

Effect of Transglutaminase Cross Linking on the Functional Properties as a Function of NaCl Concentration of Legumes Protein Isolate

Nahid A. Ali, Salma H. Ahmed, ElShazali A. Mohamed, Isam A. Mohamed Ahmed and Elfadil E. Babiker

Abstract—The effect of cross linking of the protein isolates of three legumes with the microbial enzyme transglutaminase (EC 2.3.2.13) on the functional properties at different NaCl concentration was studied. The reduction in the total free amino groups (OD_{340}) of the polymerized protein showed that TGase treatment cross-linking the protein subunit of each legume. The solubility of the protein polymer of each legume was greatly improved at high concentration of NaCl. At 1.2 M NaCl the solubility of the native legumes protein was significantly decreased but after polymerization slightly improved. Cross linked proteins were less turbid on heating to higher temperature as compared to native proteins and the temperature at which the protein turns turbid also increased in the polymerized proteins. The emulsifying and foaming properties of the protein polymer were greatly improved at all concentrations of NaCl for all legumes.

Keywords—Functional properties, Legumes, Protein isolate, NaCl, Transglutaminase.

I. INTRODUCTION

In recent years, there has been a concerted effort toward harnessing the potential of lesser-known legumes to minimize problems of protein malnutrition in Africa [1]. This is because animal protein is beyond the reach of a large percentage of the people in developing countries. In order to develop plant proteins for use as ingredients in the food industry, there is the need to determine the physicochemical and functional properties of these proteins. Because of inadequate supplies of food proteins, there has been a constant search for unconventional legumes, as new protein sources, for use as both functional food ingredients and nutritional supplements [2]. Proteins have unique surface properties due to their large molecular size and their amphiphilic properties.

Nahid A. Ali is with the Department of Food Science, Faculty of Applied Sciences, University of Juba, Sudan.

Salma H. Ahmed and ElShazali A. Mohamed were with the Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan.

Isam A. Mohamed Ahmed is with the United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan (e-mail: isamnawa@yahoo.com).

Elfadil E. Babiker is with the Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North 13314 Shambat, Sudan (phone: +2499122992425; e-mail: elfadilbabiker@yahoo.com).

However, the industrial applications of food proteins are limited, because proteins are generally unstable with heating, organic solvents and proteolytic attack. Therefore, if proteins could be converted into stable forms, their applications would be greatly broadened. Molecular forces such as hydrophobic and electrostatic interactions can be manipulated by pH and ionic strength to produce proteins with varying structural conformations and hence functional properties. Modification of food proteins has been investigated to improve their physical functionality, i.e. gelation, viscosity, emulsification and foaming [3]. The ability of transglutaminase (TGase; E.C. 2.3.2.13) to modify the functional properties of food proteins has been extensively reviewed [3]–[5]. By acyl group transfer between the ϵ -amino group of lysine and the γ -carboxamide group of glutamine residues in proteins/peptides, TGase catalyses the formation of an ϵ -(γ -glutamyl) lysine isopeptide bond. In the absence of free ϵ -groups, water acts as the acyl acceptor, resulting in the deamidation of glutamine to glutamic acid. Food proteins are often denatured during processing, so there is a need to understand the protein both as a biological entity with a predetermined function, and as a randomly coiled biopolymer. Protein cross-linking has profound effects on their structure, which affects the functional attributes of these proteins. Food processing often involves high temperature as in baking and low pH as in beverage industry. Such conditions can result in the introduction of protein cross links producing substantial changes in the structure of proteins and which can be reflected in the final product profile [6]. The formation of this cross link does not reduce the nutritional quality of the food, as the lysine residue remains available for digestion. Chemical and physical methods are commonly used. Food proteins can have their functionality altered by temperature and other chemical means. Specific functional attributes could be obtained by enzymatic polymerization of proteins and such enzymatic reaction could be controlled for desired time to enhance the functionality to the desired level [7]. Work on enzymes, especially mammalian and microbial transglutaminases have been employed to modify proteins for functionality. The covalent cross linking of proteins catalyzed by transglutaminases can cause significant changes in the size, conformation, stability and other properties of the proteins by

enhancing protein–protein interaction. The enzymes have been used for modifying the functionalities of various proteins. In this study, an attempt was made to investigate the effect of transglutaminase cross-linking on the functional properties of protein isolate of two legumes.

II. MATERIALS AND METHODS

A. Materials

Pigeon pea (*Cajanus cajan*) and hyacinths bean (*Dolichos hyacinth* L.) were obtained from the Agricultural Research Corporation, Wad Medani, Sudan. Refined corn oil was brought from Bitar Company, Khartoum, Sudan. Transglutaminase was donated by Professor Akio Kato, Yamaguchi University, Faculty of Agriculture, Department of Biological Science, Japan. Unless otherwise stated all chemicals used in this study were reagent grade

B. Protein isolate preparation

The protein isolate was prepared by the method of Iwabuchi and Yamauchi [8]. A sample of defatted meal (100 g) was extracted once with 2 l of 0.03M Tris-HCl buffer (pH 8) containing 10 mM 2-mercaptoethanol (2-ME) at 20 °C. After centrifugation, the supernatant was acidified to pH 4.8 with 2N HCl and then centrifuged. The precipitated protein was dissolved in water at 4 °C and the pH adjusted to 8. After centrifugation (8000 rpm), the clear supernatant was dialyzed against distilled water for 24 h at 4 °C and then freeze-dried.

C. Transglutaminase treatment

The protein isolate of each legume was dissolved in 0.1 M phosphate buffer (pH 7.5; 10 mg/ml) and then reacted with TGase (0.5 mg/ml). The mixture was incubated at 55 °C for 60 min. The enzyme was inactivated by N-ethylmaleimide (0.1 ml; 0.1%) [9]. The treated samples were dialyzed against distilled water and then freeze-dried.

D. Change in free amino groups

Changes in free amino groups of 0.1% protein solutions were determined by spectrophotometric assay (OD₃₄₀) using o-phthalaldehyde as described by Church et al. [10]

E. Measurement of solubility

The samples of the native protein and that polymerized by transglutaminase (0.2%) were used for the determination of solubility at different NaCl concentration (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2M). Samples were dissolved in the buffer and shaken with a vortex mixer (Scientific Industries, adjusted on digit 4 to work on touch) for 10s, and the turbidity was measured at 500 nm. Values obtained are means of triplicate samples.

F. Heat stability

Heat stability was determined by the method described by Kato et al. [11]. The samples were dissolved at a protein

concentration of 2 mg/ml in 50 mM Tris-HCl buffer (pH 7.0) and heated at 50-90 °C for 20 min. Protein turbidity was measured at 500 nm.

G. Measurement of emulsifying properties

The emulsifying properties of the samples were determined by the method of Pearce and Kinsella [12]. To prepare emulsions, 1.0 ml of corn oil and 3.0 ml of protein solution (0.1%) in 0.1 M phosphate buffer (pH 7.0) were shaken together and homogenized in an Ultra Turrax instrument (Hansen & Co. West Germany) at 12000 rpm for 1 min at 20 °C. A 50-ml sample of the emulsion was taken from the bottom of the container at different times and diluted with 5 ml of a 0.1% sodium dodecylsulfate solution. The absorbance of the diluted emulsion was then determined at 500 nm. The emulsifying activity was determined from the absorbance measured immediately after the emulsion formation (0 min). The emulsion stability was estimated by measuring the half time of the initial turbidity of the emulsion.

H. Measurement of foaming properties

Foaming capacity of the sample was determined by following the procedure described by Lawhon et al. [13]. About 2.0 gm of the sample were blended with 100 ml buffer at different NaCl concentration (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2M) in a Moulinex blender at "HI" speed for 2 minutes. The mixture was poured into a 250 ml measuring cylinder and the foam volume was recorded after 30 sec.

$$FC \% = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100 \quad (1)$$

FC% was determined as a function of NaCl concentration (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2M). The foam stability (FS) was conducted according to Ahmed and Schmidt [14]. The FS was recorded at 15 minutes interval for the first 15 min after pouring the material in a cylinder. FS was determined using the following formula:

$$FS \% = \frac{\text{Foam volume after 15 min}}{\text{Initial foam volume}} \times 100 \quad (2)$$

FS% was determined as a function of NaCl concentration (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2M).

III. RESULTS AND DISCUSSION

A. Effect of transglutaminase (TGase) treatment on the free amino groups and solubility of legumes protein isolate

The native protein of each legume polymerized by TGase showed changes in free amino groups of the protein as shown in Fig. 1. The free amino groups (OD₃₄₀) of the protein isolate

of the two legumes were greatly reduced after TGase treatment. It was observed that after TGase treatment about 49% of the free amino groups of pigeon pea and about 47% of hyacinth bean free amino groups were cross-linked. Results revealed that most of the protein molecules of each legume were cross-linked by TGase. The result indicated that TGase might have catalysed the transfer reaction between an amide group in a protein-bound glutamine and a ϵ -amino group in a protein-bound lysine side chain, resulting in cross-links between the protein molecules [15], [16]. Similar results were obtained when soy protein and chymotrypsin digests were polymerized by TGase [17].

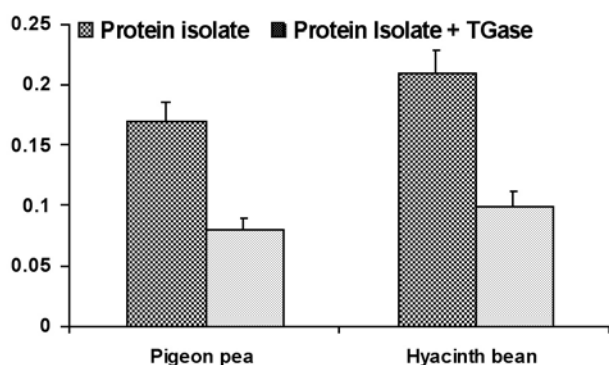


Fig. 1 Changes in free amino groups of the protein isolate of selected legumes treated with transglutaminase (TGase). Error bars indicate the standard deviation of three replicates.

The effect of NaCl concentration on solubility of the protein isolate of two legumes with and without TGase treatment was investigated (Fig. 2). The results showed that the flour and the protein isolate of the legumes had low solubility before addition of salt and at high salt concentration. However, after being polymerized by TGase, the protein isolate solubility was greatly improved even at high salt concentration. The turbidity (OD_{500nm}) of the flour was 0.8 and 0.54 for pigeon pea and hyacinth bean, respectively while that of the protein isolate was 0.3 and 0.31 for the legumes, respectively. Addition of NaCl at different concentration greatly improved solubility of the flour and the protein isolate for both legumes with maximum turbidity obtained at 2.0 M NaCl which was found to be 0.42 and 0.35 for the flour of the legumes, respectively while that of the isolate was 0.36 and 0.31.

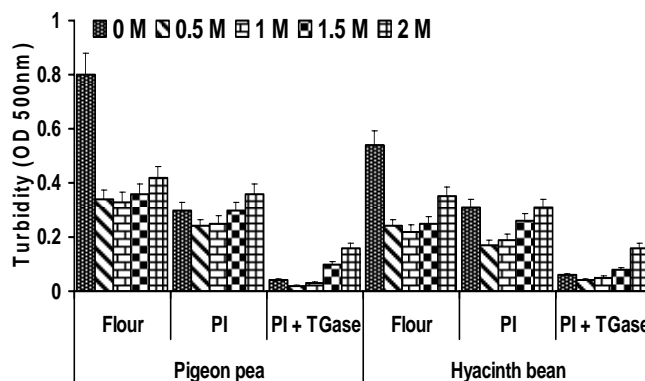


Fig. 2 Solubility of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different NaCl concentration (M). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

Moreover, polymerization of the protein isolate caused further improvement in solubility even at high concentration of NaCl. The turbidity (OD_{500nm}) of the polymerized proteins was 0.02 and 0.04 at 0.5M NaCl for the two legumes, respectively while at 2.0M NaCl it was slightly increased to 0.16 for the two legumes. The improvement in the solubility due to TGase treatment of the protein isolate at different NaCl concentration is mainly due to the fact that the protein isolate had recognizable sites for TGase reaction and the resultant polymer had lower level of free amino groups. Also, TGase treatment decreases the surface hydrophobicity of the proteins molecules and increases the electrostatic repulsion as a result of partial deamidation of glutamine and asparagine [18]. Addition of NaCl at high concentration causes negatively charged chloride ions to interact with the positively charged proteins, thereby decreasing electrostatic repulsions and enhancing hydrophobic interactions. The increase in hydrophobic interactions would result in a higher tendency for the protein to form insoluble aggregates, thus decreasing solubility [19]. However, polymerization of the native protein isolate might increased the net negative charge on the protein, coupled with the salting-in effect of NaCl on the hydrophobic interactions dissociates the protein aggregates, and solubility increases. The solubility (OD_{500nm}) of the protein isolate of the legumes decreased as the heating temperature increased (Fig. 3) and the turbidity at 90 oC was observed to reach 0.97 and 1.17 for the flour of pigeon pea and hyacinth bean, respectively while that of the isolate was 0.92 and 1.02 for the legumes, respectively. However, after polymerization the turbidity was decreased to 0.64 and 0.68 for the two legumes, respectively. The results indicated that TGase treatment was found to be effective in improving heat stability of legumes protein compared to the native protein. Proteins treated with transglutaminase form more compact structures which make it more heat stable. Similar increase in the thermal stability of oat globulin has been attributed to the formation of aggregates with compact network [20]. Transglutaminase often increase

thermal stability by intramolecular or intermolecular interaction. Cross-linking of sodium caseinate with transglutaminase resulted in the lower turbidity at 140 oC. This indicated that the cross linked products were more heat stable than the unmodified sodium caseinate [21].

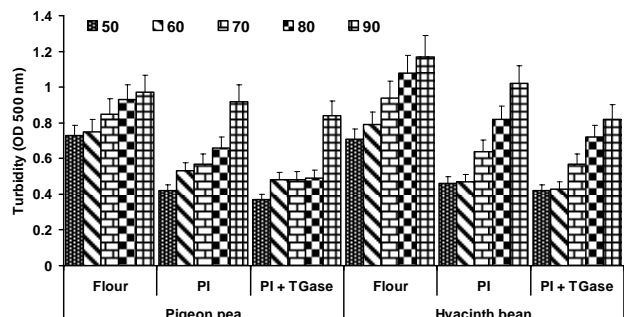


Fig. 3 Heat stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different temperature (Legend values in °C). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

B. Effect of TGase treatment on physical functionality of legumes protein isolate.

The physical functionalities such as the emulsification and foaming properties of the flour and the protein isolate of the legumes are poor and in order to improve these physical properties, the effect of TGase treatment was investigated. As shown in Fig. 4, the emulsifying activity of the protein isolate polymers was improved. The emulsifying activity of the flour and the protein isolate of the legumes, which is estimated as a percentage of emulsion, was greatly improved as NaCl concentration increased up to 0.5M at which the emulsifying activity was found to be high for both the native and polymerized protein isolate of the legumes. Further increase in NaCl concentration was observed to decrease the emulsifying activity for the flour and the native protein even after polymerization. The emulsion stability (the half time of the initial turbidity) of the flour of the legumes was 5 min and that of native protein isolate was 5.5 and 4.5 min for pigeon pea and hyacinth bean, respectively. However, after polymerization the stability of the isolate increased to 7 and 7.5 min for the legumes, respectively (Fig. 5). Addition of NaCl improved the emulsion stability of the flour and native protein isolate up to 1.0M NaCl and further improvement was observed after polymerization of the isolate. Also it was observed that TGase treatment alleviated the effect of high concentration of NaCl. Similar results were reported by Olayide [22].

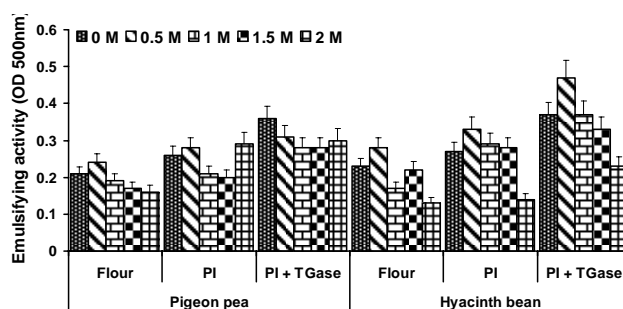


Fig. 4 Emulsifying activity of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different NaCl concentration (M). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

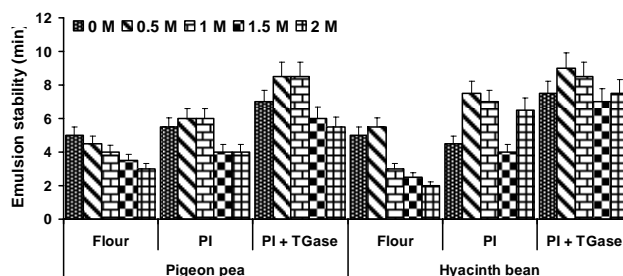


Fig. 5 Emulsion stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different NaCl concentration (M). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

The improvement in the emulsifying properties is likely due to an increase in the negative charges which result from the hydrolysis of the amide groups in glutamine and asparagine, as reported for millet protein [18]. The results obtained show that polymerization of legumes protein was very effective in the improvement of the emulsifying properties. It has been reported that addition of NaCl increased both emulsifying activity and stability. However, further increase in ionic strength progressively reduced both emulsifying activity and emulsion stability. Increase in ionic strength up to 0.2 M encouraged unfolding of protein molecules and subsequent increase in protein solubility [23]. This increase in protein solubility enhanced a rapid migration to the oil– water interface and improved emulsifying activity of the protein. With further increase in ionic strength (>0.2 M), screening of the surface charges increased and this encouraged protein–protein interaction but reduced protein–oil interaction.

The foaming properties of the legumes protein isolate were also improved after polymerization by TGase (Fig. 6 & 7). Increase in concentration of NaCl favoured the foaming capacity of the flour and protein isolate of both legumes up to 0.5M concentration, while further increase in concentration, from 0.5M to 2.0M reduced the foaming capacity of the flour and the protein isolate of both legumes (Fig. 6). The foaming capacity of the proteins was high at 0.5M concentration and

after polymerization was further increased for both legumes protein isolate. The foam stability (Fig. 7) of the protein was determined as a percentage of total whipping volume after the mixture was stands for 15 min. As shown in Fig. 7, the foam stability of the protein isolate stand for 15 min was high before addition of NaCl. Addition of NaCl greatly reduced the foam stability of the flour and the protein isolate even after polymerization. However, transglutaminase treated protein when compared to untreated one had high foam stability at all NaCl concentration levels for both legumes. Akintayo *et al.* [24] reported initial increase in foam capacity and stability of pigeon pea protein concentrate up to 0.5 M NaCl protein solution, while further increase in ionic strength markedly reduced foam capacity and stability. The improvement of the foaming properties of the protein polymers reflects the importance of protein association or polymerization as a structural factor governing the foaming property. The higher emulsion and foaming attributes of the treated protein could have been due to increased ability to form a interfacial protein film, since its high molecular size and cross linked structure are more resistant to excessive denaturation than the native protein at the high speed of the homogenization used to make emulsions and foams. Moreover, reduced electrostatic repulsion as the result of decrease in the number of amino groups could have enhanced protein-protein interaction and therefore protein adsorption on the interface. Transglutaminase catalysed polymers of cowpea proteins were also found to form better foam and emulsion forming ability than the native protein, results that were attributed to increase in strengthening of the interfacial protein film by the polymerized proteins [23].

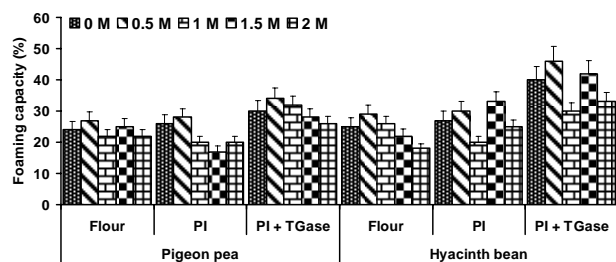


Fig. 6 Foaming capacity of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different NaCl concentration (M). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

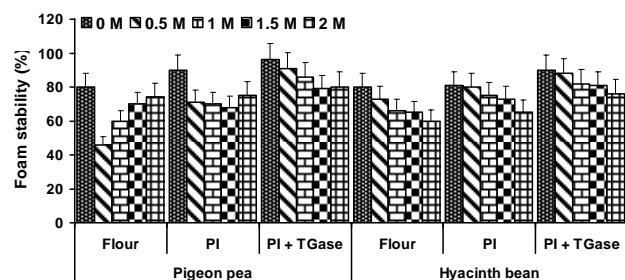


Fig. 7 Foam stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different NaCl concentration (M). PI, protein isolate. Error bars indicate the standard deviations (n = 3).

IV. CONCLUSION

The protein-protein complexes obtained from cross linking of legumes protein isolate had improved functional properties in the presence of high salt concentration. The solubility and heat stability of the polymers was enhanced significantly than the unpolymers. Thus transglutaminase could be used to improve the functional attributes of proteins with varied applications in food products.

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