

Experimental Studies on the Mineral Content of Solojo Flour and Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (*Vigna Unguiculata L. Walp*)

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ABSTRACT

Studies on the Mineral Content of Solojo Flour and Protein Isolates from the two varieties (DAS and BS) of Nigeria cultivated solojo cowpeas were conducted to determine their nutritional value. These inorganic elements or minerals were classified into 3 categories, the ultra- trace minerals, which is the third category; the micro elements, also known as the trace minerals is the second category; while the first category are the macro elements, also known as major minerals. Some of the macro-elements are Ca, P, Na and Cl, the second category, micro-elements include, iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, F, Cr, Se and S. Results show that the proportion of Sodium (Na) which is ingested into the body in the form of NaCl through food intake maintenance of body pH and to retain water ranged from 728.97 to 253.37 ppm (72.90 to 25.34 mg/100 g); 715.24 to 235.45 ppm; 735.28 to 270.37 ppm; 726.59 to 264.35ppm, for FFDAS, FFBS, DAS and BS respectively with all values of the germinated samples all bellow the control. While FFDAS iron content ranged from 4.25 to 13.50 mg/100 g; FFBS ranged from 3.15 to 12.56 mg/100 g; DAS ranged from 3.81 to 12.90 mg/100g; BS ranged from 3.42 to 9.40 mg/100 g. The values of the germinated flours were all greater than the ungerminated flour. Iron helps to transport oxygen round the body and also helps in red blood cells building and to convert food into needed energy by the body. While Manganese an element that is needed in micro quantity but necessary to convert food into energy, is also crucial for healthy bone and cartilage creation. Results also show that zinc quantity increased as germination proceeded and the values ranged from 38.80 ppm to 230.00 ppm (3.880 mg/100 g to 23.00 mg/100 g; 0.003880% to 0.0230%); 40.84 to 250.01 ppm; 32.85 to 93.41 ppm; 37.07 to 115.00 ppm, for FFDAS, FFBS, DAS and BS respectively. The Ca content improved significantly ($p < 0.05$) with sprouting, the value extended from 250.56 ppm to 760.03 ppm (25.056 to 76.00 mg/100g or 0.0251 to 0.0760 %); 400.40 to 998.22 ppm; 116.87 to 195.69 ppm; 113.48 to 220.75 ppm, for FFDAS, FFBS, DAS and BS respectively. Zinc element although needed at the micro level in the body, is essential for a strong immune system to keep the body in good health. It is also crucial for the maintenance of a healthy sense of taste and odor, while Calcium is critical for strong bones and teeth, blood coagulation, and muscle tightening and relaxation. Magnesium is needed to build enzyme and antioxidants and also for healthy bones while Potassium is needed to maintain; water balance, muscle movement, and nerve impulses. It functions in conjunction with Na to regulate blood pressure.

Keywords: Solojo Cowpea, Under-Utilized Legumes, Protein Isolates, BS, DA, Gelation Property, Un-Germinated

Introduction

All body tissues and fluids contain inorganic substances known as minerals whose existence is essential for the support of some physico-chemical activity that are critical to life. They are chemical compounds utilized by man for various processes. Even though no energy is given by them, their activities in the body is very crucial [1]. These inorganic elements or minerals are needed

by every living matter only in minute quantity for the sustenance of life. Their classification can be into 3 categories, the ultra- trace minerals, which is the third category; the micro elements, also known as the trace minerals is the second category; while the first category are the macro elements, also known as major minerals. Some of the macro-elements are Ca, P, Na and Cl, the second category, micro-elements include, iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, F, Cr, Se and S [2]. Table 4.80 to 4.83 represents the results of mineral analysis for the flours and protein isolates of Solojo cowpea samples.

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Sodium (Na) is ingested into the body in the form of NaCl through food intake sodium helps in the maintenance of body pH and to retain water. Yellavila et al. reported a value range of 19.99 to 21.33 mg/100 g for lima beans flour accession which is lower than the obtained result for lima beans flour by Ezeagu et al. [3,4].

Iron helps to transport oxygen round the body and it helps in red blood cells building and to convert food into needed energy by the body [5]. Devi et al. reported an improvement in value of iron with germination for the three cultivars of cowpea (PL-1 -3) they worked on even after 24 h. The values ranged from 4.86 - 6.78 mg/100 g. Yellavila et al. expressed result range of 2.45 to 2.67 mg/100g for the five lima accessions worked upon [3,6]. Al-Numair et al. recorded values ranging between 5.97 to 7.08 mg/100g for one faba bean cultivar and 6.47 to 7.47 mg/100 g for another cultivar [7]. They also reported a value range of 10.67 to 14.53 mg/100 g for one cultivar of white beans and 8.60 to 10.20 mg/100 g for another cultivar. They also observed an increase in iron quantity with germination time and cultivar variation was also noticed as was for our solojo varieties. Aremu et al. also reported a value of 5.5 mg/100 g and 6.7 mg/100 g for moderate brown and small white beans, this also shows varietal difference [8]. Ghavidel and Prakash reported a value of 6.5 mg/100 g for raw cowpea, and 5.87 mg/100 g after germination, even though the value for the raw was higher than that after germination, the bioavailable iron in the germinated cowpea was 19.7% while that of the raw was 11.2% [9].

Manganese is an element that is needed in micro quantity but necessary to convert food into energy. It is also crucial for healthy bone and cartilage creation [5]. Singh et al. reported for soy protein isolate 0.002% (2 mg/100 g), soy concentrates 0.005% (5 mg/100 g) and the flour 0.003 to 0.004% (3-4 mg/100 g) [10]. Al-Numair et al. observed a rise in value with germination of the two cultivars of faba beans 2.69 to 4.72 mg/100 g (0.269 to 0.472%) and 2.01 to 3.06 mg/100 g (0.00201 to 0.00306%) [7]. Likewise, the cultivars of white beans also gave the same pattern of increase with germination. Aremu et al. reported values of 1.2 mg/100 g (0.12%) for the two cowpea varieties [8]. although these values were found to be lower than that reported for Soy beans and faba beans, it was within the spread of that observed by Aremu et al. for two varieties of cowpea they worked upon [8]. The low result could also have been due to draining into water at the time of soaking, germination and rinsing. On the contrary, Seena et al. recorded a value of 1.36 mg/100 g for raw *Canavalia cathartica* which reduced in value to 0.79 mg/100 g with roasting and cooking [11].

Zinc is an element that is although needed at the micro level in the body but very essential for a strong immune system to keep the body in good health. It is also crucial for the maintenance of a healthy sense of taste and odour [5]. Al-Numair et al. noted a similar increase in zinc quantity of the legumes worked upon with increase in germination [7]. They reported a value range of 2.22 to 2.73 mg/100 g (0.00222 to 0.00273%) for faba bean accession and 2.46 to 2.82 mg/100 g (0.00246 to 0.00282%), while Aremu et al. had a value of 5.7 and 6.0 mg/100 g (0.0057 and 0.0060%) for the two cowpea varieties and Singh et al. recorded a value of 0.004 to 0.009% (4.0 mg/100 g to 9.0 mg/100 g) for soy protein isolates [8,10]. These values were found to fall

within the values obtained for the Solojo samples in the present work. The increase in value of Zinc with germination could be due to the reduction in the anti-nutrients content in the legume due to germination.

Calcium. This element is critical for strong bones and teeth, blood coagulation, and muscle tightening and relaxation [5].

Magnesium is needed to build enzyme and antioxidants and also for healthy bones [5].

Potassium is needed to maintain; water balance, muscle movement, and nerve impulses. It functions in conjunction with Na to regulate blood pressure [5]. Potassium apart from its importance in cellular water balance, it is also very important for protein and carbohydrate metabolism [12].

Generally, the values obtained for each mineral was lower than reported by other researchers, this may be as a result of leakage of the mineral into the water, and this was similarly detected by Desalegn, for soaked and germinated chickpea flour [13]. Similar reduction in minerals (Fe, Zn and Ca) content was also observed for germinated chickpea and quality protein maize based complimentary food [14].

Materials and Methods

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).



Figure 1: Brown Solojo Cowpea



Figure 2: Dark-Ash Solojo Cowpea.

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, 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 for, 24 h, 36 h, 48 h and 72 h (Figures 3).

plastic pack and preserved in a fridge freezer at 4°C pending utilization.

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

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 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 for various hours such as 24 h, 36 h, 48 h and 72 h. 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.

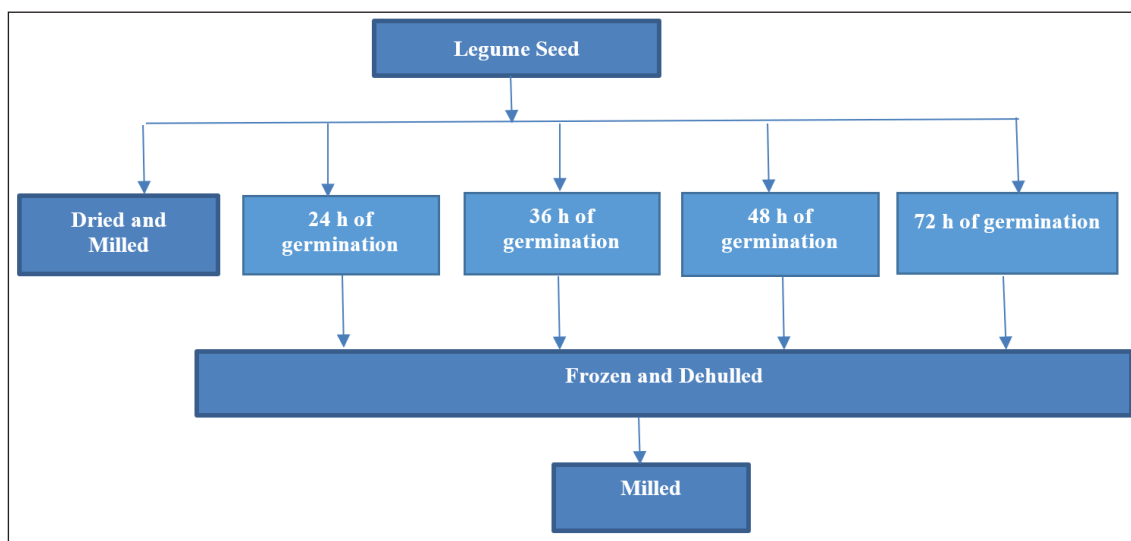


Figure 3: Preparation of Beans Flour/ Schematic representation

Results and Discussion

The values of Sodium (Na) ranged from 728.97 to 253.37 ppm (72.90 to 25.34 mg/100 g); 715.24 to 216.05 ppm; 735.28 to 270.37 ppm; 726.59 to 264.35ppm, for FFDAS, FFBS, DAS and BS as shown in tables (1-4) respectively with all values of the germinated samples all bellow the control. The low values for all the germinated samples are very good because, Na even though important should not be too much in the body. Germination reduced the Na concentration in the sample, thereby giving room for the added salt in food.

Table 1: Elemental Analysis of Dark Ash Solojo Flour (ppm)

FFDAS (ppm)	Ca	Na	Zn	Fe	Mg	K	Cu	Mn	Cd	Pb	Cr
Raw	250.56	728.97	38.80	4.25	103.0	206.99	0.42	0.36	0.30	ND	0.21
6 h	439.36	721.19	59.90	5.20	116.12	427.13	16.99	7.20	0.24	ND	0.21
24 h	614.39	704.66	78.90	6.90	125.98	547.45	23.01	8.52	0.20	ND	0.73
36 h	642.38	634.61	95.10	7.12	137.00	628.31	27.05	16.93	0.11	ND	0.89
48 h	684.62	560.56	139.900	9.97	146.12	1041.23	32.65	16.99	0.06	ND	0.60
72 h	760.03	253.37	230.01	13.59	200.88	1118.57	40.42	18.33	0.04	ND	0.94

FFDAS iron (Fe) content ranged from 4.25 to 13.50 mg/100 g; FFBS ranged from 3.15 to 12.56 mg/100 g; DAS ranged from 3.81 to 12.90 mg/100g; BS ranged from 3.42 to 9.40 mg/100 g as shown in tables (1-4). The values of the germinated flours were all greater than the un-germinated flour. This buttresses the fact that germination makes the macronutrient to be more available. This could be due to decrease in the anti- nutrient level which was binding to the iron before germination, which has now released the iron to be bioavailable for utilization. The values obtained in this work fell within the range observed by other researchers.

Table 2: Elemental analysis of FFBS

FFBS	Ca	Na	Zn	Fe	Mg	K	Cu	Mn	Cd	Pb	Cr
Raw	400.40	715.24	40.84	3.15	113.03	398.39	0.39	0.38	0.26	ND	0.42
6 h	571.06	653.72	65.47	5.39	123.88	547.45	4.32	10.60	0.24	ND	0.52
24 h	836.94	460.41	85.47	6.13	166.81	728.34	4.95	11.74	0.23	ND	1.73
36 h	870.75	320.48	105.06	8.28	187.10	948.44	5.30	12.66	0.11	ND	1.85
48 h	953.42	235.12	148.12	10.26	197.19	1096.65	8.18	14.10	0.08	ND	1.92
72 h	998.22	216.05	250.01	12.56	219.26	1268.98	9.39	15.95	0.10	ND	1.98

FFBS: Full fat brown solojo

ND: Not detected

The manganese (Mn) quantity in the flour of DAS spread between 0.36 ppm to 18.33 ppm (0.036 mg/100 g to 1.833 mg/100 g) after germinating for 72 h; 0.38 to 15.95 ppm; 1.25 to 4.11 ppm; 0.98 to 3.10 ppm, for FFBS, DAS and BS respectively. This value rose with rise in germination time.

Zinc (Zn)

The zinc quantity increased as germination proceeded and the values ranged from 38.80 ppm to 230.00 ppm (3.880 mg/100 g to 23.00 mg/100 g; 0.003880% to 0.0230%); 40.84 to 250.01 ppm; 32.85 to 93.41 ppm; 37.07 to 115.00 ppm, for FFDAS, FFBS, DAS and BS respectively. On the contrary, Desalegn reported significant ($p < 0.05$) decrease in value of Zinc with germination, the value decreased from 2.55 to 2.22 mg/100 g, but phytate zinc ratio, was found to increase with germination. This also goes to buttress the fact that germination reduces the anti-nutrient content of legumes [13].

Table 3: Elemental Analysis of Dark Ash Solojo Isolate

DAS	Ca	Na	Zn	Fe	Mg	K	Cu	Mn
Raw	116.87±0.56	735.28±0.43	32.85±0.36	3.81±0.47	38.37±0.47	123.34±0.32	0.89±0.04	1.25±0.25
6 h	126.44±0.70	688.24±0.90	42.96±0.49	5.21±0.34	51.91±0.49	154.92±0.26	1.32±0.03	1.55±0.35
24 h	139.10±0.40	620.19±0.75	57.73±0.39	6.35±0.47	74.04±0.53	203.38±0.60	1.45±0.03	1.89±0.21
36 h	159.09±0.37	524.40±1.38	61.91±0.39	7.42±0.63	100.42±0.45	258.73±0.43	1.72±0.04	2.73±0.26
48 h	175.76±0.47	317.17±1.14	79.11±0.18	9.02±0.60	104.04±0.36	412.05±0.47	1.84±0.04	2.95±0.26
72 h	195.69±0.84	270.37±6.08	93.41±0.41	12.90±0.50	114.22±0.56	771.73±0.34	1.87±0.07	4.11±0.30

DAS: Dark ash solojo cowpea protein isolate

The Calcium (Ca) content improved significantly ($p < 0.05$) with sprouting, the value extended from 250.56 ppm to 760.03 ppm (25.056 to 76.00 mg/100g or 0.0251 to 0.0760 %); 400.40 to 998.22 ppm; 116.87 to 195.69 ppm; 113.48 to 220.75 ppm, for FFDAS, FFBS, DAS and BS respectively. Al-Numair et al. Devi et al. and Bhartiya et al. observed similar trend (faba bean, 427 to 634 mg/100 g; white beans, 321 to 379 mg/100 g and cow pea cultivars, 105.99 to 148.15 mg/100 g) respectively [6,7,15]. Bhartiya et al. similarly recorded a value of 238 mg/100g in whole grain horse gram and 223 mg/100 g for dehulled horse gram [15]. The obtained results in this project was observed to be less than the values recorded by the researchers; these could be due to leaching into water of the nutrient due to the mode of germination employed.

The magnesium (Mg) content increased with germination time. The value ranged from 103.0 to 200.80 ppm (10.3 to 20.08 mg/100 g; or 0.0103 to 0.0201%); 113.03 to 219.26 ppm (11.30 to 21.93 mg/100 g); 38.37 to 114.22 ppm (3.84 to 11.42 mg/100 g); 55.89 to 124.17 ppm (5.59 to 12.42 mg/100 g) for FFDAS, FFBS, DAS and BS respectively. Aremu et al. obtained a higher value (67.7 and 54.6 mg/100 g) for the two cowpea varieties, Al-Numair et al. reported (255 to 312 mg/100 g) for Faba beans [7,8]. Lou et al. reported actual decrease in value, with germination for soybeans, the bioavailability was greater in the germinated beans than the ungerminated for the legume. Singh et al. reported a higher value (0.03 to 0.09%; 30 to 90 mg/100 g) for soy isolate [10]. Seena et al. also recorded a value of 5.3 mg/100 g which reduced to 3.58 mg/100 g with treatment for *Canavalia cathartica* [11]. Bhartiya et al. reported a value range of 1.64 to 1.73 mg/g for Horsegram [15]. The obtained result in this project was close in value to that obtained for horse gram and *canavalia cathartica*. The increase observed could be as a result of decrease in phytate content with germination.

Table 4: Elemental Analysis of Brown Solojo Isolate

BS	Ca	Na	Zn	Fe	Mg	K	Cu	Mn
Raw	113.48±0.51	726.59±1.99	37.07±0.39	3.42±0.47	55.89±0.71	137.02±0.62	0.82±0.04	0.98±0.05
6 h	138.17±0.62	696.95±3.12	62.66±0.45	4.22±0.62	69.91±0.50	248.11±0.48	1.09±0.03	1.24±0.09
24 h	156.54±0.60	547.96±2.58	84.43±0.28	5.46±0.56	71.85±0.38	497.58±0.86	1.27±0.03	1.28±0.17

36 h	169.43±0.97	417.81±0.80	108.27±0.28	7.37±0.63	82.54±0.41	580.66±0.13	1.38±0.06	1.39±0.06
48 h	175.08±0.26	387.68±1.48	115.00±0.38	8.47±0.62	100.78±0.53	792.85±0.47	1.50±0.02	2.30±0.06
72 h	220.75±1.21	264.35±1.48	120.57±0.48	9.40±0.67	124.17±0.69	925.97±0.83	1.61±0.05	3.10±0.21

BS: Brown solojo cowpea protein isolate

The potassium (K) content was observed to rise with germination with value ranging from 206.99 ppm to 1118.57 ppm (20.70 to 111.86 mg/100 g); 398.39 to 1268.98 ppm; 123.34 to 771.73 ppm; 137.02 to 925.97 ppm for FFDAS, FFBS, DAS and BS respectively. Al-Numair et al. observed increase in potassium content with germination time for the Faba bean and the white beans cultivars and higher values (1037 to 1169 mg/100 g; 1319 to 1497 mg/100 g for Faba beans and white beans respectively) [7]. Seena et al. and Desalegn, on the contrary recorded reduction in potassium quantity with treatment for *Canavalia cathartica* and germinated chickpea respectively, this, like observed in the case of solojo samples, could be due to leaking of the mineral into processing water during germination [11,13].

Conclusion

Biochemical modification which involves the activation of the intrinsic enzymes of the Solojo cowpea seed itself by germination was carried out for different hours for the two varieties, i.e. the dark-ash and the brown solojo beans.

Germination was also found to improve foaming capacity of flour and the protein isolate of the seed. The improvement brought to foaming capacity as a result of the addition of salt, further reiterate improvement in their ability to be useful for the production of cakes and pastries among other food. The capability of protein to form gels and produce a structure matrice able to hold water, flavours, sugars and food ingredients is important in food applications and in the development of new products, thereby contributing an added magnitude to protein functionality. The low gelation concentration noticed may be an advantage in the use of these flours for the formation of cord or as an added ingredient to other gel forming materials in food products as little quantity will produce the desired gelation needed and this help in product acceptability.

Recommendation

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 flavor retention and mouth feel of foods will be greatly enhanced if used in food formulation.

This research work also shows that biochemical modification (germination/malting/sprouting) had an enormous impact on the nutritional composition, functional properties, mineral bioavailability, 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 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.

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