Effect of Chemical Reagents on Functional Properties of Mungbean Protein Products

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ABSTRACT

Mungbean protein products were modified to various degrees by succinic anhydride, acetic acid and sodium sulfite. Changes in functional properties such as nitrogen solubility, water holding capacity, oil binding capacity, foaming activity and stability, and emulsion activity and stability of modified mungbean protein products were determined. Mungbean protein products significantly decreased the nitrogen solubility and increased the water holding capacity and oil binding capacity at all levels of modification compared to untreated mungbean protein product. Foaming activity was significantly increased up to 0.2-0.4% acetic acid. Modification mungbean protein products significantly increased the emulsion activity at 0.4% succinic anhydride, 0.2-0.4% acetic acid and 0.2-0.4% sodium sulfite compared to untreated mungbean protein product.

Key words: mungbean, protein product, chemical modification, functional properties

INTRODUCTION

Mungbeans [*Vigna radiate* (L.) Wilczek, *Phaseolus aureus Roxb*., Vigra mungo (L) Hepper.], commonly known as green gram, an important crop in Thailand and a popular legume in Asian countries is an excellent source of high-quality protein (Mendoza *et al.*, 2001). They contain 20% to 27% protein (El-Adawy, 1996) and about 1.0% to 1.2% fat. Mungbeans are consumed as a food, boiled and cooked with vegetables and meat, as well as dessert, cooked in syrup, or incorporated in bread or cake. They are also popularly used to make spouts for egg rolls, other vegetable dishes and starch noodles (Mendoza *et al.*, 2001). Mungbean residue, which is another waste material from the production of mungbean noodles, is also used for animal feed (Prabhavat, 1988). In Thailand, mungbean protein from the production of mungbean noodles is at low value, has a bad smell and poor functional properties. Mungbean protein has been shown to perform many desirable functions in processed foods, such as foaming, emulsification, water holding capacity, and oil binding capacity. However, improvements in those functions would make mungbean protein more desirable as a food component.

Protein modification using chemical modification is an important tool for tailoring food proteins into products with different functional properties (Li-Chan *et al.*, 1979). Chemical modification, the acylation of protein with acetic or succinic anhydride is one of the most convenient and most frequently used methods for altering the functional properties of many plant proteins. A great number of food proteins have been investigated with regard to the improvement of their functional properties by acylation. These

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comprise wheat gluten (Barber and Warthesen, 1982), proteins from oat (Ma, 1984), peanut (Beuchat, 1977), cottonseed (Choi *et al*., 1981), soybean (Franzen and Kinsella, 1976), sunflower seed (Kabirullah and Wills, 1982), field pea (Johnson and Brekke, 1983), rapeseed (Ponnampalam *et al*., 1990) and mungbean (El-Adawy, 2000), among other proteins. Moreover, chemical modification procedures used in the manufacture of processed food include general non-specific modifications. An example is oxidation-reduction of disulfide bonds with sulfite (Howell, 1996).

The objectives of this study were to improve the production and the functional properties of mungbean protein and product. Therefore the effect of acetic acid, succinic anhydride and sodium sulfite on mungbean protein products, the functional properties of mungbean protein products from waste liquor, and the chemical composition of mungbean protein products were presented.

MATERIALS AND METHODS

Materials

Cracked mungbean seeds were obtained from the Amonpun market, Bangkean, Jatujuk, Bangkok, Thailand. The seeds were cleaned by hand to remove the foreign materials.

Succinic anhydride, acetic acid and sodium sulfite were obtained from E. Merck, Germany. All other chemicals used were of reagent grade.

Preparation of mungbean protein solution

Mungbean protein solution was extracted from cracked mungbean seeds by a water extraction process. The cracked mungbeans were washed with water 2-3 times or until clean, after that they were soaked in water (bean: water = 1:1) at room temperature for 3 hours. Then the mungbeans were blend with water in the ratio of bean: water 1: 6 (by weight) by using a Waring blender to obtain a slurry which was then filtered through a fine cloth. A mungbean protein-starch solution and a mungbean residue were obtained. The mungbean protein-starch solution was collected to stand for 3 hours to precipitate the starch. The protein solution was decanted. In this way mungbean protein solution was obtained. The mungbean protein solution was proximate by analyzed.

Modification of mungbean protein

The mungbean protein solution were acylated and oxidation-reduction of disulfide bonds by reaction with acetic acid, succinic anhydrides and sodium sulfite separately, by adding different concentrations of these reagents (0.2, 0.4, 0.6 and 0.8 g/mol of protein solution) at pH 8. The slurry was left for 90 min at room temperature, then it was coagulated at the isoelectric point with HCl, followed by separation by centrifugation at 3,781 x g for 10 min with Sorvall RC 5C Plus. The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to spray-drying. Control was treated in the same manner except that no modifying reagents were added. All extractions were carried out in triplicate. The mungbean protein products were stored in containers at 5 °C for further analysis.

Chemical analysis

Moisture, crude protein, lipid, ash, and carbohydrate contents (by difference) of samples were determined in duplicate by standard methods analysis (AOAC, 2000).

Functional properties determinations

Nitrogen solubility (NS) of protein isolate was determined according to the method of AACC (1976). Mungbean protein samples (5g) were dispersed in 200 ml distilled water, shaken at 200 rpm for 2 hours at room temperature and centrifuged for 10 min at 3,781 x g using Sorvall RC 28S centrifuge (rotor F16/250). The protein of dispersion after centrifugation supernatant was determined using the Kjeldahl method AOAC

(2000). The solubility was expressed as percent ratio of the protein content of the supernatant to that of the suspension.

Emulsion activity and stability (EA/ES) of protein isolate were determined according to the modified method of Beuchat (1977), 20 ml of pure soy oil were added to the mungbean protein samples dispersions 1.0% (w/v, 30 ml) in distilled water. The mixture was then homogenized for 1 min at 4,700 g using a Ultra Turax T-25 homogenizer. Immediately afterwards, the mixture was transferred into a 100-ml measuring cylinder to stand for 1 hour and the emulsion volume was recorded. Emulsion activity was calculated by the equation :

$$EA\ (\%) = H_1/H_T \times 100$$

where H_1 = the high of emulsified layer, H_T = the high of total content in the tube.

Foaming activity and stability (FA/FS) of protein isolate were determined according to the modified method of Liceage-Gesualdo and Li-Chan (1999), as described by Johnson and Brekke (1983). Distilled water was added to mungbean protein samples to give 3.0% protein (w/v). The solution was adjusted to pH 6.5-7.0 with 0.1 N NaOH and mixed gently. Fifty milliliters of protein solution were homogenized for 3 min using a Waring blender at high speed. Immediately afterwards, the mixture was transferred into a 100-ml measuring cylinder and the foam volume was recorded. The percentage ratio of volume increase to that of the original volume of protein solution was calculated and expressed as foam activity. Foam stability was expressed on the basis of 50 ml of a 3% (w/v dispersion) as the volume of the foam remaining after 30 min of quiescent period.

Water holding capacity (WHC) was determined by the modified method of Quinn and Paton (1979). Mungbean protein samples (1g) were weighed into 30 ml centrifuge tubes. Distilled water (10 ml) was added, followed by mixing with stirrer to form a homogeneous paste. The tubes were centrifuged for 15 min at 8,175 x g (Sorvall

RC 28S centrifuge , USA). The small amount of supernatant was carefully removed by decanting and the tubes were weighed to determine the weight difference between the weight of the hydrated pellet and the original product/g dry product.

Oil binding capacity (OBC) was determined by the modified method of Sosulski (1976). Mungbean protein samples (1g) were weighed into 30 ml centrifuge tubes. Soybean oil (10 ml) was added, followed by mixing with stirrer to form a homogeneous paste. The tubes were centrifuged for 15 min at 8,175 x g (Sorvall RC 28S centrifuge, USA). The free oil was decanted and the tubes were weighed to determine the weight difference between the weight of the oil pellet and the original product/g dry product.

Gelation of protein isolate was determined according to the method of Coffmann and Garcia (1977). Mungbean protein samples were dispersed in distilled water to make 100 ml total volume and protein concentrations of 2-20% (w/v). The mixtures were stirred and distributed into test tubes in 5 ml aliquots and evaluated for gel formation by the least concentration endpoint. A series of protein concentrations were heated in a Memmert water bath at 100 °C for 30 min. After heating, the samples were cooled at 4 °C in a cooler and the strength of the coagulum were evaluated by inverting the tube. The lowest protein concentration which formed a stable gel (remained in an inverted test tube) was considered to be the gelation endpoint.

Statistical analysis

All experiments were replicated at least three times. Mean values with standard deviation (S.D.) were reported when and where necessary. Analysis of variance (ANOVA) was performed and differences in mean values were determined using DMRT test at P<0.05 and employing ANOVA and DMRT procedures of SPSS, repectively.

RESULTS AND DISCUSSION

Chemical analysis

      The original composition of cracked mungbean seed was 10.44% moisture, 22.49% (db) crude protein, 0.67% (db) crude fat and 3.66% (db) ash. The yield of total protein in solution ranged from 88.5 to 90.7% of raw cracked mungbean. Incomplete recovery of protein may, in part, be due to their loss during the washing process or their retention in the residue, due to complication with other seed components. Mungbean protein solutions are extracted from cracked mungbean seeds by water extraction process. The water extracted mungbean protein solution had 67.96% dry basis protein and 23.66% dry basis carbohydrates (Table 1). From these data, it may be used as a protein source by the product of protein isolate. The compositions of mungbean protein product are shown in Table 2.

The mungbean protein products modified with 0.4% (of solution) acetic acid and 0.4% (of solution) sodium sulfite had highest protein (85.77% dry basis) and the mungbean protein products modified with 0.2-0.4% (of solution) chemical reagent had more protein than the mungbean protein products

Table 1 Chemical composition of mungbean protein solution.[1]

Constituent	Mungbean protein solution
Extract recovery, %	90.72
Moisture, %	94.85
Protein, %db	67.96
Lipid, %db	0.27
Ash, %db8.11	
Carbohydrates, %db	23.66

[1]   Results are means of three determinations, on a dry weight basis, ± standard deviation.

Table 2 Chemical compositions of mungbean protein product.[1]

% of solution		Moisture,%	Protein, %db	Lipid, %db	Ash, %db	Carbohydrates, %db
	0.0	5.9 ^{e}	84.2^b	0.25 ^{a}	5.8 ^{ab}	9.6 ^{c}
Succinic anhydride	0.2	5.9^e	82.5^c	0.37^b	6.4^{cd}	10.6^d
	0.4	5.9^e	82.2^c	0.23^a	7.2^{ef}	10.2^{cd}
	0.6	5.7^d	81.6^c	0.31^a	7.6^f	10.4^d
	0.8	5.0^a	77.1^e	0.36^b	9.1^g	13.3^e
Acetic acid	0.2	7.3^i	85.4^{ab}	0.72^f	5.8^{ab}	8.0^{ab}
	0.4	6.5^f	85.7^a	0.47^d	5.6^a	8.0^{ab}
	0.6	7.5^j	84.6^{ab}	0.39^c	5.7^a	9.1^{bc}
	0.8	5.4^b	81.4^c	0.52^e	7.1^e	10.8^d
Sodium sulfite	0.2	5.0^a	84.2^b	0.33^a	6.5^{cd}	8.8^{ab}
	0.4	5.6^c	85.1^{ab}	0.38^b	6.8^{de}	7.6^a
	0.6	7.1^h	84.6^{ab}	0.36^b	6.2^{bc}	8.7^{ab}
	0.8	6.6^g	80.1^d	0.49^d	9.1^g	10.2^{cd}

[1]   Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different letters in each column are significantly ($P \leq 0.05$) different from one another.

with 0.6-0.8% (of solution) chemical reagent. The mungbean protein modified with 0.2-0.4% (of solution) chemical reagent had lower carbohydrates content than the mungbean protein product modified with 0.6-0.8% (of solution) chemical reagent and ash was significantly increased (P≤0.05) due to modification of mungbean protein product with 0.8% (of solution) chemical reagent.

Nitrogen solubility

The protein solubility indices of succinylated, acylated and oxidation-reduction of disilfite mungbean protein products in distilled water are shown in Figure 1. Oxidation-reduction of disulfite mungbean protein products had a higher nitrogen solubility than acylated and succinylated mungbean protein products. In acylated, succinylated and oxidation-reduction of disulfite mungbean protein products there was a significant decrease (P≤0.05) of the nitrogen solubility at all levels of modification compared to the untreated
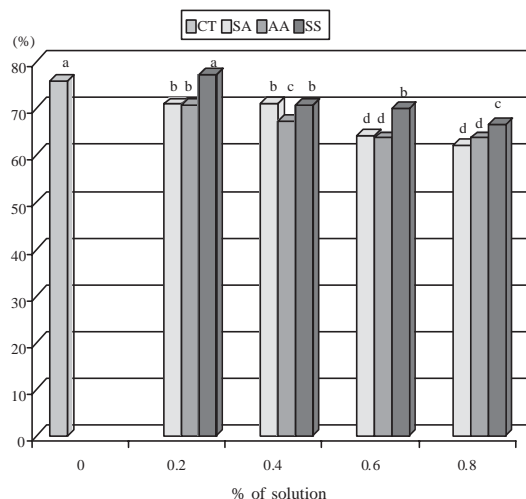

Figure 1 Effects of various concentrations of control treatment (CT), succinic anhydride (SA), acetic acid (AA), and sodium sulfite (SS) on nitrogen solubility of mungbean protein products. Values followed by the same letter are not significantly different (P<0.05).

mungbean protein product. Generally, the better nitrogen solubility of succinylated mung bean protein products than acylated ones can be explained by the fact that succinylation introduces longer side chains compared with acylation, produces more electrostatic repulsion in the protein and produces greater change in conformation, which results in better protein-water interactions (El-Adawy, 2000).

Emulsion activity and stability

The emulsion activity and stability of modified mungbean protein products are shown in Table 3. Modification of mungbean protein products significantly increased the emulsion activity at 0.4% succinic anhydride, 0.2-0.4% acetic acid and 0.2-0.4% sodium sulfite compared to untreated mungbean protein product. Emulsion activity was significantly increased (P≤0.05) due to acylation and oxidation-reduction of disulfite of mungbean protein products by acetic acid and sodium sulfite. From these data, the improvement of emulsion activity can be used to optimize chemical reagent (Franzen and Kinsella,1976; Liu and Hung, 1998; El-Adaway, 2000). The observed action of increasing in emulsion activity of acylated and oxidation-reduction of disulfite mungbean protein products when compared to an untreated mungbean protein product due to acylation and oxidation-reduction of disulfite tends to cause unfolding of protein chains. Thereby exposing hydrophilic residues of peptides (Feeny *et al*., 1982; El-Adaway, 2000) causes an improvement in emulsion activity of the protein (Kinsella, 1979; Voutsinas *et al*., 1983). Emulsion activity was also depended on pH and solubility of protein (Sathe *et al*., 1982). It is well known that emulsion activity of soluble protein depends upon the hydrophilic-lipophilic balance which is affected by pH (Sosulski, 1977).

Foaming activity and stability

The effects of modification of mungbean

Table 3 Effects of various concentrations of succinic anhydride, acetic acid, and sodium sulfite on the emulsion activity and stability and foaming activity and stability of mungbean protein products.[1]

	% of solution	Emulsion activity, %	Emulsion stability, %	Foaming activity, %	Foaming stability, %
	0.0	88.0[d]	63.5	78.1[c]	74.6
Succinic anhydride	0.2	82.0[f]	57.0	76.0[cd]	70.4
	0.4	95.6[a]	95.6	63.0[f]	49.1
	0.6	82.3[ef]	82.3	76.0[cd]	71.7
	0.8	83.6[e]	83.6	66.0[f]	45.8
Acetic acid	0.2	94.0[ab]	65.0	99.0[a]	75.2
	0.4	93.6[b]	60.0	86.0[b]	77.9
	0.6	89.6[c]	59.0	71.0[e]	76.0
	0.8	94.0[ab]	58.5	62.0[gh]	71.0
Sodium sulfite	0.2	95.6[a]	58.0	63.5[f]	45.1
	0.4	95.3[ab]	58.0	72.0[de]	40.3
	0.6	90.0[c]	58.6	53.0[h]	41.3
	0.8	93.6[b]	59.0	58.0[h]	33.6

[1] Results are means of three determinations, ± standard deviation. Means followed by different letters in each column are significantly ($P \leq 0.05$) different from one another.

protein products are shown in Table 3. Foaming activity was significantly increased up to 0.2-0.4% acetic acid. The foaming activity and stability of acylated mungbean protein product was high compared to the oxidation-reduction of disulfite and succinylated mungbean protein products. Foam stability reduced with acylation because of the negative charges imparted during modification causing the protein molecule to unfold. Excessive modification leads to increased net charge density which prevents protein-protein interaction in the foam lamellae, causing foam destabilization and poor stability (El-Aldaway, 2000).These observations agree well with these reported by Dua *et al.* (1996) for acylated rapeseed meal. The low foaming capacity could also be due to inadequate electrostatic repulsions, and hence, excessive protein-protein interaction to form aggregates that were detrimental to foam formation. Increase in foam expansion in certain protein isolate might be due to increasing solubility, rapid unfolding at the air-water interface, limiting intermolecular cohesion and flexibility of the protein surfactant molecular (Kinsella *et al.*, 1985).

Water holding capacity

The water holding capacity of succinylated and acylated mungbean protein products are shown in Figure 2. Acylation and oxidation-reduction of disulfite bonds significantly increased ($P \leq 0.05$) the water holding capacity at all levels of modification compared to untreated protein product. Further, oxidation-reduction of disulfite bonds decreased water holding capacity at high
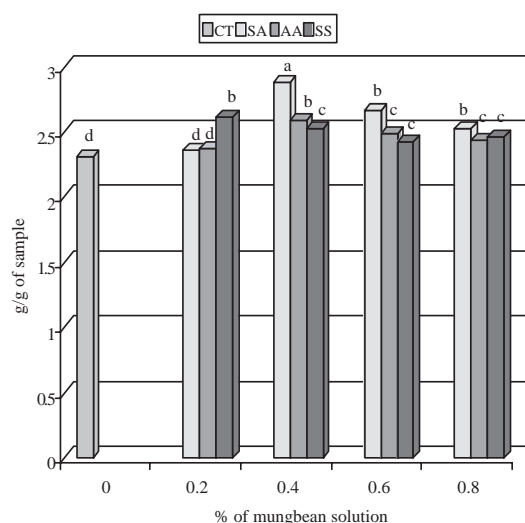
Figure 2 Effects of various concentrations of control treatment (CT), succinic anhydride (SA), acetic acid (AA), and sodium sulfite (SS) on water holding capacity of mungbean protein products. Values followed by the same letter are not significantly different (P<0.05).

sodium sulfite concentrations (0.6-0.8 g/ml of protein solution) and acylation decreased water holding capacity; however, it was still higher than untreated mungbean protein product. Acylation and oxidation-reduction of disulfite bonds can cause dissociation and unfolding of the protein. This might expose more hydrophilic groups than hydrophobic, thereby increasing the hydrophilic binding sides. The lower water holding capacities of succiniylated protein product and oxidation-reduction of disulfite bonds than acylated protein product may be due to higher solubility of the succinylated protein. It has been reported that highly soluble protein exhibits poor water holding (Hemansson, 1973). The increasing in water holding capacity by acylation has been reported by Liu and Hung (1998) for chickpea protein, Dua *et al*. (1996) for rapeseed flour, and El-Adawy (2000) for mungbean protein.

Oil binding capacity

The effects of the type of chemical reagent and concentration on oil binding capacity of mungbean protein products are shown in Figure 3. Significant increasing (P≤0.05) was observed in oil binding capacity at all levels of acetic acid, succinic anhydrides and sodium sulfite compared to untreated mungbean protein product. The high oil binding capacity of modified mungbean protein products is attributed to the degree of denaturation due to chemical modification (El-Adaway, 2000). However, oil binding capacity was not markedly affected at high levels of succinic anhydride, it increased with increasing levels of acetic acid. Chemical reagent modification increased oil absorption capacity of cottonseed flour (Choi *et al*., 1981) and peanut flour (Beuchat, 1997). The oil binding capacity has been attributed to physical entrapment of oil within the protein isolate, the protein content, the surface area, non-covalent
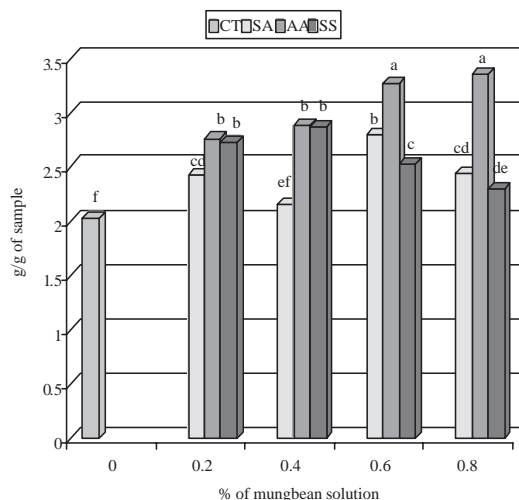

Figure 3 Effects of various concentrations of control treatment (CT), succinic anhydride (SA), acetic acid (AA), and sodium sulfite (SS) on oil binding capacity of mungbean protein products. Values followed by the same letter are not significantly different (P<0.05).

bonds such as hydrophobic, electrostatic and hydrogen bonding are forces involved in lipid-protein interaction, the charge and topography, and the liquidity of the oil the method used (Kinsella, 1976).

Gelation

The gelation properties of chemical reagent modification of mungbean protein products are shown in Figure 4. The observed decreasing in gelation properties of modification of mungbean protein products when compared with untreated mungbean protein product due to chemical reagent modification tends to cause unfolding of protein chains due to part of degree of denaturation of protein and protein content (Doi, 1993). Also, the modification with acetic acid to increase the negative charges and succinic anhydride and sodium sulfite to increase positive charges of protein during modification causing the electrostatic repulsions between net charges of

Figure 4 Effects of various concentrations of control treatment (CT), succinic anhydride (SA), acetic acid (AA), and sodium sulfite (SS) on gelation of mungbean protein products. Values followed by the same letter are not significantly different (P<0.05).

protein, and poor gelation (Barman *et al.*, 1977). Voutsinas *et al.* (1983) reported that protein gelation was affected by exposed hydrophobicity and the square of sulfhydryls of protein. The roles of hydrophobicity and solubility are used for predicting the gelation properties of protein. Gelation of protein depends on protein concentration, pH, balance of cations and anions, and the absence or presence of interfering substances (Wiseman and Price, 1987).

CONCLUSION

Mungbean protein products were modified by different chemical reagents to show the differences of the functional properties like nitrogen solubility, water holding capacity, oil binding capacity, foaming activity and stability, emulsion activity and stability and gelation in comparison to the untreated mungbean protein products. In general, modified mungbean protein products reduced nitrogen solubility and gelation but increaseed the other functional properties. The mungbean protein products treated with succinic anhydride improved the water holding capacity. The modification with acetic acid results for the best properties in foaming activity and stability, oil binding capacity and gelation. If the mungbean protein products were modified with sodium sulfite, they showed the best results in nitrogen solubility, emulsion activity and also gelation. Generally, the obtained results indicated that modified mungbean protein products had more desirable and improved functional properties than untreated mungbean protein products.
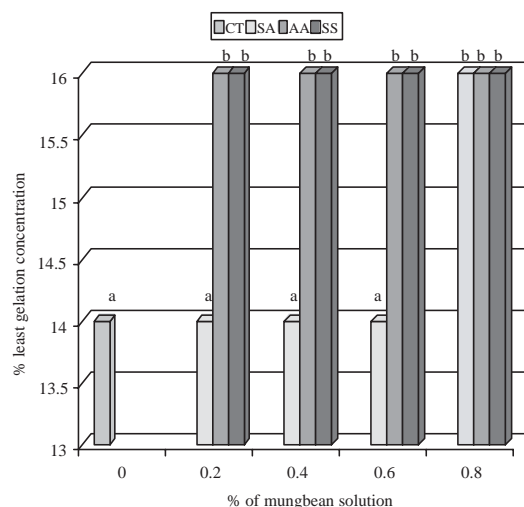
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