IMPROVEMENT OF FUNCTIONALITIES OF SOYMILK RESIDUE PROTEIN BY PAPAIN HYDROLYSIS

Yuporn puechkamut* and Porchanan Thiewtua

Agro-Industry Faculty , King Mongkut's Institute of Technology Ladkrabang
Ladkrabang, Bangkok 10520, Thailand

ABSTRACT

Protein was extracted at pH 9 from fresh soy milk residue (okara) received from soy milk factory, and was modified by papain hydrolysis. The hydrolysis was done at the concentration of 1 gm of enzyme per 100 gm of protein and incubated for 30, 60 and 90 mins The electrophoresis pattern showed a decrease in molecular size of the proteins by the enzyme hydrolysis. Surface hydrophobicity of papain modified okara protein was significantly increased with increase in reaction time. This indicated the unfolding of the molecule. The poor solubility of okara protein was clearly improved by partially hydrolysis with papain, particularly at 90 min. Other functionalities such as emulsion activity and foaming properties of okara protein were also enhanced by papain hydrolysis.

KEYWORDS: soy protein, okara

1. INTRODUCTION

In the manufacturing of soymilk and tofu, soymilk residue or okara is produced as a by-product with limited market values. However, okara contains about 27% protein (dry basis) with good nutritional quality and superior protein efficiency ratio [1] . Therefore, okara protein is suggested as a potential source of low cost vegetable protein for human consumption The protein could be extracted from okara at alkaline pH ([2] and [3]). The resulting protein isolate was found to possess functionalities and nutritional quality similar to that of a commercial soy isolate. However, during soymilk production, the severe heat treatments cause extensive protein denaturation and the resulting okara protein has poor solubility, limiting its use into the food system. Moreover, the approximate analysis showed that the okara protein contained 56-60% protein, 38-40% carbohydrate and 3-4% ash [3]. This carbohydrate might be conjugated with partially denatured protein, therefore, it was difficult to eliminate the carbohydrate during extraction.

Solubility and other functional properties of protein are closely related to the size of protein, structural conformation and level and distribution of ionic charges [4]. Treatments that could cause alteration of these properties include reactions which either induce a new functional groups to the protein or remove a component part from the protein such as reaction of succinylation, phosphorelation, deamidation (limited hydrolysis) have been used to improve functional properties of the protein [5]. Chan and Ma [6] reported that the solubility of okara protein which was extracted at alkaline pH and was modified by mild acid treatments was increased. Limited hydrolysis by enzyme have found to be an effective method to improve functional properties of soy protein ([7] and [8]). In the present study, therefore, the functional properties of okara protein were modified by papain which natural extracted from papaya. The change in the physicochemical properties of the okara protein were also studied.

2. MATERIALS AND METHODS

Fresh okara was received from soymilk factory (Greenspot Co.,Ltd, Thailand). Enzyme papain (EC.3.4.22.2) (3 units/mg solid) was purchased from Sigma Inc.. Other chemical reagents were analytical reagent grade.

* Corresponding author: Tel : 01-6424428, Fax: 01-66-2-3736718
E-mail: cayongyuth@yahoo.com

2.1 Extraction of okara protein

Protein was extracted from the okara according to the method of Supichayangkul [3]. The okara was mixed with distilled water at the ratio of 1:8 and pH was adjusted to 9 by 2 N NaOH. The mixture was stirred at 80°C for 1 hour and was then centrifuged at 9000 rpm for 20 min. The extracted protein in the supernatant was precipitated by adjusted the pH to 4.5, and recovered by centrifugation at 12000 rpm for 20 min. The precipitated protein was neutralized by using 2 N NaOH. The protein was freeze-dried and defatted by hexane. The defatted okara protein was kept and applied to the following experiments.

2.2 Preparation of papain modified okara protein

The okara protein was hydrolyzed by papain according to the method of Ortiz and Wagner [9]. The okara protein (2%) was incubated with papain at the concentration of 1 gm of enzyme per 100 gm of protein. After the mixture was incubated at 40°C for 30 60 and 90 min, the protein was precipitated by adjusted pH to 4.5. The precipitated protein was neutralized by 2 N NaOH and freeze-dried.

2.3 Physicochemical properties

The surface hydrophobicity of protein samples was determined by the fluorescence probe method using 1-anilino-8-naphathalenesulfonate ([10] and [11]). Polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS) was proformed with 12% gels according to the method of Laemmli [12]. The sample buffer contained 2% 2-mercaptoethanol. The standard marker with molecular weight ranging from 14000 to 70000 daltons was used.

2.4 Functional properties

Protein solubility was determined according to Voutsinas *et al* [13]. Emulsion activity was determined by a turbidimetric method [14]. Foam ability and foam stability were determined according to Phillips *et al* [15].

The analyzed for physicochemical and functional properties were performed in triplicates. Analysis of variance and Duncan's multiple range tests were used to established the significance of difference among samples at the $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of papain modified okara protein

Figure 1 shows the SDS-PAGE patterns of okara protein that modified from papain at various reaction times. The majors bands, according to the estimated molecular weight, correspond to α'-, α- and β-subunit of β-conglycinin (7S globulin), AP (acidic polypeptide) and BP (basic polypeptide) of glycinin (11S globulin), similar to those observed by other workers ([3] , [16] and [17]). When the incubation time was increased from 0 to 90 min, the intensities of the major bands of 7S and 11S globulin were decreased whereas small subunits with molecular weight less than 14000 dalton were increased. It indicated that the compact structure of globular okara protein was partially hydrolyzed to be small subunits. Compared to the previous data of a alkalase [3], at the same enzyme concentration and reaction time the major bands of 7S an 11S globulin on the SDS-PAGE disappeared and only small molecules were found. This can be explained by their specificity of both enzymes. Papain has more specificity, therefore, the hydrolysis was limited. The size of these protein molecules effected their functional properties.

Table 1 Surface hydrophobicity of okara protein modified by papain at various reaction times.

Sample	Surface hydrophobicity
Okara protein	355.9 ± 1.3^{a}
PMO 30 min	393.4 ± 5.6^{b}
PMO 60 min	432.1 ± 13.7^{c}
PMO 90 min	466.7 ± 15.0^{d}

^{abcd} the same letter is not significantly different ($p \leq 0.05$)

PMO Papain modified okara protein

Table 1 shows the surface hydrophobicity of okara modified protein. The surface hydrophobicity was found to increase as the degree of modification increased. When the okara protein was hydrolyzed, the structure was unfolded leading to the exposure of hydrophobic groups. The changes in surface hydrophobicity would affect the solubility and other functionalities

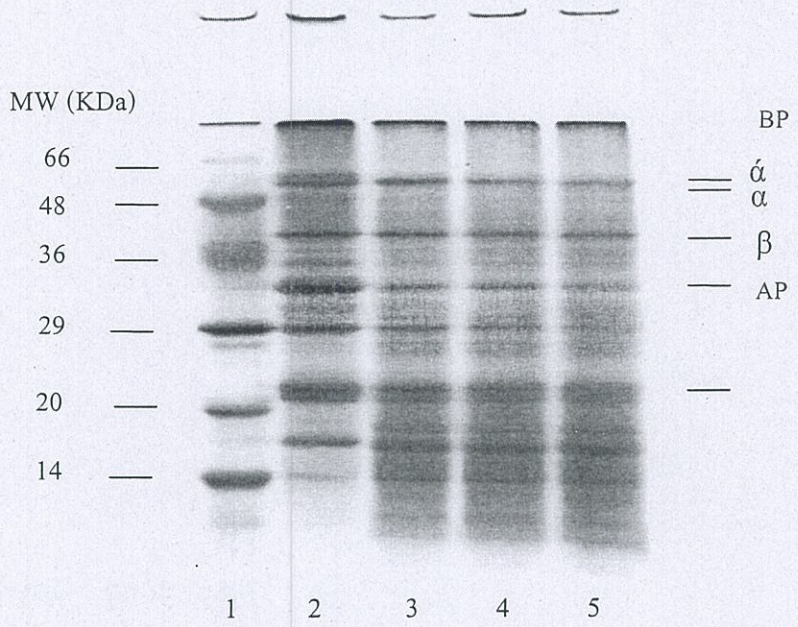

Figure 1 SDS-PAGE pattern of papain modified okara protein. Lane1: Standard weight marker; Lane 2: okara protein; Lane 3 to Lane5 indicate okara protein incubated with papain for 30 60 and 90 min, respectively; α', α and β are α', α and β subunits of β-conglycinin, respectively; AP an BP are acidic and basic polypeptide of glycinin, respectively.

Table 2 Solubility and emulsion activity of okara protein modified by papain at various reaction times.

Sample	Solubility (%)	Emulsion Activity
Okara protein	48.7 ± 2.8^{a}	2.61 ± 0.04^{a}
PMO 30 min	57.2 ± 2.6^{b}	2.59 ± 0.06^{a}
PMO 60 min	62.8 ± 2.0^{bc}	2.67 ± 0.05^{b}
PMO 90 min	68.1 ± 2.4^{c}	2.76 ± 0.05^{c}

^{abcd} the same letter in the same column is not significantly different ($p \leq 0.05$)
PMO Papain modified okara protein

3.2 Functional properties of papain modified okara protein

The solubility and emulsion activity of papain modified okara protein were described in Table 2. The solubility of okara protein was progressively improved with increase in the hydrolysis time, particularly at 90 min. This result was correlated with the previous result of Mahmoud [18] and Govinaraju and Srinivas [19]. The improvement in solubility could be due to both a decrease in molecular size and increase in net charge Rupture of peptide bond would also result in exposure of

more charged and polar groups to the surrounding water, promoting protein-water interaction and hence increase solubility ([20] and [21]).

The emulsion activity of papain modified okara protein was also gradually increased with increase in hydrolysis times, particularly at 90 min. The hydrolysis of protein caused the hydrophobic group which placed interior of the molecule exposed to the surface corresponding to the increase in surface hydrophobicity. Therefore, the interaction between oil droplet and the protein improved. Moreover, increase in solubility enhanced a better oil-protein interaction [20].

Table 3 shows the foaming activity and stability of okara protein modified by papain at various reaction times. The foaming activity and stability were gradually increased with increase in the hydrolysis time, particularly after 60 min of reaction time. The foaming ability of papain modified okara protein would be enhanced by the increase in the solubility of protein since soluble proteins contribute to the foaming [21]. Increase in charge may induce protein-protein interactions and promote the formation of cohesive films at air-water interface, hence increasing foam stability [21].

Table 3 Foaming ability and stability of okara protein modified by papain at various reaction times.

Sample	Foaming Activity	Foaming Stability (%)	
		10 min	30 min
Okara protein	2.4 ± 0.1^{b}	70.4^{a}	66.6^{a}
PMO 30 min	2.1 ± 0.1^{a}	83.9^{b}	71.2^{b}
PMO 60 min	2.9 ± 0.1^{b}	91.5^{c}	83.6^{c}
PMO 90 min	2.8 ± 0.1^{c}	95.5^{d}	88.9^{d}

[a]the same letter in the same column is not significantly different ($p \leq 0.05$)

PMO Papain modified okara protein

4. CONCLUSION

The present result shows that the properties of okara protein were significantly affected by papain hydrolysis. This would be due to the peptide bond hydrolysis, resulting in the decrease in molecular size. The solubility of papain modified okara protein was incrased from 48% to 68% when incubated with papain for 90 min. Moreover, the emulsion activity and foaming properties of the proteins were also improved when modified by papain. The improvement of their solubility and other functionalities would enhance the utilization of okara protein as a food ingredient.

5. REFERENCES

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