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Acetylated and Succinylated Derivatives of African Yam Bean (*Sphenostylis sternocarpa* Hochst. Ex A. Rich.) Harms) Protein Isolates

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Abstract: Protein isolate was prepared from African yam bean (*Sphenostylis sternocarpa* Hochst. Ex A. Rich.) *Harms*). This was modified by acetylated using acetic anhydride and succinylated with succinic anhydrides. Proximate analysis revealed that moisture and ash content increased following acetylation and succinylation. On the other hand, both acetylation and succinylation reduced percentage crude fat and protein. Acetylation and succinylation reduced protein solubility in the acidic pH range below the isoelectric point (4.5) of the protein concentrate, but improved the solubility of the unmodified protein concentrate at the isoelectric point and pH range alkaline to the isoelectric point. Both acetylation and succinylation increased the water absorption capacity of native protein isolates at all levels of ionic strength investigated (0.1-1.0 mol L^{-1}). Acetylation improved oil absorption capacity but reduced on succinylation. Maximal emulsifying activity of native and modified proteins were obtained at pH 10. Emulsion stability of acetylated and succinylated proteins were higher than those of native proteins in the range of pH 4-10, but lower when the pH was 2. Foam capacity and stability of both native and modified proteins increased with increase in protein concentration. Foam capacity of modified proteins also, increased progressively with increase in pH from 2-10. Gelation capacity of both native and modified proteins was maximal at the region of isoelectric point (pH 2.0-4.0) and at ionic strength between 0.2-0.5 mol L^{-1}.

Key words: African yam bean protein isolate, acetylation, succinylation, functional properties

INTRODUCTION

Legumes are important ingredients of a balanced human diet in many parts of the world due to their high protein and starch contents (Czuchajowska *et al*., 1998). They have been consumed traditionally as whole seeds or as ground flour after dehulling. The rapidly growing food industry, which constantly demands new ingredients has drawn researchers to legume components i.e., starch and proteins (Czuchajowska *et al*., 1998). The legumes have been subjected to protein isolation (Soetrisnot and Holmes, 1992; Clemente *et al*., 1998; Adebowale *et al*., 2005). The increasing demand for plant protein in lieu of expensive animal protein has been emphasized in the earlier publications (Adebowale *et al*., 2005, 2007; Adebowale and Adebowale, 2007, 2008). Several researchers have also expounded of this development (Dzudie and Hardy, 1996; Chel-Guerrero *et al*., 2002). Therefore, the need to intensify research efforts aimed at identifying new legume sources.

African yam bean (*Sphenostilis sternocarpa* Hochst. Ex A. Rich.). Harmsreadily comes to mind. It belongs to the family, Papilionaseae, which is sometimes classified in the sub family Leguminosae. It is usually cultivated for its edible seeds and tuberous roots. It is an important crop in western, central and some parts of East Africa. It is cultivated for both its seeds and tubers. The seeds have protein content which ranged from 21.0-29.0% with about 50% carbohydrate mainly starch (Eromosele *et al*., 2008). The seeds vary in size and colour (marbled, brown, dark brown, black and grey). The protein content is however, lower than soybean (38%), but its amino acid spectrum indicated that lysine and methionine which are limiting amino acids in most vegetable proteins are better than in most legumes including soybean (Evans and Haismer, 1979). *S. Sternocarpa* has exceptional ability of adapting to low land tropical soil conditions with high yields. It is generally, regarded as underutilized crop due to its low social esteem and lack of detailed information on its compositional analysis.

Seed proteins provide essential amino acids, but they should also possess requisite functional characteristics for their successful utilization in various formulations. The important functional properties required in food ingredients include solubility, oil/fat and water absorption

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capacity, emulsion capacity and stability, foaming capacity and stability, gelation characteristics, whippability and good sensory attributes. Maximal utilization of proteins requires the matching of variety of functional and nutritional characteristics with the complex needs of different food products. However, native proteins have limited functionality and this has necessitated the need for development of processes to improve plant protein functional characteristics. Against this background, research has been reported on different modification methods of various proteins. These processes include physical modification (Heinzelmann *et al*., 1994), genetic (Creamer, 1994) enzymatic (Casey *et al*., 1991; Thomas and Leoffler, 1994), chemical (Wagner and Gueguen, 1999).

The schematic diagram of the acylation of protein is shown in Fig. 1.

Chemical derivatisation through acylation of amino acids residues with acetic and succinic anhydrides have been used to improve functional properties of many plant proteins. These includes wheat (Grant, 1973), Soybean (Franzen and Kinsella, 1976), peanut (Beuchat, 1977), sunflower (Kabirrulah and Wills, 1982); pea (Johnson and Brekke, 1983) winged bean (Narayana and Narasinga Rao, 1984) rapeseed (Dua *et al*., 1996) cotton seed (Rahman and Narasinga Rao, 1983) and Chickpea (Liu and Hung, 1998). Succinylation and acetylation relations involve chemical conversion of groups such as ε-amino groups of lysine in protein with acetic anhydride and succinic anhydrides. Reaction with acetic acid results in the elimination of the positive charges of lastly residues and the corresponding increase in electronegativity. Acylation with succinic or other dicarboxylic anhydrides results in the replacement of the positive charge with negative charge of the lysly residues (Johnson and Brekke, 1983). This leads to tremendous increase in electronegativity of the proteins resulting in unfolding of the protein chain. Earlier researchers have observed, an increase in the nitrogen solubility, emulsifying activity and stability and foaming capacity of the protein with acylated leaf proteins (Franzen and Kinsella, 1976). Similar observation have been reported for succinylated and acetylated winged bean protein (Narayana and Naransinga Rao, 1984) succinylated peanut protein (Shyama and Rajageopal Rao, 1978) sun flower proteins (Kabirrulah and Wills, 1982).

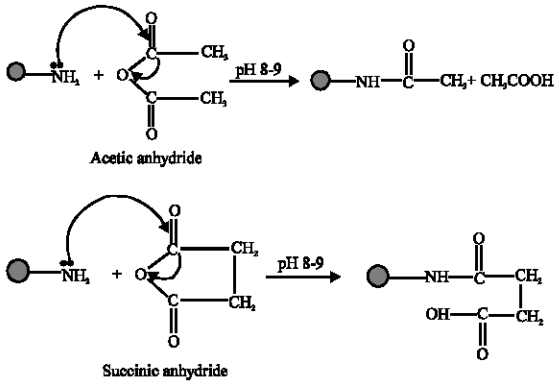


Fig. 1: Schematic diagram of the acetylation and succinylation reaction of protein

Adebowale *et al*. (2009) has just reported the heat moisture treatment and annealing of African yam bean starch. As far as we know, no report is available in literature on the acylation of African yam bean protein isolate. However, acylation technique have been employed in protein modification for certain food products. These include coffee whiteners (Melnychyn and Stapley, 1973), carbonated beverages (Creamer *et al*., 1971), mayonnaise (Evans and Irons, 1971) and margarine (Evans, 1970). A reversible acylation could be advantageous in that during the digestion, amino groups such as those of lysine would be deacylated and lysine would become nutritionally available in the medium. Deacylation of soybean after modification have been studied by Jones and Tung (1983) and it was established that substantial reversibility of the amino acids took place in mild acidic conditions.

The objective of this study was to evaluate, the effect of acetic anhydride and succinic anhydride on some functional properties of African yam bean (*Sphenostylis stenocarpa*) protein isolate. The solubility profile, water and oil absorption capacity, foaming and emulsifying properties of the native and modified protein will be examined in relation to the influence of ionic strength, pH and presence of other salts.

It is hoped that the data will provide information on the application of the modified protein as food hydrocolloids.

MATERIALS AND METHODS

Preparation of flours: Cleaned seeds of African yam bean seeds were cracked with a hammer mill followed by winnowing of the seed coats before milling into flour in a hammer mill. The meal was sieved to pass 0.5 mm mesh sieve and kept in air-tight plastic container in a refrigerator at 4°C prior to use. The fraction collected (<0.5 mm) is referred to as flour in this study. A portion of the flour was defatted by extracting with n-hexane in a soxhlet extractor for 9 h followed by air-drying in the fume cupboard for 24 h. The full fat and defatted samples were then used for the analysis.

Preparation of protein isolates: The procedure for isolate preparation was as described by Lqari *et al.* (2001). The basic steps are as follows:

The slurry (1:20, flour-to-water ratio) at pH 6.37 was first extracted for 10 min at room temperature, thereafter the slurry was further stirred for 2 h using a Gallenhamp magnetic stirrer and the pH was adjusted to the desired pH using 1 M NaOH. This was followed by centrifuging in a Sorvall RC5C automatic super speed refrigerated centrifuge at 10,000×g for 30 min at 5°C. After centrifugation and recovery of supernatant, 3 additional extractions were carried out with half of the volume of the initial water. The supernatants were pooled and precipitated at pH 5.0, the Isoelectric Point (IEP). The precipitate formed was subsequently recovered by centrifugation at 10,000×g for 15 min at 5°C. The precipitate was washed twice with distilled water adjusted to pH 5.0 with HCl. The precipitate was neutralized by the addition of 1 M NaOH. The final protein isolate was obtained by lyophilization. A schematic diagram of the isolation procedure is shown in Fig. 2.

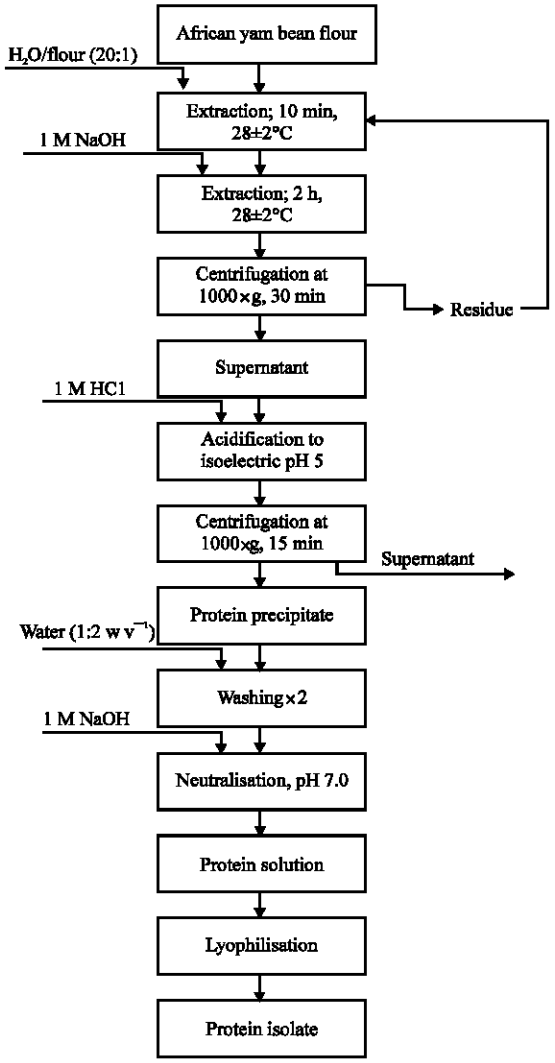


Fig. 2: Preparation of protein isolate

Modification of the protein isolates

Succinylation: This was determined according to the method described by Beuchat (1977). A 15% (w v^{-1}) suspension of the isolates was prepared in water and adjusted to a pH 7.7. Succinic anhydride was added to the suspension over a 2 h. Period at levels 10, 40, 70, 100 and 130% of the weight of proteins in the suspension. The pH was maintained at 7.7±0.3. Slurries were dialysed against distilled water at 2°C for 24 h freeze dried and passed through a Wiley mill twice.

Acetylation: This was prepared according to the method of Wanasundara and Shahadi (1997). The proteins was acetylated at room temperature with three levels of acetic anhydride (0.05, 0.10 and 0.20 g g^{-1} of protein equivalents) at pH 8.5±0.1. After the acylation reaction was completed proteins were precipitated and dispersed in distilled water, neutralised and then dialysed against distilled water over a 24 h period at 4°C. Dialysed protein solutions was then lyophilised and stored at 4°C.

Determination of the degree of acylation: The degree of acetylation or succinylation of free amino acid groups was determined according to the trinitrobenzene sulphonic acid TNBS already described by Habeeb (1966). Protein solution (1 mL) was added to 1 mL of 0.1% TNBS and 1 mL of 4% $NaHCO_3$. The solution was allowed to react at 40°C for 2 h. Then 1 mL of 10% dodecyl sodium sulphate was added to solubilize the protein followed by addition of 0.5 mL of 1 M HCl. The absorbance was then recorded at 335 nm using spectrophotometer against a reagent blank. The absorbance of the control protein isolate was set to 100% free amino groups and the degree of acylation of the modified samples was calculated based on the decrease in absorbance because fewer amino groups will be able to react with the TNBS reagent.

Functional properties of protein isolates

Protein solubility: Protein solubility was determined by the method of Sathe *et al.* (1982) with some modifications stated below. The suspension (0.2%) of the flour in distilled water was adjusted to different pH values of between 2 and 11 using either 1 M HCl or 1 M NaOH. Percent nitrogen in each supernatant was determined by micro Kjedahl method according to the method already described by AOAC (1990). Percent soluble protein was calculated as percent nitrogen multiplied by 6.25 on wet basis.

Water and oil absorption capacity: Water absorption capacity was determined using the method of Sathe and Salunkhe (1981) with slight modifications. The 10 mL of distilled water was added to 1.0 g of the sample in a beaker. The suspension was stirred using a magnetic stirrer for 5 min. The suspension obtained was thereafter centrifuged at 4000×g for 30 min and the supernatant measured in a 10 mL graduated cylinder. The density of water was taken as 1.0 g cm^{-3}. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The same procedure was repeated for oil absorption except that oil was used instead of water. Studies were conducted to investigate the influence of ionic strength on the water and oil absorption capacity by varying the ionic strength of the medium using 0.1, 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0 MKCl solutions.

Determination of the gelation concentration: The least gelation concentration was determined by the method of Sathe *et al.* (1981). Test tubes containing suspensions of 2, 4, 6, 8 up to 20% (w v^{-1}) flour in 5 mL distilled was heated for 1 h. In boiling water, followed by cooling in ice and further cooling for 2 h at 4°C. The least gelation concentration was the one at which the sample did not slip when the test tube was inverted. Effect of ionic strength was investigated by preparing sample solutions (2-20% w v^{-1}) at various concentrations of KCl solutions of varying ionic strength ranging from 0.1-1.0 M. Effect of carbohydrates was also studied by adding maltose, sucrose, lactose and starch at 0.25 g g^{-1} of the flour and isolate. LGC was determined as described earlier.

Foaming properties: The foaming characteristics of the protein isolates were investigated using the methods of Coffmann and Garcia (1977) and Vani and Zayas (1995). The procedure involved blending of 50 mL of a protein suspension (1%, adjusted to the required pH level) in an Ultra-Turrax homogeniser (Janke and Kunkel, Staufen, FRG) at 12,000 rev min^{-1} for 1 min at about 25°C and determining the volume of foam (mL) that was present above the surface of the liquid contained in a glass cylinder of 100 mL. Foam expansion was expressed as follows:

$$\text{Foam expansion}\,(\%) = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

$$\text{Foaming stability}\,(\%) = \frac{\text{Foaming volume after time t}}{\text{Initial foam volume}} \times 100$$

Emulsifying properties: The emulsifying properties of the samples were determined by measuring the Emulsion Activity Index (EAI) and the Emulsion Stability Index (ESI) by the turbidimetric technique as described by Pearce and Kinsella (1978) with some modifications.

Emulsion activity index: Emulsion of each protein dispersion was prepared according to the method described by Lqari *et al.* (2001). Protein sample (3.5 g) was homogenised for 30 sec in 50 mL water using a model A polytron homogeniser (Brinkmann, Wesbury, New York) at 10,000 rpm). Canola oil, 25 mL (Nobisco Foods, Winston-Salem, N.C., USA) was added to the mixture and homogenised again for 30 sec. Then, another 25 mL of canola oil was added and the mixture homogenised for 90 sec. One gram of the emulsion was weighed into a 100 mL standard flask and made up to mark with 0.1% solution of dodecyl sodium sulphate. The solution (1 mL) was withdrawn and 4 mL of the 0.1% dodecyl sodium sulphate (0.002%) was added. The absorbance of the diluted emulsions was measured by a spectrophotometer (UV/VIS Spectrophotometer Lambda 3B; Perkin-Elmer Norwalk, Conn., USA) at 500 nm in 1 cm path length cuvettes. The absorbance was read initially and turbidity, T, was calculated using the formula:

$$T = \frac{2.303A}{I}$$

Where:

A = Absorbance at 500 nm
I = Path length of cuvette (cm)

The oil volume fraction of emulsion was estimated by drying 5 g of the emulsion in an IsoTemp oven (Fischer Scientific) to a constant weight at 105°C. The oil volume fraction was calculated from:

$$\phi = \frac{W_d - (E \times W_1)}{W_d + W_1\left[\left(((1+E) \times D_o)/D_m\right) - E\right]}$$

Where:

D_o = Density of oil
D_m = Density of protein solution
E = Concentration of solutes (mass per unit mass solvent)
W_1 = Loss of weight of emulsion on heating/weight of emulsion
W_d = Dry weight/weight of emulsion

The Emulsion Activity Index (EAI) was then calculated as follows:

$$EAI = \frac{2T}{\varphi C}$$

Where:

T = The turbidity (calculated from the equation above)

ϕ = The oil volume fraction (mL)

C = The weight (g) of the protein per unit volume of aqueous phase before emulsion is formed

EAI has units of area of interphase stabilised per unit weight of protein (i.e., $m^2 g^{-1}$).

Emulsion stability index: The emulsions were held at 4°C for 24 h and reanalyzed for emulsion activity as described above. An emulsion stability index was calculated by the formula:

$$ESI = \frac{T\,\Delta t}{\Delta T}$$

Where:

T = The turbidity value at 0 h

ΔT = The change in turbidity during the 24 h period

Δt = The time interval (24 h)

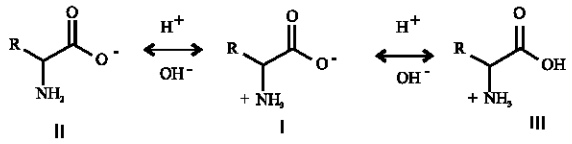
Statistical analysis: All experiments in this study are reported as mean of 3 replicate analysis. One-way Analysis of Variance (ANOVA) was carried out to compare between the mean values of different species of the seeds. Differences in the mean values were determined at $p<0.05$ (SAS, 1990).

RESULTS AND DISCUSSION

The chemical composition of native and acylated [Acetylated (AYB-Acet) and Succinylated (AYB-Succ)] African yam bean protein isolate is presented in Table 1. There was no significant differences ($p<0.05$) in the moisture content of the native and acylated protein isolate. However, ash content increased significantly ($p<0.05$) following modifications. This observation agrees with previous report of Naczk *et al.* (1986) on green pea protein isolates and grass pea protein isolate (Sumner *et al.*, 1981). Crude fibre were not detectable in the wheat germ protein reported by Tomoskozi *et al.* (1998). Variations might be due to isolation procedures employed for the proteins. The protein content of the proteins ranged between 91.25-92.50%. Sumner *et al.* (1981) earlier reported protein content ranging from 91-98% in different types of isolates prepared from field pea while, Deshpande and Campbell (1992) reported that grass pea protein isolate contained 83.3-92.1% protein depending on the solvent used in their preparation. Increase in ash content of the proteins after modification can be attributed to salts used during the modification process. Reduction in the fat content of the proteins after modification might be due further washing of the isolate after the precipitation while, the reduction in the pH after the modification process might be due to the acid anhydrides employed for the modification process.

Protein solubility: The pH-dependent protein solubility profile of the acetylated and succinylated African yam bean protein isolate is presented in Fig. 3. The solubility profile of the native African yam bean protein isolate showed that the solubility decreased as the pH was increased from 2.0-4.0. Subsequent increase in the pH increased protein solubility progressively. Highest solubility was observed at the extremes of the acidic alkaline pH ranges. Similar observation was reported for winged bean and Chickpea by Sathe *et al.* (1982) and Clemente *et al.* (1998).

This observation can be explained in terms of the prevalent charge on the constituent amino acids of proteins at various pH values. According to Kinsella (1979), amino acid exists in three different forms depending on the pH value, as shown:



where, I is a zwitterion or dipolar ion which predominates at the region of isoelectric point in protein. At this pH, minimum solubility takes place because of minimum repulsion among the constituent amino acids. The balance in positive and negative charges minimises the electrostatic repulsion and this reduces solubility of proteins at isoelectric pH. When, the pH of the solution

Table 1: Proximate composition of native, acetylated and succinylated African Yam bean protein isolate

	Parameters (g/100 g)							
Samples	Moisture	Ash	Crude fibre	Fat	Protein	Carbohydrate	Yield	pH
AYB-N*	6.05±0.05^a	2.07±0.04^a	0.02±0.00^a	0.30±0.01^a	92.50±0.45^a	ND	35.12	5.8
AYB-Acet	6.33±0.12^a	3.28±0.03^b	0.01±0.00^a	0.10±0.01^b	91.70±0.39^a	ND	98.86	5.0
AYB-Succ	6.45±0.06^a	3.77±0.05^b	0.03±0.00^a	0.10±0.01^b	91.25±0.55^a	ND	97.21	4.9

AYB-N*: African Yam Bean Native protein isolate, AYB-Acet: African Yam Bean Acetylated protein isolate. AYB-Succ: African Yam Bean Succinylated protein isolate. All values are expressed as Mean±SD of three determinations. Means values within columns with different superscripts are significantly different at $p<0.05$. Degree of acetylation 85%; Degree of succinylation 87%; Percentage yield calculated based on native protein isolate basis

Table 2: Effect of ionic strength on water absorption capacity of native, acetylated and succinylated African yam bean protein isolates

	Ionic strength of KCl solution (mol L^{-1})						
Samples	0.00	0.1	0.2	0.4	0.6	0.8	1.0
AYB-N	3.90±0.16^{a}	4.45±0.21^{a}	4.78±0.18^{a}	3.39±0.19^{a}	3.65±0.17^{a}	2.30±0.16^{a}	2.04±0.12^{a}
AYB-Acet	4.43±0.16^{b}	5.32±0.19^{b}	6.05±0.24^{b}	4.78±0.17^{b}	3.65±0.23^{a}	3.20±0.14^{b}	2.34±0.15^{a}
AYB-Succ	5.11±0.13^{c}	5.67±0.15^{c}	6.50±0.20^{ab}	4.97±0.18^{b}	3.95±0.25^{b}	3.58±0.16^{c}	2.84±0.11^{a}

AYB-N*: African Yam Bean Native protein isolate, AYB-Acet: African Yam Bean Acetylated protein isolate, AYB-Succ: African Yam Bean Succinylated protein isolate. All values are expressed as Mean±SD of three determinations. Mean values within columns with different superscripts are significantly different at $p<0.05$. Degree of acetylation 85%; Degree of succinylation 87%

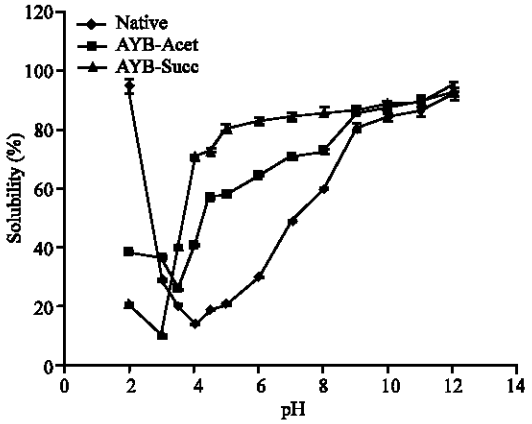


Fig. 3: pH dependent protein solubility profile of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation: 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

reduces below the isoelectic point, cation III predominates while, anion II dominates in alkaline medium. In both extremes of the pH scale, electrostatic repulsion improves and this enhances solubility; as observed in the current study at pH 2 and 12.

Acetylation and succinylation reduced protein solubility below their isolectric point (pH 3.0 for acetylated; pH 3.5 for succinylated protein isolate). Reduction in solubility following modification has been reported in earlier publications on wheat gluten and soy protein (Barber and Wartheson, 1982; Franzen and Kinsella, 1976). According to Paulson and Tung (1987), the solubility at both extremes of the pH region can also be explained in terms of the intra and intermolecular charge repulsion which, promotes protein unfolding resulting in fewer protein-protein interaction and more protein-water interactions. Succinylated was more soluble at isoelectric point compared with the actylated counterpart after the isoelectric point. Succinylation introduces longer side chains compared to acetylation. This involves the introduction of short chain repulsive forces, which results in better solubility of the succinylated moiety compared to acetylated derivative.

The effect of ionic strength on the water absorption capacity of the native, acetylated and succinylated is presented in Table 2. The water absorption capacity of the native protein isolate ranged from 2.04-4.78 mol L^{-1}. These values compared favourably with the water absorption capacity of similar legumes and in some cases higher values were obtained in this study. Idouraine *et al.* (1991) reported a water absorption capacity of 3.84 g g^{-1} for soy isolate; 5.28 g g^{-1} for *P. calcaturus*, 5.08 g g^{-1} for *D. lablab* 5.05 g g^{-1} for *P. angularis* (Dzudie and Hardy, 1996).

At low concentrations (0-0.2M KCl), the water absorption capacity is high. This is because hydrated salt ions binds weakly to charged groups on the protein molecule. The binding of ions to the protein molecule does not affect the hydration shell of the charged groups on the protein. Therefore, the increase in the water absorption capacity results from water associated with the bound ions. Upon further increase in the ionic strength (exceeding 0.2 M), larger amount of the water remains essentially bound to the salt ions thereby leading to the dehydration of the protein molecule with subsequent decrease in the water absorption capacity. A similar observation was reported by Idouraine *et al.* (1991). The increased water absorption capacity by acylated proteins over that of the control has been attributed to charge effect of the added acyl groups (Barber and Warthesen, 1982). In addition, after chemical modifications, high molecular weight protein may dissociate, thus facilitating enhanced water absorption as a result of increase in surface area.

Oil absorption capacity: Oil absorption capacity of native and modified protein isolates are presented in Fig. 4. The oil absorption capacity of the native protein isolate was 4.8 g g^{-1}. This result compares well with values of 4.77 and 4.38 g g^{-1} for *D. lablab* and *P. angularis,* respectively (Idouraine *et al.*, 1991) and 4.9 g g^{-1} reported for jack bean protein concentrate (Lawal, 2005). It is higher than the value of 3.29 g g^{-1} oil reported for soy bean protein isolate; 2.54 g g^{-1} reported for hyacinth bean; 0.64 g g^{-1} 0.82 g g^{-1} for beach pea; 0.90-0.96 g g^{-1} for Woodstone pea and 0.90 and 1.27 g g^{-1} for field pea (Mwasaru *et al.*, 2000; Subiago, 2006; Chavan *et al.*, 2001).

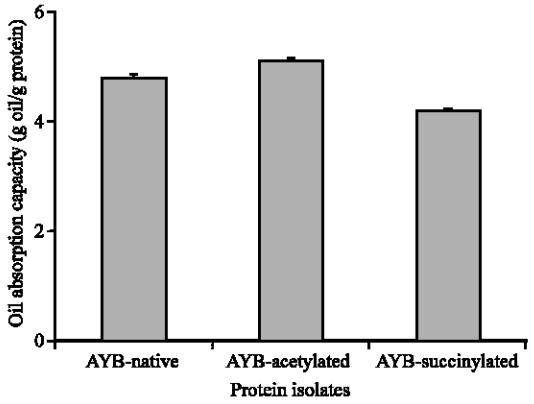


Fig. 4: Oil absorption capacity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

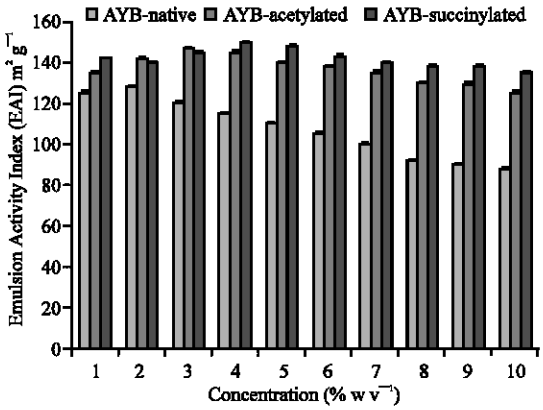


Fig. 5: The effect of concentration on the emulsion activity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

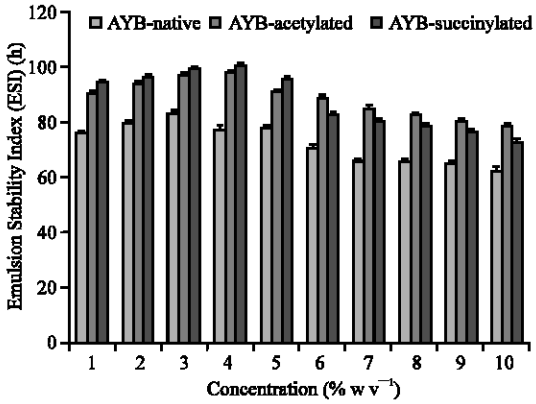


Fig. 6: The effect of concentration on the Emulsion stability of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

Acetylation improved the oil absorption capacity of the protein whereas, succinylation reduced the oil absorption capacity. This result is in agreement with result reported on soy protein isolate by Achouri *et al.* (1998). Acetylation of protein leads to the introduction of lipophilic groups into the protein molecules while, succinylation introduces hydrophilic groups. Succinylation increased the net negative charge and decreased lipophilicity of protein isolate and this causes reduction of oil absorption capacity of protein (Narayana and Narasinga Rao, 1984). Oil absorption capacity is the ability of fat to bind non polar side chains of proteins. According to Kinsella (1976), the mechanism of fat absorption have been attributed to physical entrapment of oil as well as mechanisms which, affects the surface area, size of macromolecule, the charge and the hydrophobicity of the proteins. Thus, any change in any of these parameters would affect the oil absorption capacity. This property is of great importance because of its effect on the emulsifying capacity.

Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI): Effect of concentration of protein solution on emulsifying activity index EAI of native, acetylated and succinylated African yam bean protein isolate is presented in Fig. 5 while, the effect of concentration on the emulsion stability of the isolates is presented in Fig. 6. The result indicates an initial increase in the EAI with increase in concentration. For the native isolate, the EAI increased only to 2% concentration of the protein, while for the acetylated protein, there was an increase up to 3-4% concentration of the protein. Also, for succinylated derivative there was an increase in the EAI up 4-5% protein concentration. There was steady reduction in the EAI with further increase in the concentration of the proteins. It is noteworthy that even at the lowest reduction of EAI due to ionic strength, levels were still higher than that of unmodified protein isolate Kinsella (1976) has studied the relationship between emulsion activity and the concentration of

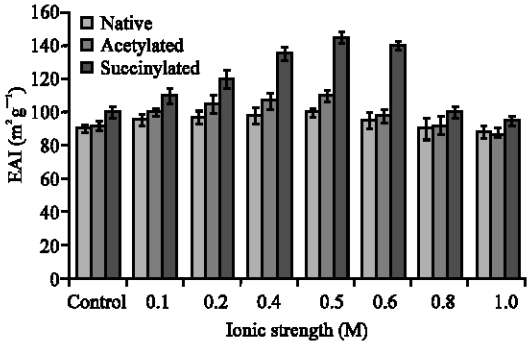


Fig. 7: The effect of ionic strength on the emulsion activity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

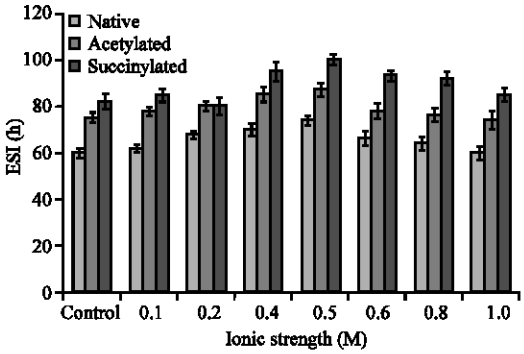


Fig. 8: The effect of ionic strength on the emulsion stability of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

proteins. It is believed that at low concentration of proteins, the adsorption of proteins on oil-water interphase is diffusion controlled, whereas at high concentration of proteins, the mechanism of diffusion is not favoured due to activation energy barrier. Initial increase in protein concentration facilitated enhanced interaction between the oil phase and the aqueous phase. However, as the concentration increased, a point was reached where further increase in protein concentration led to accumulation of proteins in the aqueous phase, this development resulted in decrease in emulsifying activity.

Similarly, Emulsion Stability Index (ESI) also, increased with increase in the concentration of proteins. For the native protein isolate, the ESI increased up to 3% protein concentration, while in the acetylated and succinylated derivatives, the ESI increased up to 4-5% protein concentration. This observation is consistent with the report on mung bean protein isolate (El-Adawy, 2000). Franzen and Kinsella (1976) have also reported a 3 fold improvement in emulsifying activity and stability with succinylated soy protein. Also, succinylated sunflower protein isolate had improved emulsifying activity and stability (Canella *et al*., 1979). Acylation (acetylation and succinylation) causes unfolding of protein chains, thereby exposing hydrophilic residues of peptides. This produced an improvement in solubility of the protein and a rapid migration to the water-oil interface. This phenomenon eventually enhanced emulsifying stability.

The effect of ionic strength of solution on emulsifying activity and stability indices of native and modified proteins are presented in Fig. 7 and 8. Emulsifying activity indices increased with increase in ionic strength progressively up to 0.5 M, but reduced as the ionic strength was increased further. Influence of ionic strength on emulsifying properties of cowpea protein had earlier been reported by some researchers. Aluko and Yada (1995), Chavan *et al*. (2001) and Wagner and Gueguen (1999) have reported the effect of ionic strength on some legumes. They all attributed higher emulsion stability of the protein at low ionic strength to dissociation of Oligomeric structure of 11S-glycinin and subsequent improvement of surface behaviour. It was observed that emulsifying properties of proteins depend basically on two effects. A substantial decrease in interfacial energy due to the adsorption of the protein at the oil-water interface and the electrostatic, structural and mechanical energy barrier caused by the interfacial layer that opposes destabilization processes (Wagner and Gueguen, 1999). The enhancement of the emulsion activity and stability at low ionic strength (up to 0.5 M) is due to the improvement in formation of charged layers around the fat globules, which resulted in mutual repulsion among them. Also, at low ionic strength, formation of hydrated layer around the interfacial material resulted in lower interfacial energy and retarded droplet coalescence thus, facilitating stability. At higher ionic strength (beyond 6.0 M), protein unfolding was decreased. This development will probably limit adsorption of the protein at the oil-water interface.

The effect of concentration on the Foam Capacity (FC) and Stability (FS) of native and modified proteins are presented in Fig. 9 and 10. Significant ($p \leq 0.05$) increase in foam capacity was observed following modifications as the protein concentration was increased from 4% w v^{-1} up to 10% w v^{-1}. Increase in concentration enhances greater protein-protein interaction, which increases viscosity and

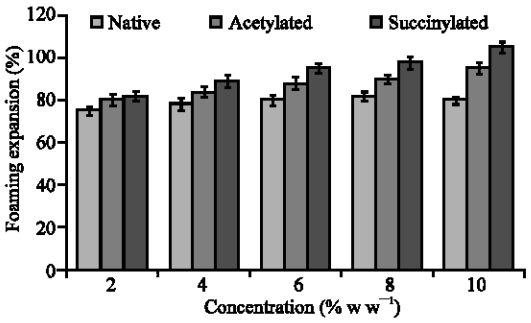


Fig. 9: The effect of concentration on the foaming capacity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

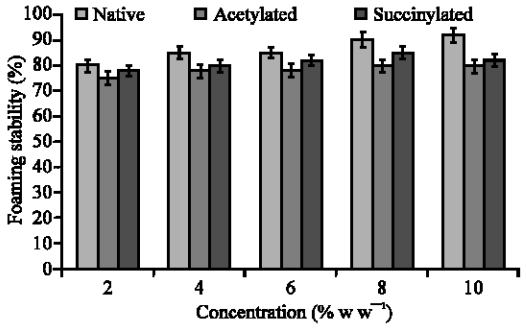


Fig. 10: The effect of concentration on the foaming stability of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

facilitates formation of multilayer cohesive protein film at the interface. The formation of cohesive multilayer film offers resistance to disproportionation and coalescence of bubbles thus, enhancing foam stability. In addition, increase in concentration could lead to the formation of thicker films, which limits the effect of drainage of protein from films. The availability of more protein as the level of protein concentrate increases in the aqueous dispersion enhances foam formation, which accounts for increase in foam capacity. The results also indicate that acylated protein derivative had better foam capacity compared with native proteins. In addition, at all concentrations, succinylated protein derivatives had improved foam capacity over the acetylated protein. It was however,

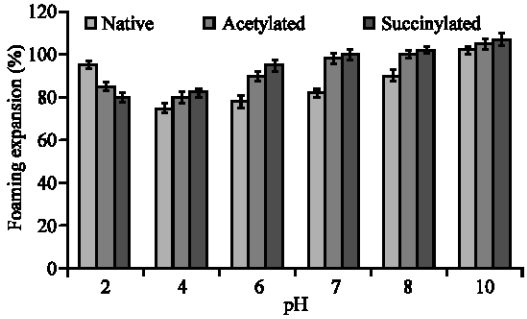


Fig. 11: The effect of pH on the foaming capacity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

observed that foam stability reduced following acetylation and succinylation significantly ($p \leq 0.05$) as the protein concentration increased. Earlier researchers reported a similar increase in the foam capacity as the concentration of the proteins was increased (El-Adawy, 2000; Franzen and Kinsella, 1976). Acylation can cause unfolding of the protein, which increases protein-water interaction. Also, the increased net negative charge of succinylated proteins would especially promote protein-water interaction, which facilitates improved foaming capacity. Decrease in foam stability following modifications might be as a result of increased charge density of succinylated proteins which inhibits the protein-protein interactions (Townsend and Nakai, 1983). Effect of pH on foam capacity and stability is presented in Fig. 11 and 12. High foam capacity observed at pH 10 may be due to an increase in the net charge of the protein molecules, which weakens hydrophobic interactions and increases protein flexibility. This allowed them to spread to the air water interface more quickly thus, encapsulating air particles and increasing foam formation.

Protein stability is high in the neighborhood of isoelectric pH than at any other pH. This observation lends credence to similar results that have been reported earlier by Buckingham (1970). Increase in the foam stability at the region of isoelectric pH might be due to the formation of stable molecular layers in the air-water interface of the foams. Protein adsorption and viscoelasticity at an air-water interface is high at isoelectric pH because protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air-water interface, a development that improves foam stability.

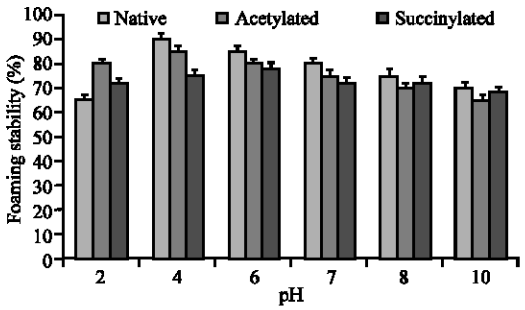


Fig. 12: The effect of pH on the foaming stability of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

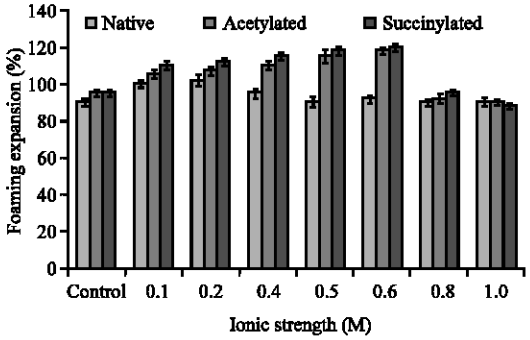


Fig. 13: The effect of ionic strength on the foaming capacity of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

Effect of ionic strength on foaming capacity and stability of native and modified proteins are presented in Fig. 13 and 14. Foam capacity and stability of native protein increased with increase in ionic strength from the control to 0.2 M while, those of acetylated and succinylated protein isolates increased progressively from the control to 0.6 M.

Thereafter, foaming capacity and stability decreased with further increase in ionic strength of the media. Increase in ionic strength of the protein concentrates solutions improved solubility and protein dispersion, thus, leading to enhanced whippability and formation of stable cohesive films around the air vacuoles. However, when the increase in ionic strength exceeds 0.1 M, charge-screening occurred and thus, hydrophobic

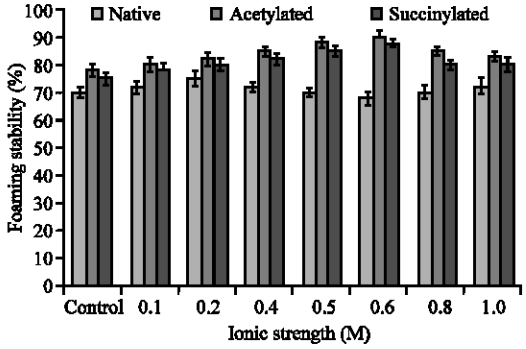


Fig. 14: The effect of ionic strength on the foaming stability of native, acetylated and succinylated African yam bean protein isolate. Degree of acetylation 85%; percentage yield calculated on native protein isolate basis. Degree of succinylation 87%; percentage yield calculated on native protein isolate basis

interaction and intermolecular cohesion between protein molecules improved and these led to reduction in flexibility of the protein surfactant molecules.

Data on the gelation capacity as a function of the pH of African yam bean protein isolates are presented in Table 3. The Least Gelation Concentration (LGC) was taken as a measure of the gelation capacity. The lower the LGC the better the gelation characteristics of the protein isolates. Gel formation of protein is the result of a two step process involving the partial denaturation of individual proteins to allow more access to reactive side groups within the protein molecules and the aggregation of these proteins by means of reactive side groups into a three-dimensional network structure capable of retaining significant amount of water. This phenomenon is of importance in the food industry since, it contributes significantly to the textural and rheological properties of various foods. It was also observed that the LGC increased as the pH value moves away from the isoelectric region. Differences in gelation capacity at various pHs is due to prevalent surface charge of the proteins. At pH close to the isoelectric point, the net surface charge is reduced, which significantly reduces the repulsive interactions between the protein molecules (Elofsson *et al*., 1997). Gelation is therefore enhanced at this pH due to greater interaction of protein molecules. On the other hand, at pH far removed from the isoelectric points, the surface charge on the properties is large and significant repulsive forces prevent aggregation of protein molecules and formation of ordered network structure. Contrary to observations on native proteins, lowest LGC for acylated protein derivatives were obtained at pH 2. It is reasonable that at this pH, inter and intramolecular

Table 3: Effect of pH on gelation properties of native, acetylated and succinylated African yam bean protein isolate

	pH						
Sample	2.0	4.0	5.0	6.0	7.0	8.0	10.0
AYB-N	14	10	12	14	14	14	14
AYB-Acet	10	12	12	12	16	16	16
AYB-Succ	8	10	12	12	12	14	14

AYB-N: African Yam Bean Native protein isolate, AYB-Acet. African Yam Bean Acetylated protein isolate, AYB-Succ: African Yam Bean. Succinylated protein isolate. Values expressed as the Least Gelation Concentration (LGC)

Table 4: Effect of ionic strength on gelation properties of native, acetylated and succinylated African yam bean protein isolate

	Ionic strength of KCl solution (mol L^{-1})							
Samples	Control	0.1	0.2	0.4	0.5	0.6	0.8	1.0
AYB-N	14	10	8	12	14	14	14	18
AYB-Acet	12	10	8	8	8	14	14	16
AYB-Succ	12	10	10	12	14	14	14	16

AYB-N: African Yam Bean Native protein isolate, AYB-Acet: African Yam Bean Acetylated protein isolate, AYB-Succ: African Yam Bean Succinylated protein isolate

repulsion would be minimal since it is near their isoelectric points and this probably resulted in enhanced aggregation of protein molecules.

Effect of ionic strength on gelation capacity of proteins is presented in Table 4. Initial increase in ionic strength of the media from the control to 0.6 M improved the gelation capacity of both native and modified proteins. Akintayo *et al.* (1999) had earlier reported ionic strength-dependent gelation properties for *Cajanus cajan* protein. In their report, the gelation capacity improved at low ionic strength (0.5 M), while, it reduced at high ionic strength solution (1.0 M). The researchers attributed the improvement in gelation capacity at low ionic strength to enhanced protein solubilization in the salt solution, which created an effective overlapping of the functional groups between adjacent protein molecules, a condition necessary for a network of gel formation. Van Camp *et al.* (1997), postulated that addition of sodium chloride to whey protein solution provided a shielding effect that strongly reduces the repulsive forces acting among proteins. However, increase in LGC particularly at 0.5 M may be a result of reverse in protein unfolding with increasing ionic strength as earlier stated. Decrease in protein unfolding limited access to reactive side groups within the protein molecules a condition necessary for the formation of a three-dimensional network structure as observed in protein gels.

CONCLUSION

Physicochemical and functional characteristics of native, acetylated and succinylated African yam bean protein isolates were investigated.

Solubility of the protein isolates improved following acetylation and succinylation and this improved both emulsifying and foaming properties of the native protein concentrate. Good emulsifying and foaming properties suggest that these proteins will find good applications as additives for food products such as cakes, breads, marshmallow, whipped toppings ice-cream and deserts. Equally, improved water absorption capacity implies the modified isolates could be potentially useful in flavour retention, improvement of palatability and extension of shelf life in meat products.

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