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EFFECT OF IONIC STRENGTH ON EMULSION ACTIVITY (EA) OF ISOLATES FROM TWO VARIETIES (DAS & BS) OF NIGERIAN CULTIVATED SOLOJO COWPEA (VIGNA UNGUICULATA L. WALP)

Olubamike Adetutu ADEYOJU¹, Henry Okwudili CHIBUDIKE², Kayode Oyebode ADEBOWALE³, Bamidele Iromidayo OLU-OWOLABI⁴, and Chinedum Eunice CHIBUDIKE⁵

¹Production, Analytical and Laboratory Management, Federal Institute of Industrial Research, Oshodi, F.I.I.R.O., Lagos-Nigeria

²Chemical, Fiber and Environmental Technology Department, Federal Institute of Industrial Research, Oshodi, Lagos-Nigeria

³Department of Chemistry, Indistrial Unit, University of Ibadan, Ibadan-Nigeria ⁴Department of Chemistry, Analytical Unit, University of Ibadan, Ibadan-Nigeria ⁵Planning, Technology Transfer and Information Management, Federal Institute of Industrial Research, Oshodi, F.I.I.R.O., Lagos-Nig.

KEYWORDS: Concentration Effect on activity of emulsion (EA) and stability of emulsion (ES) of the various samples was investigated. Emulsifying Activity (EA) of raw (native/ control) and germinated Dark-ash Solojo Cowpea (FFDAS, DFDAS, FFBS and DFBS flours; DAS and BS protein isolate) were all concentration dependent. The 6h DFDAS flour and raw DAS isolate both had the emulsifying activity increasing up to 2% w/v before initial fall of activity with increase in concentration. FFDAS Raw, 24 h, 48 h and 72 h; DFDAS 24 h, 48 h, 72 h; FFBS 6 h, 24 h, 48 h, 72 h; DFBS 6 h; 24 h; DAS 48 h; BS 36 h, 48 h and 72 h all had their EA increasing with rise in concentration up to 4%w/v; while FFDAS 36 h; DFDAS Raw, 36 h; FFBS Raw, 36 h; DFBS 36 h, 48 h, 72 h; DAS 6 h, 36 h; BS Raw, 6 h and 24 h germinated flour and protein isolates went up to 6% w/v before additional rise in concentration brought about a decrease in value. Only DAS 24 h germinated protein isolate gave a rise of up to 8% w/v before decline in emulsifying activity took place. The values of the emulsifying activity ranged between 42.45±2.54 and 49.30±0.96%; 43.48±4.35 and 47.68±0.37%; 43.75±0.55 and 60.57±2.10%; 44.37±1.57 and 47.74±0.36%; 41.36±3.66 and 78.26±4.35%; 42.22±2.41 and 67.26±1.96%, for FFDAS, DFDAS, FFBS, DFBS, DAS and BS respectively. The increase in EA with germination shows increased solubility. Germination caused the exposure of the functional groups inside the protein matrix due to the unfolding of the protein matrix and this brought about increased synergy at the protein oil interface. Protein being a surfaceactive agent is capable of quickly moving to the oil – water point of linking where it is adsorbed to create a shielding membrane via intermolecular interaction. The solubility of the protein in the aqueous phase makes this possible. Once protein quantity is small, protein adhesion at the oil-water border is by moving from area of high concentration to low concentration (Diffusion controlled). Decrease in emulsifying activity was observed among DFBS Raw, DAS 72 h, and FFDAS 6 h up to 2%, 4% and 8% respectively. Germination was observed to improve EA of the Solojo flours and isolates. Varietal difference was also observed in the effect of germination on EA, FFBS was found to have a higher EA than FFDAS. The isolate had the DAS having a better EA than the BS. This

could be due to the degree of uncoiling of the protein molecule and also the quantity of hydrophilic and non-polar constituent on the interface of the molecule. The foaming capacity (FC) for FFDAS increased with germination except at lower concentration of 2-4%. That of DFDAS showed a better response. The FC was higher than that of the FFDAS, this could be as a result of the exposure of more hydrophilic sights as a result of defatting. The DFBS likewise exhibited a higher FC compared to that of FFBS. The FC of the flours of brown solojo was found to be higher than that of the dark-ash solojo cowpea. The high foaming capacity (FC) at highly acidic and alkaline pH was probably as a result of the rise in total charge on the protein, bringing about the lowering of the nonpolar inter- actions but increasing protein pliability, making the protein to disperse speedily to the junction between air and water enclosing particles of air thereby encouraging formation of foam. This observation proved the authenticity that foaming property depends on protein solubility.

KEYWORDS: Nutritional composition, Solojo Cowpea, Under-utilized legumes, essential amino acids, Nutraceuticals, food industry

INTRODUCTION

Cowpea (Vigna unguiculata) is among the pulse's species of greatest economic and social importance. This legume is strategic for the food security and health of millions of people in the world. Cowpea is rich in nutraceuticals compounds such as dietary fibre, antioxidants and polyunsaturated fatty acids and polyphenols, whose health benefits and use in the food industry have been extensively studied. However, research on the identification of functional proteins from cowpea, their metabolic functions and applications in the food, health and other industries are still scarce. Cowpeas are classified into five cultivar groups: biflora, melanophthalmus, sesquipedalis, textilis and unguiculata (Pasquet, 2000). Among the cultivated crop plants, the cowpea is one of the most variable species in terms of its plant growth, morphology, maturity and grain1 types (Singh, 2014). The cowpea has a long taproot and adaptation mechanisms such as turning the leaves upwards to prevent them from becoming too hot and closing the stomata that help give it drought tolerance. As a legume crop, the cowpea fixes atmospheric nitrogen through symbiotic interactions with soil rhizobia (Sarr, Fujimoto and Yamakawa, 2015).

Processing of cowpeas and legumes, in general, is essential to make them nutritious, nontoxic, palatable and acceptable. The cowpea is utilised either whole or decorticated or dehulled. It is decorticated by soaking in water (at room temperature) for about 30-60 min, and the seed coat removed by squeezing between the palms or by gentle abrasion using grinding stones. The seed coat is separated by subsequent filtration (Adebooye and Singh, 2007). The constraints to maximum utilisation of cowpeas can be overcome by appropriate processing technology. For example, these techniques include dehulling, grinding, soaking, germination, fermentation, addition of salts, wet and dry heat treatments, cooking and roasting (Uzogara and Ofuya, 1992; Adebooye and Singh, 2007). Irradiation by gamma rays can also be used to sterilise cowpea flours and pastes but high levels of irradiation can reduce food quality (Abu et al., 2005).

Cowpeas are a source of essential minerals, calcium, magnesium, potassium, iron, zinc and phosphorus (Tables 3.8 and 3.9). Low availability of soil phosphorus is a primary constraint to cowpea production in developing countries

(Burridge et al., 2016). Levels of grain phosphorous, potassium and manganese vary widely due to environmental conditions (Adebooye and Singh, 2007). Most minerals are at higher concentrations in leaves (Gerrano et al., 2015a) and immature green pods (Gerrano, Jansen van Rensburg and Adebola, 2017b) compared to grain (Belane and Dakora, 2012; Madode et al., 2011).

Cowpeas are a source of thiamin and niacin, and also contain reasonable amounts of other water-soluble vitamins such as riboflavin. Vegetative tissues including germinated grain tend to have higher levels of niacin, thiamin and riboflavin than grain (Nnanna and Phillips, 1989; Goncalves et al., 2016). Seed coat removal results in up to 30% loss in niacin content, while thiamin is reduced 41% by cooking (Nnanna and Phillips, 1989). Vitamin C values are higher in leaves than grains and increased by 4 to 38-fold after grains sprout (Devi, Kushwaha and Kumar, 2015; Goncalves et al., 2016). Cooking in an alkaline solution containing "kanwa" (naturally-occurring rock-salt) decreases thiamin, niacin and riboflavin levels compared to cooking without "kanwa" (Uzogara, Morton and Daniel, 1991). Fermentation results in a significant increase in the levels of thiamin and niacin (Akinyele and Akinlosotu, 1991).

However, studies on the anticancer and anti-inflammatory properties of cowpea have produced conflicting results. Some studies support a protective effect of cowpea on the progression of cancer and inflammation, while other studies demonstrate no effect. Since there are only a few studies carried on this regard, further studies in this area are suggested. In addition, despite the so far reported favorable effects of cowpea on diabetes, hyperlipidemia and hypertension, a long-term epidemiological study investigating the association between cowpea consumption and diabetes, cardiovascular disease and cancer is also recommended. In this study, a critical review of the most recent and important research about functional cowpea proteins was carried out.

The objective was to identify and systematize information about the nature and functions of these proteins, as well as their ionic strength effect on WAC, foaming capacity, pH and foaming stability of both the full and defatted Dark–ash and Brown Solojo cowpea.

Experimental

Materials and Methods

Two varieties of the underutilized cowpea (V. unguculata) found in South west region of Nigeria where it is called 'Solojo' were used. 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 sterilised by soaking in 0.07 % Sodium hypochlorite [29] for 30 min, then, it was 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 [30] for, 24, 36, 48 and 72 h.

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 μ m mesh screen. The resulting flour was packaged in plastic pack and preserved in a fridge- freezer at 4°C pending utilization.

Germination of seed: This was implemented by the method of Mubarak A.E. 2005 with minor adjustment. The seeds for germination were disinfected by soaking in 0.07 % Sodium hypochlorite Rumiyati, A.P. and James V. J. 2012 for 30 mins, then, it was rinsed painstakingly. The Solojo seeds were then immersed for 6 hours at ambient temperature in water in the ratio (1:10 w/v) (seed/water) (~25°C), then placed in a colander and germinated under subdued light in an open laboratory Rusydi, M. R., Noraliza, C. W., Azrina, A. and Zulkhairi, A. 2011 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 40°C for 48 h,

cooled, milled and packaged in an air tight plastic bag in the refrigerator pending analysis.



Figure 1: Brown Solojo Cowpea



Figure 2: Dark-Ash Solojo Cowpea

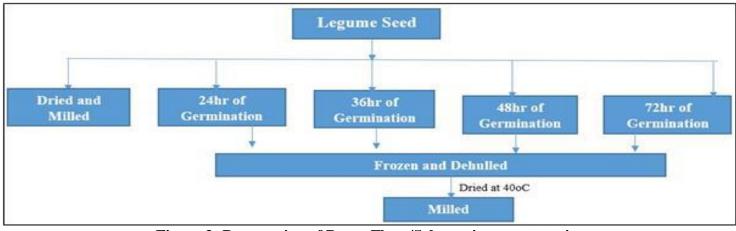


Figure 3: Preparation of Beans Flour/Schematic representation

RESULT AND DISCUSSION

Effect of Ionic Strength on Emulsifying activity (EA) and stability (ES) Addition of NaCl brought about initial increase of EA and ES to various degree of ionic strength. Effect of ionic strength on EA and ES is presented in Tables 1 - 12 for FFDAS, DFDAS, FFBS, DFBS flours; DAS and BS isolate. Emulsifying activity (EA) generally rose for all the flour samples as the ionic strength is rising but each had a maximum. FFDAS, DFDAS, FFBS and DFBS flour

samples all had the Emulsifying activity for all the samples increasing to between 0.2 M and 0.6 M before further increase brought about decrease in emulsifying activity. The EA of the isolates also increased and decreased within 0.2 M and 0.6 M concentration of the added salt. The commercial protein isolate also had its EA decrease with rise in common-salt quantity up to 0.8 M. This was comparable to the findings of Lawal et al. (2005) for the Parkia biglobosa

albumin, globulin, defatted and undefatted flours. It was reported that EA rose with rise in NaCl quantity up to 0.4, 0.6 M, 0.2 M, respectively before further increase led to a decrease in EA value (50.4 ± 2.5 - $64.2\pm0.9\%$; 53.2 ± 1.8 – $71.8\pm1.0\%$; 30.4 ± 1.0 – $41.2\pm1.0\%$; 20.4 ± 0.6 – $33.7\pm1.8\%$ respectively). Ahmed et al. (2012) likewise observed a related trend for the result of ionic strength on the EA of Vigna unguiculata flour.

	Table 1: Effect of Ionic Strength on Emulsifying Activity (EA) of FFDAS									
FFDAS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M				
Raw	43.07± 2.28°	$44.07 \pm 3.50^{\circ}$	$45.30\pm0.26^{\circ}$	43.94± 2.62°	44.14±1.14°	$43.28\pm2.28^{\circ}$				
6 h	45.42 ± 0.36^{b}	47.26±0.37 ^a	46.03±0.93 ^b	44.31±0.35°	43.92±0.35°	43.65±0.35°				
24 h	45.63±0.35 ^b	45.78 ± 1.05^{b}	47.39±0.35 ^a	44.98±0.35 ^b	45.63±0.35 ^b	47.22±0.35 ^a				
36 h	46.18±0.35 ^{ab}	46.18±0.35 ^{ab}	46.75±0.35 ^a	45.38±0.95 ^{bc}	44.44±0.35°	44.44±0.35°				
48 h	45.63±0.35 ^{bc}	45.63±0.35 ^{bc}	46.18±0.35 ^{ab}	46.59±0.35 ^a	45.10±0.34°	45.10±0.34°				
72 h	46.18±0.35 ^{bc}	46.83±0.35 ^{ab}	47.39±0.35 ^a	45.63±0.35°	45.35±1.01°	46.27 ± 0.34^{bc}				

FFDAS- Full fat dark- ash Solojo cowpea Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

The EA ranged between $43.07 \pm 2.28 - 47.39 \pm 0.35\%$; $43.47 \pm 1.24 - 47.69 \pm 1.28\%$; $43.21 \pm 0.53 - 47.69 \pm 0.74\%$; $42.87 \pm 4.47 - 49.16 \pm 0.97\%$ for FFDAS, DFDAS, FFBS and DFBS. The obtained values of the FFDAS; DFDAS; FFBS and DFBS all fell within the region of the flour and the albumin of African locust bean. The DAS and BS isolate ranged between $43.72\pm4.99 - 54.67\pm2.25\%$; $44.68\pm1.84 - 57.87\pm1.66\%$ respectively. Commercial isolate $43.27\pm0.36 - 47.76\pm2.27\%$.

Table 2: Effect of Ionic Strength on Emulsifying Activity (EA) of DFDAS

DFDAS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
DFDAS	0.1M	0.2M	0.4141	0.01/1	0.814	1.014
Raw	43.94±2.62 ^b	$45.71{\pm}2.18^{b}$	44.07±3.50 ^b	46.31±3.34 ^{ab}	43.62±5.58 ^C	46.44±3.30 ^a
6 h	44.77±0.29 ^b	$45.80{\pm}0.94^{ab}$	46.41±0.99 ^a	44.73±0.37 ^b	44.73 ± 0.37^{b}	$45.80{\pm}0.94^{ab}$
24 h	44.58±0.37 ^b	45.80 ± 0.94 ^b	47.67±0.98ª	45.99±0.37 ^b	$45.34{\pm}0.95^{b}$	$45.15 \pm 0.37 ^{b}$
36 h	44.68±1.00 ^b	44.74 ± 1.26^{b}	$46.67{\pm}0.36^{ab}$	47.69±1.28 ^a	$45.34{\pm}0.95^{b}$	45.16±1.25 ^b
48 h	43.47±1.24 ^c	44.73 ± 0.92^{bc}	47.01 ± 0.96 ^a	46.70±0.93 ^a	$46.03{\pm}0.94^{ab}$	$45.34{\pm}0.95^{ab}$
72 h	43.88±0.37 ^b	45.99±0.37 ^a	47.01±0.96 ^a	46.13±0.92 ^a	46.89±1.05 ^a	47.09±1.26 ^a

DFDAS- Full fat dark- ash Solojo cowpea Germ- Soaked and germinated Means in column not followed by same alphabet(s) are significantly different at 5% level (P<0.05

The ES of the flour samples, FFDAS, DFDAS, FFBS and DFBS generally showed an increase in ES with rise in NaCl quantity between 0.2 M and 0.6 M except for FFDAS, Raw flour which showed a decrease as ionic strength increased. The ES of protein isolate from DAS (Raw, 24 h and 48 h) and BS (24 h-72 h) increased with rise in NaCl concentration

at the same time, the ES of same samples at other germination period decreased. The commercial protein isolate also had its ES increase with ionic strength up to 0.8 M. The initial increase in EA and ES with imput of NaCl may be due to improved dissolution which was brought about by unfolding of the molecules, which promoted easy migration of the protein to the oil- water intersection

thereby causing a growth in the EA of the protein. With increase in the ionic strength, there is increase in surface charge screening, which brought about protein with protein inter-action instead of protein with oil inter-action, thereby bringing about reduction in emulsifying activity and emulsion stability at high salt concentration (Salam et al., 2010).

The ES values ranged between $42.46 \pm 0.36 - 49.02 \pm 0.34\%$; $43.70 \pm 0.63 - 51.38 \pm 5.58\%$. $43.75 \pm 0.55 - 48.11 \pm 0.55$ 0.61%; $43.75 \pm 0.56 - 48.74 \pm 0.63\%$; $42.20 \pm 1.28 - 54.05 \pm 1.58\%$; $42.43 \pm 1.95 - 58.84 \pm 2.88\%$, for FFDAS, DFDAS, FFBS, DFBS, DAS and BS respectively. The commercial isolate $46.74 \pm 1.91 - 49.58 \pm 4.81$

Table 3: Effect of Ionic Strength on Emulsifying Activities (EA) of FFBS cowpea									
FFBS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M			
Raw	44.14 ± 1.14^{a}	44.93±2.51 ^a	44.86±3.56 ^a	45.59±2.18 ^{ab}	45.71±2.18 ^a	46.38±2.51 ^a			
6 h	$45.00{\pm}~0.56^{a}$	$45.23{\pm}0.92^a$	46.05 ± 0.75^{a}	45.00 ± 0.56^{ab}	44.96± 0.63 ^a	44.11 ± 1.85^{a}			
24 h	$45.42{\pm}0.36^a$	$45.57{\pm}0.58^a$	$\begin{array}{c} 46.25 \pm \\ 0.58^a \end{array}$	$43.21{\pm}0.53^{b}$	$\begin{array}{c} 45.00 \pm \\ 0.56^a \end{array}$	$46.25{\pm}0.58^a$			
36 h	45.67 ± 1.66^{a}	$46.25{\pm}0.58^a$	$\begin{array}{c} 46.67 \pm \\ 0.36^{a} \end{array}$	$47.11{\pm}0.34^a$	$45.0{\pm}0.56^a$	44.44 ± 0.55^{a}			
48 h	$45.42{\pm}0.36^a$	46.25±0.58 ^a	$\begin{array}{c} 46.25 \pm \\ 0.58^{a} \end{array}$	46.69± 1.59 ^a	45.00± 0.56 ^a	$45.00{\pm}0.56^a$			
72 h	45.83±0.98 ^a	46.66± 1.34 ^a	47.69 ± 0.74^{a}	45.67± 1.66 ^{ab}	45.00 ± 0.56^{a}	$45.00{\pm}0.56^a$			

FFBS- Full fat brown Solojo cowpea flour

Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Initial increase in EA and ES with an increased ionic strength was also reported by Adebowale and Lawal (2004) where they reported that raising the ionic concentration to 0.2 M brought about increase in EA and ES of the protein concentrate of African locust bean. Salam et al. (2010), likewise noticed a great enhancement in the EA of both native and transglutaminase (TGase) treated cowpea with addition of NaCl to 0.4 M after which additional increase in salt concentration brought about a decrease in emulsifying activity.

Table 4: Effect of Ionic Strength on Emulsifying Activity (EA) of DFBS cowpea

DFBS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	$42.87{\pm}4.47^{a}$	$46.38{\pm}2.51^{ab}$	$46.97{\pm}8.37^{a}$	44.32± 3.17°	46.38± 2.51ª	47.04 ± 1.37^{a}
6 h	$46.03{\pm}~0.70^{a}$	$48.52{\pm}0.37^a$	$46.63{\pm}0.75^{a}$	$45.56{\pm}0.69^{ab}$	$45.23{\pm}0.92^a$	$44.73{\pm}~0.37^{b}$
24 h	$45.57{\pm}0.58^{\rm a}$	$46.84{\pm}0.59^{ab}$	$46.86{\pm}0.63^a$	$47.32{\pm}0.36^{ab}$	$45.38{\pm}~1.31^{a}$	$47.08{\pm}~0.36^{a}$
36 h	$45.84{\pm}1.55^{\mathrm{a}}$	$46.83{\pm}0.67^{ab}$	$47.92{\pm}0.36^{a}$	$49.16{\pm}0.97^{a}$	$46.67{\pm}0.36^{a}$	$46.03{\pm}~0.70^{ab}$
48 h	$45.34{\pm}0.95^a$	$45.80{\pm}~0.94^{b}$	$47.09{\pm}~1.26^{a}$	$46.41{\pm}0.99^{abc}$	$45.42{\pm}0.36^a$	$46.41{\pm}~0.99^{ab}$
72 h	$44.77{\pm}0.93^a$	$46.41{\pm}0.99^{ab}$	$48.75{\pm}~1.08^{\rm a}$	46.84± 1.10 ^{abc}	$44.77{\pm}0.93^a$	46.02 ± 1.31^{ab}

DFBS- Defatted brown Solojo cowpea flour Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Lawal and Dawodu (2007) also reported a rise in EA and ES of equal to 0.4M NaCl for both native and maleic anhydride and acylated Parkia biglobosa protein isolate. Related result was observed for soya bean, with gradual increase in ES with rise in salt concentration with highest value of 89.7% at 5% NaCl inclusion. Arogundade et al. (2009) also observed a notable increment of EA of up to 0.8 mol/dm3 for cashew nut protein isolate. High ES of soy protein at reduced salt concentration has been connected to break-down of oligomeric structure of 11S-glycinin and consequent betterment of surface behavior (Lawal and Dawodu 2007).

Table 5: Effect of ionic strength on Emulsifying Activity (EA) of DAS and Soy Protein Isolate

Sample	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	47.04±1.37 ^{bc}	44.73±3.51 ^a	43.72±4.99°	44.93±2.51 ^b	46.25±1.37 ^{ab}	46.44±3.30 ^b
6 h	45.60±2.89°	47.79 ± 4.03^{a}	44.85±0.70°	47.04 ± 1.37^{b}	47.39 ± 2.79^{ab}	47.44 ± 0.67^{b}
24 h	49.97 ± 2.22^{b}	48.95±4.93ª	46.38 ± 1.26^{bc}	47.37 ± 2.17^{b}	47.83 ± 2.17^{ab}	48.86±3.00 ^{ab}
36 h	48.53 ± 2.72^{bc}	49.63±0.64 ^a	50.00 ± 2.17^{ab}	51.45 ± 3.32^{a}	50.72±2.51 ^a	$48.16{\pm}1.69^{ab}$
48 h	46.31±1.32 ^a	47.10±1.26 ^{bc}	47.86±2.17 ^{ab}	$54.67{\pm}2.25^a$	$48.55{\pm}1.26^a$	$49.28{\pm}1.26^{ab}$
72 h	48.52 ± 1.28^{bc}	49.63±0.64 ^a	$53.27{\pm}2.25^{a}$	$46.44{\pm}1.21^{b}$	48.02 ± 4.32^{a}	52.85 ± 4.63^{a}
soy isolate	47.76±2.27 ^{bc}	46.24±2.39 ^a	44.80±1.14 ^c	43.93±1.36 ^b	43.27 ± 0.36^{b}	44.93±2.51 ^b

DAS- Dark-ash Solojo cowpea protein isolate Soy Isolate- Commercial Soy protein isolate Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

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0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
55.94±3.73ª	56.06±3.65 ^a	57.50±0.41ª	56.26±4.13 ^a	54.76±2.55ª	53.66±1.55ª
49.61±3.78°	48.21±0.59 ^b	44.68±1.84°	46.82±1.00 ^b	47.42±2.80 ^b	51.05±1.04 ^{ab}
54.75±0.70 ^{ab}	56.94±0.73ª	57.87±1.66ª	54.60±2.66ª	51.48±4.49 ^{ab}	48.23±3.71 ^b
50.35 ± 1.67^{bc}	46.46±1.11 ^b	47.13±1.28°	47.50 ± 2.20^{b}	47.52±1.23 ^b	48.20±0.64 ^b
46.13±1.52 ^b	47.16±1.15°	47.49 ± 1.66^{b}	48.94±1.84°	48.90±0.03 ^b	48.98±3.17 ^b
51.42±1.23 ^{abc}	53.22±2.83ª	53.64±2.27 ^b	55.14±1.20 ^a	51.45±1.26 ^{ab}	48.55±1.26 ^b
	55.94 ± 3.73^{a} 49.61 ± 3.78^{c} 54.75 ± 0.70^{ab} 50.35 ± 1.67^{bc} 46.13 ± 1.52^{b}	55.94 ± 3.73^{a} 56.06 ± 3.65^{a} 49.61 ± 3.78^{c} 48.21 ± 0.59^{b} 54.75 ± 0.70^{ab} 56.94 ± 0.73^{a} 50.35 ± 1.67^{bc} 46.46 ± 1.11^{b} 46.13 ± 1.52^{b} 47.16 ± 1.15^{c}	55.94 ± 3.73^{a} 56.06 ± 3.65^{a} 57.50 ± 0.41^{a} 49.61 ± 3.78^{c} 48.21 ± 0.59^{b} 44.68 ± 1.84^{c} 54.75 ± 0.70^{ab} 56.94 ± 0.73^{a} 57.87 ± 1.66^{a} 50.35 ± 1.67^{bc} 46.46 ± 1.11^{b} 47.13 ± 1.28^{c} 46.13 ± 1.52^{b} 47.16 ± 1.15^{c} 47.49 ± 1.66^{b}	55.94 ± 3.73^{a} 56.06 ± 3.65^{a} 57.50 ± 0.41^{a} 56.26 ± 4.13^{a} 49.61 ± 3.78^{c} 48.21 ± 0.59^{b} 44.68 ± 1.84^{c} 46.82 ± 1.00^{b} 54.75 ± 0.70^{ab} 56.94 ± 0.73^{a} 57.87 ± 1.66^{a} 54.60 ± 2.66^{a} 50.35 ± 1.67^{bc} 46.46 ± 1.11^{b} 47.13 ± 1.28^{c} 47.50 ± 2.20^{b} 46.13 ± 1.52^{b} 47.16 ± 1.15^{c} 47.49 ± 1.66^{b} 48.94 ± 1.84^{c}	55.94 ± 3.73^{a} 56.06 ± 3.65^{a} 57.50 ± 0.41^{a} 56.26 ± 4.13^{a} 54.76 ± 2.55^{a} 49.61 ± 3.78^{c} 48.21 ± 0.59^{b} 44.68 ± 1.84^{c} 46.82 ± 1.00^{b} 47.42 ± 2.80^{b} 54.75 ± 0.70^{ab} 56.94 ± 0.73^{a} 57.87 ± 1.66^{a} 54.60 ± 2.66^{a} 51.48 ± 4.49^{ab} 50.35 ± 1.67^{bc} 46.46 ± 1.11^{b} 47.13 ± 1.28^{c} 47.50 ± 2.20^{b} 47.52 ± 1.23^{b} 46.13 ± 1.52^{b} 47.16 ± 1.15^{c} 47.49 ± 1.66^{b} 48.94 ± 1.84^{c} 48.90 ± 0.03^{b}

BS- Brown solojo cowpea protein isolate Means in column not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Contrary-wise, Aremu et al. (2008) observed a general reduction in emulsification property. They reported that there was general decrease in emulsion capacity of seed flours as NaCl concentration increases in all the samples investigated by them. Ahmed et al. (2012) also detected substantial drop in ES of some chosen legume flours such as Navy bean, Cajanus cajan, Vigna unguiculata and Lablab purpureus, with addition of salt. According to Berhanu and Amare (2013) for defatted Brebra seed (Millettia ferruginea) seed flour and

defatted Soybean flour, emulsion stability decreased with increase in ionic strength for Brebra seed flour, with zero ionic strength being 84.6% and 0.5% NaCl inclusion having a value of 78% and the value decreased further with rise in salt concentration.

Emulsion property of protein could be said to depend on two factors; A considerable reduction in surface energy as a electronic charges on the protein surface, structure and mechanical energy barrier produced by the surface layer that opposes destructive process (Adebowale and Lawal, 2004.

FFDAS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	$46.97{\pm}2.62^{b}$	45.59±2.18 ^{cd}	44.14 ± 1.14^{d}	45.45±4.55 ^{cd}	46.16±3.41ª	47.69±2.27 ^a
6 h	44.84±0.35 ^d	46.83±0.35 ^b	48.63±0.34ª	46.83±0.35 ^b	46.04 ± 0.89^{bc}	45.20±0.60 ^{cd}
24 h	46.83±0.35 ^b	47.22±0.35 ^b	49.02±0.34ª	46.83±0.35 ^b	46.03±0.35°	$44.84{\pm}0.35^{d}$
36 h	45.63±0.35ª	46.03±0.35 ^a	45.24±1.03 ^a	43.25 ± 0.35^{b}	42.46 ± 0.36^{b}	42.80±0.60 ^b
48 h	46.42±0.64 ^a	47.23±0.89ª	44.44 ± 0.35^{b}	43.91±1.29bc	43.14±0.35	42.74±0.35°
72 h	45.63±0.35°	46.66±0.92 ^b	48.63±0.34ª	46.83±0.35 ^b	45.63±0.35°	$43.14{\pm}0.35^{d}$

Table 7: Effect of Ionic Strength on Emulsion Stability (ES) of FFDAS

FFDAS- Full fat dark- ash Solojo cowpea Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

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DFDA	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
S						
Raw	45.59±2.1 8 ^a	46.44±3.3 0 ^a	45.59±2.1 8 ^a	50.00±2.3 8 ^a	51.38±5.58ª	47.04±1.37 ^a
6 h	44.30±1.1 0 ^c	45.73±0.3 7 ^{ab}	46.67±0.3 6 ^a	44.73±0.3 7 ^{bc}	44.73±0.37 ^{bc}	44.44±0.38 ^c
24 h	43.94±1.5 4 ^c	45.42±0.3 6 ^{bc}	47.92±0.3 6 ^a	45.73±0.3 7 ^b	45.53±0.64 ^b	45.34±0.95 ^{bc}
36 h	43.70 ± 0.6 3 ^d	45.39±1.5 5 ^{cd}	$47.72{\pm}0.9$ 4^{ab}	48.96±0.3 6 ^a	45.95±1.00 ^{bc}	44.30±1.10 ^{cd}
48 h	44.02±0.3 8 ^c	44.81±1.2 9°	$47.28{\pm}0.9$ 4^{ab}	48.54±0.9 6 ^a	46.86±0.63 ^b	45.34±0.40 ^c
72 h	44.17±0.3 7 ^c	46.86±0.6 3 ^{ab}	47.90±1.3 1 ^ª	48.32±0.9 5 ^a	47.09±1.26 ^{ab}	45.83±3.700.37 ^b

Table 8: Effect of Ionic Strength on Emulsion Stability (ES) of DFDAS

DFDAS- Full fat dark- ash Solojo cowpea

Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Ionic strength at lower concentration improved protein dissolution which facilitated a quick movement to the oil-water surface of the protein, this enhanced EA and ES; with further rise in salt concentration, charge shielding took place on the protein molecules, a process that encourages protein with protein cooperation but reduced protein dissolution, decline in protein solvation and decreased EA and ES.

Germination generally improved EA with the addition of salt, this could be because of better protein uncoiling which encouraged better reaction between the protein and the oil, but decrease in ES was brought about by the addition of salt.

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Table 9: Effect of Ionic Strength on Emulsion Stability (ES) of FFBS cowpea

FFBS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	$45.59{\pm}\ 2.18^{ab}$	$47.04{\pm}~1.37^{ab}$	$46.25{\pm}1.37^{a}$	45.71 ± 2.18^{a}	45.58 ± 0.22^{a}	44.73± 3.51 ^a
6 h	$45.82{\pm}~1.36^{ab}$	$47.08{\pm}0.36^{ab}$	$46.02{\pm}0.40^{a}$	$45.57{\pm}0.58^{a}$	$46.25{\pm}0.58^a$	45.57 ± 0.58^{a}
24 h	$43.75{\pm}0.55^{\text{b}}$	$45.00{\pm}~0.56^{\circ}$	$46.16{\pm}0.59^{a}$	$44.58{\pm}~0.99^{\rm a}$	43.75±0.55 ^b	45.00 ± 0.56^{a}
36 h	$46.25{\pm}0.58^{a}$	48.11±0.61ª	45.00±0.56 ^{ab}	45.00 ± 0.56^{a}	45.83 ± 0.98^{a}	45.00 ± 0.56^{a}
48 h	$45.57{\pm}0.58^{ab}$	$46.25{\pm}0.58^{bc}$	43.75 ± 0.55^{b}	45.00±0.56 ^a	46.05±0.75 ^a	45.00±0.56ª
72 h	45.00±0.56 ^{ab}	46.84±0.59 ^{ab}	44.31±0.56 ^b	45.00±0.56ª	45.00±0.56ª	45.68±0.56ª

Table 10: Effect of Ionic Strength on Emulsion Stability (ES) of DFBS cowpea

DFBS	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	$45.59{\pm}\ 2.18^{a}$	$46.97{\pm}2.62^a$	45.71± 2.18°	46.38 ± 2.51^{a}	$46.97{\pm}\ 2.62^a$	$45.59{\pm}\ 2.18^{a}$
6 h	44.58 ± 0.37^{a}	46.86±1.65 ^a	45.80±0.70°	44.73±0.37 ^a	$45.42{\pm}0.36^a$	46.00±0.93ª
24 h	45.23±0.92ª	46.00 ± 0.93^{a}	$48.74{\pm}0.63^{a}$	$45.23{\pm}0.68^{a}$	$46.67{\pm}0.36^a$	46.67 ± 0.36^{a}
36 h	46.41±0.37 ^a	$46.50{\pm}0.36^a$	$47.92{\pm}0.36^{ab}$	$45.83{\pm}0.37^{a}$	$45.42{\pm}0.36^a$	$45.43{\pm}1.25^{a}$
48 h	44.58±0.37 ^a	$45.99{\pm}0.37^{\mathrm{a}}$	$46.25{\pm}1.08^{bc}$	$45.15{\pm}1.56^a$	$46.25{\pm}1.08^a$	46.67 ± 0.92^{a}
72 h	46.03±0.70 ^a	47.32 ± 0.36^{a}	44.31±0.56°	$45.23{\pm}0.68^{a}$	$45.42{\pm}0.36^a$	45.42±0.36ª

DFBS- Defaed brown solojo cowpea flour

Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

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Table 11: Effect of Ionic Strength on Emulsion Stability (ES) of DAS protein isolate

Sample	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	45.59±1.11 ^{bc}	48.91±1.09 ^a	47.46±1.65 ^a	46.58±1.20 ^{ab}	47.81±2.90 ^{ab}	49.28±1.68 ^b
6 h	47.04±1.37 ^{ab}	$44.80{\pm}1.14^{ab}$	$43.70{\pm}0.66^{ab}$	42.20±1.28 ^b	45.89±0.11 ^{ab}	46.31±3.34 ^{bc}
24 h	43.70±1.28 ^{bc}	44.20±1.26 ^b	44.93±2.51ª	46.45±1.37 ^{ab}	46.26±0.70 ^{ab}	44.46±0.99°
36 h	49.26±0.64ª	48.57 ± 5.11^{ab}	47.46±3.12ª	46.38±3.32 ^{ab}	$45.97{\pm}3.52^{ab}$	43.38±1.11°
48 h	44.80±1.14 ^{bc}	45.59±2.18 ^{ab}	47.44±0.67ª	45.60±1.00 ^{ab}	$44.20{\pm}1.26^{ab}$	44.19±1.77°
72 h	45.65±3.77 ^{bc}	44.99±1.69 ^{ab}	46.84±2.80ª	49.27±6.28ª	49.48 ± 5.46^{a}	54.05±1.58ª
Soy isolate	42.87±1.05°	44.14±1.14 ^b	40.58±2.51b	42.87 ± 1.05^{b}	43.53±1.89 ^b	44.14±1.14°

DAS – Dark -ash solojo cowpea protein isolate

Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Table 12: Effect of Ionic Strength on Emulsion Stability (ES) of BS protein isolate

Sample	0.1M	0.2M	0.4M	0.6M	0.8M	1.0M
Raw	58.84 ± 2.88^{a}	52.07±3.65 ^{ab}	51.30±2.07 ^a	54.64±4.27 ^a	50.59 ± 0.89^{ab}	49.22±3.25 ^a
6 h	44.59±1.92°	42.75±1.28 ^e	42.43±1.95°	45.42±2.14 ^b	46.44 ± 1.21^{bc}	$47.07{\pm}1.23^{ab}$
24 h	53.62±3.32 ^b	53.92 ± 2.74^{a}	55.48±1.09 ^a	52.81±1.59 ^a	52.44±4.03 ^a	$48.84{\pm}3.95^{ab}$
36 h	45.55±1.80°	49.65±0.61 ^{bc}	42.75±1.26 ^c	43.50±2.01 ^b	46.78±2.83 ^{bc}	$45.65{\pm}2.17^{ab}$
48 h	44.29±1.18°	45.40±2.22 ^{de}	46.04 ± 0.67^{b}	46.08±2.10 ^{bc}	45.33±2.25 ^b	44.44 ± 0.61^{b}
72 h	$46.74{\pm}1.91^{b}$	46.79 ± 1.13^{b}	47.76 ± 2.27^{cd}	47.83 ± 2.17^{bc}	49.58±4.81 ^{bc}	$48.58{\pm}1.23^{ab}$

BS – Brown solojo cowpea protein isolate

Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.

CONCLUSION AND RECOMMENDATION

This research work shows that biochemical modification (Germination/Malting/ Sprouting) had an enormous impact on the nutritional composition, functional properties, mineral bioavailability, anti-nutrient content and amino assay of Solojo bean, thus, it could be used as protein supplement in infant, young children and geriatric foods. Efforts should be increased to promote the cultivation, encourage the consumption and industrial application of this underutilized legume by the Government, especially in the south-western region where it can survive the rain fall level. Large scale production of this legume which is gradually going into extinction should be encouraged in order to fight the menace of malnutrition in developing countries where animal protein price is exorbitant; This will ensure food security and also creation of jobs, because people can engage in different aspects of the production process and thereby reducing the rate of unemployment

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