

## Research Article

### Emulsifying and Foaming Properties of Soy Protein Isolates with Covalent Modification by (-)-Epigallocatechin-3-Gallate

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**Abstract:** Soy Protein Isolates (SPI) with covalent modification by (-)-Epigallocatechin-3-Gallate (EGCG) were prepared under the alkaline condition. The effects of covalent modification on the emulsifying and foaming properties of SPI were evaluated. The Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) profiles of the modified SPI revealed that EGCG treatment caused cross-linking of subunits. Emulsifying activity of modified SPI significantly increased when compared with that of control ( $p < 0.05$ ) at the concentration of 35 mg/mL. Modification of SPI by EGCG caused a decrease in the foam volume initially. In the range of 15 to 35 mg/mL, the foaming activities of modified SPI were found to be less than those of the control ( $p < 0.05$ ). The foaming stabilities of modified SPI were significantly higher when compared to those of control ( $p < 0.05$ ). The results obtained in this study indicated the modification by EGCG resulted in the enhancement of emulsifying activity and foaming stability of SPI. This modification may serve as a promising approach for improving functional properties of SPI.

**Keywords:** EGCG, functional properties, modification, soy protein isolates

## INTRODUCTION

Soy Protein Isolates (SPI) is the most highly refined soy products commercially available and represent the major protein fraction of the soy bean. SPI as a valuable food ingredient with important functional properties is gaining acceptance as functional food ingredient. Emulsifying and foaming properties are important parameters generally for the application of SPI. There is a need to improve the functional properties of SPI to maximize the efficiency of its use in the food industry. SPI can be designed for enhancing functional properties by altering the protein composition and/or modifying the proteins. Chemical modification has been developed to improve the functional properties of SPI and consequently to promote its utilizations in food products (Babiker *et al.*, 1998; Matemu *et al.*, 2011).

Phenolic Compounds (PCs) are defined as compounds possessing one or more aromatic rings bearing a hydroxyl substituent (s). Dietary PCs have received much attention in recent years due to their biological activity. PCs may be oxidized in an alkaline solution to corresponding quinones (Rawel *et al.*, 2005). The quinones may react with cysteine, lysine, methionine and tryptophan residues in protein (Rawel *et al.*, 2002, 2004). (-)-Epigallocatechin-3-Gallate (EGCG) is the most abundant and biologically active

polyphenol in green tea. Several reports have confirmed that EGCG could covalently bind to the amino acid residues in proteins by means of its autoxidation (Ishii *et al.*, 2008; Mochizuki *et al.*, 2002). Chemical modification has been focused on an effective method to improve the functional properties of protein. However, such interactions have not yet been emphasized enough for their role played in affecting the functional properties of SPI. The purpose of the present study was to underscore the influence of covalent modification with EGCG on the emulsifying and foaming properties of SPI.

## MATERIALS AND METHODS

**Materials:** SPI was procured from Wonderful Technol. Co. Ltd. (Shandong Province, China). Soybean oil was purchased from a local supermarket in Hangzhou (China). EGCG and other reagents for electrophoresis were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Sample preparation:** SPI (35.0 g) was suspended in 950 mL of water and the pH value was adjusted to 9 using 0.5 M NaOH. EGCG (0.2 g) dissolved in 20 mL of water were added. After 24 h of reaction time under continuous stirring at 35°C with free exposure to air, neutralization was done using 1 M HCl. The total

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volume of reaction system was adjusted to 1 L with water. The modified SPI by EGCG was obtained with the concentration of 35 mg/mL. The control was prepared under the same conditions but without addition of EGCG.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE):** Gel electrophoresis was conducted according to the method described by Laemmli (1970) using 12% acryl amide separating gel and 1% acryl amide stacking gel. Protein samples were mixed directly with the SDS-PAGE sample buffer solution containing 5% β-mercaptoethanol, 2% SDS, 5% glycerol and 0.05% bromphenol blue. About 50 μg of protein was loaded per well. The gel electrophoresis was carried out at 120 V constant voltages. The gel was stained with 0.1% Coomassie Brilliant Blue-R250. Molecular weight marker proteins were run along with test samples.

**Determination of emulsifying properties:** Emulsion Activity Index (EAI) was determined according to the method of Aewsiri *et al.* (2009), with a slight modification. Soybean oil (5 mL) and sample suspension (15 mL) were homogenized using a dispersing homogenizer at a speed of 20,000 rpm for 1 min. Emulsions were pipette out at 0, 10 and 20 min and 200-fold diluted with 0.1% SDS, respectively. The mixture was mixed thoroughly for 10 sec using a vortex mixer. Absorbance of the resulting dispersion at 500 nm was measured using a spectrophotometer. EAI were calculated by the following formula:

$$EAI (m^2 / g) = \frac{(2 \times 2.303 A)DF}{l\phi C}$$

where,

*A* = Absorbance at 500 nm

*l* = Path length of cuvette (0.01 m)

*DF* = The dilution factor (200)

*φ* = Oil volume fraction

*C* = Protein concentration in aqueous phase (g/m<sup>3</sup>)

**Determination of foaming properties:** Foaming activity and foaming stability were measured according to the method described by Thewissen *et al.* (2011) with some modification. An equivalent of 20 mL of sample suspension with different concentration was accurately weighted out and the original volume of liquid was measured. This was quantitatively transferred into a blender and whipped for 3 min at high speed. The mixture was poured immediately into a 100 mL measuring cylinder and the total volume of the foam was measured immediately after 30 sec. The foaming activity was expressed as the percentage of foam volume relative to original volume of liquid. Foaming stability was defined as the percentage of foam volume remaining after 30 min relative to initial foam volume.

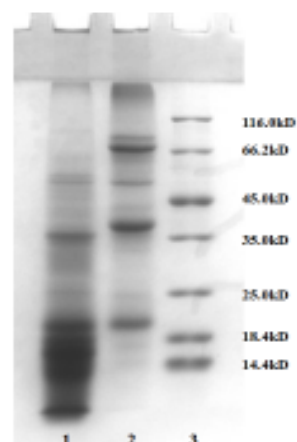


Fig. 1: SDS-PAGE patterns for SPI and modified SPI  
Lane 1: SPI; Lane 2: Modified SPI; Lanes 3: Molecular weight standard

**Statistical analysis:** The experimental data were expressed as means±standard deviations. One-way Analysis of Variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences (*p*<0.05) between the means by SPSS (version 10.1 for Windows 98, SPSS Inc.).

## RESULTS AND DISCUSSION

**SDS-PAGE analysis:** SDS-PAGE was employed to monitor structural changes of SPI subunits after reaction. Figure 1 shows SDS-PAGE patterns of SPI and modified SPI. The SDS-PAGE patterns of the control SPI sample (Fig. 1, lane 1) exhibited the characteristic bands for the constituents of SPI. These bands are in good agreement with the results reported by Walsh *et al.* (2003). The SDS-PAGE profiles of modified SPI (Fig. 1, lane 2) revealed that EGCG treatment caused cross-linking of subunits, as evidenced by the disappearance on the intensity of the bands with low molecular weight. At the same time, the formation of new bands was observed at the top of the separating gel.

**Emulsifying properties:** Proteins are the main emulsifying agents in many foods. Emulsifying activity is important parameter generally used to investigate the emulsifying properties of proteins in food emulsion systems. Emulsion Activity Index (EAI) expressed as the turbidity of emulsion at the wavelength of 500 nm is shown in Table 1. Modified SPI showed significant increase in EAI when compared with the control (*p*<0.05). Emulsifying activity of a protein emulsifier depends on its ability to form adsorption films around the oil globules and to lower the interfacial tension at the oil-water interface. The improved emulsifying activity of modified SPI could be attributed to changes in the conformational and surface properties of protein structures (Kuan *et al.*, 2011). Protein changes on the conformational structure would affect surface hydrophobicity and subsequently lead to better

Table 1: Emulsifying Activity Index (EAI) for SPI and modified SPI standing for 0, 10 and 20 min, respectively

Sample	EAI (m <sup>2</sup> /g)		
	0 min	10 min	20 min
SPI	5.4±0.29 <sup>a</sup>	3.2±0.38 <sup>a</sup>	2.3±0.23 <sup>a</sup>
Modified SPI	6.2±0.44 <sup>b</sup>	5.5±0.69 <sup>b</sup>	3.9±0.45 <sup>b</sup>

Different letters within the same line indicate significant differences (p<0.05)

Table 2: The foaming activity for SPI and modified SPI at different concentration

Sample	The foaming activity (%)		
	35 mg/mL	25 mg/mL	15 mg/mL
SPI	194.5±4.95 <sup>a</sup>	136.2±6.34 <sup>a</sup>	139.4±7.23 <sup>a</sup>
Modified SPI	67.5±11.76 <sup>b</sup>	76.7±9.42 <sup>b</sup>	54.8±6.69 <sup>b</sup>

Different letters within the same line indicate significant differences (p<0.05)

Table 3: Experimental results of the central composite design for optimization of enzymatic hydrolysis of mackerel processing byproduct

Sample	The foaming stability (%)		
	35 mg/mL	25 mg/mL	15 mg/mL
SPI	30.6±4.51 <sup>a</sup>	44.4±3.91 <sup>a</sup>	7.6±1.74 <sup>a</sup>
Modified SPI	62.6±3.75 <sup>b</sup>	89.5±4.12 <sup>b</sup>	80.1±4.31 <sup>b</sup>

Different letters within the same line indicate significant differences (p<0.05)

adsorption of the oil-in-water emulsion system (Jambrak *et al.*, 2009). Protein cross-linking is indicated by the result obtained from SDS-PAGE. The results suggest that conformational changes of SPI would have given rise to an improvement of the emulsifying properties.

**Forming properties:** Foam formation is an important requirement in the manufacture of foods such as ice cream, cakes and meringues. Therefore, the ability of SPI to form foams could be essential to their application in the manufacture of nondairy foods. The foaming activities of samples at different concentration are presented in Table 2. Modification of SPI with EGCG caused a decrease in foam volume initially. The foaming activities of modified SPI were found to be less than those of the control (p<0.05). The foaming stability was determined 15 min after whipping. The foaming stability of SPI and modified SPI was presented in Table 3. In the range of 15 to 35 mg/mL, the foaming stabilities of modified SPI were significantly higher (p<0.05) when compared to those of control.

## CONCLUSION

In conclusion, the results obtained in this study indicated the possibility of EGCG treatment to induce cross-linking of SPI. The modification by EGCG resulted in the enhancement of emulsifying activity and foaming stability. The modification may serve as a promising approach for improving functional properties

of SPI, because of the safety due to the naturally occurring reaction.

## REFERENCES

- Aewsiri, T., S. Benjakul, W. Visessanguan, J.B. Eun, P.A. Wierenga and H. Gruppen, 2009. Antioxidative activity and emulsifying properties of cuttlefish skin gelatin modified by oxidised phenolic compounds. *Food Chem.*, 117: 160-168.
- Babiker, E.E., A. Hiroyuki and N. Matsudomi, 1998. Effect of polysaccharide conjugation or transglutaminase treatment on the allergenicity and functional properties of soy protein. *J. Agric. Food Chem.*, 46: 866-871.
- Ishii, T., T. Mori, T. Tanaka, D. Mizuno, R. Yamaji, S. Kumazawa *et al.*, 2008. Covalent modification of proteins by green tea polyphenol (-)-epigallocatechin-3-gallate through autoxidation. *Free Radic. Biol. Med.*, 45: 1384-1394.
- Jambrak, A.R., V. Lelas, T.J. Mason, G. Krešić and M. Badanjak, 2009. Physical properties of ultrasound treated soy proteins. *J. Food Eng.*, 93: 386-393.
- Kuan, Y.H., R. Bhat and A.A. Karim, 2011. Emulsifying and foaming properties of ultraviolet-irradiated egg white protein and sodium caseinate. *J. Agric. Food Chem.*, 59: 4111-4118.
- Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Matemu, A.O., H. Kayahara, H. Murasawa, S. Katayama and S. Nakamura, 2011. Improved emulsifying properties of soy proteins by acylation with saturated fatty acids. *Food Chem.*, 124: 596-602.
- Mochizuki, M., S. Yamazaki, K. Kano and T. Ikeda, 2002. Kinetic analysis and mechanistic aspects of autoxidation of catechins. *Biochim. Biophys. Acta*, 1569: 35-44.
- Rawel, H.M., K. Meidtner and J. Kroll, 2005. Binding of selected phenolic compounds to protein. *J. Agric. Food Chem.*, 53: 4228-4235.
- Rawel, H.M., D. Czajka, R. Sascha and J. Kroll, 2002. Interactions of different phenolic acids and flavonoids with soy proteins. *Int. J. Biol. Macromol.*, 30: 137-150.
- Rawel, H.M., H. Ranters, S. Rohn and J. Kroll, 2004. Assessment of the reactivity of selected isoflavones against proteins in comparison to quercetin. *J. Agric. Food Chem.*, 52: 5263-5271.
- Thewissen, B.G., I. Celus, K. Brijs and J.A. Delcour, 2011. Foaming properties of wheat gliadin. *J. Agric. Food Chem.*, 59: 1370-1375.
- Walsh, D.J., D. Cleary, E. McCarthy, S. Murphy and R.J. FitzGerald, 2003. Modification of the nitrogen solubility properties of soy protein isolate following proteolysis and transglutaminase cross-linking. *Food Res. Int.*, 36: 677-683.