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Functionality of native and succinylated Lablab bean (*Lablab purpureus*) protein concentrate

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Abstract

Lablab bean protein concentrate was acylated with succinic anhydride and resultant changes in some functional properties such as protein solubility index; oil absorption capacity, emulsifying properties and foaming properties were investigated. The result indicate that addition of succinic anhydride at 0.1, 0.25, 0.5, 0.75, and1.0 g/g of protein acylated 42.6, 61.3, 84.6, 89.1 and 92.4%, respectively of the ε-amino groups. Succinylation reduced solubility of native protein (nLPC) at pH values below its isoelectric point (4.5). However, marked improvement in solubility were observed at pH 4.5–10 following succinylation. Emulsifying activity (EA) increased with increase in concentration up to 4 and 6%, w/v for nLPC and succinylated protein (SLPC) respectively but further increase in concentration put the EA on the decline. Increase in ionic strength of the media to 0.2 M and 0.4 M significantly ($P < 0.05$) increased the EA of nLPC and SLPC respectively. Further increase in ionic strength however reduced the EA of protein solutions. Succinylation reduced the emulsion stability (ES) of nLPC at pH 2. However, in the pH 4–10, all succinylated protein concentrates showed improved ES over the native protein concentrate. A direct relationship was observed between increase in concentration of the protein concentrate and increase in foam capacity. Foam stability reduced after succinylation at all concentration studied. Succinylation improved foam capacity at pH 4–10 but reduced it at pH 2. Native lablab protein had better foam stability compared with the succinylated derivatives.

Keywords: Lablab; Succinylation; Protein Concentrate

1. Introduction

Insufficient protein in human nutrition has been plaguing many parts of Africa and the rest of the underdeveloped countries (Adebowale & Lawal, 2003). This problem has stimulated research on improving the quality of conventional protein sources and on developing new unconventional sources (Chel-Gurero, Perez-Florez, Bentacur-Ancona, & Davilla-Onitz, 2002). Legumes have been used as a source of protein in the diets of people from many cultures, especially where animal proteins are scarce or expensive. Lablab bean (*Lablab purpureus*) is one of the underexploited legumes in Africa. In particular, it is an unconventional source of food protein (Deka & Sarkar, 1990).

In addition to providing essential amino acids, the ultimate success of using any food protein as food ingredient depends largely upon its desirable functional properties (Chau & Cheung, 1998). In recent years, studies on the functional properties of protein of various plants such as cowpea (Aluko & Yada, 1995), lupin seed (Sathe, Deshpande, & Salunkhe, 1982), faba bean (Cepada, Villarain, & Arangiuz, 1998) and soy (Wagner & Gueguen, 1999) have been reported.

Many native proteins possess limited functionality; and modification such as succinylation is often performed to expand the range of functional properties available. Succinylation involves chemical derivatization of groups such as ε-amino group of lysine in proteins with succinic anhydride. Such modifications have been applied to various plant and non-plant proteins with resultant improvement in functional properties. Research have revealed that succinylation of leaf protein increased the nitrogen solubility, emulsifying capacity and foam capacity of the protein (Franzen & Kinsella, 1976). Similar observations were reported with succinylated peanut protein (Shyama & Rajagospel, 1978). Also, succinylation was found to increase emulsifying capacity, emulsion stability, oil

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holding and foam capacity of sunflower protein isolates (Kabirrulah & Wills, 1982). Improved functional characteristics of cottonseed protein isolate following succinylation has also been reported (Choi, Lusas, & Rhee, 1981). In addition, effect of succinylation on functional properties of some plant proteins has also been reported. These include rapeseed (Ponnampolam, Delisle, Gagne, & Amiot, 1990), pea (Johnson & Brekke, 1983) and soy (Achouri, Zhang, & Shying, 1998).

The author is not aware of any previous report dealing with the effect of succinylation on functional properties of lablab protein concentrate. Therefore, the objective of this work was to prepare a protein concentrate from lablab bean (*L. purpureus*), modify it with succinic anhydride and investigate resultant changes in functionality. A further objective was to determine effect of concentration, pH and ionic strength on the functional properties of the protein, with a view to providing these as bedrock information to various food industries.

2. Materials and methods

2.1. Materials

Lablab bean seeds (*L. purpureus*) were gifts from Dr N.Q. Ng, head genetic resources unit, International institute of Tropical Agriculture, Ibadan, Nigeria. The seeds were ground to pass through a BS-60-mesh screen, using a household flourmill (Braun Multimix Deluxe, Germany). The flour was kept in a refrigerator at about 4 °C until used.

2.2. Sample preparation

Two kilograms of lablab bean flour was dispersed in 10 l of distilled water The pH was adjusted to 8.0 with 0.5 M NaOH to enhance protein solubilisation and it was stirred for 5 h. at 30 ± 2 °C. The pH of the supernatant obtained after centrifuging at 4000*g* for 30 min was adjusted to 4.5 with 0.5 M HCl. The protein concentrate was recovered by centrifugation at 5000*g* for 30 min. It was dispersed in distilled water at pH 7, following which the pH was readjusted to 4.5 and protein concentrate recovered by centrifuging at 5000*g*. The average yield of protein concentrate from mucuna bean flour was 30.2% while the percentage protein content of the concentrate was 80.4% using Kjeldahl method, (AOAC, 1985).

2.3. Succinylation of protein concentrate

Succinylation was carried out according to the method described by Groninger (1973) Lablab protein concentrate (LPC) was made into slurry of 25%, w/v with distilled water. The pH of the solution was adjusted to 9.0 using 1 M NaOH and cooled in an ice bath to reduce the temperature to 4–5 °C. Succinic anhydride was added to the solution at the levels of 0.1, 0.25, 0.5, 0.75 and 1.0 g/g of protein. The pH of the solution was maintained at about 8.0–8.5 with constant stirring. The reaction was completed when the pH of the protein solution stabilized. Succinylated lablab protein concentrate (SLPC) was precipitated isoelectrically before centrifuging at 19,200*g* for 20 min. The slurry was dialyzed against distilled water for 16 h before drying.

2.4. Degree of succinylation

The trinitrobenzene sulphonic acid (TNBS) method of Habeeb (1966) was used to determine the extent of acylation of lablab proteins. One milliliter of 0.1% TNBS solution was added to protein suspension. The samples were heated in a 60 °C water bath for 2 h and then cooled to room temperature. One milliliter of a 10% sodium dodecyl sulphate and 0.5 ml of 1 M HCl were added to the protein solutions. The absorbance of the solution was read at 335 nm in a spectrophotometer against a reagent blank. The absorbance of the control lablab protein concentrate was set equal to 100%. Free amino groups and the degree of acylation of the modified samples were calculated based on the decrease in absorbance because fewer amino groups were able to react with the TNBS reagent.

2.5. Solubility profile

Hundred milligrams of protein samples were dispersed in 20 ml of distilled water and the pH of the suspension was adjusted to 2–10 with 0.5 M NaOH or 0.5 M HCl. The suspensions were agitated with a magnetic stirrer for 30 min at room temperature before centrifuging at 1200*g* for 20 min at 4 °C. The amount of protein content in the supernatant was determined. Studies were conducted in triplicates and the solubility profile was obtained by plotting averages of protein solubility (%) against pH.

$$\text{Solubility}\,(\%) = \frac{\text{Amount of protein in the supernatant} \times 100}{\text{Amount of protein in the sample}}$$

2.6. Oil absorption capacity

Oil absorption capacity was determined using the method of Beuchat (1977). One gram samples was mixed with 10 ml oil (Executive Chef Oil, Unilever Nig. PLC) for 30 s. The samples were then allowed to stand at room temperature (30 ± 2 °C) for 30 min before centrifuging at 5000*g* for another 30 min. The volume of the supernatant was noted in a 10 ml graduated cylinder.

2.7. Emulsifying properties

Emulsifying activity (EA) and stability were determined using the method described by Neto, Narain, Silva,