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Rheological characterization of acylated and dextran conjugated African yam bean (*Sphenostylis stenocarpa* Hochst. Ex A. Rich.) proteins

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ABSTRACT

The molecular weight distribution and rheological properties of acetylated, succinylated and dextran conjugated African yam bean (*Sphenostylis stenocarpa*) proteins dispersion were studied. Succinylation of the protein showed the three prominent electrophoretic bands of the unmodified protein but all the bands disappeared with acetylation. Immobile band characterized dextran conjugated *S. stenocarpa* electrophoregram. The flow behavior indices (n) of these modified *S. stenocarpa* protein dispersions were in the range 0.03–0.22. This is an indication that they were pseudoplastic in nature. This pseudoplastic nature was maintained in ionic media 0.05–0.5 mol dm^{-3}, pH 3–8 and temperature range of 27–75 °C. The yield stresses were 0.270, 0.302 and 0.320 Pa for acetylated, succinylated and dextran conjugated protein respectively. Activation energy of acetylated and succinylated protein were in the range 6.2–8.2 and 2–5.4 J mol^{-1} respectively. Thus acetylation of *S. stenocarpa* protein made its dispersion viscosity more susceptible to temperature change than succinylation. These results suggest that acylation and dextran conjugation of African yam bean (*S. stenocarpa)* protein produce protein species with different rheological properties.

1. Introduction

Food produced on industrial scale is a considerable part of human diet and the increasing consumers' preference for highly nutritious products challenge the food processing industry to choose healthier ingredient as functional agent for their formulated foods. Protein has often been considered as healthy ingredient for improving industrial food products quality and acceptability because of its unique nutritional and functional properties. The preferential increase in the demand for plant protein over animal protein has been emphasized (Adebowale, Adeyemi, Oshodi, & Niranjan, 2007)

In the food industry, rheological study has many applications in the fields of food acceptability, processing, and handling (Barbosa-Cánovas, Kokini, Ma, & Ibarz, 1996). Rheological measurement is a tool for physical characterization of raw material prior to processing, intermediate products during manufacturing, and finished foods. Rheological data are useful in estimating velocity, shear and residence time distribution in extrusion and continuous mixing (Rao, 1999). In rheological study, effects of intrinsic and extrinsic factors (e.g. amino acid composition, molecular weight, protein concentration, pH, temperature, ionic strength, type of salts present, pressure, etc.) must be given due consideration since both can affect protein's rheological performance.

To broaden vegetable protein application as food functional agent, chemical, physical and enzymatic modifications have been recommended. These modifications were found to improve protein functionalities. Chemical modification can be done by attaching low molecular weight molecules to protein, eliminating low molecular weight moiety from protein, and by conjugating high molecular weight molecules with the protein moiety (Kobayashi et al., 2001). Low molecular weight modification can be in different forms i.e. methylation, acetylation, alkylation, esterification, amidation, deamidation and succinylation. Modification by acylation has been reported to have positive impact on protein functionality and adaptable to food formulations. The use of acylated proteins in preparation of some food products such as carbonated beverages, coffee whiteners, cheese-like gels, mayonnaise and salad dressings, margarine and ice cream have been reported (Chen, Richardson, & Amundson, 1975; Creamer, Roeper, & Lohrey, 1971; Evans, 1970; Evans & Irons, 1971; Melychyn & Stapley, 1973). Acylation simply involves the interaction of the amino acid residue particularly lysine with acetic acid anhydride (acetylation) or succinic anhydride (succinylation). The functional improvement

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resulting from this reaction of dicarboxylic anhydrides with the ε-amino group of lysine and the amino-N-terminal α-amino group of proteins, has been attributed to conformational changes in the protein molecule, which is a function of degree of acylation and protein source (El-Adawy, 1996; Kim & Rhee, 1989). Modification by high molecular weight is by conjugation of protein with another polymer like polysaccharide. This is carried out by Maillard-type reaction involving covalent cross-linkage between ε-amino groups in protein and the reducing-end of carbonyl group in polysaccharide (Kato, Shimokawa, & Kobayashi, 1991).

African yam bean (*Sphenostylis stenocarpa*) which belongs to the family of Papilionaseae is an important crop in west, central and some parts of east Africa (Okigbo, 1973). Increasing interest in production African yam bean for human consumption is being stimulated for number of reasons: ease of cultivation, even in acid and highly leached sandy soil of the humid lowlands of tropics; lysine and methionine levels which limits the nutritive value of many vegetable protein are equal to or better than those of soybean; good emulsifying and gelling tendencies (Adebowale, Henle, & Schwarzenbolz, 2009; Arogundade, Eromosele, Ademuyiwa, & Eromosele, 2009; Evans & Boulter, 1974).

Enhanced functional performance of acylated *S. stenocarpa* protein was reported by Adebowale et al. (2009) but effect of such modification on its rheological properties has not been reported. This study thus reports molecular weight distribution and rheological properties of acylated (acetylated and succinylated) and dextran conjugated African yam bean (*S. stenocarpa*) protein. Rheological attributes of coating solution made from these modified proteins were also assessed. The information presented here was meant to expand the existing knowledge and effort geared toward developing protein based food and non-food products from this African legume resource.

2. Materials and methods

2.1. Sample preparation

S. stenocarpa seeds were obtained from local dealers in Ekiti and Kogi States (Nigeria). After a careful removal of the extraneous materials and the damaged ones, the seeds were milled and sieved with a 0.4 mm sieve (Eromosele, Arogundade, Eromosele, & Ademuyiwa, 2008). The protein and moisture content of this *S. stenocarpa* flour were 21.1% and 12% respectively (Eromosele et al., 2008).

2.2. Preparation of S. stenocarpa protein isolates

Isoelectrically precipitated protein was obtained as described by Arogundade et al. (2009). The alkaline extracts of African yam bean (*S. stenocarpa*) obtained by extracting the flour for 45 min on Stuart orbital shaker (S01 model) at flour solvent ratio 1:10 in distilled water at pH 10 was clarified by centrifugation at 4000 rpm for 45 min. The protein in the clear supernatant was precipitated by acidification to pH 5 to obtained isoelectrically precipitated isolate.

2.3. Preparation of acetylated and succinylated S. stenocarpa proteins

Succinylation and acetylation of *S. stenocarpa* protein were performed by the method of Groninger (1973) as modified by Paulson and Tung (1987) and Lawal, Adebowale, and Adebowale (2007). Twenty percent protein dispersion was made with 200 g of *S. stenocarpa* protein in distilled water. The pH of the solution was adjusted to 9.0 using 1 mol dm^{-3} NaOH. One hundred grams of succinic anhydride or acetic acid anhydride was added in small increment over a period of 1½ h. After each incremental addition of acylating agent, the pH was maintained between 8.0 and 8.5 with 4 mol dm^{-3} NaOH. After the final addition, the reaction was judge completed when the pH of protein solution stabilized and the dispersions were further left for 1 h at room temperature then isoelectrically precipitated, frozen, lyophilized and kept for use.

2.4. Preparation of S. stenocarpa protein–dextran conjugate

Protein–dextran dispersion was made as described by Hassan, Osman, and Babiker (2007). *S. stenocarpa* protein and dextran were mixed in the weight ratio of 2:1. The mixture was dissolved in distilled water to make 30% (w/v) concentration. The protein–dextran dispersions were stirred on orbital shaker (Stuart scientific SO1) at 150 rpm for 125 min; the pH adjusted to pH 3.5, frozen and lyophilized (Lillard, Clare, & Daubert, 2009). Protein–dextran conjugate were prepared from the lyophilized powder by the Maillard-type reaction as described by Akhtar and Dickinson (2003) with the modification of Lillard et al., (2009).

2.5. Reduced SDS-PAGE gel electrophoresis of modified S. stenocarpa protein

Molecular sizes of acylated and dextran conjugated S. *stenocarpa* proteins were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970). Electrophoresis was performed in a vertical slab (MiniProtein II Bio-Rad laboratories, Hercules, USA). The resolving (12%, pH 8.8) and stacking (4%, pH 6.8) gels were prepared using acrylamidebisacrylamide solution, Tris–HCl buffer, ammonium-persulphate solution and tetramethyl-ethylenedamine (TEMED) as described by BIO-RAD Miniprotein II electrophoresis instruction manual.

Five hundred milligrams of the protein were solubilized with 1 ml reducing (β-mecaptoethanol) sample buffer solution. Ten microliters of the protein sample solutions were loaded. On completion, gels were stained with 0.1% Coomassie brilliant blue in acetic acid/ethanol/water solution (10:40:50, v/v) and destained in the same staining solution without Coomassie brilliant blue. Sigma Marker low range molecular weight standards ranging from 6500 to 66,000 Da were used as protein standard and developed along with each electrophoretic analysis. The molecular weights of the protein in the test samples were computed from the R_f values of scanned images as described by Sogi, Arora, Garb, and Bawa (2002). SDS-PAGE gel electrophoresis experiment was repeated trice.

2.6. Rheological flow behavior of modified S. stenocarpa proteins

The rheological flow properties of modified *S. stenocarpa* protein dispersions were determined as described in our previous study (Arogundade, Eromosele, Eromosele, & Ademuyiwa, 2011). Briefly, the 10% protein dispersions placed in the small sample adaptor (SC4-45Y) of LVT Brookfield Synchro-lectric viscometer (Brookfield Engineering Laboratory INC., Middleboro, MA02346, USA) were sheared with SC4-18 spindle at shear rate in the range 0.8–15.9 s^{-1}. The flow properties of the protein dispersion were obtained by fitting the experimental data into some rheological models i.e. Power law, Bingham and Casson models. Rheological properties of coating solutions made from these proteins (10% protein dispersion and glycerol plasticizer) were determined as described by Kaya (2001) with the modifications of Arogundade et al. (2011). Shearing of each of the protein dispersions was carried out at least in triplicate.