Functional modifications of enzyme-hydrolyzed albumin fractions from legumes

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Abstract: The quality improvement of plant food stuff can be achieved through modification of its protein content by various means including enzymatic hydrolysis. In this study, the functional properties of enzyme-hydrolyzed water soluble protein fraction (albumin) from four dehusked legumes viz, Bengal gram (Cicer arietinum), Green gram (Phaseolus aureus), Red gram (Cajanus cajan) and Lentil (Lens esculenta) were considered for any desirable changes. The enzymatic treatment of albumin fractions of legumes influenced the functional properties to a varying extent. While minor differences were noted for bulk density, fat absorption, protein solubility and foaming property, a great reduction was observed in water absorption capacity of treated samples.

Key words: Albumin, enzymatic food processing, legume, Bengal gram, Green gram, Red gram, Lentil.

Introduction

Proteins play a major role in influencing physical and chemical properties of food systems. They have been used as additives in foods for improving the rheological, surfactant, flavour, structure and functional properties. Functional properties are related to physico-chemical properties, which give information on how a particular component (such as protein or carbohydrate) will behave in a food system. The functional properties of plant foods are determined by the molecular composition and structure of the individual component and their interactions with one another (Kinsella, 1976). These can be modified to alter their behaviour as required for various processed end products. For proteins three types of modifications, viz, thermal, chemical and enzymatic, have been experimented with and in some cases, successfully utilized for improving the quality of end products. Thermal modifications include dry heat, wet heat and microwave treatments. Succinylation and acetylation have been frequently reported as part of chemical modification. Partial denaturation and hydrolysis of proteins by enzymes have been used for changing their solubility and related properties (Beuchat,1977; Beuchat et al., 1975; Sekul et al., 1978; Bhagya & Srinivasan, 1989; Geethalakshmi & Prakash, 2000; Nagmani & Prakash, 1997).

Protein functionality can be altered or extended by the action of enzymes through partial hydrolysis of the polypeptide backbone, incorporating inter- or intra-molecular cross links and attaching specific groups. Enzymatic modification of food proteins by controlled hydrolysis decreases the chain length of polypeptides and brings about changes in functional properties which are dependent on particle size, molecular weight, hydration and surface properties (Kinsella, 1981; Prakash & Rao, 1986). Specific properties of the hydrolysates are dependent upon the degree of hydrolysis, physical and chemical character of the protein substrate and reaction conditions. There is an optimum degree of hydrolysis beyond which any improvement gained in functional behaviour can be lost. Partial proteolysis facilitates unfolding of polypeptides and thereby enhance certain functional properties such as solubility and foaming properties. Proteolysis generally leads to reduced tendency for gelation, increased foam volume upon whipping and decreased foam stability and enhanced thermal stability. Factors which affect the enzymatic hydrolysis of proteins include enzyme concentrations, pH, ionic strength, temperature and absence or presence of inhibitory substances. Enzymatic hydrolysis of proteins is accompanied by three distinct effects that directly affect the functional properties - a decrease in molecular weight, an increase in the number of ionizable groups and the exposure of hydrophobic groups (Panyam & Kilara, 1996). The use of proteolytic enzymes to improve protein functionality is most promising to increase its application and also in the formulation of nutritious foods that have identical or more desirable functional characteristics than the traditionally accepted foods.

Based on the classical fractionation originally suggested by Osborne (1865), proteins are classified as albumins (water soluble), globulins (soluble in salt solution), prolamin (soluble in alkali solution) and glutelins (soluble in 70% ethanol). Legumes generally have a larger fraction of albumins and globulins. They differ in their amino acid composition and conformational
structure. Hence there is a possibility that these fractions may also differ in their functional properties. While there are reports on the functional properties of legume flours as such (Nagmani & Prakash, 1997; Geethalakshmi & Prakash, 2000; Ghavidel & Prakash, 2006; Puyed & Prakash, 2006), there are limited studies on functional properties of isolated protein fractions. Effects of enzymatic hydrolysis on functional properties of these fractions have also not been studied. The present investigation was planned to study the functional properties of the water soluble protein fraction from legumes. Since enzyme modification can alter some of these functional properties, an attempt was also made to study the effect of a proteolytic enzyme pancreatin on the functional properties of these isolated albumin fractions.

Materials and methods

Four commonly used legumes in decorticated form (without outer husk) viz, Bengal gram (Cicer arietinum), Green gram (Phaseolus aureus), Red gram (Cajanus cajan) and Lentil (Lens esculenta) were procured from the local market of Mysore city, cleaned and ground into a fine powder and sieved through a 40-mesh sieve to obtain the water soluble protein fraction. The chemicals used for the study were procured from Qualigens fine chemicals and SD fine chemicals, Mumbai, India. The enzyme used for hydrolysis was pancreatin (Batch no. RM083, from pig pancreas) obtained from Himedia Laboratories Ltd. Mumbai. Glass double distilled water (5.0ml) was added in the same tube for washing the particles adhering to the glass rod. The tubes were weighed and allowed to stand for 30 min. The suspension was centrifuged at 3500 rpm for 30 min. The extra water was drained from the tube and the tubes were weighed again. The difference in weight of the tubes before and after was taken as water absorbed and expressed as the amount of water bound by 100 g of sample.

Fat absorption capacity (FAC) was determined by the method of Sosulki et al., (1976). To 1.0 g of sample, 10 ml of refined sunflower oil (Sunpure Brand, procured from local market, Mysore) was added. After 30 min, the slurry was centrifuged at 3500 rpm for 30 min. The oil was completely drained by inclining the tube at a 45° angle for one hour and measured. The amount of oil retained was calculated by difference in initial and final volume. FAC was described as the amount of oil bound by a 100 g of sample.

Nitrogen solubility (NS) of the albumin fractions was determined in the pH range 2-10. A weighed amount (250mg) of sample was added to 25 ml of distilled water. The pH was adjusted and maintained by the addition of 1 N hydrochloric acid or 1 N sodium hydroxide over a period of 60 min with continuous agitation in a shaker bath at 27°C. The slurry was centrifuged at 3500 rpm for 30 min and nitrogen was estimated in supernatant by micro-Kjeldahl procedure (AOAC, 1984). NS was expressed as protein solubilized per 100g of sample by
converting the value to protein and taking into consideration the original protein content of the sample.

**Foam properties**

For determination of foam capacity (FC) and foam stability (FS) the foam was prepared by dispersing and mixing 2 g of the sample in 100 ml water. The suspension was whipped in a blender for 3 min, before pouring into a 250 ml measuring cylinder. The volume of foam was recorded after 30 sec. Foaming capacity was expressed according to Lawhon et al. (1972), as increase in volume in percent. The liquid or leakage from syneresis of the foam was observed at 10, 20, 30, 45 and 60 min time intervals for a period of 1 h at room temperature (27°C). This was denoted as foam stability and was expressed in volume percent as suggested by Ahmed and Schmidt (1976).

**Emulsion properties**

Emulsifying activity was determined using the method of Yasumatsu et al. (1972) by measuring the volume of the emulsified layer in relation to the total height of the contents after centrifugation at low speed. To 100 mg sample, 10 ml distilled water was added and blended for 30 sec in a standard blender. Refined groundnut oil (10 ml) was added to the slurry and blended again for 5 min. These samples were centrifuged for 20 min at 3000 rpm. The emulsifying activity was calculated by using the equation given below:

\[
\text{Emulsifying activity} = \frac{\text{Height of the emulsified layer after centrifugation}}{\text{Height of the total contents in centrifuge tube}}
\]

The emulsifying activity was expressed as percent. For determining emulsifying stability, the emulsions prepared as described above were heated at 80°C in a hot water bath for 30 min and centrifuged again at 3000 rpm for 5 min. Emulsifying stability was also calculated using the above equation.

**Statistical analysis**

All analyses were done in duplicate with four replications and average values are reported. Data were subjected to statistical analysis using software Minitab 11.32 on an IBM compatible computer to determine significant differences with probability levels of \( p < 0.05 \) as marginally significant and \( p < 0.001 \) as highly significant.

**Results and discussion**

The protein content of isolated albumin fractions from the legumes, and bulk density, water and fat absorption capacities are presented in Table 1. The isolated albumin fraction was very rich in protein content ranging from 81.3% for red gram to 89.1% for lentils indicating that most of the protein could be extracted in water. Green gram and Bengal gram had intermediate values of 85.9% and 84.9%, respectively. Pretreatment as enzymatic hydrolysis resulted in an improvement of protein fraction in all 4 samples ranging from 3.8 to 5.6 g/100g. Clearly such higher protein value in extraction could be attributable to better extractability of protein after enzyme treatment, which causes breakage of peptide bonds and converts protein to smaller fractions.

Bulk Density of the albumin fractions ranged between 74.4 to 93.8 g/100ml and was least for the Bengal gram fraction and highest for lentil. After enzyme hydrolysis, the fraction from Bengal gram was similar to the control with no variations but major differences were observed in Green

<table>
<thead>
<tr>
<th>Albumin fractions</th>
<th>Protein (g/100g)</th>
<th>Bulk density (g/100ml)</th>
<th>Water absorption capacity (ml/100g)</th>
<th>Fat absorption capacity (ml/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green gram</td>
<td>85.9 ± 2.21</td>
<td>84.9 ± 1.37</td>
<td>144.6 ± 2.33</td>
<td>97.5 ± 2.26</td>
</tr>
<tr>
<td>Bengal gram</td>
<td>84.9 ± 0.93</td>
<td>74.4 ± 0.74</td>
<td>135.0 ± 1.51</td>
<td>82.2 ± 1.03</td>
</tr>
<tr>
<td>Red gram</td>
<td>81.3 ± 0.00</td>
<td>88.2 ± 0.00</td>
<td>144.0 ± 1.93</td>
<td>92.4 ± 1.37</td>
</tr>
<tr>
<td>Lentil</td>
<td>89.1 ± 0.09</td>
<td>93.8 ± 0.00</td>
<td>143.4 ± 0.58</td>
<td>86.4 ± 0.00</td>
</tr>
<tr>
<td>Enzyme hydrolyzed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green gram</td>
<td>91.5 ± 0.0</td>
<td>93.7 ± 0.0</td>
<td>80.3 ± 0.51</td>
<td>82.6 ± 2.12</td>
</tr>
<tr>
<td>Bengal gram</td>
<td>89.6 ± 0.0</td>
<td>75.0 ± 0.0</td>
<td>122.7 ± 1.85</td>
<td>84.9 ± 0.32</td>
</tr>
<tr>
<td>Red gram</td>
<td>85.1 ± 0.0</td>
<td>88.1 ± 0.17</td>
<td>91.5 ± 2.64</td>
<td>76.5 ± 0.11</td>
</tr>
<tr>
<td>Lentil</td>
<td>94.1 ± 0.0</td>
<td>88.2 ± 0.00</td>
<td>100.8 ± 0.88</td>
<td>81.5 ± 1.18</td>
</tr>
<tr>
<td>P value</td>
<td>0.000522 ***</td>
<td>0.38787 ns</td>
<td>0.0154*</td>
<td>0.0792 ns</td>
</tr>
</tbody>
</table>

**Table 1. Protein content, bulk density, water and fat absorption capacities of albumin fractions**

nc – Not Significant, * - \( p < 0.05 \), *** - \( p < 0.001 \).
gram and Lentil fractions. The bulk density of the enzyme-hydrolyzed fraction of Green gram rose to 93.7 g/100ml and that of Lentil to 88.2 g/100ml.

The water absorption capacity of the albumin fraction ranged from 135 to 144.6 ml/100g. In enzyme hydrolyzed samples, a significant decrease in water absorption was observed. The extent of lowering was different for each sample. In case of Green gram, the water absorption was 80.3 ml/100g, indicating a decrease of 44.5% compared to control sample (without enzymatic pretreatment). Same way, the enzyme hydrolyzed albumin fraction of Bengal gram, Red gram and Lentil was 9.1%, 36.5% and 29.8% lesser than control, respectively. Clearly enzyme hydrolysis increased in protein extraction due to enzyme hydrolysis, but lowered the water absorption quality of the protein indicating a lesser number of hydrophilic groups on the surface of the protein molecule. The degree of such changes also varied widely among samples indicating that the enzymatic treatment did not impose the effect uniformly in all samples. Probably, the type of protein configuration of a given sample can be attributed for the variation (Prakash & Rao, 1986).

The fat absorption capacity of albumin fractions of legumes was much lower than that of water absorption. Among the samples, Green gram albumin absorbed highest amounts of fat (97.5%) while it was least in Bengal gram (82.2%). The enzyme hydrolysis lowered the fat absorption capacity by 15.3% (82.6ml/100g) in Green gram and by 17.2% in Red gram (76.5ml).

In contrast, the fat absorption capacity in the Bengal gram increased by 2.7% (84.9ml/100g). This phenomenon of increased fat absorption capacity after enzyme hydrolysis was exhibited only in Bengal gram, which may be attributed to a higher number of exposed hydrophobic groups. It may be noted that Bengal gram has the highest amount of total fat content, i.e., 5.6% when compared to other legumes such as Green gram (1.2%), Lentil (0.7%) and Red gram (1.7%) (Gopalan et al., 1976).

A study conducted by Nagmani and Prakash (1997) showed that the water absorption and fat absorption of thermally treated and untreated legumes exhibited a large variation. According to them, the water absorption for Bengal gram, Green gram and Lentil were 138, 186 and 180ml/100g, respectively, while Black gram absorbed an enormous amount of water, i.e., 441ml/100g. After thermal treatment, some of the samples showed a difference in water absorption capacity, which was higher for Bengal gram and Black gram and lower for Green gram.

The protein solubility profile of different samples analyzed is presented in Fig. 1-4. The protein solubility curves exhibit the typical bell shaped patterns of oil seed proteins (Prakash & Rao, 1986). However the curves are characterized by a large range of isoelectric pH between 4.0 and 6.0 indicating a wide precipitation range of these proteins. In Green gram sample, at acidic pH 2.0, 39.1% of protein could be solubilized and the value increased to 54.3% at pH 3.0 (Fig.1). However, on increasing
the pH to 4.0 a sharp drop of 6.4% was observed and the values were low up to pH 6.0 after which it increased dramatically at pH 7.0 and continued in the alkaline pH range. The enzyme hydrolyzed sample also exhibited a similar curve with slight variation in values in the acidic range (pH 2.0 - 3.0). There was a slight decrease, but in the pH range of 4.0 - 5.0, the values were a little higher than the control sample, between pH 6.0 and 7.0. The values were similar at the higher pH range of 8.0-11.0. Protein in the enzyme hydrolyzed sample was more solubilized.

The protein solubilized from two samples of Bengal gram is presented in Fig. 2, which shows a lesser extent of digestibility in comparison to Green gram samples. At pH 2.0, 33.5% of protein was soluble but when the same sample was subjected to enzyme hydrolysis, the solubility dipped to 29.3%. Similar trend but slightly higher value (38.6% and 36.7%, respectively) was noticed at pH 3.0. The values at pH 4.0 and 5.0 were almost similar irrespective of the pretreatment (4.3 and 6.3%). At pH higher than 6, differences continued to exist with an exception at pH 8.0. Even in the alkaline range the solubility values of Bengal gram were slightly lower than Green gram sample.

In Red gram, (Fig. 3), differences were clearly shown between the solubility curves of the albumin fraction with or without enzyme hydrolysis. The control sample (without enzymatic hydrolysis) for the entire range exhibited slightly higher values compared to samples subjected to enzymatic hydrolysis. The differences were great in the lower pH range. In the acidic pH range, the values of the control sample at pH’s 2.0 and 3.0, were 39.8% and 46.3%, respectively; while it was 33.9 and 38.8%, respectively for the enzyme hydrolyzed samples. At the isoelectric pH, the solubility was lowest for the control sample (7.4%), whereas the enzyme treatment still lowered it to 6.9 - 3.8%. At pH 7.0 and above, an increase in solubility was observed, ranging from 42.6% to 44.0%. In the enzyme hydrolyzed samples, the values were between 36.7 to 43.8%.

The albumin fraction from Lentil also clearly demonstrated the effect of enzyme treatment. The control sample exhibited a slightly higher solubility at all pH ranges from 2.0-11.0 (Fig. 4). This sample was different from other legumes at the isoelectric pH. It had the least solubility at pH 5.0, and at pH 4.0 the solubility was 18.6% for control and 14.4% for the enzyme treated samples, which was highest among the samples analyzed. Other samples exhibited a low solubility even at pH 4.0. The protein solubility at a pH 7.0-11.0 was very similar, ranging from 47.9 to 49.5%.

Youssef et al., (1995) reported protein solubility of chickpea and white bean. They recorded the minimum protein solubility for chickpea, white bean and fat free sesame flours at pH 4.5. The solubility of protein was reported to increase especially in the alkaline range (pH 9.0).

Foam capacity and stability of all four albumin fractions as well as their enzyme hydrolyzed counterparts are presented in Table 2.

**Table 2. Foam capacity and stability of albumin fractions from legumes**

<table>
<thead>
<tr>
<th>Albumin fractions</th>
<th>Control (% foam volume)</th>
<th>Enzyme hydrolyzed (% foam volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Green gram</strong></td>
<td>150</td>
<td>130</td>
</tr>
<tr>
<td><strong>Bengal gram</strong></td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td><strong>Red gram</strong></td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td><strong>Lentil</strong></td>
<td>135</td>
<td>130</td>
</tr>
</tbody>
</table>

Youssef et al., (1995) reported protein solubility of chickpea and white bean. They recorded the minimum protein solubility for chickpea, white bean and fat free sesame flours at pH 4.5. The solubility of protein was reported to increase especially in the alkaline range (pH 9.0).

Foam capacity and stability of all four albumin fractions as well as their enzyme hydrolyzed counterparts are presented in Table 2.
2. It might be noted that the protein samples were not able to exhibit any foaming capacity in water medium at pH 7.0. This is quite likely since legume flours are known for very low foaming property, with exception of Black gram and soybean. Hence experiment was also conducted with 0.2M NaCl at neutral pH as suggested by Giami (1993). This methodology helped in the formation of foam in all samples. The foaming capacity of albumin fractions of all legumes ranged between 135-150ml. The Green gram albumin fraction exhibited foaming stability up to 45 min. Bengal gram did not exhibit any foaming stability. The foam disappeared in Red gram immediately after the initial recorded value. Lentil also followed a similar pattern with a slightly higher foam value at 10 min. After enzyme hydrolysis, a reduction of foaming capacity was observed for all samples which ranged between 110-135%. These samples did not exhibit any foam stability with the exception of Green gram where some foam could be seen at 10 min. It can be concluded that the albumin fractions of legumes, though exhibit some foaming properties in NaCl media, showed considerable reduction after enzyme hydrolysis.

The emulsion activity and stability of the protein samples given in Table 3 showed a range of 48-56%. Green gram and Red gram were in lower range while Bengal gram exhibited higher value. On enzyme hydrolysis, Green gram and Red gram showed an insignificant increase in emulsion activity in contrast to a great decrease (49%) in Bengal gram. The emulsion stability of these samples was also determined by holding the emulsions in a water bath at 80°C for 30 min. It was seen that none of the samples showed any reduction on application of heat, hence it can be said that the emulsion formed was stable. Legume flours are known for forming moderate amounts of stable emulsion and contribute to food systems.

<table>
<thead>
<tr>
<th>Albumin fractions</th>
<th>Control (%)</th>
<th>Enzyme hydrolyzed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emulsion activity</td>
<td>Emulsion stability</td>
</tr>
<tr>
<td>Green gram</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Bengal gram</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Red gram</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Lentil</td>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>

From the results of the present study, it is observed that the enzyme treatment of albumin fractions of legume influenced the functional properties to a varying extent. While minor differences were seen in bulk density, fat absorption capacity, protein solubility and foaming property, major differences were observed in water absorption capacity. Information of such a kind may find application in food processing industries to incorporate desired qualities.

**References**