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Transglutaminase cross-link of legumes protein isolate: changes in functional properties as a function of pH

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Abstract: The protein isolates of pigeon pea and hyacinth bean were polymerized by transglutaminase (EC 2.3.2.13) and the effect of such polymerization on the functional properties at different pH levels was studied. The reduction in the total free amino groups (OD340) 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 a wide range of pH level. Cross linked proteins were less turbid on heating to higher temperature as compared to untreated samples and the temperature at which the protein turns turbid also increased in the polymerized samples. The emulsifying and foaming properties of the protein polymers were greatly improved at a wide range of pH level compared to the flour and the native protein of the two legumes.

Keywords: transglutaminase, pigeon pea, hyacinth bean, functional properties, pH

Introduction

The use of plant proteins in the formulation of new food products or as a replacement for more expensive animal proteins in conventional foods has been the focus of various research efforts. 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. 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. Modification of food proteins has been investigated to improve their physical functionality, i.e. gelation, viscosity, emulsification and foaming (Motoki and Seguro, 1998). The ability of transglutaminase (TGase; E.C. 2.3.2.13) to modify the functional properties of food proteins has been extensively reviewed (Kuraishi et al., 2001; Lorenzen, 2000; Motoki and Seguro, 1998). By acyl group transfer between the ε-amino group of lysine and the γ-carboxyamide 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 (Gerrard, 2002). 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 (Singh, 1991). 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

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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 (Babiker et al., 1998; Babiker, 2000; Amro et al., 2007; Salma et al., 2010). 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 as a function of pH.

Materials and Methods

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.

Methods

Protein isolates preparation

The protein isolate was prepared by the method of Iwabuchi and Yamauchi (1987). 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 20oC. After centrifugation, the supernatant was acidified to pH 4.8 with 2N HCl and then centrifuged. The precipitated protein was dissolved in water at 4oC and the pH adjusted to 8. After centrifugation (8000 rpm), the clear supernatant was dialyzed against distilled water for 24 h at 4 oC and then freeze-dried.

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 55oC for 60 min. The enzyme was inactivated by N-ethylmaleimide (0.1 ml; 0.1%) (Kato et al., 1991). The treated samples were dialyzed against distilled water and then freeze-dried.

Changes in free amino groups

Changes in free amino groups of 0.1% protein solutions were determined by spectrophotometric assay (OD340) using o-phthaldiadehyde as described by Church et al. (1983).

Measurement of solubility

The samples of the native protein and that polymerized by transglutaminase (0.2%) were used for the determination of solubility at various pHs: pH 2-3, 0.05 M citrate buffer; pH 4-5, 0.05 M acetate buffer; pH 6-8, 0.05 M phosphate buffer; pH 9-11, 0.05 M carbonate buffer and pH 12, 0.05 M NaOH slightly adjusted with 0.05 M HCl. 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.

Heat stability

Heat stability was determined by the method described by Kato et al. (1995). The samples were dissolved at a protein concentration of 2 mg/ml in 50 mM Tris-HCl buffer (pH 7.0) and heated at 50-90oC for 20 min. Protein turbidity was measured for each sample at 500 nm.

Measurement of emulsifying properties

The emulsifying properties of the samples were determined by the method of Pearce and Kinsella (1978). 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 and Co. West Germany) at 12000 rpm for 1 min at 20 oC. 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.

Measurement of foaming properties

Foaming capacity (FC) of the sample was determined by following the procedure described by Lawhon et al. (1972). About 2.0 gm of the sample were blended with 100 ml buffer at different pH levels (2, 4, 6, 8, 10, and 12) in a Moulinex blender at "HI" speed for 2 min. The mixture was poured into a 250 ml measuring cylinder and the foam volume was recorded after 30 sec.

$$FC\% = \frac{\text{Volume after whipping – volume before whipping}}{\text{Volume before whipping}} \times 100$$

FC% was determined as a function of pH (2, 4, 6, 8, 10 and 12).

The foam stability (FS) was conducted according to Ahmed and Schmidt (1979). The FS was recorded at 15 min interval for the first 15 min after pouring the material in a cylinder. FS was determined using the following formula:

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

FS% was determined as a function of pH (2, 4, 6, 8, 10 and 12).

Results and Discussion

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 Figure 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 may have catalysed the transfer reaction between an amide group in a protein-bound glutamine and an ε-amino group in a protein-bound lysine side chain, resulting in cross-links between the protein molecules (Sakamoto et al., 1995; Sergo et al., 1995). Similar results were obtained when soy protein and chymotrypsin digests were polymerized by TGase (Babiker, 2000) and also when two legumes protein isolate were polymerized by TGase (Salma et al., 2010). The pH dependence of the solubility of the protein isolate of the two legumes with and without TGase treatment was investigated (Figure 2). The results showed that both the flour and protein isolate of each legume was markedly insoluble at pH 4 and 6. However, after being polymerized by TGase, the protein isolate of both legumes was observed to be soluble at pH 4 and 6, while at pH 2 and alkaline pH (pH 8-12) it was completely soluble. The improvement in the solubility due to TGase treatment of the protein isolate at various acidic and alkaline pH values is mainly due to the fact that the protein isolate had recognizable sites for TGase reaction and the resultant polymer, as shown in Figure 1, 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 (Amro et al., 2007). Flanagan et al. (2003) reported that limited and extensive TGase cross-linking resulted in significant improvements in solubility at low pH (pH 2.0 and 3.0). Improvements in solubility

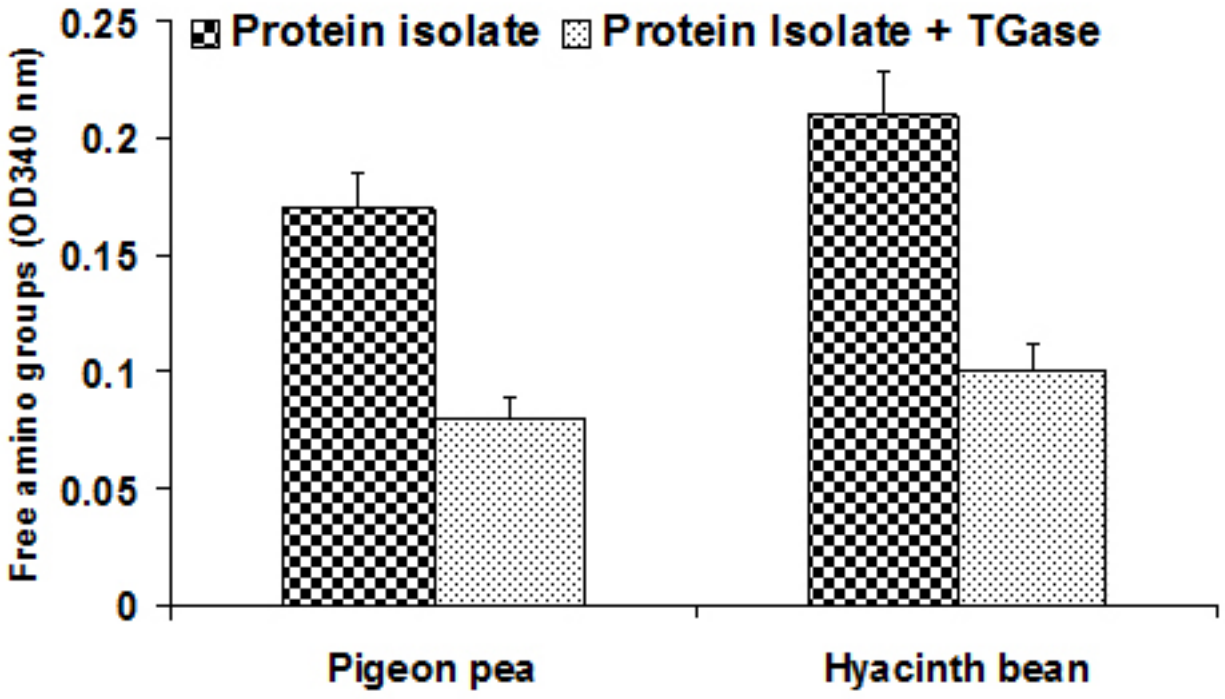

Figure 1. Changes in free amino groups of the protein isolate of selected legumes treated with transglutaminase (TGase). Error bars indicate the standard deviation of three replicates.

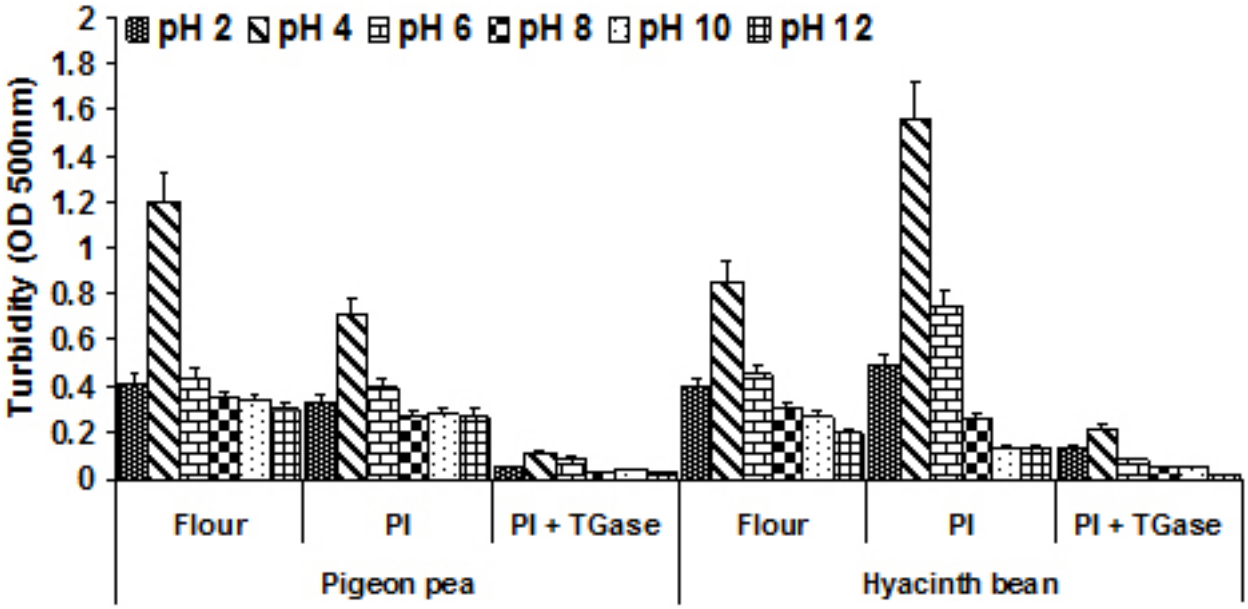

Figure 2. Solubility of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3).

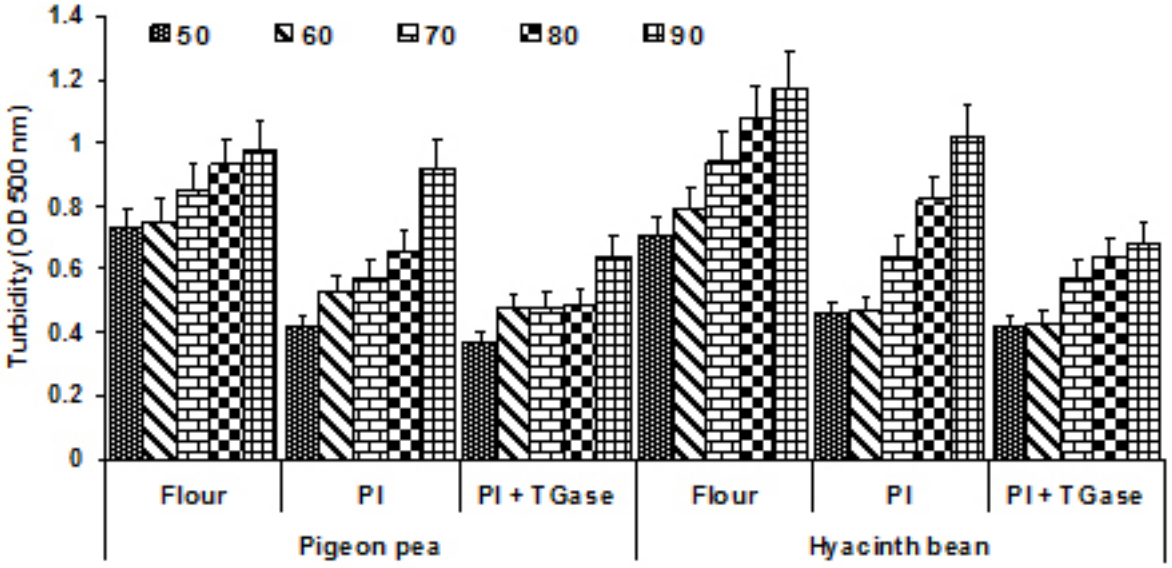

Figure 3. Heat stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3). Legend values are in ^{o}C (Temperature).

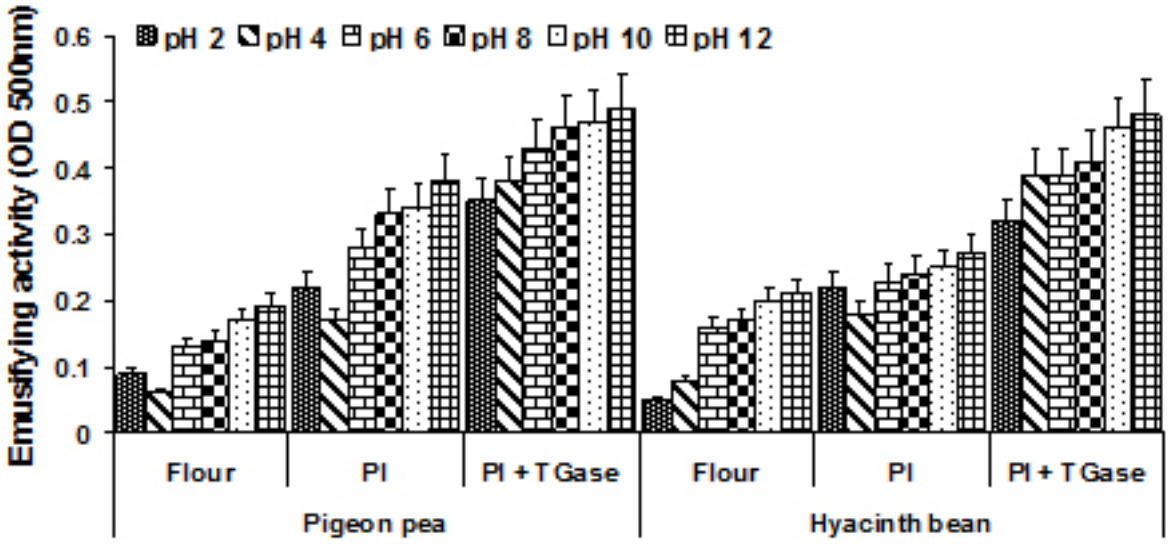

Figure 4. Emulsifying activity of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3).

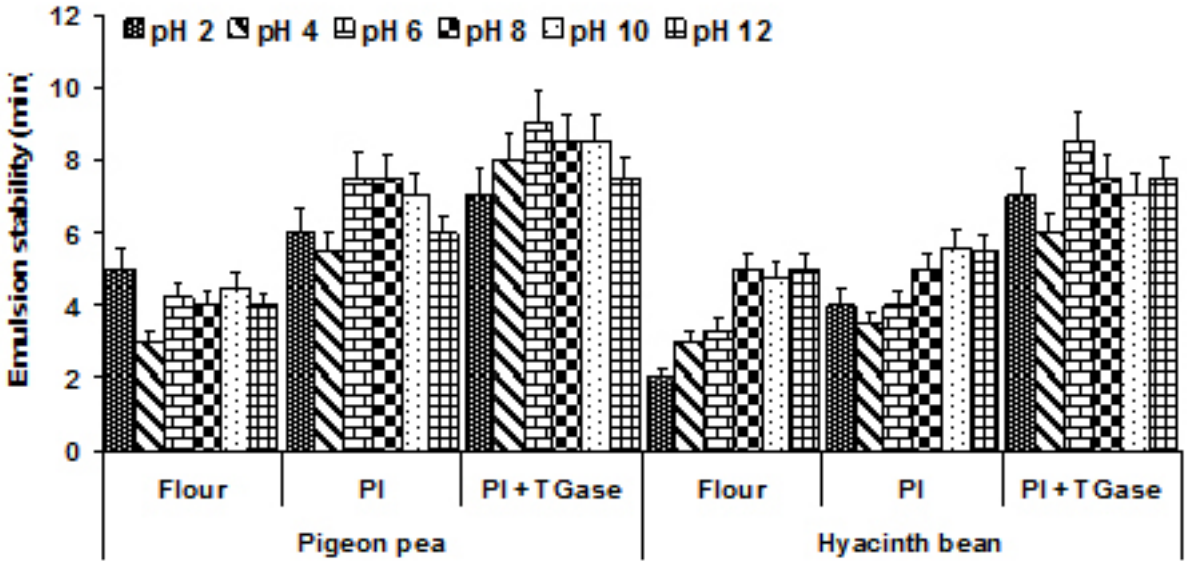

Figure 5. Emulsion stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3).

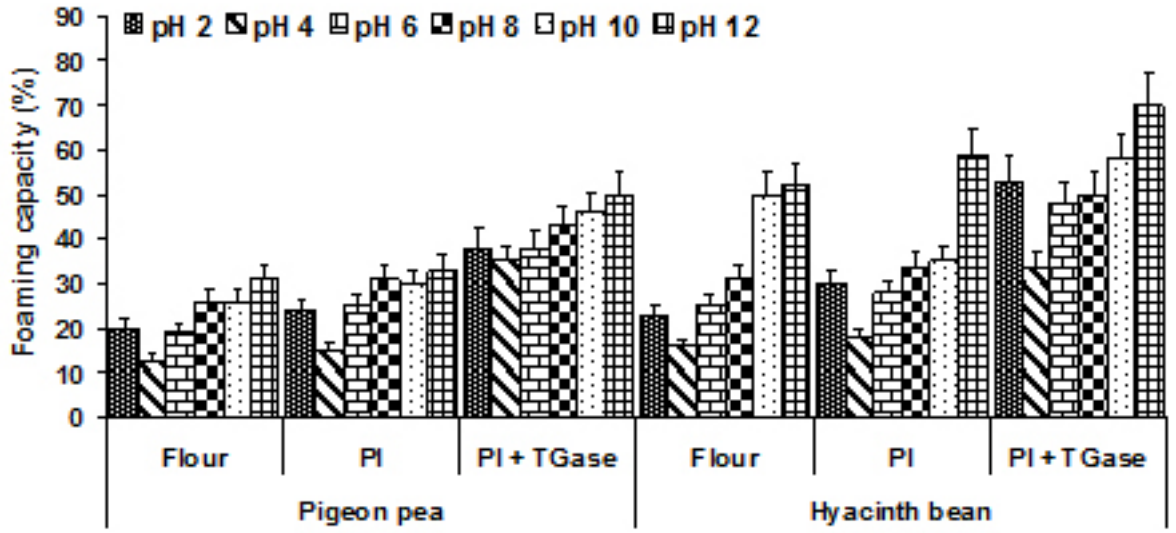

Figure 6. Foaming capacity of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3).

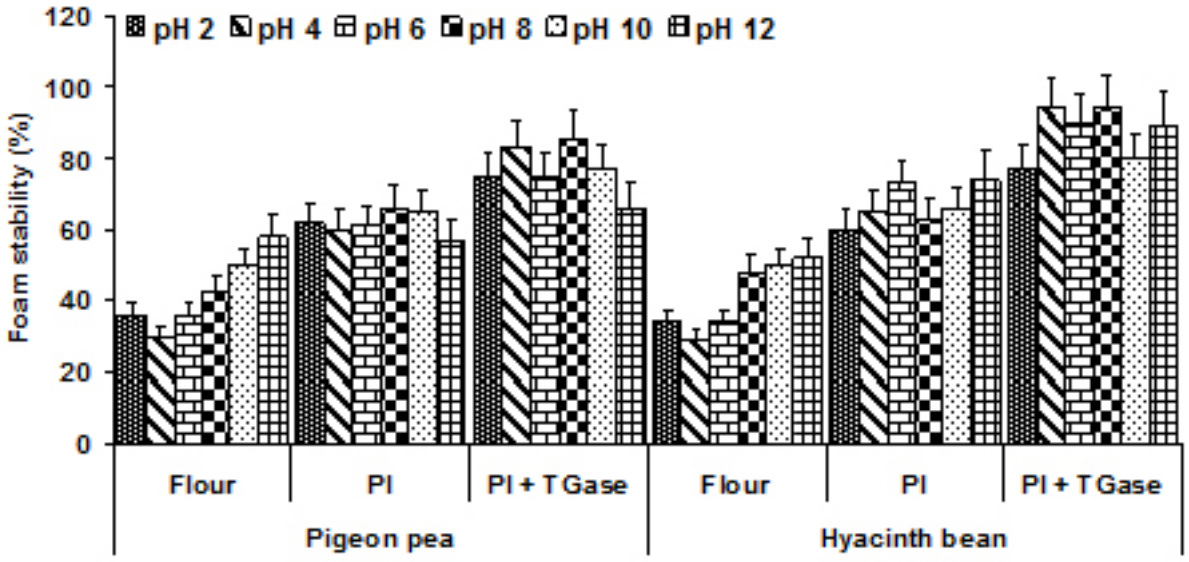

Figure 7. Foam stability of legumes flour and protein isolate polymerized by transglutaminase (TGase) at different pH levels. PI, protein isolate. Error bars indicate the standard deviations (n = 3).

were also observed at pH 5.0 for the minimal and extensively cross-linked samples (Flanagan et al., 2003). The solubility (OD500nm) of the flour and the protein isolate of the legumes under investigation decreased as the heating temperature increased (Figure 3) but after TGase treatment the stability against heat was greatly improved for the protein isolate of both legumes. The turbidity (OD500nm) was observed to reach 0.97, 0.92 and 0.64 for the flour, protein isolate and protein isolate treated with TGase at 90oC, respectively of pigeon pea while that of hyacinths bean was 1.17, 1.02 and 0.68 for the treatments, respectively. The results indicated that TGase treatment was found to be effective in improving heat stability of legumes protein. Moreover, 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 (Nai et al., 2002). 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 (Flanagan et al., 2003).

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

The physical functionalities such as the emulsification and foaming properties of the protein isolate of legumes are poor and in order to improve these physical properties, the effect of TGase treatment was investigated. As shown in Figure 4, the emulsifying activity of the protein isolate polymers was improved. The emulsifying activity of the protein isolate, which is estimated as a percent of emulsion, was greatly improved at pH 2 and alkaline pH values. However, at pH 4 the emulsifying activity was found to be very low for the flour and the protein isolate of both legumes but after TGase treatment it was greatly improved. The emulsion stability (the half time of the initial turbidity) of the flour and the protein isolate of both legumes was found to be low especially at pH 4 but after polymerization by TGase the emulsion stability of the protein isolate was greatly improved for both legumes at all pH levels (Figure 5). 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 (Amro et al., 2007). The results obtained show that polymerization of legumes protein was very effective in the improvement of the emulsifying properties. The foaming properties of the legumes protein isolate were also improved after polymerization by TGase (Figures 6 and 7). The foaming capacity (Figure 6) of the flour and the protein isolate of the two legumes was low at pH 4 and after polymerization; the foaming capacity of the protein isolate of both legumes was greatly improved. Compared to that of the flour, the foaming capacity of the native protein isolate of both legumes was found to be high at pH 2 and 12 compared to other pH levels. Further improvement in foaming capacity of both legumes protein at pH 2 and 12 was observed after TGase treatment. The foam stability (Figure 7) of the flour and the protein isolate of the legumes was determined as a percentage of total whipping volume after the mixture was stands for 15 min. As shown in Figure 7, the foam stability of the flour and the protein isolate of both legumes stand for 15 min was slightly high at all pH levels except at pH 4. Transglutaminase treatment greatly improved the foam stability of the protein isolate of both legumes especially at pH 4. The improvement in 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 an 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 (Aluko and Yada, 1995).

Conclusion

The protein–protein complexes obtained form cross linking of legumes protein isolate had improved the functional properties at different pH levels than the unpolymerized proteins. The solubility and heat stability of the polymers was enhanced significantly than the unpolymerized proteins. Thus transglutaminase could be used to improve the functional attributes of proteins with varied applications in food products.

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