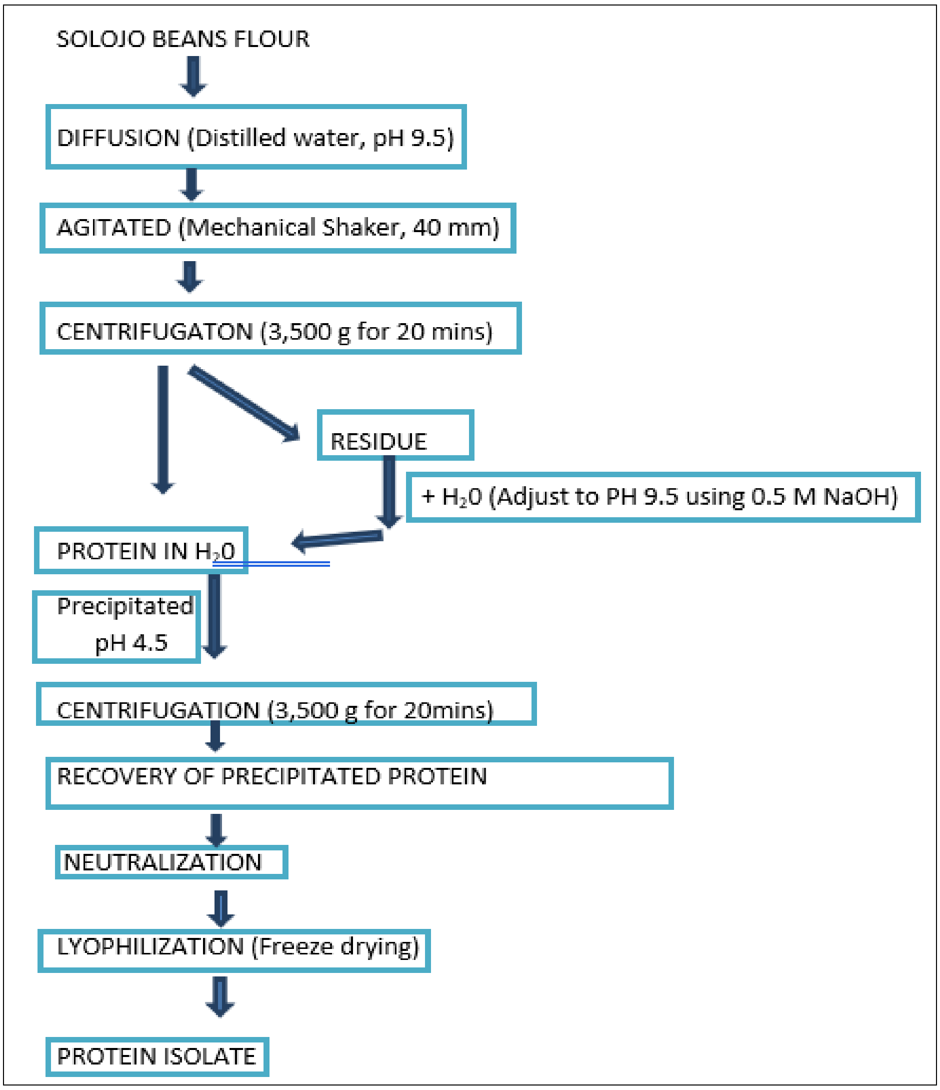

Fig 6: Schematic diagram for the processing of protein isolate (Adebowale *et al*., 2007modified)

The bulk density of all the other samples generally increased with germination time. This is in line with the report of Akaerue and Onwuka, (2010) for germinated Mung bean flour. This increase in BD is very good for increased ease of dispersion bringing about reduction in paste thickness, which is an important factor in production of food for recuperating children; it will also be good as thickner (Akaerue and Onwuka, 2010). Ragab *et al.* (2004) obtained 0.64 g/cm^3 for bulk density of cowpea flour. Lima beans had bulk density varying between 0.66 g/mL to 0.83 g/mL for different accessions of Lima beans. (Yellavila *et al.*, 2015) [24]; bulk density ranging from 0.42 to 0.61 g/cm3 for full fat and 0.72 to 0.88 g/cm3 for defatted flours was reported for Mucuna beans, which is in line with our finding that, defatting increases bulk density (Adebowale *et al* 2005) [2], likewise for taro (Colocasia esculenta), rice (Oryza sativa) flour, pigeonpea (Cajanus cajan) flour blends (Kaushal *et al*., 2012). Butt and Batool (2010) however, observed that defatting process reduced the bulk density which they attributed to porous texture of the flour. This they also deduced will be advantageous in compounding complementing foods. Other legume flours such as, peas and pigeon pea showed BD of 0.55 and 0.46 g/cm3 respectively. Similar report was obtained for chickpea and winged bean flours (Kaur and Singh 2007) [10]. Ojo *et al.* (2015) [17] obtained a value of 0.546 g/cm^3 and 0.627 g/cm^3 for both LBD and PBD of one species of Vigna subterranean. The various values obtained for the bulk densities of both the DAS and BS were between the range 0.66 g/mL to 0.83 g/mL, obtained by Yellavila *et al*. (2015) [24], for five Lima bean accessions and 0.536 to 0.971 g/mL reported by Kaur and Singh (2006) for Chickpea cultivars.

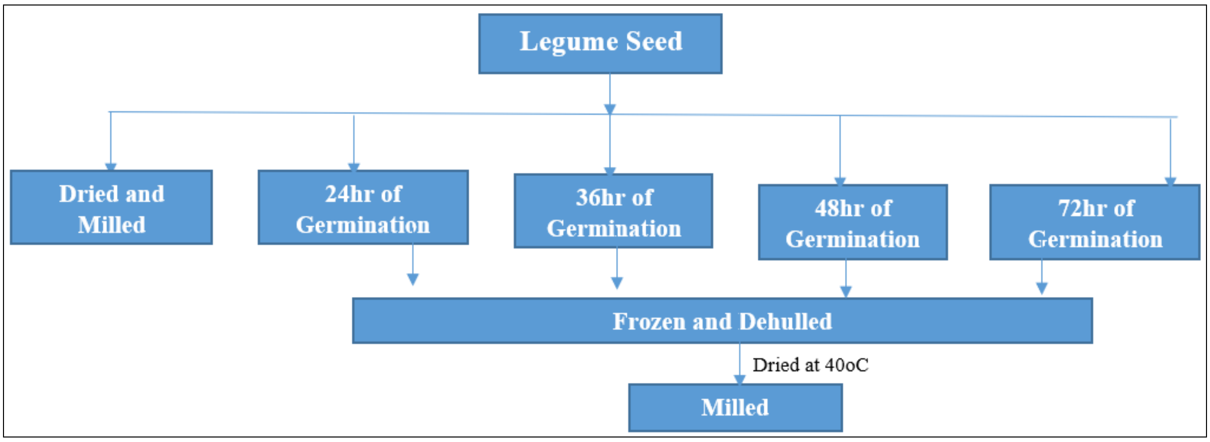

Fig 7: Preparation of Beans Flour/Schematic representation

Table 2: Bulk Density full fat and defatted brown solojo cowpea (FFBS) and (DFBS) flour

FFBS	Raw	6 h	24 h	36 h	48 h	72 h
LBD (g/cm^{3})	0.44± 0.01^{b}	0.59± 0.04^{a}	0.64± 0.05^{a}	0.63± 0.02^{a}	0.63± 0.05^{a}	0.64± 0.03^{a}
PBD (g/cm^{3})	0.59± 0.01^{c}	0.81± 0.02^{b}	0.83±0.04^{ab}	0.86± 0.02^{a}	0.86± 0.01^{a}	0.88± 0.03^{a}
LBD (g/cm^{3})	0.54± 0.02^{b}	0.65± 0.03^{a}	0.65± 0.02^{a}	0.65± 0.04^{a}	0.65± 0.03^{a}	0.67± 0.03^{a}
PBD (g/cm^{3})	0.69± 0.03^{b}	0.91± 0.02^{a}	0.87± 0.02^{a}	0.87± 0.03^{a}	0.87± 0.02^{a}	0.87± 0.04^{a}

Loose bulk density - LBD
Packed bulk density - PBD
Full fat dark ash Solojo Cowpea - FFBS
Defatted dark ash Solojo Cowpea - DFBS
Means in rows not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Factors affecting BD are, particle size, intensity of attractive interparticle forces and number of contact points (Suliman *et al*., 2006). High bulk density also indicates greater compactness ability of the particles, this is because, particle size and bulk density have an inverse relationship (Falade and Olugbuyi, 2010; Adediran *et al*., 2013) [7]. The high BD shows the flour will be very useful in food formulation for easier dispersion and reduced thickness of paste, which is very significant in the production of infant foods (Suliman *et al*., 2006). Flours with low bulk density are said to be desirable for the preparation of weaning foods because they give reduced/ low paste thickness and viscosity on reconstitution (Abass *et al*., 2009; Ojo *et al*., 2015) [17]. However, flours that have high bulk density, have been said to enhance fat absorption, even though this is not very good for weaning food, it is a very good property for flours used for baked and pastry products (Abass *et al*., 2009; Ojo *et al*., 2015) [17].

Table 3: Bulk Density Dark-ash (DAS) and Brown (BS) Solojo Cowpea protein isolate

DAS	Raw	6 h	24 h	36 h	48 h	72 h
LBD (g/cm^{3})	0.36±0.01^{c}	0.42± 0.01^{b}	0.50± 0.02^{a}	0.39± 0.02^{c}	0.43± 0.01^{b}	0.49± 0.02^{a}
PBD (g/cm^{3})	0.50± 0.02^{c}	0.55± 0.02^{b}	0.58± 0.02^{a}	0.47± 0.03^{c}	0.55± 0.02^{b}	0.59± 0.01^{a}
LBD (g/cm^{3})	0.38±0.01^{c}	0.40±0.02^{bc}	0.45±0.02^{a}	0.35±0.03^{d}	0.47±0.01^{a}	0.42±0.01^{b}
PBD (g/cm^{3})	0.50±0.02^{d}	0.55±0.02^{bc}	0.56±0.01^{b}	0.45±0.02^{e}	0.59±0.01^{a}	0.53±0.01^{c}

Loose bulk density - LBD
Packed bulk density - PBD
Dark ash Solojo Isolate - DAS
Brown Solojo Isolate - BS
Means in rows not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

The isolates had the bulk density varying between 0.36 to 0.50 g/cm3 for the LBD of DAS and 0.47 to 0.59 g/cm3 for PBD; while the BS isolate had for LBD 0.35 to 0.47 g/cm3 and 0.45 to 0.59 g/cm3 for PBD. Butt and Batool, (2010) had values of 0.71, 0.68, 0.55 and 0.53 g/cm3 for cowpea, pea, mungbean and pigeon pea protein isolates respectively. Sibt-e-Abbas *et al*. (2015) [16] obtained for two varieties of peanut, Golden 0.51±0.01 g/cm3 and Bari 0.54±0.01 g/cm3 which are comparable to our result. When compared with enzymatic hydrolysis, Wani *et al*. (2015) observed a decreased in BD with enzymatic hydrolysis using Indian black gram cultivars (Phaseolus mungo L.) with value range between 0.21- 0.17 g/mL. Rajesh and Prakash (2008) also reported that bulk density of albumin fraction of lentil went down from 0.94 g/mL to 0.88 g/mL on hydrolysis. Their results were higher than that reported in this study.

Conclusion

In food formulation, the high bulk density of germinated flours and protein isolate, shows that the flour and protein isolate will be very useful for infant food and geriatric food formulation for this will allow for higher ease of dispersion and also reduce paste thickness, which is a very important attribute in this class of food product. Better protein solubility at higher and lower pH was also observed with germination, this is important because protein solubility is a useful guide for the conduct of protein in the food system. Solubility of a protein is one of the crucial functional properties needed by the food industry, because it greatly affects other properties such as emulsification, gelation and foaming, indicating that Solojo germinated flour and protein isolate can be utilised in various food type. Water absorption capacity is another important functional attribute in foods, such as sausages, custards and doughs, germination brought about the improvement in water absorption capacity of the flour and protein isolate. The addition of a pinch of salt brought about greater protein solubility and therefore increased the desired water absorption properties for food formulation. The increase in OAC with germination means that the flavour retention and mouth feel of foods will be greatly enhanced if used in food formulation.

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