

## Types and concentrations of coagulants affected protein and anthocyanin content in soft tofu produced from black bean

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### Abstract

Black bean (*Phaseolus vulgaris*) is the legume for seed, which is considered important in the nutritional quality and economy of Thailand. The beans contain high protein, amino acid and antioxidant especially anthocyanin. Normally, a soft tofu is made from soybeans but these beans are insufficient for consumption in Thailand. Therefore, this research used black beans to produce soft tofu. Different types (CaSO<sub>4</sub> and glucono delta lactone, GDL) and concentrations (1, 2 and 3%) of coagulants were used to investigate their effects on protein and anthocyanin contents of black bean soft tofu (BBST). GDL 3% coagulated the greatest amount of protein (6.97%) ( $p<0.05$ ) and anthocyanin contents (1.292 mg 100 g<sup>-1</sup>) from black bean milk. The BBST was sensory evaluated. The GDL at 3% resulted in the following scores of sensory evaluation: color (5.83), odor (5.67), flavor (6.00), texture (6.17) and overall likeness (5.93). BBST contained greater protein (6.62%) and anthocyanin (1.423 mg 100 g<sup>-1</sup>) and lower lipid content than soybean soft tofu (SBST) ( $p<0.05$ ).

**Keywords:** black bean, soft tofu, anthocyanin, protein content, calcium sulphate, glucono delta lactone

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## Introduction

Black bean is a relatively small (7-13 g 100 g<sup>-1</sup>) and it is rich in dietary fiber, minerals, particularly calcium, and phenolic compounds exhibiting strong antioxidant activity essential for the development of novel food products (Oomah *et al.*, 2010). In addition, the beans are rich in protein and antioxidant especially, anthocyanin which can control and prevent various metabolic diseases such as diabetes, coronary heart disease and cancer (López-Barrios *et al.*, 2016). The most recognized soy food in the west is tofu whereas soybeans production has not been enough for consumption. They were imported more than 1 million tons per year (Food Intelligence Center Thailand, 2009).

There was a report that the texture and microstructure of the soybean tofu were greatly influenced by the types of coagulant used. The curd produced by GDL and CaSO<sub>4</sub> gave a much finer and a more uniform honeycomb-like structure than the curd prepared with CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub> (Deman *et al.*, 1986). Therefore, this research aimed at investigating the effects of different types and concentrations of coagulants on protein and anthocyanin contents of soft tofu produced from black bean.

## Methodology

Black beans (*Phaseolus vulgaris* L.) from local market in Phitsanulok province were washed with tap water 3 times and soaked for 16 h at temperature 4 °C. The beans were blended with a blade mixer, pressed through a filter bag and boiled for 20 min with stirring. After heating, both coagulants (CaSO<sub>4</sub> or GDL) were added at concentrations of 1, 2 and 3% while stirring for 5 s and poured into the mold. The black bean milk was stored at room temperature for 20 min. After coagulation, the soft tofu samples were stored at 4 °C.

### Protein content

Five-gram samples were weighed and dried in an oven at 105 °C until constant weight. The ground BBST was used to analyze for protein by Kjeldahl method using a protein conversion factor of 6.25 according to method 955.04 of AOAC (2000).

### Anthocyanin content

The total anthocyanin content was determined using the pH-differential method (Steed and Truong, 2008). Five gram of ground dried BBST samples was extracted with methanol 50 mL. The extracts were performed on each sample. Firstly, the potassium chloride (0.025 M) at pH 1 was used and secondly was the sodium acetate (0.4 M) at pH 4.5. Samples

were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 min before absorbance at 530 and 700 nm was recorded using a spectrophotometer calibrated with distilled water as the blank. The difference in absorbance between pH values and wavelengths was calculated:

$$A = (A_{530} \text{ nm} - A_{700} \text{ nm}) \text{ pH 1.0} - (A_{530} \text{ nm} - A_{700} \text{ nm}) \text{ pH 4.5}$$

#### Sensory evaluation

Sensory evaluation was done on freshly made tofu. Fifteen semi-trained panelists evaluated the sensory attribute of the fresh tofu. Panelists were familiar with product sensory evaluation, most having participated in previous related projects. Panelists were given no time limit for the evaluation, though most panelists took 10-15 min to complete the rating of all the samples. Tofu was cut into cubic samples and placed on a plastic plate with a random number. The attributes evaluated were color, odor, flavor, texture and overall likeness. For each sample, panelists scored their liking of these characteristics using the nine-point hedonic scale (1 = extremely dislike, 2 = highly dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = moderately like, 8 = highly like, and 9 = extremely like (Obatolu, 2008).

#### Statistical analysis

The completely randomized design (CRD) was used in this experiment. All measurements were replicated three times and experimental results were expressed as means. The data were analyzed by analysis of variance (ANOVA) and the means separation was done by the use of Duncan's multiple range tests (DMRT) in SPSS version 16.0.

#### Results and discussion

##### Protein content

Protein content of BBST at different coagulant types and concentrations are presented in (Table 1). Protein of BBST coagulated with GDL (6.19-6.97) was higher than that of BBST added  $\text{CaSO}_4$  (5.21-6.19). These results indicated that the increase in titratable acidity by GDL can affect curd formation during tofu making by interacting with coagulants and protein (Kong *et al.*, 2008). The protein content of BBST added 3% GDL was the greatest (6.97%) ( $p<0.05$ ). Recent results from others reported that the protein content of soft tofu from soy milk and egg ranged 5.6-8.3% (Murad *et al.*, 2013). BBST contained the protein content less than that of soybean tofu 9.6-19.3% (Noh *et al.*, 2005). However, BBST protein compared with the Thai industrial standard of soybean soft tofu which the BBST

proteins were higher than 5% (Thai Industrial Standard Institute, Ministry of Industries, 1990).

#### Anthocyanin content

Anthocyanins are the most important group of phenolic compounds present in black bean. They contribute to the characteristic color and have been linked to antihyperglycemic, anticancer, and antimutagenic health benefits

(Steed and Truong, 2008; Oomah *et al.*, 2010).

Anthocyanin content of BBST at different coagulant types and concentrations are presented in (Table 1). Total anthocyanin of BBST samples ranged from 1.29-1.71 mg 100 g<sup>-1</sup>. BBST added CaSO<sub>4</sub> contained anthocyanin higher than that of BBST added GDL ( $p<0.05$ ) at the same concentration.

**Table 1** Protein and anthocyanin contents of black bean soft tofu affected by types and concentrations of coagulants.

coagulants		protein content	anthocyanin
type	concentration (%)	(%)	(mg 100 g <sup>-1</sup> )
CaSO <sub>4</sub>	1	6.19 ± 0.35 <sup>b 1/</sup>	1.68 ± 0.14 <sup>a</sup>
	2	5.21 ± 0.34 <sup>c</sup>	1.71 ± 0.07 <sup>a</sup>
	3	5.36 ± 0.19 <sup>c</sup>	1.67 ± 0.18 <sup>ba</sup>
GDL	1	6.19 ± 0.25 <sup>b</sup>	1.14 ± 0.07 <sup>c</sup>
	2	6.19 ± 0.26 <sup>b</sup>	1.30 ± 0.18 <sup>b</sup>
	3	6.97 ± 0.18 <sup>a</sup>	1.29 ± 0.16 <sup>b</sup>

<sup>1/</sup> In a column, means followed by the same letter are not significantly different ( $p<0.05$ ) by DMRT.

#### Sensory evaluation

The sensory evaluation of tofu produced by using various types and concentrations of coagulants is shown in (Table 2). The results were expressed on a 9-point hedonic scale. BBST added 2 and 3% GDL had higher texture score than that of other samples. The BBST

using 3% GDL obtained the highest color and texture score. The protein content of BBST added 3% GDL had the highest protein that increased number of cross-linking in the gel network and acid induced gel protein stabilized network may influence water holding capacity (Tay and Perera, 2004).

**Table 2** The sensory evaluation of black bean soft tofu produced by using different types and various concentrations of coagulants.

coagulants		sensory evaluation score				
type	concentration (%)	color	odor	flavor	texture	overall likeness
CaSO <sub>4</sub>	1	5.03 <sup>c 1/</sup>	5.20 <sup>c</sup>	5.60 <sup>bc</sup>	5.33 <sup>b</sup>	5.23 <sup>d</sup>
	2	5.43 <sup>b</sup>	5.47 <sup>abc</sup>	5.40 <sup>c</sup>	5.43 <sup>b</sup>	5.50 <sup>bcd</sup>
	3	5.53 <sup>b</sup>	5.27 <sup>bc</sup>	5.67 <sup>bc</sup>	4.97 <sup>c</sup>	5.60 <sup>bc</sup>
GDL	1	5.40 <sup>b</sup>	5.57 <sup>ab</sup>	5.60 <sup>bc</sup>	5.43 <sup>b</sup>	5.77 <sup>ab</sup>
	2	5.47 <sup>b</sup>	5.30 <sup>bc</sup>	5.83 <sup>ab</sup>	6.00 <sup>a</sup>	5.43 <sup>cd</sup>
	3	5.83 <sup>a</sup>	5.67 <sup>a</sup>	6.00 <sup>a</sup>	6.17 <sup>a</sup>	5.93 <sup>a</sup>

<sup>1/</sup> In a column, means followed by the same letter are not significantly different (p<0.05) by DMRT.

### Conclusion

Three percentage of GDL was the best type and concentration for coagulating protein of black bean milk which provided protein content of 6.97% and anthocyanin content of 1.292 mg 100 g<sup>-1</sup> in BBST. In addition, the BBST sensory evaluation score were; color (5.83), odor (5.67), flavor (6.00), texture (6.17) and overall likeness (5.93). Comparing between BBST and soy bean soft tofu (SBST), BBST contained greater protein (6.62%) and anthocyanin (1.423 mg 100 g<sup>-1</sup>) and lower fat than that of SBST.

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