RAS AGRICULTURAL SCIENCE & TECHNOLOGY

Research Article: Effect of Concentration (EC) on Emulsifying Activity (EA) of Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp)

Issue Type: Volume1 Issue1

Author Name:
Olubamike A. Adeyoju[1], Henry O. Chibudike[2], Bamidele I. Olu-Owolabi[3], Kayode O. Adebowale[4] and Chinedum E. Chibudike[5]

[1] Department of Analytical and Laboratory Management, Federal Institute of Industrial Research, Nigeria
[2] Department of Chem., Fiber and Environmental Techn., Federal Institute of Industrial Research, Nigeria;
[3] Department of Chemistry, Industrial Unit, University of Ibadan, Nigeria;
[4] Department of Chemistry, Analytical Unit, University of Ibadan, Nigeria;
[5] Department of Planning, Tech. Transfer and Information Management, Federal Institute of Industrial Research, FIIRO. Nigeria

Corresponding Author:
Olubamike A. Adeyoju

Citation: Olubamike A. Adeyoju Effect of Concentration (EC) on Emulsifying Activity (EA) of Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp)

Received Date: 25th March 2024

Published Date: 20th April 2024

Abstract:
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. 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.

Keywords: Solojo Cowpea; Under-utilized legumes; Un-germinated; Emulsifying Activity; Protein Isolate.

1. Introduction:

The Emulsion Stability (ES), largely showcase the capability of the proteins to give power for durability to the emulsion for protection against pressure strain and variations and is thus controlled by the constancy of the surface expanse through a definite period of time (Sreerama et al., 2012; Du et al., 2014). Comparable view was made by Enujiugha et al. (2003) for germinated “ugba” or “ukpaka” seed flour, where it was stated that emulsion stability increased with germination going from (4.0±1.0 –8.0±2.0cm). Borijindakul and Phimolsiripol (2013) similarly observed increase in emulsion stability with germination of Lablab seed flour with value going from (46.83±3.88 -52.50±6.61%). Lawal (2005) also reported increased EA for succinylated lablab bean which he attributed to increased solubility. Succinylation was also found to reveal the hidden functional collections inside the protein matrix. Akaerue and Onwuka (2010) also reported that protein isolates from germinated legumes had higher EA. This they attributed to break down of higher molecular weight protein to lower ones due to germination, thereby causing an increase in small subunit proteins (Soestrion, 2007). Brishti et al. (2017) found the EA of Vigna radiata protein isolate to be 63.18%. However, the EA of Glycine max protein isolate showed 74.50%. The quantity of hydrophobic groups on the protein covering of different legumes has been observed to bring about variation in emulsifying activity. This observation is similar in trend to those reported by Lawal (2005) for native and succinylated Lablab protein concentrate. The values for ES ranged between 43.10±3.32 to 59.56±0.62%; 43.89±0.92 to 72.37±3.48%; 42.62 to 53.75±0.58%; 43.06±2.41 to 48.56±0.36%; 49.86±3.58 to 57.22±1.65%; 51.65±4.46 to 62.71±0.63%, for samples, FFDAS, DFDAS, FFBS, DFBS, DAS and BS respectively. Earlier researchers reported different values of ES for various legumes: Mwasaru et al. (2000) obtained a value of 54.90%, for pigeon pea; Lawal and Dawodu (2007) reported a range from 42.0 to 78.3% for African locust bean native and Maleic anhydride derivative protein isolate; Butt and Batool (2010) recorded a value of 52.20±3.64 for cowpea isolates. Nwoji (2005) also stated that the more the protein quantity, the greater the emulsifying capability, whereas Moses et al. (2012), observed a lower emulsifying property for lima bean. EA and ES were also found to increase with rise in germination time; this was also detected by Enujiugha et al. (2003) for germinated African oil bean seed. Ragab et al. (2004) observed ES figure of 82% for cowpea protein isolates. The ES values obtained for the germinated DAS and BS cowpea isolates were in the category observed for other Vigna unguiculata isolates. This means that germinated solojo cowpea will be good as an emulsifying agent which is a favorable development for food industries where high emulsion is desired.

METHODOLOGY

Materials

Two varieties of the underutilized cowpea (V. unguculata) found in South west region of Nigeria where it is called 'solojo' were used (Figure 1: Brown Solojo Cowpea and Figure 2: Dark-Ash Solojo Cowpea).

Figure 1: Brown Solojo Cowpea

Figure 2: Dark-Ash Solojo Cowpea

Methods

Preparation of flours: 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.

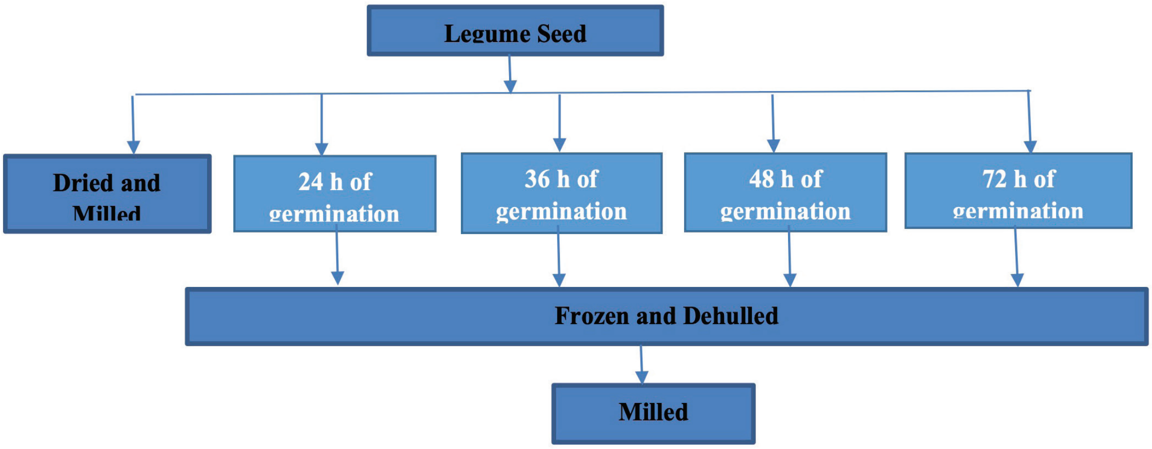

Figure 3: Preparation of Beans Flour/ Schematic representation

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.

Germination of seed: This was implemented by the method of Mubarak AE with minor adjustment. The seeds for germination were disinfected by soaking in 0.07% sodium hypochlorite for30 mins, then, it was rinsed painstakingly. The solojo seeds were then immersed for 6 hours in distilled water at ambient temperature (1:10w/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). The process of germination was terminated by freezing; the seeds were manually dehulled, dried in a draught oven at 40°C for 48 h, cooled, milled and packaged in an air tight plastic bag in the refrigerator pending analysis.

Results and Discussion

Emulsion stability (ES) followed a similar trend in previous research works reported in literature. The observed increase and reduction in Emulsifying Stability with increase in concentration are similar to those obtained in this research study as shown in Tables 1- 6.

Table 1: Effect of Concentration on Emulsifying Activity (EA) of FFDAS flour

FFDAS	1%	2%	4%	6%	8%	10%
Raw	45.59±2.17^{ab}	46.03±4.35^{a}	46.27±2.51^{a}	44.07± 4.13^{b}	43.56± 3.29^{c}	42.45± 2.54^{d}
6 h	45.83± 0.37^{a}	45.42±0.36^{ab}	44.86± 0.36^{bc}	44.31± 0.36^{c}	43.33± 0.37^{d}	45.27± 0.36^{ab}
24 h	46.09±0.36^{b}	46.25±1.08^{ab}	47.07±1.34^{ab}	44.31±0.36^{c}	45.94±0.36^{b}	47.74±0.36^{a}
36 h	46.09±0.36^{b}	47.83±0.36^{ab}	47.97±0.36^{ab}	49.30±0.96^{a}	44.86±0.36^{c}	44.86±0.36^{c}
48 h	46.67±0.36^{a}	47.32±0.36a	47.33±0.92^{a}	44.86±0.36^{bc}	44.03±0.36^{c}	45.53±0.36b
72 h	46.67±0.36^{ab}	47.32±0.36^{ab}	47.92±0.36^{a}	46.91±1.07^{ab}	45.93±0.96^{b}	43.20±1.71^{c}

EA – Emulsifying activity FFDAS- Full fat dark- ash Solojo cowpea

Concentration Effect on activity of emulsion (EA) and stability of emulsion (ES) of the various samples are recorded in Tables 1-6. Emulsifying Activity illustrates the competence and capability of protein to assist in the creation of an emulsion and is associated with the protein's power to act as a bridge between lipid and water blend

Table 2: Effect of Concentration on Emulsifying Activity (EA) of DFDAS Flour

DFDAS	1%	2%	4%	6%	8%	10%
Raw	43.48±4.35^{c}	45.59±2.18^{a}	45.76±2.27^{a}	45.88±3.52^{a}	44.90±2.52^{ab}	44.27±3.51^{b}
6 h	45.11±0.95^{b}	46.03±0.94^{ab}	44.73±0.92^{c}	45.99±0.37^{ab}	46.67±0.36^{a}	47.06±0.95^{a}
24 h	44.59±1.23^{d}	45.83±0.37^{b}	46.67±0.36^{a}	45.27±0.91^{c}	45.84±0.93^{b}	46.67±0.36^{a}
36 h	45.30±0.93^{b}	45.99±0.37^{ab}	45.99±0.37^{ab}	46.42±1.27^{a}	45.15±0.37^{c}	45.42±0.36^{b}
48 h	45.15±0.37^{c}	46.84±1.10^{b}	47.68±0.37^{a}	47.08±0.36^{a}	45.42±0.36^{c}	45.15±0.37^{c}
72 h	44.73±0.37^{c}	45.73±0.37^{b}	46.61±0.95^{a}	46.03±0.94^{ab}	45.34±0.95^{b}	44.86±0.36^{c}

EA – Emulsifying activity DFDAS- Defatted dark- ash Solojo cowpea
Means in column not followed by same alphabet(s) are significantly different at 5% level ($P<0.05$).

The observed increase and decrease in ES were within 2 – 6%. Emulsion stability was observed to reduce with rise in concentration for most of the FFDAS test materials.

Emulsion stability of both defatted and undefatted decreased with increase in flour concentration in solution. Values ranged between (52.3±1.8 – 46.5±1.0; 39.6±1.2 – 38.2±1.6; 63.6±0.9 – 56.7±1.2 percent respectively). The other treatments had their ES increased with increase in protein concentration, to various concentration levels.

Table 3: Effect of Concentration on Emulsifying Activity (EA) of FFBS cowpea

FFBS	1%	2%	4%	6%	8%	10%
Raw	45.52± 0.57^{a}	48.74± 1.65^{b}	50.81± 0.35^{b}	43.75± 0.55^{d}	52.11± 2.08^{a}	54.29± 2.18^{a}
6 h	48.54± 0.95^{a}	52.67± 0.92^{ab}	53.07± 1.81^{ab}	46.68± 1.27^{cd}	46.25± 0.58^{cd}	48.76± 0.61^{b}
24 h	51.67± 0.36^{a}	52.90± 1.99^{ab}	53.10± 1.21^{ab}	47.90± 2.55^{c}	45.00± 0.56^{d}	46.92± 0.58^{bc}
36 h	49.79± 1.30^{a}	52.91± 0.94^{ab}	53.33± 0.92^{ab}	54.58± 0.91^{b}	48.15± 0.59^{b}	46.92± 0.58^{bc}
48 h	48.76± 0.61^{a}	52.91± 0.94^{ab}	53.90± 1.24^{ab}	48.14± 1.64^{c}	46.92± 0.58^{bc}	48.15± 0.59^{bc}
72 h	50.83± 0.94^{a}	54.48±5.90^{a}	54.83±3.22^{a}	60.57±2.10^{a}	45.68± 0.56	46.35± 0.57^{c}

EA – Emulsifying activity FFBS- Full fat brown Solojo Cowpea flour
Means in column not followed by same alphabet(s) are significantly different at 5% level ($P<0.05$).

An increase in EA with concentration up to between 4-6% was observed for DAS and BS protein isolates. Increase in protein content, brought about increase in emulsifying ability for cowpea. Each treatment behaved as unique samples and followed different trends of reaction with concentration. The increase in EA observed in most of the samples increased with increase in protein concentration in solution (57.8±0.4 - 75.6±1.6 and 55.3±1.8 – 61.5±1.9% respectively).

Table 4: Effect of Concentration on Emulsifying Activity of DFBS

DFBS	1%	2%	4%	6%	8%	10%
Raw	45.10±0.22^{a}	44.92±1.26^{a}	46.50±1.15^{a}	46.31±3.34^{a}	46.25±4.75^{a}	45.71±2.18^{a}
6 h	45.34±0.71^{a}	46.00±1.27^{a}	46.67±0.36^{a}	46.41±0.37^{a}	45.42±0.36^{a}	45.42±0.91^{a}
24 h	45.15±0.93^{a}	45.84±0.93^{a}	46.67±0.36^{a}	45.42±0.36^{a}	46.67±0.36^{a}	46.09±0.36^{a}
36 h	45.34±0.71^{a}	45.80±0.70^{a}	46.67±0.92^{a}	47.09±0.94^{a}	45.83±0.37^{a}	46.03±0.70^{a}
48 h	45.15±0.93^{a}	46.22±0.63^{a}	46.67±0.92^{a}	47.09±0.94^{a}	46.67±0.36^{a}	46.87±1.21^{a}
72 h	44.37±1.57^{a}	45.99±0.37^{a}	46.09±0.36^{a}	47.74±0.36^{a}	46.67±0.36^{a}	45.84±0.93^{a}

EA – Emulsifying activity DFBS- Defatted brown Solojo Cowpea flour
Means in column not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Values of emulsion capacity for germinated Solojo cowpea processed into flours for the full fat (FFDAS) was observed to be greater than that of the defatted flour (DFDAS), but contrary for the brown solojo cowpea. They had 75.00 mL/g for the full fat flour and about 45.00 mL/g for the defatted flours.

Table 5: Effect of concentration on Emulsifying activity (EA) of DAS and SOY protein isolate

Sample	1%	2%	4%	6%	8%	10%
Raw	44.79±1.14^{b}	52.96±1.37^{a}	48.48±3.41^{a}	47.76±2.27^{ab}	47.04±1.37^{b}	46.25±1.37^{bc}
6 h	45.00±4.17^{d}	46.52±4.93^{c}	47.25±2.96^{b}	49.56±2.25^{a}	46.77±3.75^{c}	41.36±3.66^{e}
24 h	46.24±1.21^{c}	46.38±2.51^{c}	68.27±4.73^{ab}	70.83±4.17^{ab}	73.46±1.09^{a}	63.65±2.63^{b}
36 h	53.33±3.00^{e}	70.83±4.17^{b}	73.91±4.35^{ab}	78.26±4.35^{a}	66.67±2.51^{c}	62.50±4.17^{d}
48 h	46.97±2.62^{c}	47.71±0.85^{b}	49.19±0.73^{a}	45.54±1.42^{d}	43.51±1.30^{e}	41.46±0.89^{f}
72 h	47.99±0.87^{a}	45.70±0.94^{c}	44.24±0.68^{c}	45.62±0.37^{c}	46.61±1.17^{b}	46.73±1.01^{b}
Soy isolate	45.59±2.18^{b}	45.45±0.00^{b}	44.93±2.51^{c}	44.80±1.14^{c}	45.31±2.38^{b}	46.97±2.62^{a}

EA – Emulsifying activity DAS – Dark ash solojo cowpea protein isolate Soy Isolate- Soya protein isolate
Means in column not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Decrease in emulsifying activity was observed among DFBS Raw, DAS 72 h, and FFDAS 6 h up to 2%, 4% and 8% respectively. Emulsifying activity was found to decrease as concentration of flour in solution increased.

Table 6: Effect of concentration on Emulsifying activity (EA) of BS protein isolate

Sample	1%	2%	4%	6%	8%	10%
Raw	44.20±2.47^{b}	47.49±0.58^{b}	47.61±1.54^{ab}	49.01±0.46^{a}	48.55±4.53^{a}	47.10±3.32^{a}
6 h	44.37±3.68^{b}	46.72±3.49^{c}	49.75±1.85^{c}	52.27±2.53^{c}	48.81±1.41^{c}	43.18±2.14^{c}
24 h	47.25±2.96^{b}	50.01±2.29^{bc}	51.16±2.51^{c}	53.60±2.43^{ab}	42.22±2.41^{d}	46.33±2.46^{c}
36 h	47.45±0.96^{b}	53.34±0.60^{b}	57.49±2.21^{b}	56.84±1.67^{b}	55.56±1.99^{b}	55.29±2.84^{b}
48 h	49.28±2.51^{b}	53.91±1.20^{b}	57.15±2.21^{b}	51.40±2.25^{c}	46.78±2.00^{c}	42.66±1.87^{c}
72 h	58.32±4.19^{a}	62.55±1.57^{a}	67.26±1.96^{a}	63.25±2.16^{a}	62.78±3.37^{a}	65.72±2.26^{a}

EA – Emulsifying activity BS- Brown Solojo Cowpea protein isolate
Means in column not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

An improvement in the Emulsion Activity Index with increase in concentration for the protein isolate, ranging from 1.24±0.05 m2/g for 0.1% protein mixture to 3.38±0.31 m2g for 3% protein suspension was detected.

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

FFDAS	1%	2%	4%	6%	8%	10%
Raw	44.48± 1.32^{c}	43.10± 3.32^{d}	44.24± 2.27^{c}	45.52± 2.62^{a}	45.13± 3.76^{b}	45.57± 3.76^{a}
6 h	45.42±0.36^{ab}	44.73±0.37^{ab}	44.17±0.37^{b}	45.42±0.91^{ab}	44.03±0.99^{b}	46.09±0.98^{a}
24 h	46.09±0.36^{b}	47.08±0.97^{b}	48.33±0.36^{a}	44.31±0.36^{c}	46.10±0.91^{b}	43.62±0.36^{c}
36 h	46.31±1.09^{ab}	48.48±1.32^{a}	51.52±2.62^{a}	45.64±0.63^{b}	43.20±0.70^{c}	43.39±0.31^{c}
48 h	47.08±0.36^{a}	52.24±2.37^{a}	61.13±0.99^{a}	47.26±0.37^{a}	45.42±0.36^{b}	43.62±0.36^{c}
72 h	46.91±1.07^{b}	46.10±0.91^{bc}	45.42±0.36^{c}	48.29±0.37^{a}	51.53±0.36^{c}	59.56±0.62^{d}

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

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 surface-active 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.

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

DFDAS	1%	2%	4%	6%	8%	10%
Raw	43.89±0.92^{d}	46.41±0.37 ^{a}	46.41±0.37 ^{a}	45.42±0.36 ^{b}	44.62±0.98 ^{c}	44.73±0.37 ^{c}
6 h	44.30±1.10 ^{c}	46.41±0.37 ^{a}	46.61±0.95 ^{a}	46.67±0.36 ^{a}	45.43±1.25 ^{b}	45.42±0.36^{b}
24 h	44.87±1.11^{d}	46.59±1.30^{b}	47.68±0.37 ^{a}	47.09±0.94 ^{ab}	45.58±1.64^{c}	45.99±0.37^{c}
36 h	45.73±0.95 ^{c}	46.59±1.30^{b}	47.68±0.95 ^{a}	47.25±1.00 ^{a}	46.42±0.94^{b}	47.08±0.97^{ab}
48 h	45.73±0.95 ^{c}	47.26±0.37^{b}	48.51±1.33 ^{a}	47.92±0.36 ^{ab}	47.48±0.63 ^{b}	47.68±0.95^{ab}
72 h	46.25±4.75^{e}	52.24±2.27^{d}	59.09±4.55^{c}	64.23±3.69^{b}	67.09±3.24^{ab}	72.37±3.48^{a}

ES- Emulsion stability DFDAS- Full fat dark- ash Solojo cowpea
Means in column not followed by same alphabet(s) are significantly different at 5% level ($P<0.05$).

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). Howbeit, at elevated protein concentration, the energy needed to begin the reaction, (activation energy barrier) which is high, does not permit protein migration to take place in a dispersion (diffusion) related technique.

Table 9: Effect of Concentration on Emulsion Stability of full fat brown solojo cowpea (FFBS)

FFBS	1%	2%	4%	6%	8%	10%
Raw	43.00± 4.25^{b}	44.07± 3.50^{b}	45.59± 2.18^{c}	43.28± 2.28^{b}	42.62± 1.48^{c}	44.92± 1.26^{c}
6 h	51.66±0.95^{a}	52.03± 0.91^{a}	52.91±0.94^{a}	48.76±0.61^{a}	47.50±0.59^{a}	48.76±0.61^{a}
24 h	49.16±0.97^{a}	51.25±1.08^{a}	52.08±0.93^{a}	47.74±1.78^{a}	46.92±0.58^{a}	46.92±0.58^{b}
36 h	49.57±1.31^{a}	50.82±2.37^{a}	51.86±0.64^{a}	47.50±0.59^{a}	46.84±0.59^{a}	45.42±0.36^{bc}
48 h	50.84±1.57^{a}	51.67±0.96^{a}	48.97±0.36^{b}	47.50±0.59^{a}	44.45±0.55^{b}	45.68±0.56^{bc}
72 h	51.67±0.36	53.33±0.92	53.75±0.58	48.56±0.36	46.90±1.65	46.92±0.58^{b}

ES- Emulsion stability FFBS- Full fat brown Solojo Cowpea flour
Means in column not followed by same alphabet(s) are significantly different at 5% level ($P<0.05$).

Germination was observed to generally improve emulsifying action and emulsion steadiness. The detected rise in the emulsifying properties of protein with germination may be as a result of the uncoiling of protein linkage, therefore uncovering the non-polar residues of amino-acid, which causes an improvement in emulsifying capacity.

Table 10: Effect of Concentration on Emulsion Stability of Defatted brown solojo cowpea

DFBS	1%	2%	4%	6%	8%	10%
Raw	43.66±2.09^{b}	45.77±2.26^{a}	43.06±2.41^{b}	44.92±1.26^{b}	45.59±2.18^{a}	46.49±4.02^{a}
6 h	46.59±0.94^{a}	47.28±0.96^{a}	44.54±0.93^{b}	46.00±0.93^{ab}	46.67±0.36^{a}	47.26±0.94^{a}
24 h	45.99±0.37^{ab}	47.68±0.95^{a}	47.50±1.08^{a}	46.67±0.36^{ab}	45.83±0.37^{a}	46.70±0.70^{a}
36 h	45.80±0.94^{ab}	47.06±0.70^{a}	48.56±0.36^{a}	46.02±1.31^{ab}	46.67±0.36^{a}	46.48±0.69^{a}
48 h	45.34±0.71^{ab}	46.90±1.21^{a}	47.50±1.08^{a}	46.67±2.94^{ab}	46.67±2.94^{a}	46.86±0.63^{a}
72 h	44.96±1.64^{ab}	46.25±1.08^{a}	47.75±1.25^{a}	47.74±0.93^{a}	47.92±0.93^{a}	45.84±1.25^{a}

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

The decreased value of some of the treatment may be due to insufficient exposure of the (Ionized) hydrophilic protein to the water phase or due to insufficient unfolding of the hydrophobic groups of the protein to contact the lipid phase.

Table 11: Effect of Concentration on Emulsion Stability (ES) of DAS and SOY protein isolate

Sample	1%	2%	4%	6%	8%	10%
Raw	49.86±3.58^{e}	54.14±1.14^{a}	52.43±1.35^{c}	51.85±1.54^{d}	53.04±1.00^{b}	51.11±1.53^{d}
6 h	51.04±2.80^{d}	54.40±2.10^{a}	52.84±1.61^{bc}	53.38±0.42^{ab}	53.07±1.25^{b}	52.42±0.95^{c}
24 h	52.84±1.34^{c}	55.24±0.72^{a}	55.12±2.36^{a}	54.20±0.64^{b}	54.08±1.15^{b}	54.78±1.67^{ab}
36 h	53.94±2.62^{c}	54.34±1.16^{b}	55.42±0.25^{a}	54.80±1.16^{ab}	54.20±0.64^{b}	55.40±0.75^{a}
48 h	55.95±0.81^{b}	57.22±1.65^{a}	56.52±1.30^{ab}	55.21±0.78^{c}	55.19±2.03^{c}	56.96±1.57^{ab}
72 h	57.04±0.77^{a}	56.60±1.19^{b}	56.38±2.51^{b}	57.04±1.37^{a}	56.25±1.37^{b}	56.25±1.37^{b}
Soy isolate	54.44±2.41^{b}	56.38±2.51^{a}	53.66±2.09^{c}	52.87±1.05^{d}	52.62±1.48^{d}	54.14±1.14^{b}

ES- Emulsion stability DAS – Dark ash solojo cowpea protein isolate Soy Isolate- Soya protein isolate
Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

Immediately a protein molecule gets to the surface (fat/water interface) it has to be stable to uncoil sufficiently to bring out the non-polar groups in order to perform as an emulsifier. The capability to function as an emulsifying agent by protein may be related to its degree of relative hydrophobicity.

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

Sample	1%	2%	4%	6%	8%	10%
Raw	55.71±2.18^{a}	59.28±2.51^{a}	57.89±2.08^{abc}	57.76±2.27^{b}	55.58±0.22^{bc}	54.14±1.14^{bc}
6 h	55.59±2.18^{a}	59.01±2.77^{a}	57.26±1.83^{bc}	56.88±2.49^{b}	55.51±1.76^{bc}	54.74±0.95^{bc}
24 h	54.09±1.36^{a}	56.09±1.57^{a}	58.36±0.48^{ab}	56.15±1.90^{b}	54.03±1.83^{c}	51.65±4.46^{c}
36 h	54.60±2.80^{a}	56.72±1.68^{a}	57.16±0.65^{bc}	55.70±1.97^{b}	53.80±2.57^{c}	56.05±0.72^{ab}
48 h	56.49±1.16^{a}	57.64±1.54^{a}	55.59±1.34^{c}	57.19±0.65^{b}	57.98±0.84^{ab}	58.96±1.30^{a}
72 h	54.86±0.28^{a}	55.40±1.59^{a}	59.86±0.53^{a}	62.71±0.63^{a}	60.14±0.43^{a}	56.55±1.48^{ab}

ES- Emulsion stability BS- Brown Solojo Cowpea protein isolate
Means in colomn not followed by same alphabet(s) are significantly different at 5% level (P<0.05).

In conclusion, the observed increase with germination time might be due to reduced fat, higher protein component and greater inter-action of protein with other constituents existing in the germinated flour test material. Emulsifying capacity (EC) of a product is determined by the volume of oil and protein concentration. All the treatment will make better emulsifying agent than FFDAS Raw, 6 h soaked and 72 h germinated flours and 72 h DAS isolate.

Conclusion and Recommendation

Biochemical modification which involves the activation of the intrinsic enzymes of the Solojo cowpea seed itself by germination was carried out for different hours and the germination terminated by freezing for the two varieties, i.e. the Dark-Ash and the Brown. The germinated seeds were manually dehulled oven dried and milled to flour. Part of which was defatted, another part kept as full fat. Protein isolate was obtained from part of the defatted flour by the isoelectric precipitation method. The obtained flours and isolate were subjected to Proximate analysis, from which it was observed that the moisture content decreased with germination, which is good for shelf life stability; protein content increased with germination, which could be due to breakdown of the storage protein to simpler amino acid, fat content decreased with germination which could be due to it's being used as energy source during germination and is equally good for shelf life stability as it reduces the rate of inception of rancidity in food processing. Comparing the obtained result with that of Chemical modification, it was observed that the moisture content of the chemically modified isolate increased with modification and the protein content decreased with modification. Which means that, biochemically modified flour and protein isolates will do better in food processing than chemically modified isolates. This research work shows the impact of biochemical modification(Germination/Malting/Sprouting) on the nutritional composition, functional properties, mineral bioavailability, reduction in anti-nutrient content and improved amino assay of Solojo bean, confirmed by, SDS-PAGE electrophoresis, SEM, FT-IR, DSC and TGA analysis, showed that this modification method actually improved the quality of the underutilized Solojo cowpea by conferring on it qualities that make it compare favorably with existing popular and well utilized legume seeds such as soybean, groundnut, chickpea, lentil, by increasing the nutritional value like other legumes, 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 under-utilized 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.

References

Akaerue, B.I. and Onwuka, G.O. 2010. Evaluation of the yield, protein content and functional properties of Mungbean (*Vigna radiata* (L) Wilckzek) Protein isolates as affected by processing: *Pakistan journal of Nutrition*. 9 (8).728-735.

Borijindakul, L. and Phimolsiripol, Y. 2013. Physicochemical and Functional Properties of Starch and Germinated Flours from Dolichos Lablab. *Food and Applied Bioscience Journal*, 1(2): 69-80

Brishti, F. H., Zarei, M., Muhammad, S. K. S., Ismail-Fitry, M. R., Shukri, R. and Saari, N. 2017. Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. International Food Research Journal 24(4): 1595-1605

Butt, M. S. and Batool, R. 2010. Nutritional and Functional properties of some promising Legumes protein isolates. Pakistan Journal of Nutrition. 9. 4.373-379.

Du, S., Jiang, H., Yu, X. and Jay-lin, J. 2014. Physicochemical and functional properties of whole legume flour. Food Science and Technology LWT 55(1) 308 – 313

Enujiugha, V N., Badejo, A A., Iyiola, S O. and Oluwamukomi, M O. 2003. Effect of germination on the nutritional and functional properties of African Oil bean (Pentaclethra macrophylla Benth) seed flour. Food, Agriculture and Environment. 1. (3&4):72-75.

Lawal, O.S. 2005. Functionality of reactive and succinylated lab lab bean (Lab lab Purpureus) protein concentrate. *Food Hydrocolloids*. 19:63-72.

Lawal, O.S. and Dawodu, M.O. 2007. Maleic anhydride derivatives of a protein isolate: preparation and functional evaluation. European Food Research and Technology 226: 186-198

Moses O., Olawuni I., Iwouno J. O. 2012. The Proximate Composition and Functional Properties of Full- Fat Flour, and Protein Isolate of Lima Bean (Phaseolus Lunatus) // Open Access Scientific Reports. Vol. 1. N 7. 1:349.

Mwasaru, M A., Muhammad, K., Bakar, J., Che Man Y B 2000. Influence of altered Solvent environment on the functionality of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolate. *Food Chemistry* 71: 157 – 165.

Nwoji, V.C. 2005. Effect of Processing on the storage ability and functional properties of cowpea (Vigna unguiculata) flour in the production of cowpea bean (akara) and paste (moi-moi), proceeding of 29th annual conference of Nigerian Institute of Food Science and Technology.

Ragab, D.M., Babiker, E.E. and Eltinay, A. H. 2004. Fractionation, Solubility and Functional properties of Cowpea (Vigna unguiculata) proteins as affected by pH and or salt concentration. Food chemistry. 84. 207-212.

Soestrino, U. S. S. 2007. Characterization of yellow pea (Pisum sativum L. Miranda) Protein and the proteinate functional properties.Ph.D. thesis. Food resource, Nutritional Food Management Department, Oregun State univ. Corvallis, pp: 1 – 9.

Sreerama, Y. N., Sashikala, V. B., and Pratape, V. M. 2012. Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension. *Food Chemistry, 133(1), 156–162*. doi: 10.1016/j.foodchem.2012.01.011