

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials

Three varieties of soybean used in the experiment were Chaing Mai 60 (CM 60) and So Jo 5 (SJ 5) from Chiang Mai Field Crops Research Center and Sukhothai 2 (ST2) from Sukhothai Plant and Plant Production Material Technical Service Center. Surimi AA grade was obtained from Pacific Marine Food Co.,Ltd and mixed carrageenan was obtained from Burapaheep Co.,Ltd to prepare for surimi emulsion gel.

3.2 Reagents

- 3.2.1 Calcium chloride (CaCl_2 , food grade)
- 3.2.2 Calcium sulphate (CaSO_4 , food grade)
- 3.2.3 Magnesium chloride (MgCl_2 , food grade)
- 3.2.4 Magnesium sulphate (MgSO_4 , food grade)
- 3.2.5 Calcium carbonate (CaCO_3 , food grade)
- 3.2.6 Sodium dodecyl sulfate (SDS)
- 3.2.7 Glutaraldehyde

3.3 Equipments

- 3.3.1 Grinder (Washino, Thailand)
- 3.3.2 Tray dryer (Path OV663, Thailand)
- 3.3.3 Drum dryer (Saturn DD20)
- 3.3.4 Freeze dryer (Labconco, Freezone 18, USA)
- 3.3.5 Texture analyzer (Stable Micro Systems TA-XT2i, England)
- 3.3.6 Scanning Electron Microscopy (JEOL, JSM-5410LV, Japan)
- 3.3.7 Spectrophotometer (Shimadzu, 1700, Japan)
- 3.3.8 Centrifugal (Hettich, Universal 16, Germany)
- 3.3.9 Chroma meter (Minolta, CR-300, Japan)

3.4 Methods

3.4.1 Study the soybean varieties, coagulants and drying processes of tofu powder productions

3.4.1.1 The effect of soybean varieties

The effect of soybean varieties, as Chaing Mai 60, So Jo 5 and Sukhothai 2 were used to produce tofu powders. The chemical composition of these soybeans was analyzed by the method of AOAC. (2000). Soymilk and tofu powder were prepared by the method of Panyathitipong and Puechkamut (2002) as described in Figure 3.1 (Appendix A1). The properties of each soymilk and tofu powder were determined as follows:

1) Soy milk

- a) Total soluble solid by hand refractometer
- b) Total solid content (Min et al., 2005). The total solid content of soymilk was determined by drying soymilk to constant weight at 110°C in an air oven. The percent of total solid content was calculated as follows:

$$\text{Total solid content (\%)} = \frac{\text{Weight of sample after dried} \times 100}{\text{Weight of sample}}$$

- c) The color of soymilk was determined by chroma meter. The color properties of soymilk were determined by measuring the L, a and b (lightness, redness and yellowness).

2) Tofu powder

- a) The chemical compositions of tofu powder were analyzed by the method of AOAC (2000).
- b) The solubility of tofu powder were determined by the modified method of Voutsinas et al. (1983). The 1 g tofu powder samples were dispersed in 50 ml phosphate buffer pH 7.4. The dispersion was stirred for 5 min and centrifuged at 2,000 rpm for 5 min. The supernatant was pipetted out to measure by spectrophotometer at 260 nm.

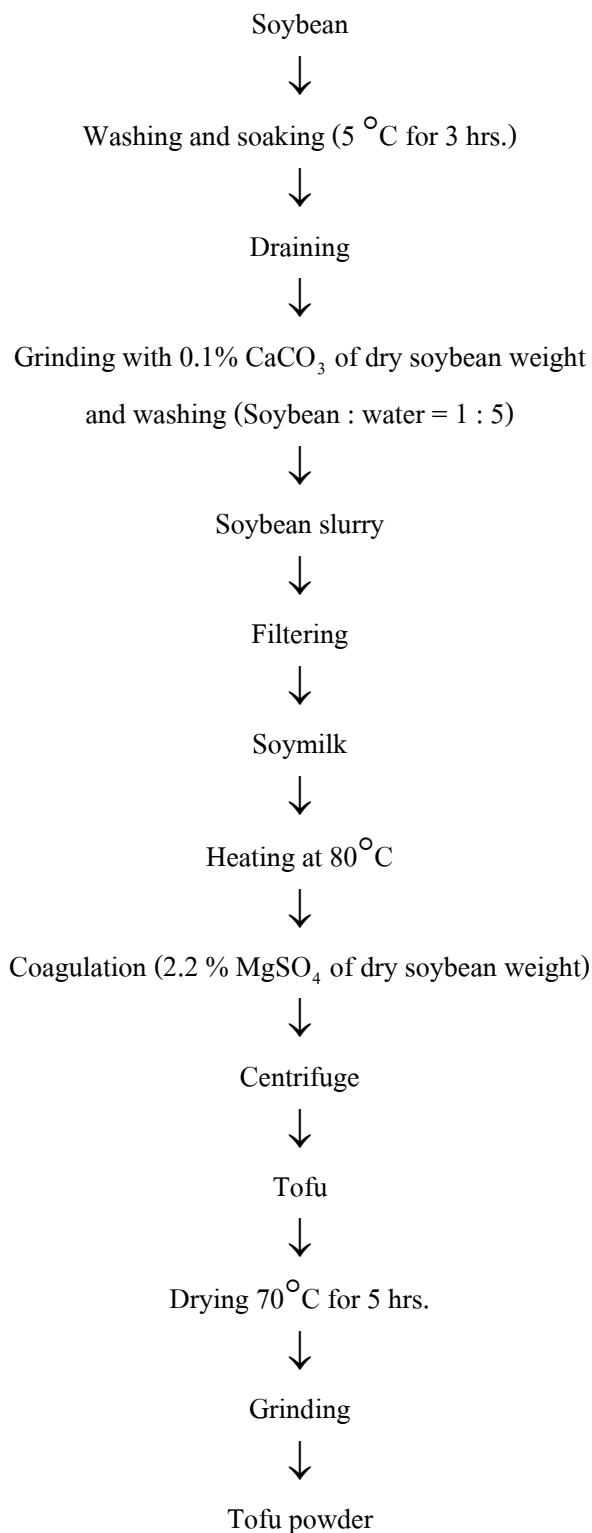


Figure 3.1 Flow diagrams for soymilk and tofu powder production

Source: Panyathitipong and Puechkamut (2002)

c) The emulsion activity and emulsion stability of tofu powder were determined by the modified method of Uruakpa and Arntfield (2005). The tofu powder samples (0.5 g) dispersed in 15 ml phosphate buffer pH 7.4 and 5 ml of corn oil. The dispersion was homogenized at 20,000 rpm for 1 min. Emulsion (50 μ l) was mixed with 5 ml of 0.1 % (w/v) SDS. Absorbance of the resulting dispersion was measured by spectrophotometer at 500 nm for emulsion activity. For the emulsion stability, 2 ml of emulsion was centrifuged at 8,000 rpm for 5 min. The supernatant was pipette out for 1 ml and mixed with 5 ml of 0.1 % (w/v) SDS. Absorbance of the resulting dispersion was measured by spectrophotometer at 500 nm for emulsion stability.

d) Yield (Noh et al. 2005). The yield is expressed as weight of tofu powder obtains from 100 g of soybean.

The experimental design of complete randomized design (CRD) was used. The significant different of all data were calculated by the method of Duncan's New Multiple Range Test. All properties of the tofu powder were compared to obtain the good condition for the next experiment.

3.4.1.2 The effect of coagulants

MgSO₄, CaSO₄, MgCl₂ and CaCl₂ were used. 2.2% CaSO₄ and 2.2% MgSO₄ are usually used for precipitate of hard tofu and 3.0% MgCl₂ and 3.0% CaCl₂ is usually used for precipitate of firm tofu. All the coagulants are added to coagulate soymilk to prepare the tofu powder as described in Figure 3.1. The properties of the tofu powder were elucidated by the methods as described in 3.4.1.1.

The experimental design of complete randomized design (CRD) was used. The significant different of all data were calculated by the method of Duncan's New Multiple Range Test. All properties of the tofu powder were compared to obtain the appropriate coagulant for the next experiment.

3.4.1.3 The effect of drying processes

From Figure 3.1, instead of tray drying, drum drying and freeze drying were used to dry tofu. For tray drying, the tofu were spread and dried at 70°C for 5 hrs. The tofu were mixed with water (1: 1.5 w/w) and dried at 135°C for drum drying. The water in tofu were sublimated in freeze dryer. The condition for freeze drying was -44°C for 18 hrs. and vacuumed between 130-140 millibar.

The resulting dried tofu powder was subjected to determine their properties as described in 3.4.1.1. The experimental design of complete randomized design (CRD) was used. The significant different of all data were calculated by the method of Duncan's New Multiple Range Test.

3.4.2. Study the physicochemical properties of the emulsion gels that are prepared from tofu powder and surimi

3.4.2.1 Study on the relation between physical and chemical properties of the emulsion gels.

The surimi emulsion gels prepared according to described in Table 3.1 was prepared by the modified method of Chotipratoom (2003) (Figure 3.3, Appendix A2). The tofu powder was substituted for surimi at 20-80% by surimi weight concentrations as pre-emulsion (Figure 3.2, Appendix A3). The pre-emulsion was made by mixing tofu powder with water and oil. Water was added to adjust the moisture content of the tofu powder to the surimi and the amount of oil was equal to the added oil of the formulation.

The setting gels were determined their properties as followed. Also, the correlation between their molecular conformation and their texture and chemical properties were elucidated.

1) Water holding capacity (WHC)

The water holding capacity of surimi emulsion gel samples were analyzed by the modified method of Hughes et al. (1997). The 10 g batter samples were placed in centrifuge tubes and heated for 15 min in a water bath at 90°C. After heating, the samples were cooled to room temperature, and centrifuged at 9,000 rpm for 20 min. the supernatant was eliminated and the WHC of the remaining pellets were calculated as follows:

$$\text{WHC (\%)} = \left\{ 1 - \frac{(\text{weight of sample before heating} - \text{weight of sample after centrifugation})}{\text{total water content in the sample}} \right\} \times 100$$

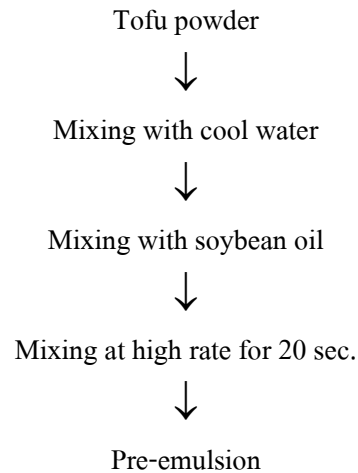


Figure 3.2 Flow diagrams for preparation of pre-emulsion from tofu powder

Source: Panyathitipong and Puechkamut (2002)

Add frozen pre-emulsion, salt, 1/2 of total ice into surimi

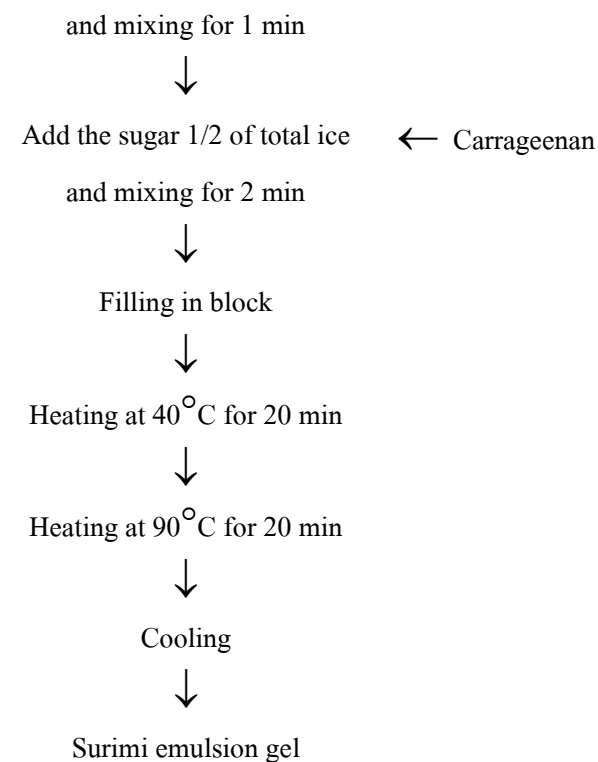


Figure 3.3 Flow diagrams for preparation of emulsion gel from tofu powder and surimi

Source: Modified method from Chotipratoom (2003)

Table 3.1 The basic formulation of surimi emulsion gel

Ingredients	Content (%w/w)
Surimi	61.00
Salt	1.50
Sugar	3.00
Ice	27.00
Soybean oil	7.50

2) Emulsion stability

The emulsion stability was analyzed by the modified method of Hughes et al. (1997). The 25 g batter samples were placed in centrifuge tubes and centrifuged at 4,000 rpm for 1 min. The samples were heated in a water bath at 70°C for 30 min. After heating, the samples were centrifuged at 4,000 rpm for 3 min. The pellets samples were removed and weighed and the supernatants were poured into pre-weight crucibles and dried overnight at 100°C the volumes of total expressible fluid (TEF) and the percentage fat was calculated as follows:

$$\%TEF = \frac{\text{weight of sample} - \text{weight of pellet} \times 100}{\text{sample weight}}$$

$$\% \text{ Fat} = \frac{\text{dried supernatant} \times 100}{TEF}$$

3) Texture profile analysis (TPA)

Texture profile analysis was performed using a texture analyzer (TA-XT2i, Stable Micro Systems). The gel samples were cut and compressed twice to 30% of their original height with 75 mm diameter aluminium platen. The condition of texture analysis as follows: pre-test speed 1 mm/s, test speed 1 mm/s and post-test speed 1 min/s. Hardness and springiness were determined.

4) Scanning electron microscopy

Small pieces of emulsion gel samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for overnight and then they were rinsed twice in phosphate buffer of 30 min/each and once in distilled water for 30 min. The samples were dehydrated using gradually series of ethanol (30 50 70 and 90% 1 hr./each and absolute ethanol 3 times, 1 hr./time). The samples were mounted on aluminium stubs and coated with a layer of gold under vacuum, allowing surface and cross section visualization. Micrographs of the samples were obtained with scanning electron microscope (JEOL, model JSM-5410LV).

3.4.2.2 Study on the physicochemical properties of the emulsion gels modified by carrageenan

Chotipratoom (2003) found that the more developed gels would be obtained by the addition of hydrocolloid as starch and gum. The amount of tofu powder could be substituted meat at higher level if the hydrocolloid was added in the mixture. Therefore, in this experiment, the effect of carrageenan concentration (0.25 0.5 and 0.75%) on the physicochemical properties of the emulsion gel was elucidated. The molecular structure, texture and emulsion stability of the gels were examined as described in 3.4.2.1. The developed gel setting conditions from the mixture of surimi and tofu powder were elucidated.

3.4.3 Develop surimi emulsion gels from tofu powder that yield product with satisfactory sensory quality

3.4.3.1 Optimization levels of soybean oil and carrageenan to formulate the emulsion gel

To make tofu powder more commercial used as the food ingredient, the product development of the gel was formed by tofu powder and surimi. The optimal ingredients levels of soybean oil (0.0-12.0% of total tofu powder and surimi weight) and carrageenan (0.0-1.0% of total tofu powder and surimi weight) were determined. Their experimental design was described in Table 3.2.

The response surface methodology (RSM) was used for the experimental design to find the optimal soybean oil and carrageenan level in producing the gel. The three code levels of soybean oil: -1 (0%), 0 (6%), 1(12%) and carrageenan: -1 (0.0%), 0 (0.5%), 1 (1.0%) were incorporated into the design and analyzed in 14 combinations with two blocks experimental

design as describes in Table 3.2. The central point of the design was repeated six times to calculate the reproducibility of the method.

The effect of these two independent variables, x_1 (soybean oil) and x_2 (carrageenan) on the responses (Y) is modeled using the second order polynomial response surface. The equation derives using RSM for the prediction of the response variables (β_n) is as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$$

Where β_0 is the value of the fixed response at the central point of the experiment that is the point (0,0); β_1 and β_2 are the linear, β_{11} and β_{22} are the quadratic and β_{12} are the interactions regression terms.

Table 3.2 Levels (%) of variables in surimi emulsion gel according to experimental design

No.	Variable levels			
	Code		Uncode	
	Soybean oil (x_1)	Carrageenan (x_2)	Soybean oil (x_1)	Carrageenan (x_2)
1	-1	-1	0	0.00
2	-1	0	0	0.50
3	-1	1	0	1.00
4	0	-1	6	0.00
5	0	0	6	0.50
6	0	0	6	0.50
7	0	0	6	0.50
8	0	0	6	0.50
9	0	0	6	0.50
10	0	0	6	0.50
11	0	1	6	1.00
12	1	-1	12	0.00
13	1	0	12	0.50
14	1	1	12	1.00

1) Texture profile analysis (TPA)

Texture profile analysis was performed using a texture analyzer (TA-XT2i, Stable Micro Systems). The gel samples were cut and compressed twice to 30% of their original height with 75 mm diameter aluminium platen. The condition of texture analysis is as follows: pre-test speed 1 mm/s, test speed 1 mm/s and post-test speed 1 min/s. Hardness and springiness were determined.

2) Sensory analysis

a) Sensory evaluations of the emulsion gels by quantitative descriptive analysis (QDA) were conducted. The trained panelists were chosen base on their ability to discriminate flavor and texture differences and to communicate their perception by using of duo-trio and ranking/rating test (Meilgaard et al., 1999). Then the 8 trained panelists selected the specified attributes to describe the characteristic of the gel. A 15-cm line scale with descriptors anchored at both ends was used to describe the intensity of the specified attributes (Appendix B1). The sample was coded with three digits random code numbers. Four sessions of sensory evaluations with two replications were carried out and seven test samples were prepared at each of the sessions (Gan et al., 2007). The panelists evaluated for all the samples by marking across the scale for intensity of each of the attributes and the mark were converted to a numerical value by measuring from left side of the line.

b) The untrained (n=42) evaluated all the samples using preference test of the 9-points Hedonic scale (score 1 = dislike extremely; score 9 = like extremely) for various quality characteristic such as colour, texture, flavour and overall acceptability for calculated response variable (Appendix B2).

Optimum basic ingredients levels of soybean and carrageenan were determined by extrapolating the plot for preference variables. The optimum formulation were selected and used for calculating the predicted values of response variables using the prediction equations derived by RSM. Verification of the optimum formulation for producing the gel was performed. The gel forming by the optimum ingredients level was experimental analyzed and the results were statistically compared to the predicted values of the mathematical model.

3.4.3.2 Consumer acceptance

The emulsion gel was prepared according to the formation that selected from 3.4.3.1. The study on the acceptance of the surimi emulsion gel was performed by a consumer

panel. The consumers were recruited in central location using local markets which are Dhevaraj market and Dhewet market around Rajamangala University of Technology Phra Nakhon. A total of 150 people participated in to separate sessions, with 75 in each group (Meilgaard et al., 1999). The consumers consisted in age between 20-60 and balance in male and female. The acceptability were evaluated using 9-point hedonic scale (score 1 = dislike extremely; score 9 = like extremely) for various quality characteristic such as color, taste, flavor, texture and overall acceptability. The scores were analyzed to determine how the product is accepted.