ISSN: 3066-716X
Research Article
Journal of Clinical and Biomedical Advances

Differential Scanning Calorimetry (DSC) Studies on Temperature-Denatured Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp)

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Submitted: 11 November 2023 **Accepted:** 15 November 2023 **Published:** 23 November 2023

https://doi.org/10.63620/MKJCBA.2023.1013

Citation: *Adeyoju, O. A., Chibudike, H. O., Oluwole, B. O., Adebowale, K. O., Olu-Owolabi, B. I., & Chibudike, C. E. (2023). Differential Scanning Calorimetry (DSC) Studies on Temperature-Denatured Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp). J of Clin Bio Med Adv, 2(4), 01-07.*

Abstract

Calorimetry is the primary method utilized for checking the heating characteristics of samples in order to ascertain a correlation betwixt heat and precise tangible qualities of materials. Found to be a straighter approach to determine the ΔH connection with the method of interest. Investigation into the Differential Scanning Calorimetry (DSC) analyses on Temperature-denatured Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp) was carried out. The Differential Scanning Calorimeter is used for the determination of the denaturation temperature (Td) of the sample which is taken as the temperature at the maximum peak. DSC measures enthalpy of unfolding resulting from heat denaturing. The transition midpoint Tm is taken as the thermal reading, in which 50% of the protein retains its indigenous configuration (shape), and the others remains denatured. Higher Tm values depicts a steadier molecule. Using DSC, additionally we can find the phase transition of the material, which is Tp (Denaturation temperature at the maximum peak). The onset temperature (To) of DAS ranged between 257.47±1.00 and 295.18±1.05oC, the end set temperature (Te) were between 293.38±0.45 and 313.85±0.62oC, while the peak temperature (Tp or Td) is the denaturation temperature. The denaturation temperature is also the temperature of thermal unfolding of the molecules of the protein. It ranged between 279.37±0.90 and 299.36±0.67 oC with the 24 h germinated isolate having the highest denaturation temperature while the 72-h germinated isolate had the lowest. The onset temperature (To) of DAS ranged between 257.47±1.00 and 295.18±1.05oC, the end set temperature (Te) were between 293.38±0.45 and 313.85±0.62oC, while the peak temperature (Tp or Td) is the denaturation temperature. The BS isolate similarly had its onset temperature ranging between 218.71±0.75 and 294.64±0.45oC, end set temperatures between 244.27±0.45 and 302.71±0.67 oC, while the denaturation temperature was between 232.36±0.5c and 298.56±0.78 oC. The raw BS however had the highest Td (Table 4. 98). DSC chart in the appendix. Enthalpy is connected with the energy needed for a transition, while Td is expressed as the temperature at which 50% of the molecules have gone through thermal transition. Heat profiles detected in the legumes were connected with disparity in the chemical configuration, thermal characteristics, and quality of the protein fractions. Noticed the existence of a denaturation peak (Td) at very high temperature which ranged between 195 and 210 °C. These values were lower than those of DAS and BS isolates [1]. The stability and the configuration of proteins were also observed to be governed by the equilibrium between the hydrophilic and hydrophobic side chains of amino acid residues of proteins.

Keywords: Solojo Cowpea, Under-utilized legumes, BS, DA, FT-IR, Un-germinated, WAC (Water Absorption Capacity)

Introduction

Differential Scanning Calorimetry (DSC) analysis detects protein denaturation which is generally an irreversible thermally induced process by the primary scanning, while, glass transition (Tg) can be reversed and is detectable in any heating or cooling scanning, as long as the total nitrogenous material has been made to attain disarranged composition. Hence, the estimate of the Tg of proteins must be derived from the next heat scanning, after the denaturization of protein have taken place and any aging development have been removed [2].

Some areas where calorimeters are used repeatedly include chemistry, biochemistry, cell biology, biotechnology, and pharmacology. It has also been found recently to be useful in nanoscience for measuring thermodynamic characteristics of the bio-molecules and Nano-sized samples [3]. The Differential Scanning Calorimeter is used for the determination of the denaturation temperature (Td) of the sample which is taken as the temperature at the maximum peak [1]. Differential Scanning Calorimeter (DSC) determines the heat absorbed or released during phase transition, which could be endothermic, like melting or exothermic like crystallization. DSC is commonly used in the determination of heat resistivity of proteins and to determine the heat specifications of unfolding of protein. DSC offers an array of operations in the assessment of factors playing crucial function in the stability of proteins [3]. In DSC, thermodynamic parameters of the compound can also be calculated. TG-DTA- studies can be used to determine the melting point, polymorphism, heat stability of the compound, and often by comparison the structure of the compound can be studied. Using TG-DTA one can tell the melting or decomposition point of the material. Degradation temperature is taken as the start of the second mass removal step in TGA, the initial step being ascribed to water removal [1]. The difference between DSC and TGA is that, DSC is used for thermal transitions, while TGA for thermal stability. Tg, liquefying temperature of the materials can be measure by DSC, while TGA will give you loss or gain of weight of any material with temperature (Degradation)

Over time, the utilization of Isolated and concentrated proteins of vegetable grains has increased tremendously due to improved information about their functional characteristics, preparation and nourishing value. Isolated total nitrogenous material oftentimes manifest enhanced character and taste in comparison to the native flour; hence, they have better utilization as nutritional and functional ingredient in most food production [4]. In addition, they contain much higher protein content than flour or meal, so the same supplementation can be obtained with lesser quantity of the isolate than the flour [5]. Although, Soybeans have enjoyed greater recognition compared to other legume seeds, it is very important to exploit other sources of concentrated plant proteins [6, 7].

Thermal Analysis

Thermal denaturation of proteins is typically an irreparable procedure discovered in the first heating scan during DSC analysis. In contrast, once the protein has been transformed to a denatured pattern; DSC can analyze for glass transition at any heating or cooling examination because it is a reversible process. The glass transition temperature of proteins is often taken after the proteins have been denatured and any aging experience expunged this is usually observed to be from the second heating scan [8]. Water is a dominant factor determining the thermal stability of proteins. According a decrease in DH (Delta H- Enthalpy) value for a protein system is indicative of partial denaturation and in the event of complete denaturation, no endotherm would appear [9]. The denaturation enthalpy represents the composite of several contributions, such as the negative heat (exothermic contribution) of disruption of hydrophobic interactions, which is maximum at 60–80oC and decreases to a negligible level at 110oC. The positive contribution (endothermic) of disruption of hydrogen bonds slightly depends on temperature and also exothermic protein aggregation. Based on these results, it can be affirmed that denaturation of protein during processing of cowpea seed to flour involved more exothermic contributions. This seems logical because the temperature employed in the drying operation (60oC) falls within the range where exothermic effects dominate. Gelatinization of starch is an endothermic phenomenon, while protein denaturation is a blend of both exothermic and endothermic reactions.

Materials and Methods

Raw Materials

Two varieties of the underutilized cowpea (V. unguculata) found in South west region of Nigeria where it is called 'solojo' were used (Figures 1 and 2). Seeds obtained from Bodija market in Ibadan, Western Nigeria, were screened to get rid of every irrelevant materials and unwholesome seeds. The beans were then portioned into six (6). The solojo seeds for germination were sterilized by soaking in 0.07% sodium hypochlorite for 30 min, then, 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 (Figure 3).

Figure 1: Brown Solojo Cowpea

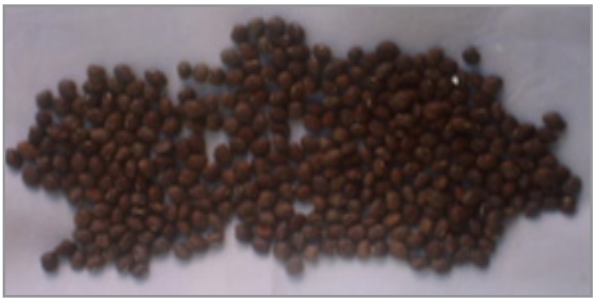


Figure 2: Dark-Ash Solojo Cowpea

Preparation of Flours

Raw Flour

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

6 h Soaked Flour

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

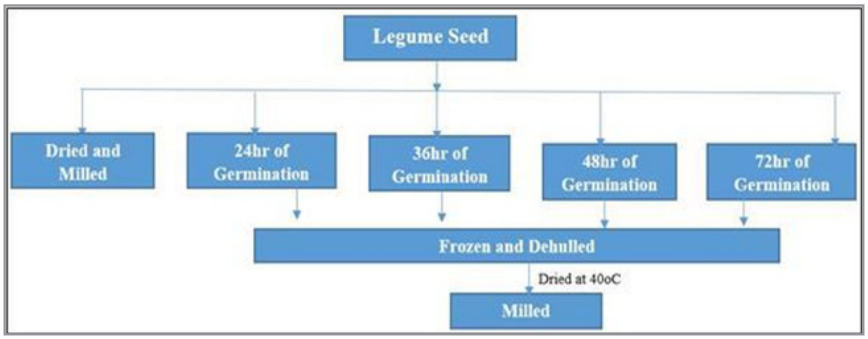


Figure 3: Preparation of Beans Flour/Schematic representation

Thermal Analysis

The thermal characteristics (stability) of the powdered sample was evaluated by differential scanning calorimetry (DSC 2013, TA instruments, New castle, USA) functioning with STAR software version 8.1, and furnished with an intercooler. Powdered sample of 5.5mg (Dry basis) was measured in an aluminum dish and analysis carried out under a dry nitrogen purge (25 mL/min) and was hermetically sealed. Heat was then applied to the test material pans from 20oC to 350oC at an interval of 10oCmin-1. The instrument

calibration for temperature and heat flow was done using standard indium and zinc of high-purity. The samples were by fast cooling brought to desired temperature (25°C) equilibrium. The curves obtained using instrument software was used to analyses for onset temperature (To), peak temperature (denaturation) (Td), end set temperature (final) (Tf), and specific heat capacity (enthalpy of denaturation) (ΔH). The mean results of triplicate determinations were reported [10, 11].

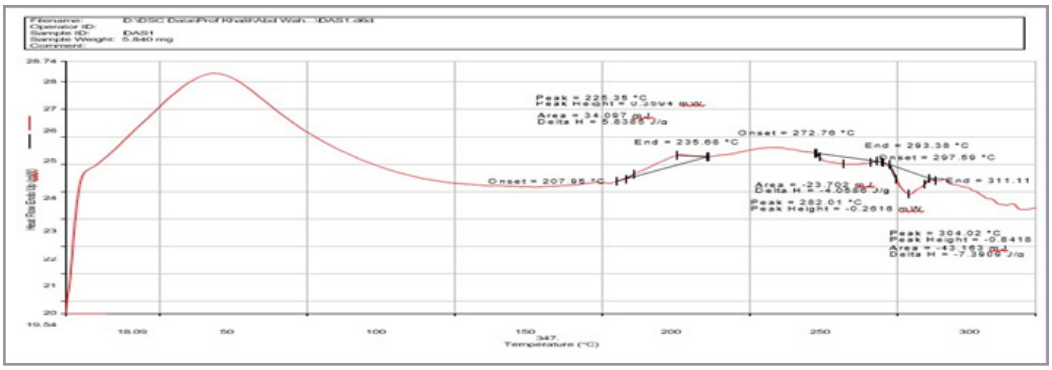
Moisture generally influence transition temperature, kinetic and reversibility of transitions, as well as the thermal capability therefore analysis was carried out at 15 % moisture content in a hermetic closed pan in order to prevent loss of moisture. Td was taken as temperature at the maximum peak.

Results and Discussion

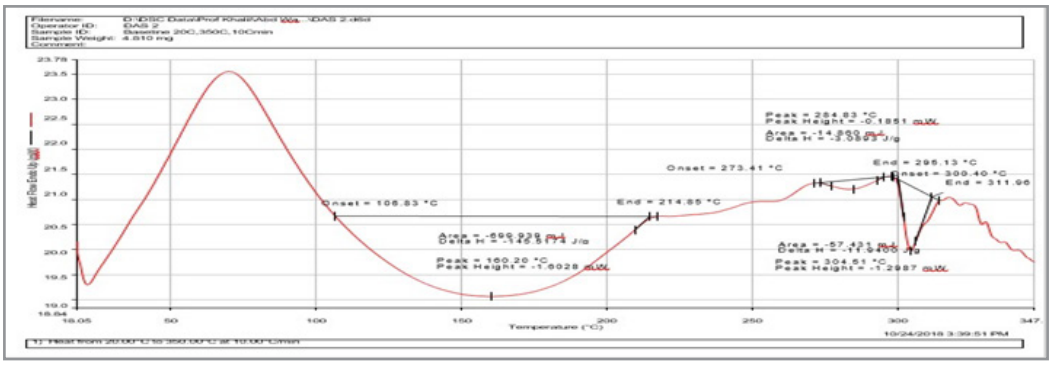
The denaturation temperature is also the temperature of thermal unfolding of the molecules of the protein. It ranged between 279.37±0.90 and 299.36±0.67 oC with the 24 h germinated isolate having the highest denaturation temperature while the 72-h germinated isolate had the lowest. The BS isolate similarly had its onset temperature ranging between 218.71±0.75 and 294.64±0.45oC, end set temperatures between 244.27±0.45 and 302.71±0.67 oC, while the denaturation temperature was between 232.36±0.5c and 298.56±0.78 oC. The raw BS however had the highest Td (Table 1).

Table 1: Differential Scanning Calorimeter DSC of DAS and BS Protein Isolates

Sample (DAS)	T_{onset} °C	T_{endset} °C	T_{peak} °C	ΔH Jg^{-1}
Raw	272.76±0.75b	293.38±0.45c	282.01±0.25b	4.06±0.03b
6 h	273.41±0.50b	295.13±0.80c	284.83±0.65b	3.09±0.06b
24 h	295.18±1.05a	303.97±0.72b	299.36±0.67a	9.95±0.04d
36 h	267.85±0.90c	313.85±0.62a	293.23±0.36a	14.73±0.10a
48 h	259.05±0.65d	294.88±0.87c	279.86±0.90b	7.57±0.06c
72 h	257.47±1.00d	294.33±1.02c	279.37±0.90b	9.92±0.10d
Sample (BS)	**T_{onset} °C**	**T_{endset} °C**	**T_{peak} °C**	**ΔH Jg^{-1}**
Raw	272.76±0.75b	293.38±0.45c	282.01±0.25b	4.06±0.03b
6 h	273.41±0.50b	295.13±0.80c	284.83±0.65b	3.09±0.06b
24 h	295.18±1.05a	303.97±0.72b	299.36±0.67a	9.95±0.04d
36 h	267.85±0.90c	313.85±0.62a	293.23±0.36a	14.73±0.10a
48 h	259.05±0.65d	294.88±0.87c	279.86±0.90b	7.57±0.06c
72 h	257.47±1.00d	294.33±1.02c	279.37±0.90b	9.92±0.10d

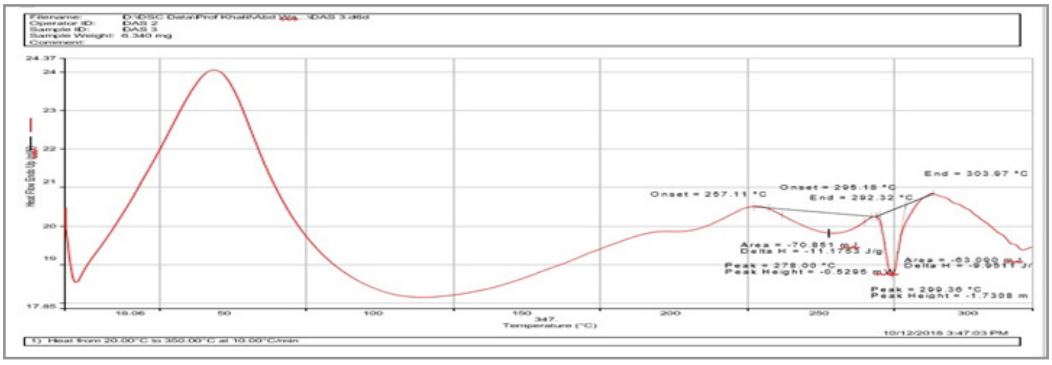


Appendix 1: DSC Thermograms of "Solojo" Cowpea showing DSC Curve of Raw DAS protein isolate



Appendix 2: DSC Curve of 6 h DAS isolate

The BS isolate similarly had its onset temperature ranging between 218.71±0.75 and 294.64±0.45oC, end set temperatures between 244.27±0.45 and 302.71±0.67 oC, while the denaturation temperature was between 232.36±0.5c and 298.56±0.78 oC.



Appendix 3: DSC Curve of 24 h DAS isolate

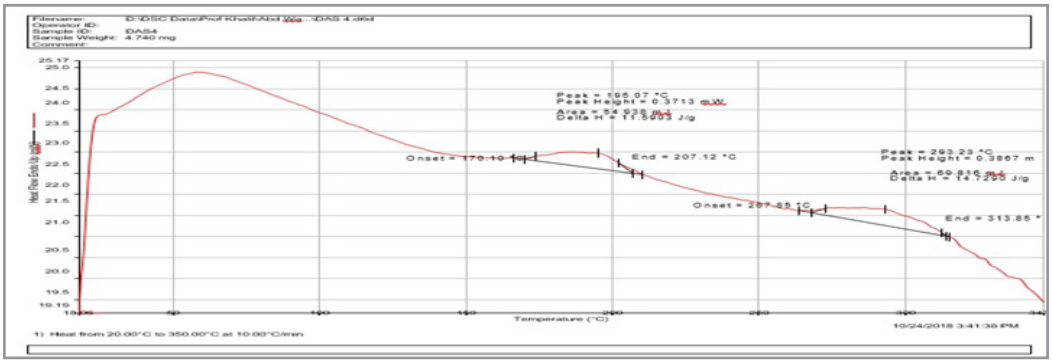
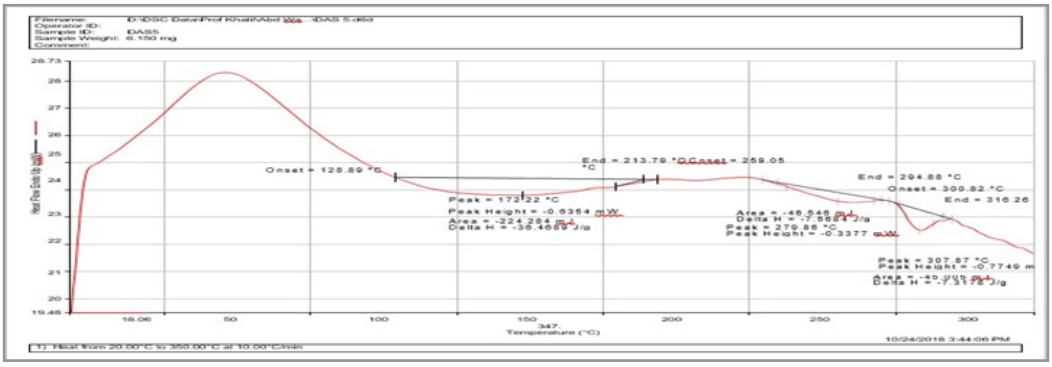
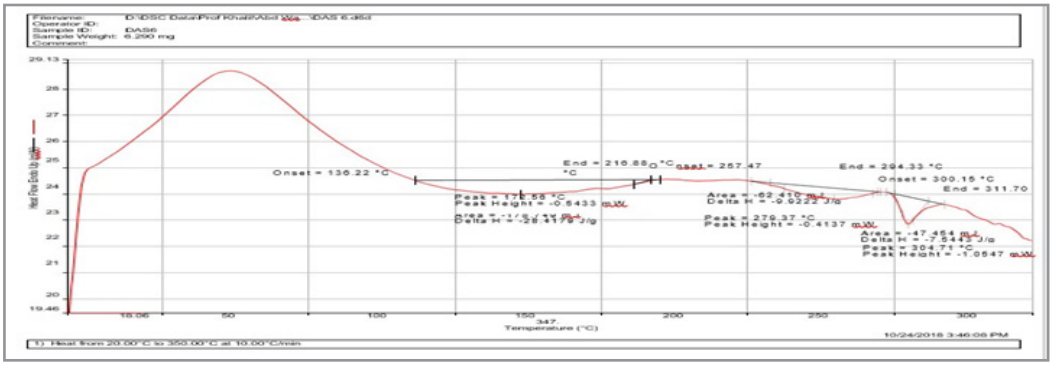


Figure 4: DSC Curve of 36 h DAS isolate

The onset temperature (To) of DAS after 24hours as seen in table 1 decreased from 295.18±1.05oC to 257.47±1.00oC after 72hours and the end set temperature (Te) increased from the raw sample 293.38±0.45 to 313.85±0.62oC after 36hours, while the peak temperature (Tp or Td) is the denaturation temperature.

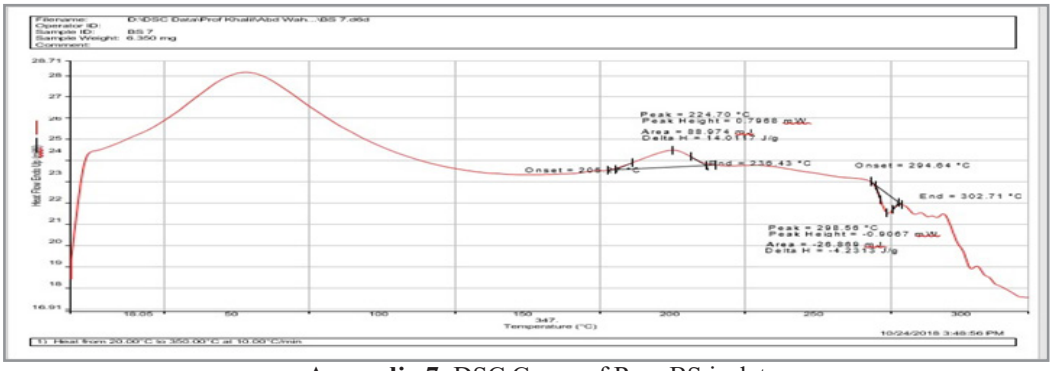


Appendix 5: DSC Curve of 48 h DAS isolate

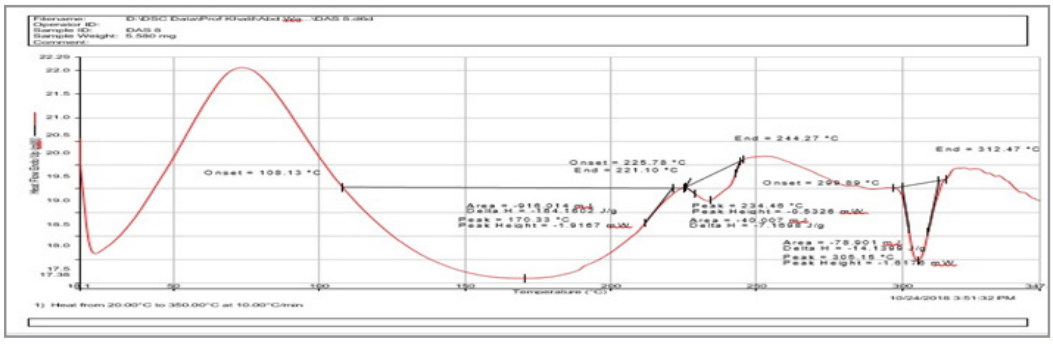


Appendix 6: DSC Curve of 72 h DAS isolate

The DSC curves as observed, exhibited the existence of only one broad endothermic peak between 63 and 74oC which coincide with the denaturation temperature (Td) of the PIs analyzed in the powder form. The only peak is that of total protein and consist of various donors as numerous protein portions are expected in the isolates.

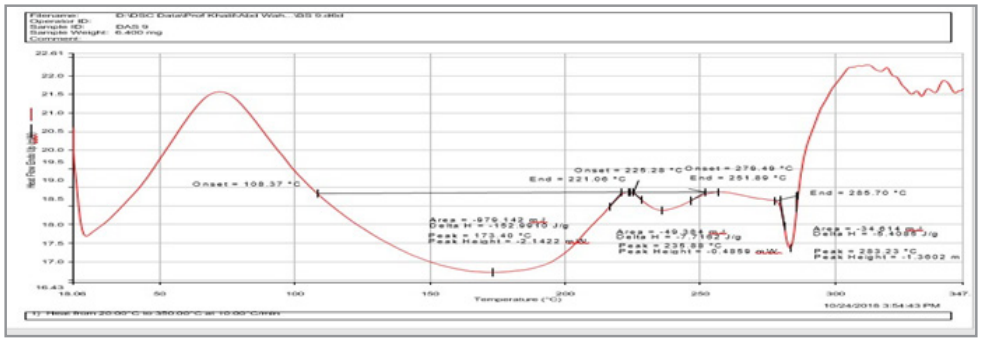


Appendix 7: DSC Curve of Raw BS isolate



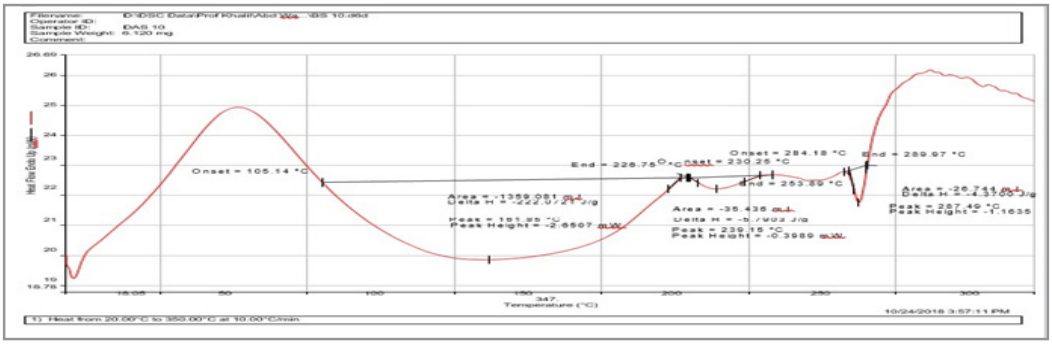
Appendix 8: DSC Curve of 6 h BS isolate

The PIs isolated at pH 10.0 and 11.0 without NaCl, exhibited reduced values of ΔH, 277.2 and 250.3 J/g respectively. pH in the alkaline region gives rise to lesser values of ΔH, indicating that at high pH situation, the protein arrangement is reformed, provoking its unfolding.

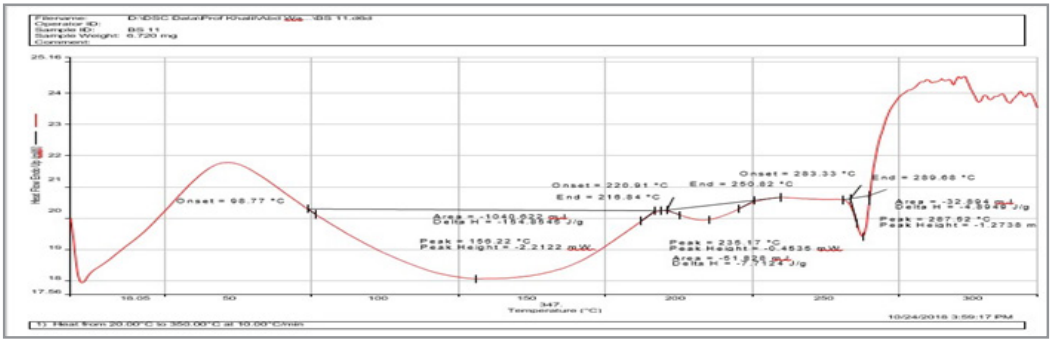


Appendix 9: DSC Curve of 24 h BS isolate

Enthalpy is connected with the energy needed for a transition, while Td is expressed as the temperature at which 50% of the molecules have gone through thermal transition. The contrast in heat profiles detected in the legumes were connected with disparity in the chemical configuration, thermal characteristics, and quality of the protein fractions

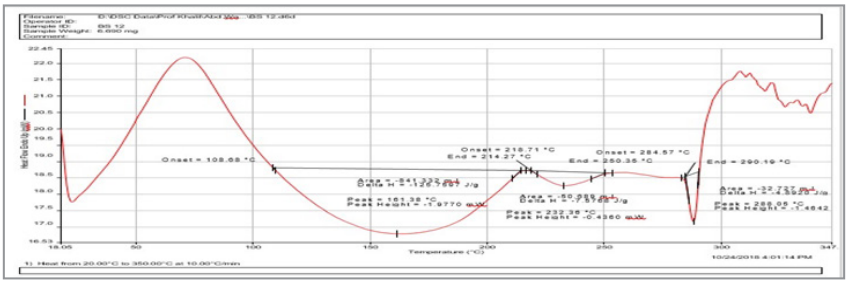


Appendix 10: DSC Curve of 36 h BS isolate



Appendix 11: DSC Curve of 48 h BS isolate

Noticed the existence of a denaturation peak (Td) at very high temperature which ranged between 195 and 210 C. These values were lower than those of DAS and BS isolates. The stability and the configuration of proteins were also observed to be governed by the equilibrium between the hydrophilic and hydrophobic side chains of amino acid residues of proteins [1].



Appendix 12: DSC Curve of 72 h BS isolate

This was observed to be within the range obtained for the solojo cowpea. It was also observed that the biggest value of ΔH 335.5 J/g, that is, the energy needed to totally denature the protein isolate (PI), was obtained for the PI isolated at neutral pH. These outcomes imply that protein isolated at close to iso-electric precipitation point pH and with enthalpy close to pH (7), continue in their natural form, retaining an entwined formation.

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 utilized in various food type [12].

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 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.

Recommendation

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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