

TOFU POWDER PRODUCTION AND ITS APPLICATION IN FISH EMULSION



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## ABSTRACT

Soy protein is incorporated in meat emulsion products to improve product quality through its emulsifying and structure forming. However, soy protein is imported from overseas and costly, while tofu powder is the soy protein product that could be substituted for soy protein isolate in meat emulsion products. The qualities of tofu powder are influenced by the processing which includes soybean quality and processing conditions such as the coagulants and drying process. Therefore, in the present study, the effect of soybean varieties and tofu powder processing on the quality of tofu powder was determined. Soybean varieties (CM 60, SJ 5 and ST 2), the commercial coagulants (2.2% CaSO<sub>4</sub>, 3.0% CaCl<sub>2</sub>, 2.2% MgSO<sub>4</sub> and 3.0% MgCl<sub>2</sub>) and drying methods (tray dryer, drum dryer and freeze dryer) were studied with respect to their effects on the quality of tofu powder. Soybean varieties influenced the quality of soybean and soymilk, and also affected the functional properties of tofu powder, whereas tofu powder obtained from CM 60 had high yield and good functional properties. Calcium ion coagulant inducing tofu powder resulted in a high solubility while magnesium ion inducing tofu powder gave a high emulsion activity. The tofu powder obtained from the freeze drying method had a high solubility and high emulsion activity but it had low emulsion stability.

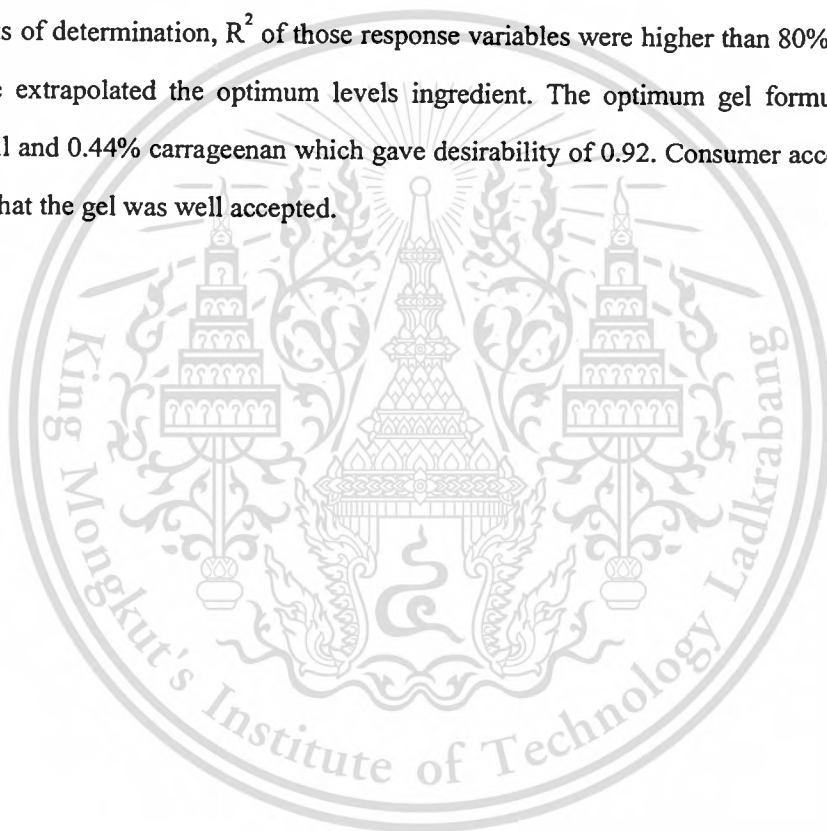
Tofu powder and carrageenan have been used as texture modifying agents in meat emulsion. Tofu powder (20, 40, 60 and 80%), a protein replacement for surimi in form of a pre-emulsion to formulate emulsion gels affected on the functionalities, textural parameters, and microstructure of the gels. The addition of tofu powder increased the emulsion stability, water holding capacity (WHC) and hardness of the gels. Microstructural observations supported the texture profile analysis (TPA) and functionality results. Evaluation was carried out on the addition

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of carrageenan at various concentrations (0.25, 0.5 and 0.75%) to the gel that was replaced by 80% of the surimi with tofu powder. Increasing the concentration of carrageenan also improved the stability of the emulsion, WHC and hardness of the gels. Microstructural observations showed that the addition of carrageenan resulting in a smoother and more compact gel network. The selected gel product was formed by incorporating 60% tofu powder for more consumers.

The response surface methodology (RSM) was used for the experimental design to determine the optimal soybean oil and carrageenan level in producing the gel. Significant regression models explained the effects of different soybean oil and carrageenan concentrations on sensory qualities (preference test and quantitative descriptive analysis) and TPA. The coefficients of determination,  $R^2$  of those response variables were higher than 80%. Peak in RSM plots were extrapolated the optimum levels ingredient. The optimum gel formula was 9.36% soybean oil and 0.44% carrageenan which gave desirability of 0.92. Consumer acceptance testing indicated that the gel was well accepted.



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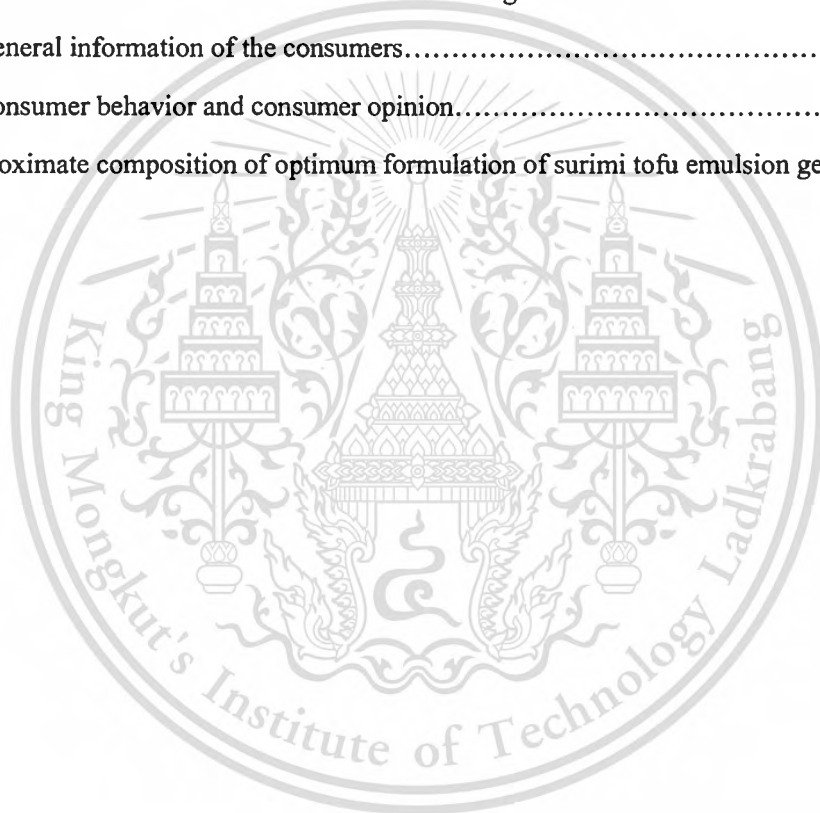
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# CHAPTER 1

## INTRODUCTION

In recent years, health and over weight have been big problems for consumers. The new food have been developed for high nutrient, low fat and calories. Soy products such as soy flour, soy protein concentrate, soy protein isolate are popularly used in many products for reducing fat and cost. In meat industry, soy protein isolate is commonly used in meat-base products for their functional properties to reduce fat and cost (Lecomte et al., 1993; Ho et al., 1997; Hin et al., 2000; Ramezani et al., 2003). However, in Thailand soy protein isolate has some disadvantages as it is imported from oversea and costly. Its manufacturing process is also complicate. Soybean has been shown to be a rich source of isoflavones, a weak form of the female hormone estrogen. Isoflavones have been shown to inhibit the growth of cancer cells, lower blood cholesterol and inhibit bone resorption. Consumption of soy food has been on the rise because the establishment of the October 1999 U.S. Food and Drug Administration (FDA) has approved soy protein claims, which links the intake of products high in soy protein with positive health benefits such as a lower risk in heart diseases.

Tofu is an important source of protein and high potential of being a satisfactory meat additive that can reduce fat and calories if substituted for the fat in meat emulsion products (Jeng et al., 1988; Ho et al., 1997). However, high moisture content in tofu causes microorganism grows rapidly. Tofu powder is the product from ground-dried tofu that can be used as the ingredient in processed meat products. It has a proximate composition of 52.54% protein, 29.78% fat and 4.65% water (Panyathitipong and Puechkamut, 2002). Tofu powder has a high protein and essential fatty acid, linolenic and linoleic acid. Panyathitipong and Puechkamut (2002) found that the emulsion made from tofu powder had good emulsion stability but the solubility of this tofu powder was very low. Chotipratoom (2003) reported that tofu powder emulsion could substitute in meat emulsion up to 90% but the products had altering flavor and cracked during frying. Because of the limitation of the tofu powder functionality, its properties should be improved. Aside from this fact the poor functional properties can be a result from the process on preparation. Therefore, the effects of processing on the quality of tofu powder will be elucidated in this experiment and new product will be made using tofu powder as a raw material. The emulsion gels formulated by different mixture of tofu powder and surimi will be developed to yield products

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with a good texture and taste. The correlation between microstructure texture and chemical properties of the gels also will be studied. All of these experiments will promote tofu powder to become one of the valuable food ingredients for meat industry.

**The objectives of this research are:**

1. To study the preparation process of tofu powder to improve its functional properties
2. To study the physicochemical properties of the emulsion gels that are prepared from tofu powder and surimi
3. To develop surimi emulsion gels from tofu powder that yield product with a satisfactory sensory quality



## CHAPTER 2

# LITERATURE REVIEWS

Soybean is the source of inexpensive and high quality protein. Soybean has been a staple of the human food in Asia, especially soymilk and tofu. In recent years, interest in soy foods has increased due to consumer concern about health. Soy foods provide protein of equal quality to other proteins and without saturated fats and cholesterol. Food and Drug Administration (FDA) has approved soy protein claims to lower serum cholesterol level as a lower risk in heart diseases and allowed food manufacturers to place a health claim on the package labels of food products containing more than 6.25 g of soy protein per serving. FDA recommends that consumers incorporate four serving of at least 6.25 g of soy protein into the diet for a total of at least 25 g of soy protein per day in order to reduce the risk of heart disease (Stein, 2000).

Tofu, a traditional soybean food in Asia, has been an important source of protein. It is a white or pale yellow soybean curd precipitated from hot soymilk with a coagulant such as  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ , and Glucono- $\delta$ -lactone (GDL). It is inexpensive, nutritious, cholesterol-free, lactose-free, lowers in saturated fat and calories. In recent years, tofu has become increasingly popular throughout the world, as the number of consumers who are looking for health foods is increasing.

The process of making tofu involves a complex interaction of many factors, including chemical composition and physical attributes of the soybean and processing techniques and conditions. Many methods are available for making tofu. There are many factors affecting tofu preparation to balance the maximum both tofu yield and quality. In general, factors affecting soymilk preparation include soybean varieties whether soybeans are ground hot or cold, whether soy slurry is heated before or after filtration, the water to bean ratio and extent of heat applied to soymilk. Factors affecting coagulation include the temperature at which a coagulant is added, the type and concentration of coagulants, the mode of adding coagulants, the duration of coagulation and molding step, the pressure and time applied to press curds.

## 2.1 Factors involved tofu making

### 2.1.1 Soybean varieties

There are great variations among soybean varieties, in terms of physical appearance and chemical composition, protein and oil content, size, amount of water absorbed at complete hydration, protein concentration of soymilk, fresh tofu yield, tofu protein content and tofu hardness (Kim and Wicker, 2005). Traditionally, soymilk and tofu manufacturers prefer large-seed soybeans with clear hilum and high protein content. Tofu beans produced tofu with whiter color and better overall quality, compared to regular field beans known as oil beans (Ang et al., 1999). The most important attributes of soymilk and tofu are flavor, texture and yield. The chemical composition of soybeans should be more important than their physical appearance (Liu, 1997). Soybean varieties and growing locations affect the chemical and physical properties of soybean, soymilk and tofu. Protein contents of soybean are the most important factor determining the qualities of soymilk and tofu. Analysis on soybean protein can be used to predict the protein content of tofu and yield (Poysa and Woodrow, 2002; Min et al., 2005b; Panyathitipong and Puechkamut, 2008).

The protein compositions that are 7S and 11S effected on the tofu quality which varies with soybean variety and environment. It should be informative to determine the effect of their ratios (Prabhakaran et al., 2006; Tay et al., 2006) and their individual sub-unit content, on soymilk and tofu yield and quality prepared using the above techniques which would emphasize differences in protein quality, as the protein quantity is being kept constant (Poysa and Woodrow, 2002). The 11S content and 11S/7S protein ratio have been reported to correlate positively with tofu gel firmness on the basis of purified soy protein system (Murphy et al., 1997; Mujoo et al., 2003). The 11S gels made in the tofu coagulants were much harder than 7S gels and 11S mainly determined the hardness of tofu gels and 11S showed greater breaking force than 7S gels (Tay et al., 2006)

The effects of soybean varieties and growing locations on the volatile compounds are significant. It was found the total volatile compound content was related to concentration of soymilk protein. The volatile contents increased as the protein content of soymilk increased (Min et al., 2005a). Moreover, the different intensity in the colors of soybeans varieties affect on the color of soymilk caused by seed coat hilum. Khatib et al. (2002) reported that the different hilum colors of soybean seeds were due to the distribution of pigments in the seed coat, which could migrate to soymilk components upon grinding but did not affect the color properties of tofu.

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### 2.1.2 Concentration of soymilk

The concentration of soymilk or total solids in soymilk is closely related to the water-to-bean ratio (Cai and Chang, 1997; Lakshmanan et al., 2006). It can be measured easily with a refractometer and expressed as °Brix. The ratio is the total weight of water added to the dried soybeans during soaking, grinding and cooking, divided by the original weight of the dry soybeans. The total water includes the water used for rewashing okara if applicable but excludes drained water and that used for washing beans. The amount of water needed to make soymilk is also important because it affects soymilk solid content and tofu texture. In general, higher solid content in soymilk correlates with a harder texture, yield of tofu and protein content of tofu (Beddows and Wong, 1987; Ohara et al., 1992; Cai and Chang, 1997; Shih et al., 1997; Poysa and Woodrow, 2002; Kong and Chang, 2009). High concentration of soymilk increased the tofu's apparent Young's modulus and reduced the syneresis rate (Liu et al., 2004) and increased the density of the network (Cheng et al., 2005). Soymilk solid content decreased when the water-to-bean ratio increased but no significant effect on protein recovery in dry tofu (Cai and Chang, 1997).

The viscosity of soymilk increased exponentially with increasing soymilk concentration (Cheng et al., 2005). In general, tofu manufacturers use soymilk with a solid content ranging between 5% and 12% (Ang et al., 1999).

### 2.1.3 Heat process of soymilk

The heating of soymilk is a prerequisite for formation of tofu gel by unfolding soybean protein molecules. The SH-groups and hydrophobic amino acid side chains are exposed. The negative charges on the protein molecules are decreased by protonation of coagulant when coagulant is added. Protein molecules come close together owing to the decrease of electrostatic repulsion leading to formation of tofu gel with a three dimensional network structure. The optimum heating time-temperature combination corresponds approximately to the maximum range of SH-groups (Liu, 1997). Heat treatment on soybean or soymilk used in tofu preparation can be one of the methods to control the texture of tofu (Yoon and Kim, 2007). It was reported that the texture and yield of tofu were affected by thermal treatments. Increasing coagulation temperature increased the tofu hardness but decreased the tofu yield (Sun and Breene, 1991; Hou et al., 1997). The hardness of tofu increased with heat treatment time. The increase in the hardness of tofu by heat treatment was explained that gel hardness increased with the amount of

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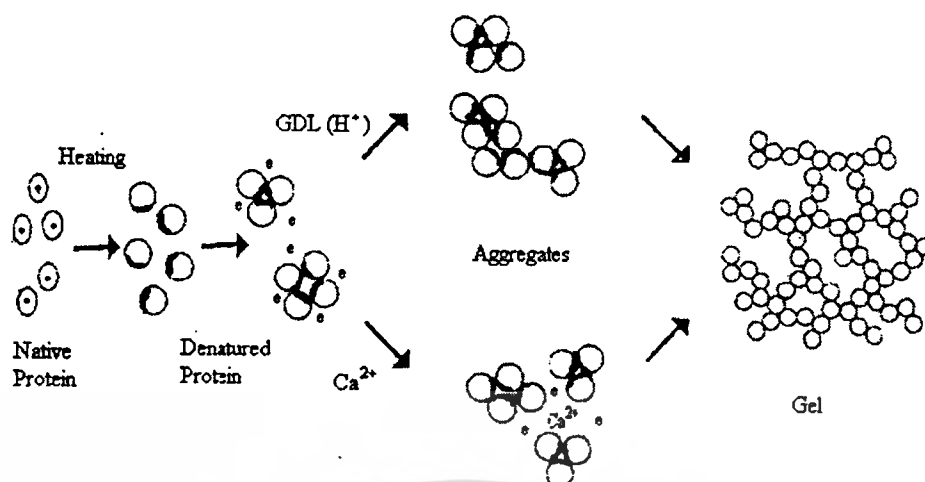
disulfide bonding. Thus, increase in concentrations of 7S globulin and sulfur amino acids can cause a stronger gel to form. Therefore, it is hypothesized that the increased formation of disulfide bonding caused by the synthesis of basic 7S by heat shock produced firmer tofu than the control (Yoon and Kim, 2007)

Soymilk heated at different conditions was different in texture. In one step heating, induced the decrease in tofu apparent breaking strength and Young's modulus. In two step heating, increased the tofu's apparent Young's modulus, breaking strength and the structure of the network of the tofu was denser, finer and more homogeneous (Liu et al., 2004; Wang et al., 2007).

The longer heat treatment may have caused more Maillard browning and hence the formation of brown color causing and increasing in the yellowness of tofu (Yoon and Kim, 2007). Regardless of whether soymilk or soy slurry is heated; it is recommended that 10 min at near 100°C is optimum for heat treatment (Ang et al., 1999).

#### 2.1.4 Type of coagulants

Coagulation is the most important step in tofu making process. At least four variables are involved in this step: the type of coagulants, the concentration of coagulants, the temperature of soymilk to which a coagulant is added, and the mode of adding and mixing the coagulant (Liu, 1997). Calcium sulfate, calcium chloride, magnesium sulfate, magnesium chloride and glucono- $\delta$ -lactone (GDL) are different type of coagulants used for industrial scale for the preparation of tofu. The tofu was formed due to the cross-linking of protein molecules in soymilk with the divalent cations such as  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  form bridges with the negatively charged protein by electrostatic interaction (Prabhakaran et al., 2006). The electrostatic interaction will cause the cations to form bridges with the proteins, which result in the formation of aggregates (Tay et al., 2006). The mechanism is shown in Figure 2.1. After the coagulation process, whey is removed for the preparation firm and hard tofu. Different coagulants used in soy gels result in products with varying flavor properties and textural characteristics. The texture of tofu should be smooth, firm and coherent (Liu, 1997).



**Figure 2.1** The gelation mechanism of soy protein with glucono delta lactone (GDL) or  $\text{Ca}^{2+}$

**Source:** Liu (1997)

Sulfate type such as  $\text{MgSO}_4$  and  $\text{CaSO}_4$  is the most widely used tofu coagulant. It is also the oldest one used in China (Ang et al., 1999). This coagulant is well suited to modern mass production methods for although they disperse slowly in water to form colloidal solutions which have a slow coagulation reaction time. It gives 15 to 20% higher bulk yields than nigari (chloride type). The tofu flavor varies from mild to bland, slightly inferior to that of nigari tofu. Sulfate type salt is sold in the form of a white powder, always stored in dry form and used in the amount of 2.2% by weight of dry soybean for making regular or firm tofu and 1.0% for making silken tofu (Shurtleff and Aoyagi, 2000). Calcium sulfate was also found to be a better coagulant than calcium chloride in terms of yield of tofu (Prabhakaran et al., 2006) due to chloride ion caused the decrease of electrostatic interaction more than sulfate ion which lead to the increase of syneresis and loss of whey from the curd (Tay et al., 2006)

Nigari or chloride type such as  $\text{MgCl}_2$  and  $\text{CaCl}_2$  is a by-product during production of table salt from seawater. It is a mixture of mineral compounds naturally found in and the sedimented from seawater, from which table salt has been mostly removed. It consists primarily of magnesium chloride plus all of the other salts and trace mineral in seawater. This coagulant has one great overriding virtue; it makes the most delicious tofu, prized for its wonderful subtle sweet flavor and aroma. Most tofu shops in Japan, used a nigari-type coagulant to make tofu. This coagulant is sold in powder, granular or flake forms and are used about 3.0 % by weight (Cai and Chang, 1997).

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GDL (glucono- $\delta$ -lactone) is a fine, white, odorless, crystalline powder with a sour taste. It is an oxidation product of glucose. Industrially made from cornstarch and followed by a fermentation process. When it is dissolved in water, it is slowly hydrolyzed to gluconic. Lactone is different from nigari and gypsum types of coagulants because the coagulation by lactone results from the action of an acid rather than a salt. The great advantage of lactone as a tofu coagulant is that, by controlling temperatures, it allows the completion of mixing and packaging before coagulation. Thus, it is particularly suitable for automation and aseptic packaging. Since each type of coagulant has advantages and disadvantages, in commercial processes, a mixture of coagulants, such as that of GDL and calcium sulfate or magnesium chloride, is sometimes used (Ang et al., 1999).

Coagulants will produce tofu of different flavor and texture due to the coagulating power. Tay et al., (2006) reported that the coagulation powers of various salts were in order of  $\text{CaCl}_2 > \text{MgCl}_2 > \text{CaSO}_4 > \text{MgSO}_4$ . Coagulants with strong coagulation power were able to form uniform curds of firmer texture compared with those of weak coagulation power.

### 2.1.5 Concentration of coagulants

One of the most difficult and most important parts of tofu making is to determine exactly how much coagulant to add per given weight of dry soybean or volume of soymilk, because the amount of a coagulant is a major factor affecting tofu quality and yield (Sun and Breene, 1991; Liu, 1997). When a proper amount of coagulant is used, the whey becomes transparent with amber or pale yellow color and a sweet taste. However, if too much coagulant is added, the whey has a slightly bitter taste, its color turns more yellowish, and curds have a coarse and hard texture. In contrast, if too little coagulant is used, whey is cloudy, and there may be some uncoagulated soymilk remained. Within a certain concentration range, as the coagulant concentration increases, the tofu bulk yield and protein recovery decrease while the tofu hardness and elasticity increase (Ang et al., 1999). The yield of tofu decreased with increasing calcium ion concentration could be due to the increasing syneresis and loss of whey from the curd as more bonding occurred thus making the protein matrix more dense and compacted. At the lower concentration, bridging of protein molecules by calcium ion is not sufficient to form a firm gel. At higher concentrations, increased bridging and the resulting compaction of protein matrix cause increased syneresis and loss of water, whey protein and other solubles (Sun and Breene, 1991).

The network of tofu obtained with lower  $\text{CaSO}_4$  concentration (0.2%) revealed a coarse and discontinuous structure with many fragments and large pores. On increasing of the  $\text{CaSO}_4$  concentration, there was a trend toward decreasing pore size and increasing regularity and uniformity. The microstructure of tofu showed the most continuous and uniform with smaller holes than those prepared with 0.2%  $\text{CaSO}_4$ . When increasing  $\text{CaSO}_4$  to 0.5% the network of tofu became too porous and too compact and not as uniform and smooth (Kao et al., 2003).

Using optimal concentration of coagulant in soymilk is the most critical step among many steps of unit operations during tofu manufacturing for achieving high quality tofu. The optimal coagulant concentration varies not only with processing condition but also with soybean materials which are affected by variety and storage conditions (Poysa and Woodrow, 2002; Mujoo et al. 2003).

The optimal concentrations of coagulant for preparing tofu decreased with increasing either  $A_w$  or storage time due to the decrease in soymilk pH and the increase in organic acid content (Liu and Chang, 2008). This speculation was in agreement with the theory of the coagulation of soymilk in acidic compounds that could coagulate soy proteins to reduce the negative charges on soy protein molecules by the protonation of the  $-\text{COO}^-$  on acidic amino acid residues. The pH reduction enhanced the extent of  $\text{Ca}^{2+}$  binding to soy proteins (Kroll, 1984). It was observe that when the respective optimal amount of coagulant was applied, pH of coagulum decreased with increasing  $A_w$  or storage time (Liu and Chang, 2008).

When soymilk was prepared by fixing the water-to-bean ratio despite the different storage conditions, the soymilk protein content or total solids content decreased with the adversity of soybean storage condition (Hou and Chang, 2003). The optimal coagulant concentration decreased with decreasing soymilk total solids content (Liu and Chang, 2008). The method of soymilk preparation with a fixed water-to-bean ratio will decrease total solids content and accordingly results in a sharper decrease in optimal coagulant concentration than the method of soymilk preparation which adjusts water-to-bean ratio for keeping a fixed total solids content. Therefore, when a fixed coagulant concentration, which may be optimal for the freshly harvested soybeans, is applied to the soymilk made form the improperly stored soybean, the soymilk would coagulate too fast and form a poor curd (Liu and Chang, 2008).

### 2.1.6 Coagulation temperature and coagulation time

The temperature of soymilk at the time of adding a coagulant affects the coagulation rate as well as the tofu texture and yield. At a high temperature, proteins possess high active energy and fast to coagulation. The tofu tends to have small network, reduce water holding capacity, hard texture and low bulk yield. When the coagulation temperature is low, the effect is just the opposite. However, if the temperature is too low, (below 60 °C) the coagulation becomes incomplete, and the tofu contains too much water and is too soft to retain its shape. Therefore, selection of an actual temperature during tofu production depends on the type and concentration of coagulants, the mode of adding coagulants, and the type of tofu to be made. The optimum temperature range is 70-80 °C. The tofu produce below 70 °C is soft and watery whereas the tofu produce above 80 °C is hard and uneven, with considerable loss of bulk yield (Beddows and Wong, 1987; Ohara et al., 1992).

After adding a coagulant, it is desirable to let the soymilk-coagulant system stands still for a while after a coagulant is added, since completion of coagulation requires a certain period of time. If the time is too short, coagulation is incomplete, if too long, the temperature of the system decreases to such extent that the subsequent molding step becomes difficult. In general, for silken tofu, the standing time should be about 30 min; for regular tofu, 20-25 min; and for firm tofu, 10-15 min (Ang et al., 1999).

## 2.2 Varieties of tofu

There are many different types of tofu on the market. Based on water content and textural properties, tofu is generally classified as soft (silken), regular, or firm tofu. Basically, all tofu is made in a similar fashion, except for variations in the water : bean ratio, the type and concentration of coagulants, the way a coagulant is added, and the amount of whey being pressed out.

### 2.2.1 Silken tofu

Soft or silken tofu has a soft cheeselike texture but is firm enough to maintain its shape after slicing. Silken tofu is normally made from rich soymilk containing 10-12 % solids. After being finely filtered, the soymilk is allowed to cool to 65-70 °C. It is then mixed with a relatively low concentration of calcium sulfate. Over a period of 30-60 min, a fine, smooth, yet firm curd

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forms. The curd is neither broken nor pressed. In China, silken tofu is normally made by pressing unbroken bean curds to remove some whey. This product has a soft texture but is firm enough to be cut into cakes and packed in containers with water. It is usually served in soups. The product is specially made by using low concentration of soymilk and a reduce amount of coagulant (Ang et al., 1999).

### 2.2.2 Regular and firm tofu

Regular and firm tofu are mostly pressed tofu, which are know as momen tofu. The difference between regular and firm tofu is that firm tofu is harder than regular tofu. Sometimes, the term extra firm is used by some manufactures. In any term, the textural difference among silken, regular, firm or extra firm is rather relative. There are no standard in absolute textural values for them and the texture of the same type of tofu may vary with manufacturers, seasons, and even batches. (Ang et al., 1999)

There are two basic features in making pressed tofu. First, the coagulant is stirred into hot soymilk and curds are thus formed upon cooling. Second, the curds are broken and then pressed while they are still warm. Also, the higher the pressure applied during pressing, the firmer is the tofu. As the texture becomes firmer, the water content becomes less. Firmer texture and reduced water content make tofu easier to handle and more similar to meat. Therefore, pressed tofu is ideal for use in pan-frying, deep-frying, grilling, freezing and then drying and dicing into an ingredient. (Ang et al., 1999)

### 2.2.3 Lactone tofu

When a tofu is made mostly with GDL, a modern coagulant, it is known as lactone tofu. The process starts with the preparation of rich soymilk (10-12 % solid content). The milk is heated and deaerated before cooling to about 30°C. Air bubbles form during milk production, and their presence affect tofu quality. This is particularly true for lactone tofu, which is characterized by a fine and smooth texture. Deaeration is carried out by passing heated milk to a vacuum evaporator, which not only reduces air bubbles, but also strips off some off-flavor volatiles. The cold soymilk is mixed with GDL at a concentration of 0.25-0.30% (based on the milk volume). The mixture is immediately run into a container and sealed. The container is then immersed in hot water (85-90°C) for 30-50 min for coagulation to occur. The resulting curd is cool in the container by immersing in cold water and then refrigerated. Lactone tofu also has three types

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based on texture : soft, regular and firm. In most cases, lactone tofu is not made by GDL alone, but by a combination of GDL and calcium sulfate or magnesium chloride (Ang et al., 1999).

### 2.3 Tofu powder

Tofu powder is the product from ground dried tofu which can be used as an alternative protein source. Tofu powder is made from soybean by the traditional tofu method. Commercial coagulants are used as calcium salt, magnesium salt and acid. The tofu powder has a high protein and good essential fatty acid, linolenic and linoleic acid. The final moisture of tofu powder is low enough to inhibit microbial growth. No yeast and molds were isolated from the tofu powder. The low values for all the microorganism counts showed that the dried tofu powder after a storage period of 4 months under refrigeration was very stable (Ho et al., 1997).

The qualities of tofu powder are influenced by the method of tofu powder processing. Vilavan and Veerothai (1997) had prepared tofu powder by using calcium chloride or calcium sulfate as protein coagulants and drying in a hot-air oven. The solubility and emulsifying properties of tofu powder were not found to be as good as the commercial SPI.

The preparation of tofu powder has an effect on its quality. Panyathitipong and Puechkamut (2002) reported that  $MgSO_4$ -tofu powder had significantly higher emulsion stability than  $CaSO_4$ -tofu powder and  $CaCl_2$ -tofu powder and found that whey separating methods and drying temperature were affected on functional properties and the yield of tofu powder. The emulsion stability of tofu powder obtained at  $70^\circ C$  drying temperature was greater and whey separation by centrifugation gave tofu powder at a higher yield and better emulsion stability compared to those of pressing method.

### 2.4 Utilization of soy protein and tofu powder in meat emulsion products

Meat emulsion products such as frankfurters, bologna are made from minced meat, water, salt and other ingredients such as phosphates, carbohydrates, non-meat protein and seasoning. The properties and quality of meat emulsion product depend on their processing and functional characteristics of salt soluble myofibrillar proteins which are extracted during ingredient mixing in the presence of salt. It was adsorbed at the product fat particle surfaces, due to their remarkable emulsifying ability (Drakos et al., 2005). The proteins in meat are coagulated during processing and cooking resulted the formation of a gel-like structure that binds muscle fibrils with the

emulsified fat particle, thus preventing liquid loosed and contributing to the development of the final product texture (Barbut, 1995; Lin and Mei, 2000).

Proteins from plant are often incorporated in meat emulsion products to improve their properties. In the meat industry, soy protein is most widely used the non-meat protein which is due to its biological value, its properties as an emulsifier, stabilizer and its capacity to increase water holding capacity (WHC). It has been used in the meat product as a binder for improving yields, as a gelling agent to enhance emulsion stability and as a meat replacer to reduce costs.

Some non-meat proteins can be used as fat replacers to bind water and to form gels, thus they respond to consumers demands for healthier and low fat products (Pietrasik and Duda, 2000). Moreover, there are also well known benefits associated with the consumption of soybean reduction of cholesterol levels and menopause symptoms and reduction of risk for several chronic diseases, cancer, heart disease and osteoporosis.

Soy protein possesses important functional properties that the constituents may interact with the meat protein in processed meat product. It appears that the properties of the system are dominated by the more functional salt soluble meat proteins while the soy protein play a rather secondary role (Mourtzios and Kiosseoglou, 2005). Mavrakis et al. (2003) reported that the texture of model comminuted meat product systems are enhanced by the presence of plant protein such as lupin seed protein indication that these proteins are involved in the development of the multicomponent comminuted meat gel structure during processing.

Feng and Xiong (2002) reported that preheated soy protein before mixed with myofibrillar protein resulted from unfolding of the protein molecules and the exposure of the hydrophobic parts of the soy protein complex. This resulted from myosin interacting with soy protein subunit due to hydrophobic aggregation between them, leading to the precipitation of heterogeneous protein aggregates that showed an improved gelling ability of their gel.

On a commercial scale, it is easier for using dry soy protein addition in meat process. However, in order to extend the functionality of soy protein in comminuted meat products, pre-emulsified fat (PEF) with soy protein as stabilizers has been employed. When PEF is used; meat proteins are saved for fat and water binding because part of the fat and water is stabilized completely by the soy protein due to the fat globules in the matrix were coated with a layer of protein membranes and dispersed in a dense protein matrix (Su et al., 2000). The fat globules contained in the protein food systems have been categorized as active or inactive filler particles depending on their interaction with the surrounding matrix (Gu et al., 2009). Active filler particles

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are covered with protein and have mainly hydrophilic surfaces that interact with the matrix whereas inactive filler particles have mainly hydrophobic surfaces and show little interaction with the matrix (Chen and Dickinson, 1999). The protein membranes surrounding the fat globules are thick. When the fat globules are confined within the denser nonmeat protein matrix, the chances for fat coalescence during cooking may be reduced so that emulsions with high fat and water binding properties are formed (Su et al., 2000).

Lecomte et al. (1993) reported soy protein was incorporated in formulations of frankfurters as PEF and as powders. Sensory analysis showed the incorporation of soy protein as pre-emulsified resulted in a reduction of specific soybean off-flavor and off-aroma. Soy protein added as pre-emulsified increased water holding capacity and yield and decreased cook losses.

Lowering fat content in meat emulsion product must be equal in quality to traditional high fat products in order to be successfully marketed. Normally, reducing fat content in meat emulsion often negatively influences the quality of meat products. Incorporation of soy protein and hydrocolloid as gums and carrageenan into processed meat has been shown stabilize emulsion and increase water holding capacity that is a good quality of meat emulsion product (Luruena-Martinez et al. 2004).

Brewer et al. (1992) reported that the addition of soy protein decreased beefy flavor and increased off-flavor score in ground beef patties. This off-flavor and aroma make the products containing soy protein unpleasant to the consumer (Das et al., 2008). Soy protein has some disadvantages not only off flavor but also its manufacturing process is complicated and costly. Traditional soy foods such as soymilk and tofu can be substituted with soy protein as soy based products. Rahardjo et al. (1994) reported that spray dried soymilks were incorporated into reduced fat pork sausage. Spray dried soymilk mixtures had less fat and lower calorie content than regular pork sausage, protein content and cooking yield were higher than regular pork sausage. Texture of lean pork sausage was improved by addition of spray dried soymilk.

Tofu has the potential of being a satisfactory meat additive that can reduce the fat and calorie contents, if it is substituted for fat in meat emulsion products. Jeng et al. (1988) studied in which two levels (15.5% and 31.6%) of tofu were substituted for fat (12%-28%) and lean (3.5%) in making bologna, a comminuted meat type product. These treatments were compared with a meat batter containing 3.5 % soy protein concentrate and an all meat bologna. They found that bologna with tofu had a higher emulsifying capacity, less moisture stability, and lower fat and calorie level

than soy protein containing bologna. Moreover, the soy protein concentrate containing bologna had more beany flavor.

Tofu is quite perishable due to its rich nutrient and high moisture content. Its spoilage is associated with bacterial growth. While tofu powder is the product from ground dried tofu which can be used as the ingredient in processed meat products. Tofu powder can reduce fat content, improve texture and increase protein content without altering flavor in meat emulsion products (Ho et al., 1997). Panyathitipong and Puechkamut (2002) found that the emulsion made from tofu powder had good emulsion stability but the solubility of this tofu powder was very low. Ho et al. (1997) reported that regular frankfurter treated with tofu powder decreased fat with no significant differences in color, texture or overall acceptability compared to a control by sensory analysis. Lean frankfurter with tofu powder had lower moisture and color, better texture and overall acceptability with no flavor differences from controls. Lean pork sausage treated with tofu powder was lower in fat, higher in protein and moisture with no differences in sensory attributes compared to controls. Many researches, however, tried to use tofu powder on the supporting ingredients for processed meat products. The researches that aimed to use tofu powder as the main ingredient have rarely been done.

## **2.5 Utilization of hydrocolloid in meat products**

Macromolecular hydrocolloids play a role in modifying the textural properties of food systems. They can be as gelling and thickening agent. The biopolymers interactions as protein-polysaccharide are importance to develop products with acceptable texture characteristics (Tolstoguzov, 1997).

Hydrocolloids are considered to influence many of the functional properties of processed meat products. They are commonly used in meat emulsion products as emulsifiers, water texture modifying ingredients (Chattong et al., 2007), fat replacer (Cierach et al. 2009) to improve the functionality. Meat protein and polysaccharide interactions play a significant role in the structure and stability of meat emulsion products (Hua et al., 2003) due to their ability of binding water and forming gel (Candogan and Kolsarici, 2003). Functional properties of food proteins such as solubility, gel forming and emulsifying capacity are affected by their interaction with polysaccharide or hydrocolloid gums. Protein and polysaccharides can form hybrid complexes with enhanced functional properties in comparison with protein or polysaccharide alone. The gelation behavior of protein-polysaccharide mixtures generally fall into three patterns: formation

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of covalent bonds between two polymers; polyanion-polycation electrostatic interactions; and formation of composite gel due to mutual exclusion of each component (Hua et al., 2003). Electrostatic complexation of oppositely charged protein and polysaccharide allows better anchoring of the newly formed macromolecular (Andres et al., 2006).

The anionic polysaccharide and protein could associate with each other through charge-charge attraction when the pH is below the isoelectric point of proteins, this interaction normally results in a precipitate of complexes, rather than formation of a gel. Most protein-polysaccharid mixtures are thermodynamically incompatible systems at neutral pH and the mixture can be separated into two liquid phases according to phase diagrams (Tolstoguzov, 1997). After gelation of the incompatible polymer mixture, the resulted gels could be regarded as filled of composite gels (Hua et al., 2003). Soy proteins and hydrocolloids have been used in meat emulsion products. They have been utilized often in modifying both the textural and sensory attributes of meat products (Flores et al., 2007).

Carrageenans are hydrocolloid that extensively use in food industry as gelling, thickening and stabilizing agent. Carrageenans and soy bean protein can be used together in food industry as gelling and viscous agents to improve textural (Ortiz et al., 2004) due to the synergistic effects between them (Baeza et al., 2002). The effect of carrageenan addition to meat emulsion products on sensory properties were increased for texture and acceptability with increasing carrageenan concentration (Cierach et al., 2009; Ayadi et al., 2009).

The microstructure of meat emulsion products can be observed by scanning electronic microscopy. The microstructure of meat emulsion products shows the meat proteins form a three dimensional compact gel network. When addition hydrocolloid as carrageenan or gum, it can forms a continuous gel network and suggesting that hydrocolloids is present in the interstitial spaces of the protein network which cause decrease in the compactness of protein network (Verbeken et al., 2005; Andres et al., 2006; Chen et al., 2007 and Ayadi et al., 2009).

## 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 ( $\text{CaCl}_2$ , food grade)
- 3.2.2 Calcium sulphate ( $\text{CaSO}_4$ , food grade)
- 3.2.3 Magnesium chloride ( $\text{MgCl}_2$ , food grade)
- 3.2.4 Magnesium sulphate ( $\text{MgSO}_4$ , food grade)
- 3.2.5 Calcium carbonate ( $\text{CaCO}_3$ , 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

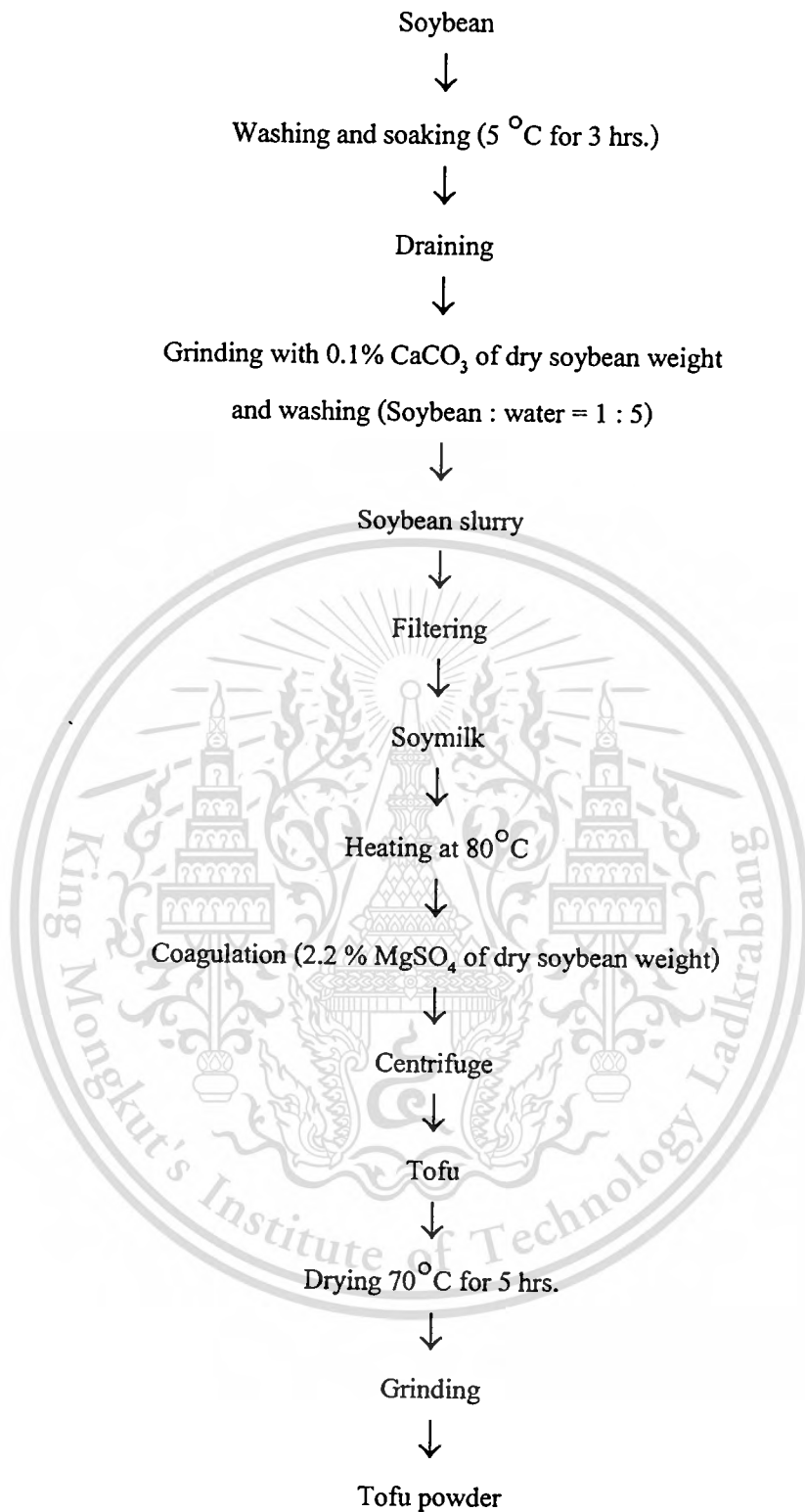
- a) Total soluble solid by hand refractometer
- b) Total solid content (Min et al., 2005). The total solid content of soymilk was determined by drying soymilk to constant weight at 110°C in an air oven. The percent of total solid content was calculated as follows:

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

- 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  $\mu$ 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

$\text{MgSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{MgCl}_2$  and  $\text{CaCl}_2$  were used. 2.2%  $\text{CaSO}_4$  and 2.2%  $\text{MgSO}_4$  are usually used for precipitate of hard tofu and 3.0%  $\text{MgCl}_2$  and 3.0%  $\text{CaCl}_2$  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.

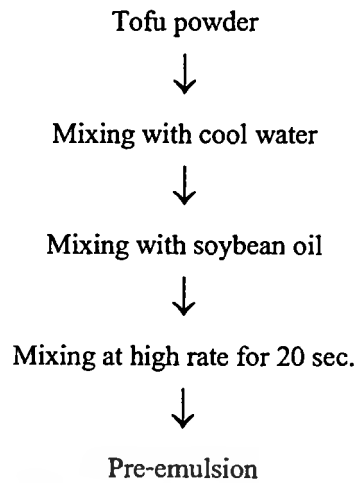
#### 1) Water holding capacity (WHC)

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:

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

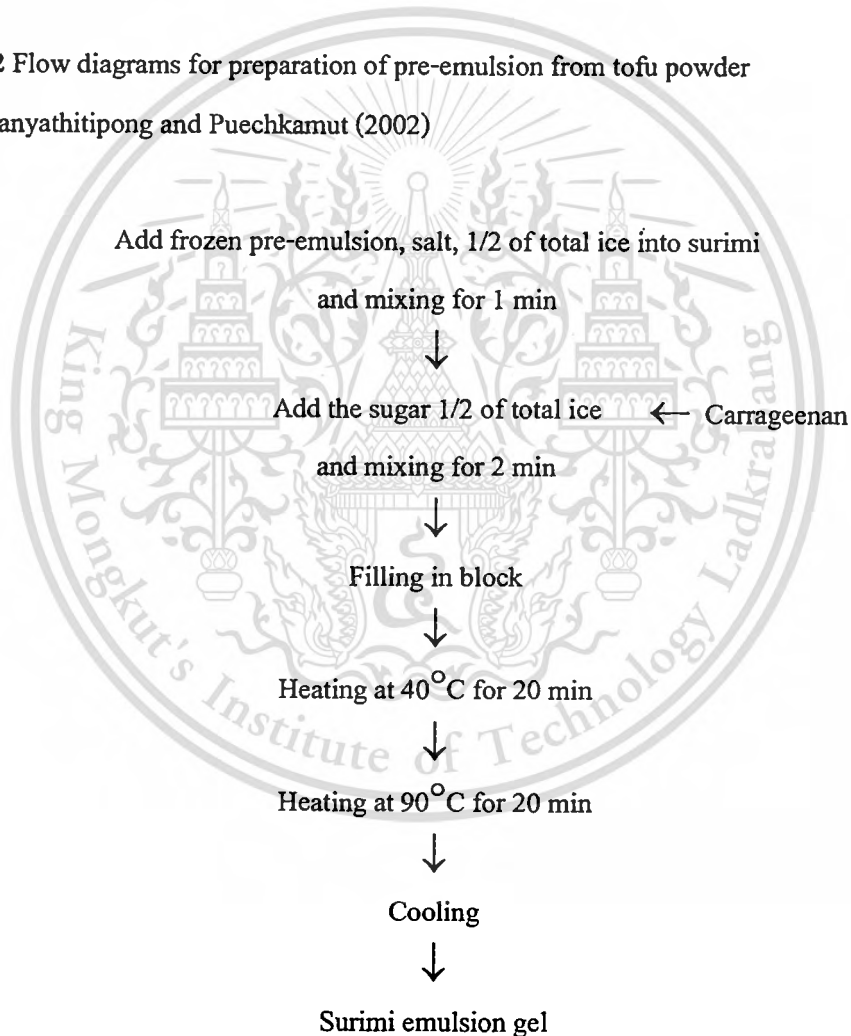
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**Figure 3.2** Flow diagrams for preparation of pre-emulsion from tofu powder

**Source:** Panyathitipong and Puechkamut (2002)



**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

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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 ( $\beta_n$ ) is as follows:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{12}x_1x_2$$

Where  $\beta_0$  is the value of the fixed response at the central point of the experiment that is the point (0,0);  $\beta_1$  and  $\beta_2$  are the linear,  $\beta_{11}$  and  $\beta_{22}$  are the quadratic and  $\beta_{12}$  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

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### 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

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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.



## CHAPTER 4

# RESULTS AND DISCUSSIONS

### 4.1 Effect of soybean varieties, coagulants and drying methods of tofu powder production

#### 4.1.1 Effect of soybean varieties

Soybean varieties affected the chemical and physical properties of soybean, soymilk and tofu. They have significant effect on the protein content of soybean. The chemical compositions of three soybean varieties are shown in Table 4.1. The protein content of SJ 5 was higher than others and the fat content of the CM 60 was highest ( $p < 0.05$ ). The protein content was negatively correlated with fat ( $r = -0.97$ ;  $p < 0.01$ ). This result agrees with Sun and Breene (1991), Wilcox and Shibles (2001) and Poysa and Woodrow (2002). The effect of soybean varieties on color and solid content of soy milk is shown in Table 4.2. The solid content of the milk from CM 60 was highest (Table 4.2). The variation of solid content of soymilk could be due to the different moisture content of soybean and extractability of some of the components (Lim et al., 1990). The solid content of soymilk was positive correlated with protein content of soybean ( $r = 0.93$ ;  $p < 0.01$ ).

**Table 4.1** Chemical composition of three soybean varieties

Varieties	Protein (%)	Fat (%)	Carbohydrate (%)	Moisture (%)
CM 60	34.27 <sup>c</sup> ±0.15	17.02 <sup>a</sup> ±0.21	34.67 <sup>a</sup> ±0.16	14.04 <sup>a</sup> ±0.05
SJ 5	37.83 <sup>a</sup> ±0.15	14.70 <sup>c</sup> ±0.12	33.82 <sup>b</sup> ±0.24	13.65 <sup>b</sup> ±0.02
ST 2	36.68 <sup>b</sup> ±0.09	15.80 <sup>b</sup> ±0.19	33.57 <sup>c</sup> ±0.21	13.95 <sup>a</sup> ±0.04

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$

CM 60 seeds were largest and lighter hilum than SJ5 and ST2; therefore, the soymilk had the highest L-value. There was a difference in soymilk color because of seed coat hilum (Khatib et al., 2002). The hilum color of soybean seed was due to the distribution of pigment in seed coat which could migrate to soy milk components during grinding (Khatib et al., 2002). However, the color of soy milk did not affect the color of tofu powder. After tofu from three soybean varieties were dried, the pale yellow color of tofu powder.

**Table 4.2** Color and solid content of soy milk prepared from three soybean varieties

Varieties	Color			Solid content (% w/w)
	L	a	b	
CM 60	88.03 <sup>a</sup> ±0.44	-2.01 <sup>b</sup> ±0.02	13.47 <sup>a</sup> ±0.35	10.20 <sup>a</sup> ±0.28
SJ 5	86.88 <sup>b</sup> ±0.49	-1.56 <sup>a</sup> ±0.25	12.77 <sup>b</sup> ±0.29	9.45 <sup>c</sup> ±0.23
ST 2	87.91 <sup>a</sup> ±0.41	-2.27 <sup>c</sup> ±0.13	13.50 <sup>a</sup> ±0.25	9.59 <sup>b</sup> ±0.03

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$

The effect of soybean varieties on the chemical composition of tofu powder is shown in Table 4.3. Tofu is curd precipitated from soy milk followed by pressing to remove whey. Most of the carbohydrate would be removed out with whey. Therefore, the main composition of tofu powder was protein. The soybeans varieties showed a significant effect on the protein content of tofu powder (Table 4.3). There was a significant correlation between protein content of soybean and protein content of tofu powder ( $r = 0.89$ ;  $p < 0.01$ ), this agrees with Lim et al. (1990) and Min et al. (2005) who reported a positive correlation between the protein content of soybean and protein content of tofu.

**Table 4.3** Chemical composition of tofu powder from three soybean varieties

Varieties	Protein (%)	Fat (%)	Moisture (%)
CM 60	56.15 <sup>c</sup> ±0.53	29.51 <sup>a</sup> ±1.91	5.95 <sup>a</sup> ±0.38
SJ 5	60.34 <sup>a</sup> ±0.75	28.60 <sup>a</sup> ±1.33	5.87 <sup>a</sup> ±0.42
ST 2	58.40 <sup>b</sup> ±1.24	30.58 <sup>a</sup> ±2.42	5.83 <sup>a</sup> ±0.28

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$

The effect of soybean varieties on functional properties and yield of tofu powder is shown in Table 4.5. The functional properties of commercial soy protein isolate (SPI) were also compared. Its solubility, emulsion activity and emulsion stability were 7.856, 1.744 and 0.778 respectively. The solubility and emulsion stability of all tofu powders were significant lower than SPI. However, emulsion activity of tofu powder is comparable to that of SPI. The emulsion activity is important functionality of SPI for industrial use. Within the three types of the tofu powder, the tofu powder which was obtained from CM 60 had the highest yield and good functional properties. Therefore, CM 60 was selected to the next experiment.

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**Table 4.4** Correlation coefficients of soybean varieties on quality of soy milk and tofu powder

	Solid content	Protein of tofu	Protein of soy milk	L-value	Solubility	Emulsion activity	Emulsion stability	Yield	Fat content	Total soluble solid
Solid content	1									
Protein of tofu	0.118	1								
Protein of soybean	0.926**	0.892**	1							
L-color	-0.083	-0.680**	-0.582	1						
Solubility	-0.554	-0.895**	-0.972**	0.645*	1					
Emulsion activity	0.094	0.210	0.126	-0.127	0.083	1				
Emulsion stability	0.087	-0.228	-0.029	-0.26	-0.097	-0.249	1			
Yield	0.082	-0.272	-0.249	0.100	0.486	0.046	0.142	1		
Fat content	-0.942**	-0.878*	-0.971**	0.762	0.926**	-0.020	0.499	0.175	1	
Total soluble solid	-0.339	-0.440*	-0.684*	0.225	0.435	0.004	0.431*	0.186	0.633	1

\*correlation is significant at  $p < 0.05$ , \*\*correlation is significant at  $p < 0.01$

**Table 4. 5** Effect of soybean varieties on functional properties and yield of tofu powder

Varieties	Solubility <sup>A</sup>	Emulsion activity <sup>B</sup>	Emulsion stability <sup>B</sup>	Yield (%w/w)
CM 60	4.69 <sup>a</sup> ±0.16	1.59 <sup>a</sup> ±0.06	0.47 <sup>a</sup> ±0.03	42.74 <sup>a</sup> ±3.00
SJ 5	2.74 <sup>c</sup> ±0.14	1.62 <sup>a</sup> ±0.05	0.44 <sup>ab</sup> ±0.04	41.04 <sup>ab</sup> ±2.71
ST 2	3.41 <sup>b</sup> ±0.26	1.62 <sup>a</sup> ±0.11	0.41 <sup>b</sup> ±0.02	39.94 <sup>b</sup> ±1.12

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$ , <sup>A</sup> absorbance at 260 nm ( $OD_{260} \times \text{dilution factor}$ ) and <sup>B</sup> absorbance at 500 nm ( $OD_{500} \times \text{dilution factor}$ )

#### 4.1.2 Effect of coagulants

Coagulation of soymilk is the most important step in tofu processing because coagulation conditions influence tofu yield and quality (Cai and Chang, 1998). The coagulation occurs due to the cross-linking of protein molecules in soymilk with the divalent cations. In this experiment, four types of coagulants that were frequently used in the industry were studied (Table 4.6).  $\text{CaCl}_2$  3.0% and  $\text{MgCl}_2$  3.0% were usually used for firm tofu with fully removed whey and  $\text{MgSO}_4$  2.2% and  $\text{CaSO}_4$  2.2% were normally used for hard tofu with partially removed whey (Liu, 1997).  $\text{CaCl}_2$  and  $\text{MgCl}_2$  were quick in coagulating than  $\text{CaSO}_4$  and  $\text{MgSO}_4$ . The result was similar to Prabhakaran et al. (2006) who reported that the chloride salts were found to be rapid in its action of coagulation the soy protein. The variation in texture and moisture content of tofu with different coagulants is due to the differences in gel network influenced by different anion and its ionic strengths towards the water holding capacity of soy protein gels. Tofu with high moisture content appeared soft and smooth while low moisture content appeared coarse and firm.

The tofu powder which was obtained from  $\text{CaCl}_2$  and  $\text{CaSO}_4$  had higher solubility than the other coagulants (Table 4.6). The aggregation and network structure formed by the calcium ions were not dense and had more space to trap water in the gel network (Sun and Breene, 1991). This would cause the more porous in the tofu powder with enhanced their solubility. However, the emulsion activity properties of tofu powders obtained from  $\text{MgCl}_2$  and  $\text{MgSO}_4$  were slightly better than the other coagulants. Since the magnesium ion has a high electro-negativity, it has a great power to attract with the polar proteins. Therefore, the non-polar residues are more flexible to bond with oil and this would effect the emulsion properties (Liu, 1997). Moreover, the emulsion stability of the tofu powder obtained from  $\text{MgSO}_4$  was significant higher than the other coagulants.  $\text{MgSO}_4$  gave the good emulsion activity and stability it was selected to the next experiment.

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**Table 4.6** Effect of the type of coagulants on function properties and yield tofu powder

Coagulants	Solubility <sup>A</sup>	Emulsion activity <sup>B</sup>	Emulsion stability <sup>B</sup>	Yield (% w/w)
3.0% CaCl <sub>2</sub>	4.42 <sup>a</sup> ±0.14	1.58 <sup>ab</sup> ±0.13	0.40 <sup>b</sup> ±0.02	43.05 <sup>a</sup> ±0.69
2.2% CaSO <sub>4</sub>	4.17 <sup>a</sup> ±0.14	1.41 <sup>b</sup> ±0.10	0.39 <sup>c</sup> ±0.04	41.67 <sup>ab</sup> ±0.55
3.0% MgCl <sub>2</sub>	3.74 <sup>b</sup> ±0.44	1.70 <sup>a</sup> ±0.03	0.30 <sup>d</sup> ±0.02	41.96 <sup>ab</sup> ±0.16
2.2% MgSO <sub>4</sub>	3.68 <sup>b</sup> ±0.56	1.62 <sup>ab</sup> ±0.11	0.49 <sup>a</sup> ±0.04	40.31 <sup>b</sup> ±0.16

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$ , <sup>A</sup>absorbance at 260 nm ( $OD_{260} \times$  dilution factor) and <sup>B</sup>absorbance at 500 nm ( $OD_{500} \times$  dilution factor)

#### 4.1.3 Effect of drying methods

Table 4.7 shows the effect of drying methods on functional properties and moisture content of tofu powder. The moisture content of the tofu powder obtained from a freeze dryer was the lowest. Moreover, the tofu powder which was obtained from a freeze dryer showed high quality in solubility and emulsion activity. At high temperatures especially with a drum dryer, the tofu powder gave low solubility and low emulsion activity. Heat treatment has the effect of causing denaturation of protein which might lead to the destruction of the functional properties of tofu powder. However, the emulsion stability of tofu powder obtained from a freeze dryer was the lowest compared to the other drying methods. The destructive of globular conformation by heat treatment may lead to the strong film formation around oil droplet then enhance the emulsion stability (Zayas, 1997).

**Table 4.7** Effect of drying methods on functional properties and moisture content of tofu powder

Drying methods	Solubility <sup>A</sup>	Emulsion activity <sup>B</sup>	Emulsion stability <sup>B</sup>	Moisture (%)
Tray drying	3.56 <sup>b</sup> ±0.16	1.70 <sup>a</sup> ±0.12	0.40 <sup>b</sup> ±0.02	3.43 <sup>b</sup> ±0.41
Drum drying	3.15 <sup>c</sup> ±0.03	1.46 <sup>b</sup> ±0.14	0.58 <sup>a</sup> ±0.05	4.07 <sup>a</sup> ±0.50
Freeze drying	3.87 <sup>a</sup> ±0.07	1.65 <sup>a</sup> ±0.12	0.28 <sup>c</sup> ±0.01	0.68 <sup>c</sup> ±0.02

<sup>a-c</sup>Different letters within same column are significantly different at  $p < 0.05$ , <sup>A</sup>absorbance at 260 nm ( $OD_{260} \times$  dilution factor) and <sup>B</sup>absorbance at 500 nm ( $OD_{500} \times$  dilution factor)

## 4.2 Physicochemical properties of the emulsion gels prepared from tofu powder and surimi

### 4.2.1 Effect of tofu powder on functionality of surimi emulsion gel

The functionality of the emulsion gel formulated with different concentration of tofu powder as pre-emulsion to replace pork is shown in Table 4.8. It showed that increasing the tofu powder cause an increase in water holding capacity (WHC) ( $p < 0.05$ ) and the correlation coefficient between the variation of tofu powder and WHC was 0.92 ( $p < 0.01$ ). Replacement of meat protein with soy protein increased WHC by reducing expressible water. The result obtained agreed with Lecomte et al., (1993) and Chin et al. (1999) who reported that the replacement soy protein in meat emulsion product increased WHC and decreased expressible moisture value. Thus, soy protein absorbed water immediately and the hydrophobic of soy protein were exposed and interacted with meat protein leading to the precipitation of protein aggregates with allowed to retain amount of water prior to form a heat stable gel (Chin et al., 1998).

**Table 4.8** Effect of tofu powder on functionality of surimi emulsion gels

Tofu powder (%)	Water holding	Emulsion stability	
	capacity (%)	TEF (%)	Fat loss (%)
Control (0)	90.90 <sup>c</sup> ±8.10	1.12 <sup>a</sup> ±0.03	1.18 <sup>a</sup> ±0.02
20	92.01 <sup>b</sup> ±5.80	1.16 <sup>a</sup> ±0.02	1.06 <sup>b</sup> ±0.05
40	92.38 <sup>b</sup> ±5.02	0.97 <sup>b</sup> ±0.07	0.97 <sup>c</sup> ±0.01
60	93.40 <sup>a</sup> ±7.11	0.86 <sup>c</sup> ±0.07	0.65 <sup>d</sup> ±0.02
80	93.68 <sup>a</sup> ±1.50	0.69 <sup>d</sup> ±0.05	0.33 <sup>e</sup> ±0.03

<sup>a-e</sup>Different letters within same column are significantly different ( $p < 0.05$ )

The emulsion stability of surimi gel formulated with various percentage of tofu powder were significant difference ( $p < 0.05$ ) (Table 4.8). The fat loss of the control was significantly ( $p < 0.05$ ) higher than other treatment, indicating that protein participated in some fat emulsification. The result was similar with Barbut (2006) who reported adding non meat protein decreased fat loss in chicken meat batters. The control and 20% tofu powder addition treatment resulted in high total expressible fluid (TEF) thus decreasing the stability of the emulsion. The TEF of the surimi emulsion decreased when increased tofu powder due to the WHC and moisture absorption of tofu powder. The WHC is dependent on both fat and moisture content which very important to maintain emulsion stability of meat emulsion product (Choi et al., 2009). Many

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proteins are used as emulsifier due to their hydrophilic and hydrophobic side chains. The characteristics of these proteins may be attributed to their particular properties which influence their adsorption capacity at the oil and water interface (Ayadi et al., 2009).

#### **4.2.2 Effect of tofu powder on texture of surimi emulsion gels**

Soy protein is often incorporated in meat product to improve their processing and final product properties, especially the textural characteristics. The effect of tofu powder incorporated in surimi on the textural properties of the emulsion gel is shown in Table 4.9. The control had the lowest hardness ( $p < 0.05$ ) and the hardness of the emulsion gel gradually increased with increasing tofu powder addition. The results indicated the inclusion of tofu powder increased the hardness over that of the control through moisture retention and increased the structural stability of the gel matrix (Chin et al., 1998), the results are similar to Hung and Zayas (1992), Su et al. (2000) and Barbut (2006) who reported the addition non meat proteins in meat emulsion products were significantly increased hardness. The mixture of meat protein and soy protein are heated making up a multiple gel complex (Chin et al., 1998) allowing the protein to interact with meat protein (Feng and Xiong, 2002; Das et al., 2008) and produced a second gel structure that acts as a filler for the primary meat protein gel network to stabilize meat emulsion system (Drakos et al., 2007) and might lead to the strengthening of structure. The hardness of surimi emulsion system was enhanced by the presence of tofu powder indicating the tofu powder was involved in the development of the multicomponent emulsion gel structure during processing. Addition of tofu powder did not significantly affect springiness. This result was similar to Shand (2000) who reported that the springiness value for the treatment with soy protein concentrate in low fat meat batters were similar to the control, indicating that addition of soy protein concentrate had minor effects on springiness value.

**Table 4.9** Effect of tofu powder on texture of surimi emulsion gel

Tofu powder (%)	Hardness (g.force)	Springiness
Control (0)	2274.44 <sup>e</sup> ±143.96	0.925 <sup>a</sup> ±0.05
20	3675.23 <sup>d</sup> ±219.81	0.932 <sup>a</sup> ±0.01
40	4908.85 <sup>c</sup> ±441.63	0.906 <sup>a</sup> ±0.06
60	6350.90 <sup>b</sup> ±393.79	0.901 <sup>a</sup> ±0.04
80	7100.82 <sup>a</sup> ±598.53	0.942 <sup>a</sup> ±0.02

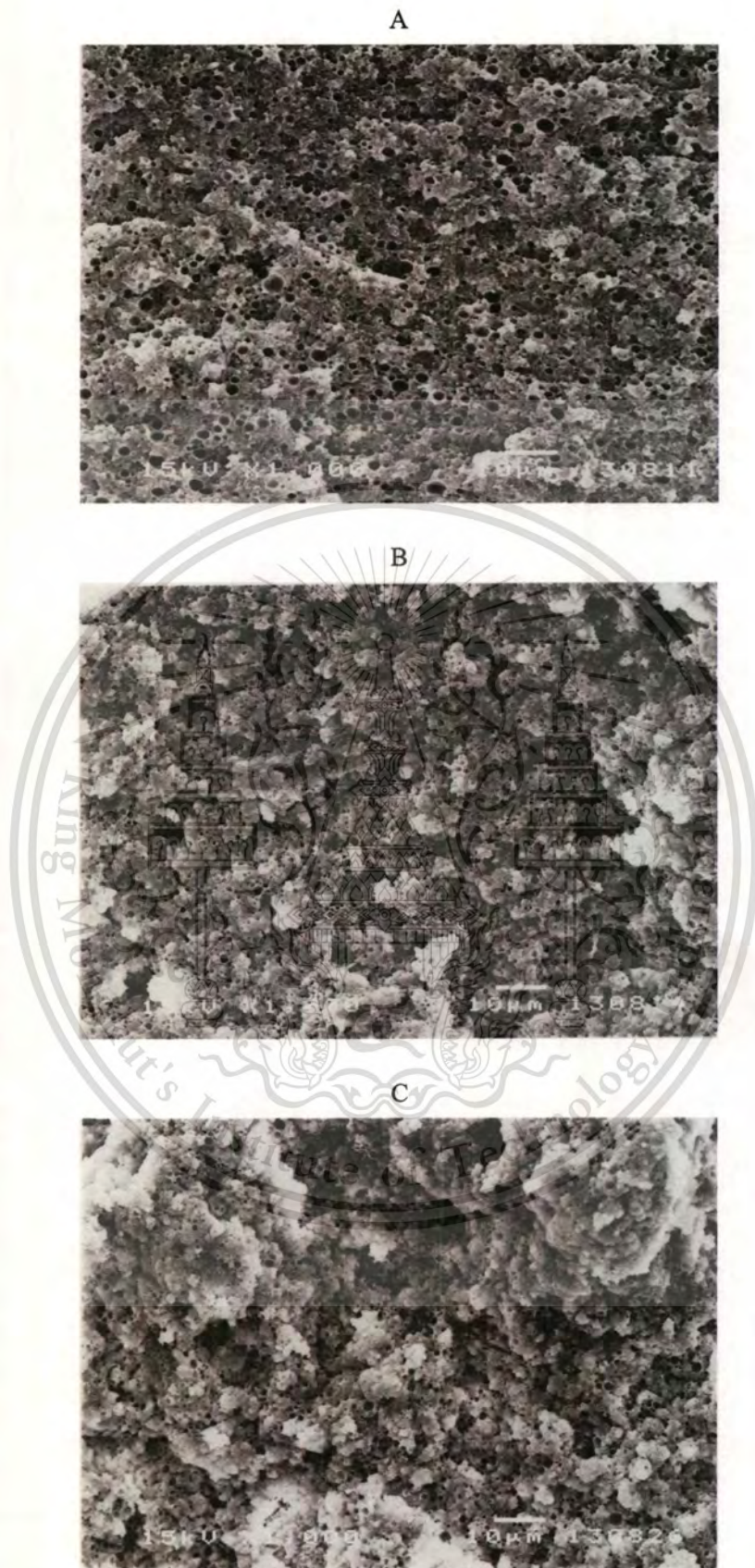
<sup>a-c</sup>Different letters within same column are significantly different (p<0.05)

#### 4.2.3 Effect of tofu powder on microstructure of surimi emulsion gel

The three-dimensional network structure of gel is an important determination texture and functional properties such as water and fat holding capacity (Chen et al., 2007). The scanning electron microscopy (SEM) was used to show differences in the three-dimensional microstructure of the surimi emulsion gel formulated with tofu powder (Figure 4.1).

The structure of meat emulsion gel showed granular aggregated structure of large open spaces within the matrix (Figure 4.1A). Increasing tofu powder concentration resulted in a more dense and rough. These microstructure support texture profile analysis (TPA) observations indicated that product with the tofu powder were more firm than the control. The micrograph of the emulsion gel (Figure 4.1B-C) showed a dense structure matrix that may cause more resistance to applied stress and great water holding capacity. These microstructural changes helped to explain functionality differences among the gels. A uniform structure with numerous small pores would probably result in more absorptive capacity and better retention of fat and water compared to structure with large pores. (Chen et al., 2007).

Replacement of meat protein with tofu powder at high level is the proposed of this research. Therefore, 80% tofu powder was selected to use for the next experiment.



**Figure 4.1** Scanning electron micrographs of surimi emulsion gel formulated with tofu powder at various concentrations (A) 0% (Control), (B) 40% and (C) 80% tofu powder.

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#### 4.2.4 Effect of carrageenan and tofu powder on functionality of surimi emulsion gel

Carrageenan is a sulphate polysaccharide extracted from red algae. It is widely used in food industry because of the water binding, thickening and gelling properties. Carrageenans at various concentrations (0.25 0.50 and 0.75 % weight of surimi) were mixed with sugar and added to the gel that replaced 80% of surimi with tofu powder. The effect of carrageenan on water holding capacity of the gels is shown in Table 4.10. The results showed that increasing the carrageenan concentration causes an increase in WHC ( $p < 0.05$ ) that is consistent with Verbeken et al. (2005) who reported that increasing carrageenan concentration in gel meat products increased gel strength and WHC. The properties between water-hydrocolloid interactions and intermolecular forces, hydrogen, hydrophobic and electrostatic bonds, lead to the formation of aggregates or three-dimensional matrix structures. These properties are related in the ability to absorb and retain large amounts of water where the network necessary for gel formation which allows to hold or retain a large amount of water (Sanchez et al., 1995).

**Table 4.10** Effect of carrageenan on functionality of the gel that replaced 80% of surimi with tofu powder

Carrageenan (%)	Water holding	Emulsion stability	
	capacity (%)	TEF (%)	Fat loss (%)
0	93.68 <sup>c</sup> ±1.10	0.69 <sup>a</sup> ±0.03	0.33 <sup>a</sup> ±0.03
0.25	93.62 <sup>c</sup> ±2.70	0.52 <sup>b</sup> ±0.04	0.30 <sup>a</sup> ±0.01
0.50	94.38 <sup>b</sup> ±1.40	0.38 <sup>c</sup> ±0.03	0.22 <sup>b</sup> ±0.01
0.75	95.47 <sup>a</sup> ±2.30	0.29 <sup>d</sup> ±0.05	0.19 <sup>b</sup> ±0.03

<sup>a-d</sup> Different letters within same column are significantly different ( $p < 0.05$ )

The stability of the surimi tofu powder emulsion were significant difference ( $p < 0.05$ ) (Table 4.10). Increasing carrageenan concentration was effective on emulsion stability. The gels without carrageenan resulted in the highest TEF and fat loss thus decreasing the stability of the emulsion. Luruena-Martinez et al. (2004) reported similar results for frankfurters made with the addition of locust bean gum and xanthan gum. Hughes et al. (1997) and Candogan and Kolsarici (2003) also reported the increased emulsion stability in frankfurters formulated with carrageenan incorporation. Protein-hydrocolloid interactions also play a significant role in the structure and stability of many processed food. They can form hybrid complexes which enhanced functional

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properties of the gel in comparison with protein or hydrocolloid alone. Electrostatic complexation of oppositely charged protein and hydrocolloid allows the better anchoring of the newly formed macromolecular amphiphile onto the oil-water interface (Andres et al., 2006).

**Table 4.11** Effect of carrageenan on texture of the gel that replaced 80% of surimi emulsion with tofu powder

Carrageenan (%)	Hardness (g.force)	Springiness
0	7113.56 <sup>c</sup> ±537.00	0.940 <sup>a</sup> ±0.016
0.25	8298.01 <sup>b</sup> ±414.41	0.908 <sup>b</sup> ±0.022
0.50	8313.36 <sup>b</sup> ±350.80	0.915 <sup>b</sup> ±0.025
0.75	8841.38 <sup>a</sup> ±414.75	0.907 <sup>b</sup> ±0.016

<sup>a-c</sup>Different letters within same column are significantly different ( $p < 0.05$ )

#### 4.2.5 Effect of carrageenan and tofu powder on texture and microstructure of surimi emulsion gel

The emulsion gels prepared from replacing 80% surimi with tofu powder and carrageenan at various concentrations were evaluated for their hardness and springiness (Table 4.11). When carrageenan was added, the increase in the emulsion gels hardness and the decrease in springiness were observed. This result is correspond with Ruusunen et al. (2003), Garcia-Garcia and Totosaus (2007) and Ayadi et al. (2009) who reported that addition of carrageenan lead to increase the hardness and low the fat content in sausages products. The textural changes could be explained in term of the influence of the presence of carrageenan on the gelling process of protein. Carrageenan protein interactions lead to compact network with resulting in the hard gel (Figure 4.2). The structure of the gel at 0% carrageenan showed gel network that looked dense and rough (Figure 4.2A). Increasing carrageenan concentration resulted in a more smooth gel matrix and increased the compactness of protein gel network which less aerated structure that might cause springiness reduction (Figure 4.2B-D). Moreover, a significant increase in the gel hardness that observed at high levels of carrageenan could be the result of additional carrageenan gel network formation (Ayadi et al., 2009). The evolution of microstructure could explain not only texture property but also the change in the WHC. The compactness of protein gel network allows more binding of water, therefore, increase carrageenan concentration the WHC was increased (Ayadi et al., 2009).

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A



**Figure 4.2** Scanning electron micrographs of surimi emulsion gel formulated with 80% tofu powder and carrageenan at various concentrations (A) 0%, (B) 0.25%, (C) 0.5% and (D) 0.75%.

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C



**Figure 4.2(CONT)** Scanning electron micrographs of surimi emulsion gel formulated with 80% tofu powder and carrageenan at various concentrations (A) 0%, (B) 0.25%, (C) 0.5% and (D) 0.75%.

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### 4.3 Development of the surimi emulsion gels from tofu powder that yield product with satisfactory sensory quality

#### 4.3.1 The effect of ingredients levels of soybean oil and carrageenan

Although, tofu powder could replace in surimi emulsion gel up to 80% but the gel has strong a tofu flavor which limited its acceptance. For consumer acceptance, therefore, the emulsion gel prepared from replacing 60% surimi with tofu powder was used in this study. (Appendix A4).

A response surface methodology (RSM) has important application in the design, development and formulation of new products, as well as in the improvement of existing product design. Moreover, the operations can be optimized, decreasing the volume of experiments, ingredients and time. In the present study the experimental design of RSM was used to determine the optimal ingredients level that are soybean oil and carrageenan concentration with two-factor central composite (face-centered) design in producing surimi tofu emulsion gel.

**Table 4.12** Central composite design arrangement and experimental result for the response variables of the emulsion gel by 9-points Hedonic scale

Trial no.	Variable levels		Responses				
	$x_1$	$x_2$	Color	Flavor	Taste	Texture	Acceptability
1	0	0	6.31	6.00	6.79	6.31	6.81
2	0	0	6.60	5.90	6.19	6.19	6.90
3	0	1	6.40	6.14	6.00	5.85	6.31
4	0	0	6.40	5.90	6.05	6.33	6.79
5	1	0	6.67	6.31	6.19	6.31	6.57
6	0	-1	6.62	6.05	6.74	5.62	6.67
7	0	0	6.69	5.86	6.29	6.31	6.88
8	1	-1	6.48	6.52	6.69	5.76	6.29
9	-1	1	6.55	5.21	6.36	5.40	5.52
10	0	0	6.71	5.95	6.86	6.21	6.90
11	0	0	6.57	6.52	6.57	6.21	6.86
12	-1	-1	6.55	5.19	6.48	5.50	5.76
13	1	1	6.36	6.31	6.64	6.21	5.92
14	-1	0	6.40	5.24	6.31	5.95	5.83

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**Table 4.13** Central composite design arrangement and experimental result for the response variables of the emulsion gel by QDA

Trial no.	Variable levels		Responses				
	$x_1$	$x_2$	Tofu Flavor	Fish Flavor	Firmness	Springiness	Juiciness
1	0	0	7.78	5.66	7.35	6.30	7.31
2	0	0	7.78	5.63	7.38	6.49	7.12
3	0	1	7.46	5.71	7.99	7.49	6.30
4	0	0	7.62	5.55	7.46	6.42	7.23
5	1	0	6.96	6.01	7.34	8.16	7.51
6	0	-1	7.56	6.07	6.38	5.30	9.23
7	0	0	7.56	5.52	7.38	6.49	7.23
8	1	-1	6.86	5.71	6.75	6.39	9.53
9	-1	1	8.83	5.34	7.56	6.36	5.47
10	0	0	7.77	5.55	7.55	6.51	7.26
11	0	0	7.68	5.58	7.41	6.54	7.34
12	-1	-1	9.22	6.42	4.65	4.65	8.38
13	1	1	6.16	4.98	7.70	9.02	6.98
14	-1	0	9.00	5.41	6.40	5.86	6.25

**Table 4.14** Central composite design arrangement and experimental result for the response variables of the emulsion gel by TPA

Trial no.	Variable levels		Responses	
	$x_1$	$x_2$	Hardness (N)	Springiness
1	0	0	6545.627	0.944
2	0	0	6416.695	0.934
3	0	1	8027.720	0.933
4	0	0	6506.545	0.940
5	1	0	6549.736	0.957
6	0	-1	5544.160	0.956
7	0	0	6524.727	0.953
8	1	-1	5986.700	0.952
9	-1	1	8643.357	0.944
10	0	0	6497.167	0.938
11	0	0	6544.932	0.938
12	-1	-1	5566.350	0.951
13	1	1	7623.106	0.941
14	-1	0	6877.540	0.945

**Table 4.15** Description and scale of the sensory attributes of the emulsion gel for QDA

Terms	Anchors		Description
	0	15	
Tofu flavor	Weak	Strong	Intensity of tofu flavor in the sample
Fish flavor	Weak	Strong	Intensity of fish flavor in the sample
Firmness	Soft	Firm	The amount of force necessary to bite completely through the sample
Springiness	Plastic	Elastic	Degree of a product return to its original shape once it has been compressed between teeth
Juiciness	Dry	Juicy	The amount of juice during chewing

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**Table 4.16** Analysis of variance (ANOVA) showing the linear, quadratic interaction and the lack of fit of the response variables

Source of variation	Df	F-value											
		Color <sup>ns</sup> (Hedonic)	Flavor (Hedonic)	Taste (Hedonic)	Texture (Hedonic)	Acceptability (Hedonic)	Tofu flavor	Fish Flavor <sup>ns</sup> (QDA)	Firmness (QDA)	Springiness (QDA)	Juiciness (QDA)	Hardness (TPA)	Springiness <sup>ns</sup> (TPA)
Regression	5	0.27	11.02**	0.35	54.50***	128.61***	76.02***	2.17	201.96***	197.11***	347.26***	862.35***	1.84
Linear													
Soybean oil	1	0.00	49.60***	0.22	68.25***	97.26***	358.87***	0.45	186.43***	483.66***	289.25***	63.14***	0.35
Carrageenan	1	0.81	0.04 <sup>ns</sup>	1.31	11.23*	32.81***	10.17*	9.53	551.60***	459.42***	1325.01***	3800.27***	5.89
Square													
Soybean oil	1	0.15	4.87 <sup>ns</sup>	0.02	3.37 <sup>ns</sup>	241.50***	8.92*	0.13	90.30***	27.99***	24.62**	44.77***	2.43
Carrageenan	1	0.04	0.20 <sup>ns</sup>	0.22	126.43***	71.89***	4.87 <sup>ns</sup>	0.47	15.41***	9.20*	118.15***	85.46***	0.00
Interaction	1	0.15	0.32 <sup>ns</sup>	0.012	15.14**	0.88 <sup>ns</sup>	1.03 <sup>ns</sup>	0.37	106.23***	13.68**	3.66 <sup>ns</sup>	228.40***	0.084
Residual	8												
Error													
Lack of fit	3	0.81	0.11 <sup>ns</sup>	0.88	1.70 <sup>ns</sup>	4.16 <sup>ns</sup>	5.35 <sup>ns</sup>	75.22***	2.85 <sup>ns</sup>	3.78 <sup>ns</sup>	2.43 <sup>ns</sup>	2227.51 <sup>ns</sup>	1.20
Pure error	5												
R <sup>2</sup> (%)	-	14.60	87.32	18.09	97.15	98.77	97.94	30.96	98.72	98.69	99.25	99.81	53.54

<sup>ns</sup> not significant, \*significant at p<0.05, \*\*significant at p<0.01, \*\*\*significant at p<0.001

The effect of two independent variables (soybean oil and carrageenan) on the response variables of surimi tofu emulsion gel by hedonic scales, quantitative descriptive analysis (QDA) and texture profile analysis (TPA) were shown in Table 4.12, 4.13 and 4.14 respectively. Description and scale of the sensory attributes for QDA is described in Table 4.15.

The independent and dependent variables were fitted to the second-order model equation and examined for the goodness of fit. The analyses of variance were performed to determine the lack of fit and the significance of the linear, quadratic and interaction effects of the independent variable on the dependent variable (Table 4.16). The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression. Coefficient of determination or  $R^2$  is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for fit model.  $R^2$  for good model for sensory should be at least 70% (Granato et al., 2010). The results showed the model for the response variables which are flavor, texture, acceptability, tofu flavor, firmness, springiness juiciness from sensory test and hardness from TPA were high adequate because they have high satisfactory levels of  $R^2$  of more than 80% and which there is no significant lack of fit in which response variables.

Estimated regression coefficients of the fitted second-order polynomial for those response variables were shown in Table 4.17. Moreover, response surface plots from these fitted equations for the response variables from sensory evaluation and TPA were also done (Figure 4.3-4.5).

**Table 4.17** Estimated regression coefficients of the fitted second-order polynomial for the response variables

Response variables	Coefficients					
	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_{11}$	$\beta_{22}$	$\beta_{12}$
Flavor (Hedonic)	5.19054	0.19543***	-0.13480ns	-0.00738ns	0.21647 <sup>ns</sup>	-0.01917 <sup>ns</sup>
Texture (Hedonic)	5.50015	0.04249***	1.80657*	-0.00214ns	-1.88824***	0.04583**
Acceptability (Hedonic)	5.71642	0.26455***	1.13461***	-0.01773***	-1.39294***	-0.01083 <sup>ns</sup>
Tofu flavor (QDA)	9.07093	-0.27357***	0.55716*	0.00751*	-0.79882 <sup>ns</sup>	-0.02583 <sup>ns</sup>
Firmness (QDA)	4.72775	0.34892***	3.69039***	-0.01491***	-0.88706**	-0.16333***
Springiness (QDA)	4.69029	0.01748***	2.61314***	0.01086***	-0.89647*	0.07667**
Juiciness (QDA)	8.39088	0.18634***	-5.40725***	-0.00770**	2.43059***	0.03000 <sup>ns</sup>
Hardness (TPA)	5556.68840	-28.8957***	2072.26974***	5.26260***	1047.02176***	-120.05008***

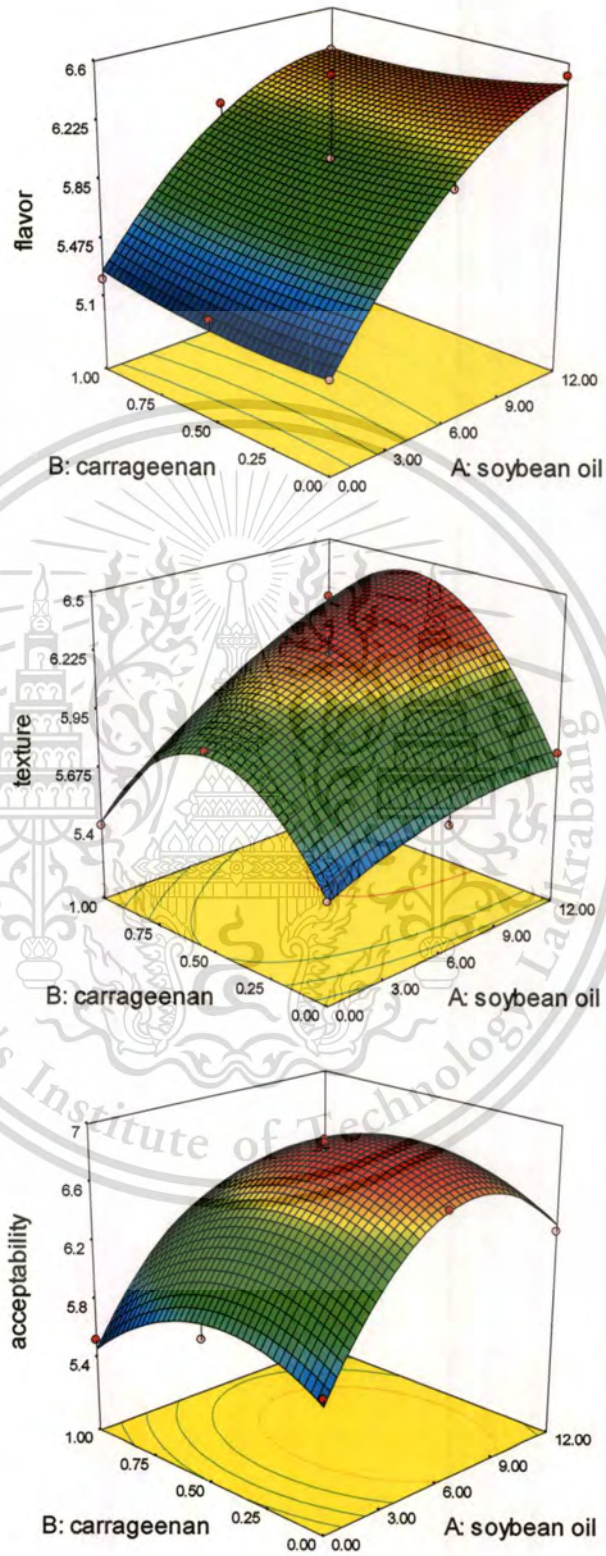
<sup>ns</sup> not significant, \*significant at  $p < 0.05$ , \*\*significant at  $p < 0.01$ , \*\*\*significant at  $p < 0.001$

The role of fat and hydrocolloid are an important consideration in any formulation because it plays a major role in the texture and sensory quality of comminuted meat products (Luruena-Martinez et al., 2004). The sensory scores of the response variables which are flavor, texture, acceptability, tofu flavor, firmness, springiness and juiciness were affected by the amount of soybean oil and carrageenan added (Table 4.17 and Figure 4.3-4.5). The tofu flavor intensity was affected by the amount of soybean oil used with negative linear ( $p < 0.001$ ) as its concentration increased. The tofu flavor intensity decreases as the amount of soybean oil was increased gradually. The tofu flavor of the emulsion gel had decreased with the increased in the soybean oil which similar result which Hughes et al. (1997) who reported that addition of fat decreases the flavor intensity of the meat emulsion gel product probably by influencing the rate of release of flavor compounds but the flavor acceptable was increased. Fat level influenced the release of volatile compounds during mastication. Increasing fat content cause a general decrease in the release of hydrophobic compounds during eating (Carrapiso, 2007) by acting as a coating

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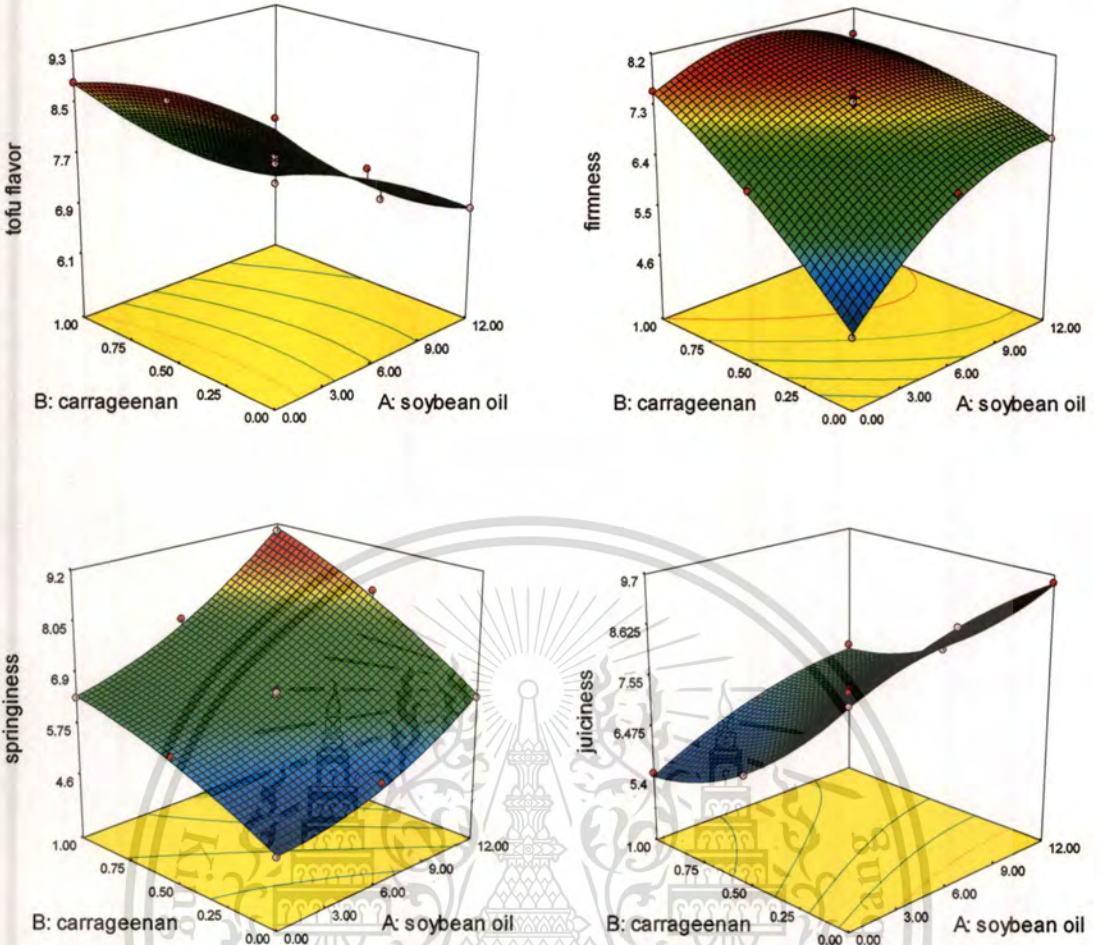
hindering flavor perception (Ventans et al., 2010) which may cause increased the sensory score in flavor acceptable.



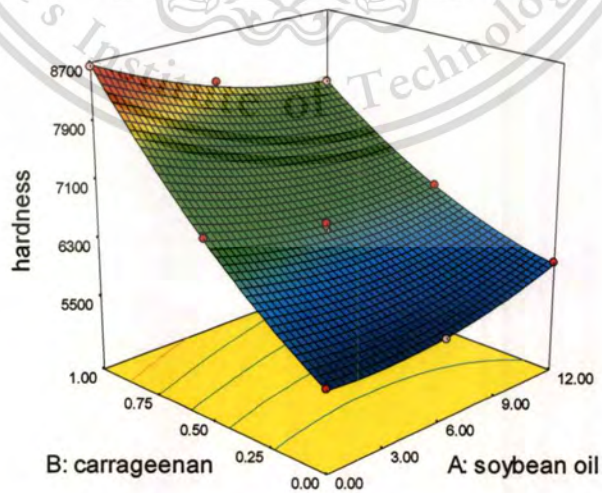
**Figure 4.3** Response surface plot of the effect of amount of soybean oil and carrageenan concentration on preference test (Hedonic scale)

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**Figure 4.4** Response surface plot of the effect of amount of soybean oil and carrageenan concentration on QDA



**Figure 4.5** Response surface plot of the effect of amount of soybean oil and carrageenan concentration on TPA

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The texture of surimi tofu emulsion varies with fat and hydrocolloids levels in formulation. The intensity of firmness by QDA significantly increased with higher carrageenan concentrations (Table 4.17). Also the hardness by TPA significantly ( $p < 0.001$ ) increased with higher carrageenan concentrations (Table 4.17). The effect of carrageenan concentration showed high positive linear ( $\beta_2 = 2072.26$ ) and quadratic ( $\beta_{22} = 1047.02$ ) on hardness. The present result was correlated with the result of Table 4.11. Carrageenan could interact with protein which leads to a compact network resulting in a hard gel (Ayadi et al., 2009).

However, the effect of soy bean oil showed negative linear on hardness of the gel by TPA (Table 4.17). The hardness of the surimi tofu emulsion gels decreased with increasing soybeans oil concentrations. Fat is bound by non-polar amino acid present in the side chains of proteins and is trapped in the gel matrix. The quantity of oil in the precipitated protein gel network which are reduced protein-protein interaction for gel matrix formation influences the hardness of surimi emulsion gels become softer (Matulis et al., 1995)

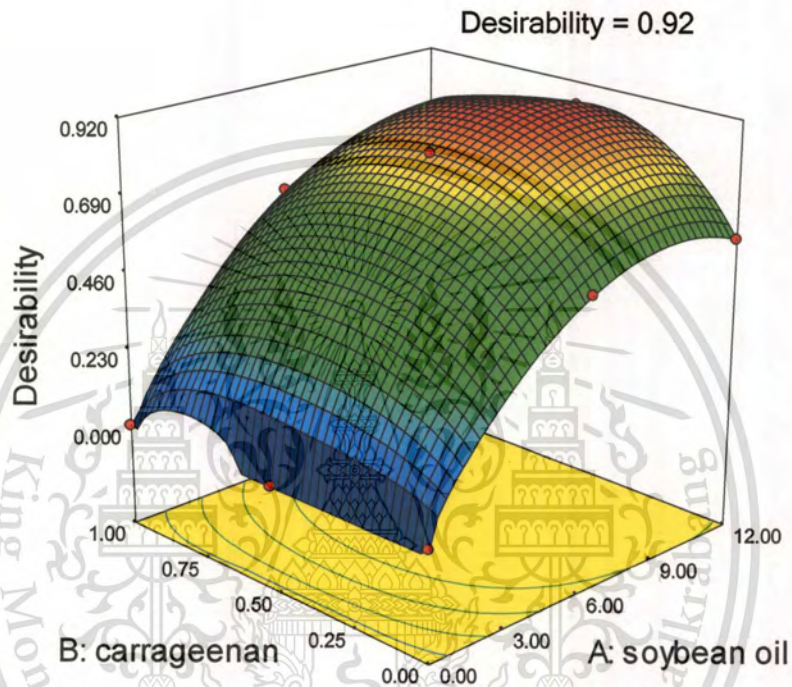
The effect of soybean oil and carrageenan did not significantly affect the gel springiness by TPA (Table 4.16). Similar results with Shand (2000) who reported that springiness values for low-fat pork bologna with carrageenan were similar to the control (0% carrageenan), indicating that addition of carrageenan concentration had minor effect on springiness.

Fat had effect on increasing juiciness intensity (Matulis et al., 1995). The juiciness intensity by QDA of the emulsion gels slightly increased with increasing soybean oil (Table 4.17). But the juiciness became decreased with increased in carrageenan concentration. This may due to the harder texture of gel when adding carrageenan leading to feeling tough during mastication for panelist.

#### 4.3.2 The optimum level of soybean oil and carrageenan to formulate the emulsion gel

Peak in RSM three-dimension plots were used to extrapolate the optimum levels of the soybean oil (0-12%) and carrageenan (0-1%). The suitability of the model equations for prediction optimum response values was tested. The optimum performance was located for the response variables which flavor, texture and acceptability. The evaluation on the optimum formulation of surimi tofu emulsion gel was presented that had a highest score accepted by panelists.

The result found that the optimum formulation of surimi tofu emulsion gel was 9.36% soybean oil and 0.44% carrageenan (desirability = 0.92) (Figure 4.6). The gel was produced and all the response variables of the final product were analyzed and compared to the predicted values using the model equation. The experimental and predicted values were not found statistically different ( $p \geq 0.05$ ) (Table 4.18). Thus, the model can be used to optimize the basic emulsion gel with tofu powder formulation.



**Figure 4.6** Desirability of the response variable optimize formulation of the surimi tofu emulsion gel

**Table 4.18** Predicted and experimental values of the responses at optimum condition

Response variable	Optimum product*	
	Predicted values	Experimental values
Flavor (Hedonic)	6.27	6.35±0.56
Texture (Hedonic)	6.33	6.21±0.76
Acceptability (Hedonic)	6.82	7.00±0.52
Tofu flavor (QDA)	7.15	7.32±0.98
Firmness (QDA)	7.47	7.55±0.47
Springiness (QDA)	7.10	7.49±0.65
Juiciness (QDA)	7.67	7.51±0.38
Hardness (TPA)	6367.37	6455.67±474.80

\*Solution: Soybean oil 9.36% and carrageenan 0.44%

Composite desirability = 0.92

#### 4.3.3 The consumer acceptability of the emulsion gel

Consumer acceptance testing is a passive measure to indicate the degree of liking of the product. The study on the acceptance of the gel prepared according to the optimum formulation was performed by consumer panel. Consumers were recruited around Rajmagala University of Technoloty Pranakhon and evaluated the gel in the attributes of appearance, taste and flavor, texture and overall acceptability by using a 9-points Hedonic scale. Demographics information for consumer acceptability is summarized in Appendix C. The ages of consumer were around 20-60 years old and 40% of consumers were 20-30 years old. The consumers about 98.70% have meat emulsion products by having it as an appetizer or an ingredient in food or by both ways. There were 72.70% consumers agreed to replace meat by tofu and 18.70% consumers were uncertain.

Consumer acceptance of the surimi tofu emulsion gel expressed as degree of liking was analyzed. Mean value of consumer degree on liking of the gel for all attributes was shown in Table 4.19. Mean degrees of liking for all attributes were 6-7. The result found that 85.30% of consumer gave degree of liking for overall acceptability greater than 5 (Table 4.20). Moreover, about more than 60% of consumers gave degree of liking for all attributes being as 7-8 (like moderately to like very much) indicated that the gel was well accepted. For all of the reason in

this experimental can elucidate that tofu powder can be a main ingredient in meat emulsion products.

**Table 4.19** Means value of consumer giving degree of liking of surimi emulsion gel

Sensory attributes	Degree of liking (1-9)
Appearance	6.83±1.31
Taste and flavor	6.06±1.47
Texture	6.46±1.66
Overall acceptability	6.90±1.55

**Table 4.20** Number of consumer giving degree of the liking for all attributes (n=150)

Degree of liking	Appearance (%)	Taste and flavor (%)	Texture (%)	Overall acceptability (%)
1	0.00	0.00	0.70	2.00
2	0.70	1.30	2.70	0.00
3	0.00	2.70	2.70	2.00
4	1.30	4.70	8.00	4.00
5	20.70	12.00	12.00	6.70
6	10.70	15.30	12.00	12.70
7	29.30	32.00	31.30	31.30
8	32.00	27.30	27.30	34.70
9	5.30	4.70	3.30	6.70

## CHAPTER 5

### CONCLUSIONS

The soybean varieties, coagulants and drying methods affected the qualities of tofu powder. The correlations between protein content of soybean and protein content of tofu powder was significant ( $p < 0.05$ ). The tofu powder which obtained from CM 60 showed good functional properties and yield. Different coagulants gave different qualities of the tofu powder.  $\text{CaCl}_2$  and  $\text{CaSO}_4$  as coagulants gave tofu powder with high solubility and  $\text{MgCl}_2$  and  $\text{MgSO}_4$  as coagulants gave tofu powder with high emulsion properties. The drying methods affected the functional properties of tofu powder. The tofu powder obtained from low heat treatment had good solubility and emulsion activity.

The influence of the addition of tofu powder and carrageenan affected the functionality textural properties and microstructure of surimi emulsion gel. The WHC, emulsion stability and hardness of the emulsion gel increased with increasing amounts of tofu powder ( $p < 0.05$ ). Microstructural observation supported the TPA and functionality results. Moreover, the addition of carrageenan caused a significant change in the gel ( $p < 0.05$ ). Increasing the carrageenan concentration resulted in greater WHC, emulsion stability and hardness. Microstructural observation showed that increasing carrageenan levels resulted in a smoother, more compact gel matrix.

The product development of the surimi tofu emulsion gel formed by replacing 60% surimi with tofu powder was determined. The response surface methodology (RSM) was used in optimization of the gel formulation. That could explain the effect of soybean oil and carrageenan concentration on response variable. The soybean oil and carrageenan concentration affected on the flavor and texture of the gel. The model equation developed could be used for predicting the quality of the emulsion gel. The formulation with desired quality could be obtained by incorporation 9.36% soybean oil and 0.44% carrageenan. Consumer acceptance testing indicated that the gel was well accepted.

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The logo of King Mongkut's Institute of Technology Ladkrabang is a circular emblem. It features a central sunburst with rays emanating from a central point. Below the sunburst are three tiered, pagoda-like structures. The entire emblem is surrounded by a decorative border with the text "King Mongkut's Institute of Technology Ladkrabang" written in a circular path.

**APPENDIX A**  
**Preparation of tofu powder, pre-emulsion and the emulsion gel**

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### A1 Tofu powder preparation (Panyathitipong and Puechkamut, 2002)

Soybean was soaked in water (soybean : water = 1:3) at 5°C for 3 hrs. The soaked beans were drained and ground in the grinder with boiled water at 80°C and 0.1% CaCO<sub>3</sub> corresponded to the water to raw bean ratio of 5:1 for extracting solids from soybean into raw soymilk. The soymilk was heated to 80°C and mixed with coagulant (2.2% MgSO<sub>4</sub>) held for 15 min to coagulate. Bean curd was centrifuged to remove whey for 5 min. Tofu was dried in a tray dryer for 5 hrs. at 70°C and ground by pin mill into powder to pass through a 0.25 mm sieve size.

### A2 Pre-emulsion and the emulsion gel preparation (Modified method from Chotipratoom, 2003)

Tofu powder was prepared to be pre-emulsion for replacing surimi in surimi emulsion gel process. Pre-emulsion was made by mixing tofu powder with water and oil. Water was added to adjust the moisture content of tofu powder to surimi and the amount of oil was equal the added oil of the formulation.

Frozen surimi was ground in commercial food processor for 1 min at low speed with salt, 50% of ice and pre-emulsion. Sugar was slowly added and the left with 50% of ice was incorporated for 2 min at high speed. The batter was stuffed in a stainless mold and heated in a temperature controlled water bath maintained at 40°C for 20 min and 90°C for 20 min. Then, samples were cooled immediately in cool water.

### A3. Formulation of surimi emulsion gel with tofu powder at various concentrations

**Table A1** The basic formulation of surimi emulsion gel with tofu powder at various concentrations

Ingredients (%w/w)	Treatment				
	0%	20%	40%	60%	80%
Surimi	60.58	44.67	31.08	19.32	9.05
Tofu powder	-	11.17	20.72	28.98	36.19
Water	-	7.82	14.50	20.28	25.33
Soybean oil	7.69	7.09	6.58	6.13	5.74
Ice	27.26	25.13	23.31	21.73	20.36
Sugar	3.03	2.79	2.59	2.41	2.26
Salt	1.44	1.33	1.23	1.15	1.07

### A4. Formulation of surimi tofu emulsion gel

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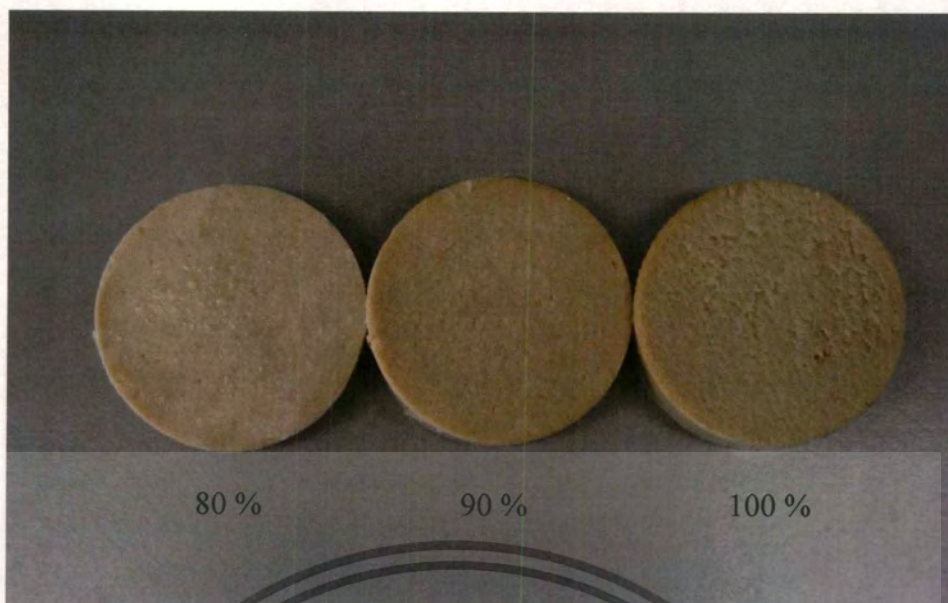
#### A4. Formulation of surimi tofu emulsion gel

**Table A2** The basic formulation of surimi tofu emulsion gel

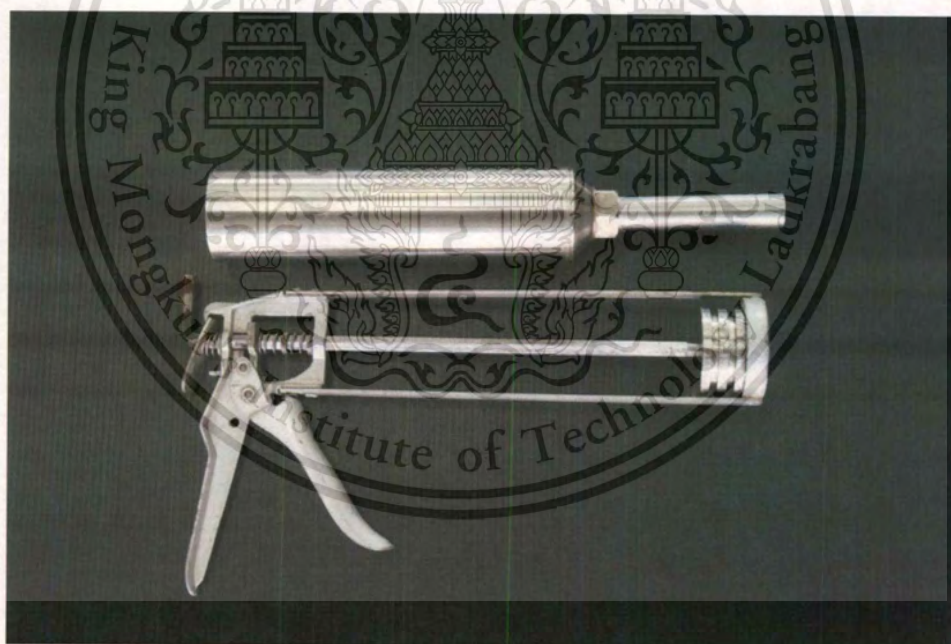
Ingredients	Content (%w/w)
Surimi	31.50
Tofu powder	21.00
Ice	23.50
Water	20.00
Sugar	2.50
Salt	1.25
Flavor enhancer	0.25



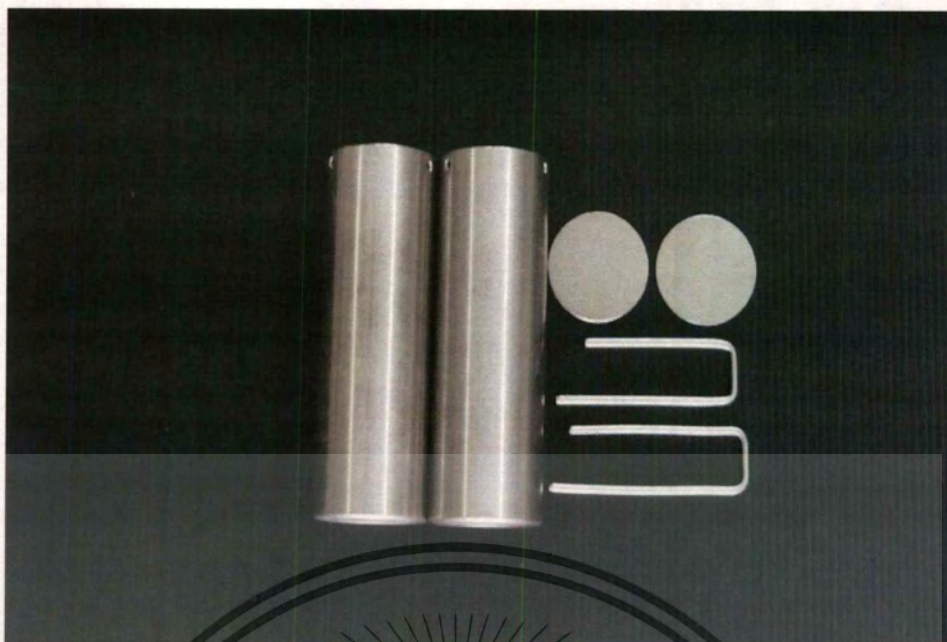
**Figure A1** The characteristic of surimi emulsion gel with tofu powder at various concentrations



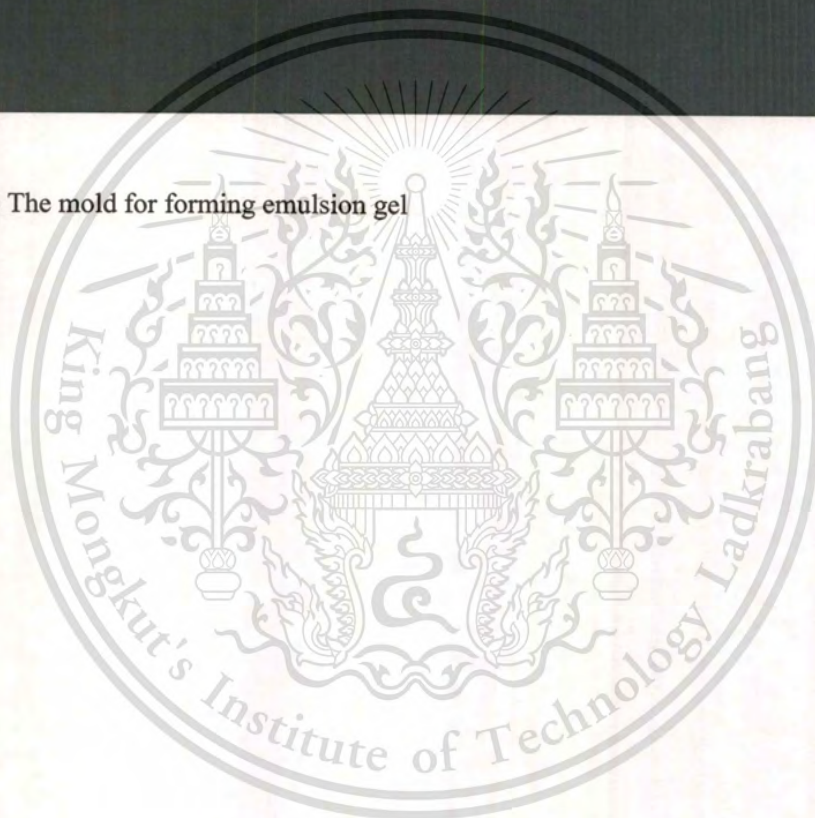
**Figure A2** Cross section of surimi emulsion gel with tofu powder at various concentrations



**Figure A3** The filler for forming emulsion gel



**Figure A4** The mold for forming emulsion gel





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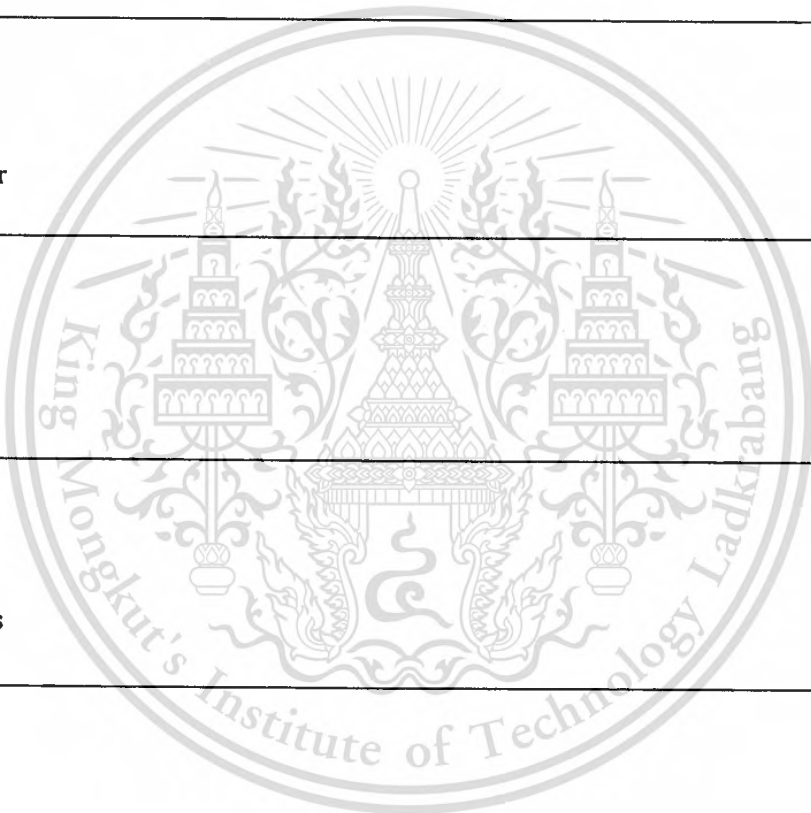
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### B1 Quantitative Descriptive Analysis

#### Descriptive analysis with QDA

Name.....Date.....

**Instruction:** Please taste the product and mark the intensity for each attribute (criteria). In between tastings, take a sip of water to clean the palate.



Tofu flavor

weak |-----| strong

Meat flavor

weak |-----| strong

Firmness

soft |-----| firm

Springiness

plastic |-----| elastic

Juiciness

dry |-----| juicy

**B2 Preference test****Preference Test Form****Product:** Surimi Emulsion Gel mixed with Tofu Powder**Instruction:** Please taste each sample and rating scale for the taste that appears on your scoring sheet. In between tastings, take a sip of water to clean the palate.

Name.....Date.....

1 = dislike extremely

2 = dislike very much

3 = dislike moderately

4 = dislike slightly

5 = neither like nor dislike

6 = like slightly

7 = like moderately

8 = like very much

9 = like extremely

Attribute	Sample label						
	.....	.....	.....	.....	.....	.....	.....
Color							
Flavor							
Taste							
Texture							
Overall acceptance							



## Part 2: Consumer behavior and consumer opinion

1. Do you eat a tofu?

- Yes  No (skip to No. 4)

2. Why do you have a tofu? (Allowing multiple responses)

- Nutrition  Healthy and benefit  
 Low cost  Reduce meat consumption  
 Delicious  Easy to buy  
 Convenient to eat  Other.....

3. How many times do you eat tofu?

- 1-2 times a week  3-4 times a week  
 more than 4 times a week  1-2 times a month  
 Other.....

4. Do you eat meat emulsion gel products?

- Yes  No (skip to No. 8)

5. How do you have meat emulsion products?

- As an appetizer  As an ingredient  
 Both an appetizer and an ingredient  
 Other.....

6. Why do you have meat emulsion products? (Allowing multiple responses)

- Nutrition  Low cost  
 Easy to buy  Many types of product  
 Delicious  Convenient to eat  
 Other.....

7. How many times do you have meat emulsion products?

- 1-2 times a week  3-4 times a week  
 more than 4 times a week  1-2 times a month  
 Other.....

8. Do you agree on replacing meat by tofu?

- Yes  No  Uncertain

### Part 3: Acceptance test of the surimi tofu emulsion gel

<u>Degree of liking</u>		<u>Score</u>
Like extremely	=	9
Like very much	=	8
Like moderately	=	7
Like slightly	=	6
Neither like nor dislike	=	5
Dislike slightly	=	4
Dislike moderately	=	3
Dislike very much	=	2
Dislike extremely	=	1

**Instruction:** Please taste sample and rating scale for the taste that appears on your scoring sheet.

In between tastings, take a sip of water to clean the palate.

1. Appearance .....
2. Taste and flavor .....
3. Texture during chewing .....
4. Overall acceptance .....



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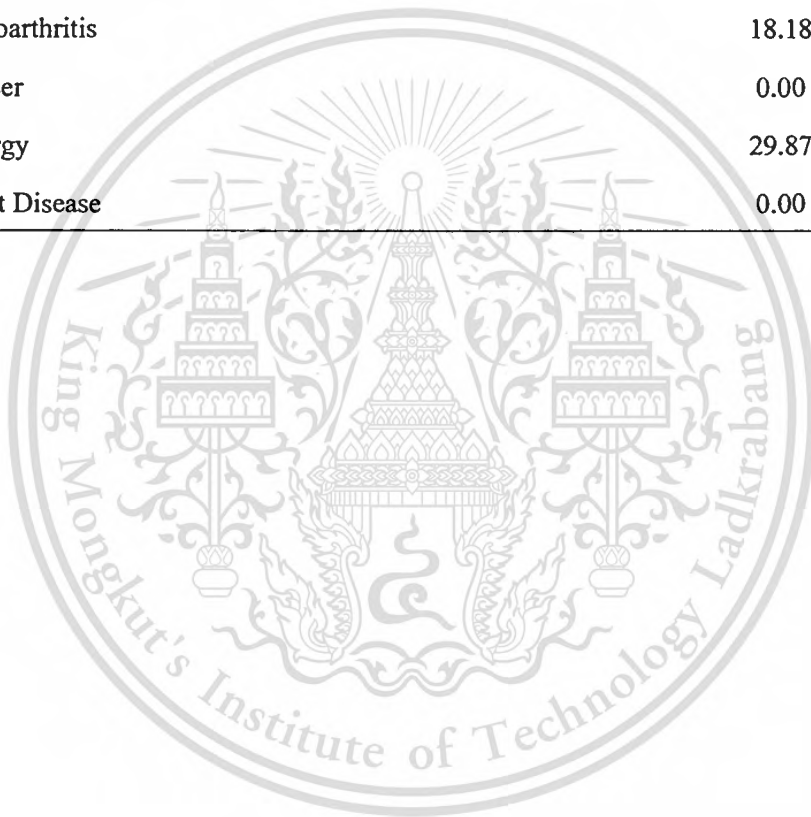
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**Table C1** General information of the consumers

Questions	Level (%)
<b>Gender</b>	
Male	50.00
Female	50.00
<b>Age</b>	
20-30 years	40.00
31-40 years	22.00
41-50 years	18.00
51-60 years	20.00
<b>Education levels</b>	
Primary school	0.00
Secondary school	0.70
High school	5.30
Diploma	4.70
Bachelor degree	68.00
Master degree	21.30
Philosophy or doctoral degree.	0.00
<b>What is your occupation?</b>	
Student	10.70
House wife	11.30
Bureau officer	41.30
State Enterprise	8.00
Employee	7.30
Entrepreneur	16.00
Labor	5.30

**Table C1 (CONT.)** General information of the consumers

Questions	Levels (%)
Do you have any annoying diseases?	
None	68.00
Have a disease	32.00
High Blood Pressure	23.38
Lipidemia	9.09
Diabetes	9.09
Obesity	10.39
Osteoarthritis	18.18
Cancer	0.00
Allergy	29.87
Heart Disease	0.00

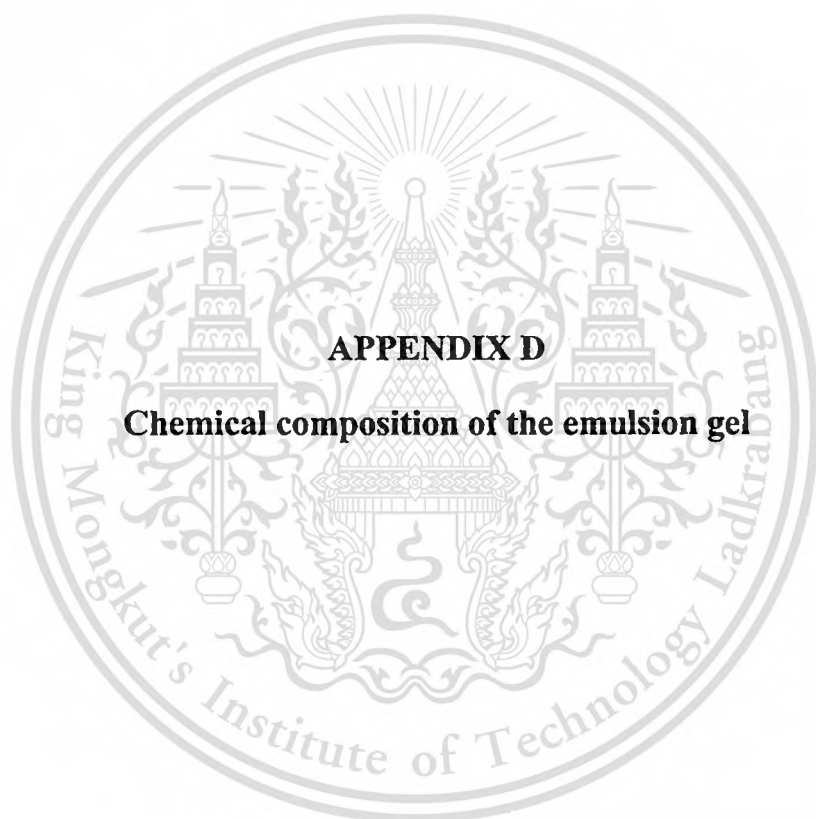


**Tabel C2 Consumer behavior and consumer opinion**

Questions	Levels (%)
<b>Do you eat tofu?</b>	
Yes	94.00
No	6.00
<b>Why do you eat tofu?</b>	
Nutrition	29.06
Healthy and benefit	23.97
Low cost	11.14
Reduce meat consumption	11.62
Delicious	12.83
Easy to buy	5.33
Convenient to eat	6.05
<b>How many times do you eat tofu?</b>	
Do not eat tofu	5.30
1-2 times a week	54.00
3-4 times a week	12.00
more than 4 times a week	2.00
1-2 times a month	26.70
<b>Do you eat meat emulsion gel products?</b>	
Yes	1.30
No	98.70
<b>How do you eat meat emulsion products?</b>	
Do not eat meat emulsion product	2.00
As an appetizer	17.30
As an ingredient	17.30
Both an appetizer and an ingredient	63.00

**Tabel C2 (CONT.) Consumer behavior and consumer opinion**

Questions	Levels (%)
<b>Why do you eat meat emulsion products?</b>	
Nutrition	30.03
Low cost	9.38
Easy to buy	26.27
Many types of product	11.53
Delicious	9.12
Convenient to eat	13.67
<b>How many times do you eat meat emulsion products?</b>	
Do not have meat emulsion product	2.00
1-2 times a week	50.00
3-4 times a week	18.00
more than 4 times a week	4.00
1-2 times a month	26.00
<b>Do you agree on replacing meat by tofu?</b>	
Yes	72.20
No	8.70
Uncertain	18.7



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**Table D1** Proximate composition of the various emulsion gels

Chemical composition	Surimi tofu emulsion gel <sup>A</sup>	Surimi-pork sausage <sup>B</sup>	Frankfurter <sup>C</sup>	Sausage <sup>D</sup>	Fish sausage <sup>F</sup>
Moisture (%)	41.51±0.05	54.03	51.26	61.41	68.64
Protein (%)	19.21±0.15	10.60	13.90	14.48	16.76
Fat (%)	26.19±0.57	16.81	29.68	23.41	5.64

<sup>A</sup>The present study,

<sup>B</sup>50% surimi in pork sausage (Muphy et al., 2004)

<sup>C</sup>3% tofu powder in frankfurter (Ho et al., 1997)

<sup>D</sup>Pork sausage (Cierach et al., 2009)

<sup>F</sup>Fish sausage (Raju et al., 2003)





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**E1. Protein content determination (Kjeldahl method) (AOAC. 2000)**

1. Weigh sample 1 g into a digestion tube
2. Add  $K_2SO_4$  anhydrous 4.5 g and  $CuSO_4 \cdot 5H_2O$  0.5 g as for a catalyst and add glass beads about 2-3 pieces
3. Add sulfuric acid 20 ml
4. Put a digestion tube on the digestion block for digesting until become clear liquid and leave for cooling
5. Add 50 ml  $H_3BO_3$  solution with indicator to graduated 500 ml Erlenmeyer titration flask and place flask under condenser tip so that is well below  $H_3BO_3$  solution surface
6. Add 75 ml 50% NaOH in digestion tube and connect to distillation bulb on condenser immediately
7. Heat until all  $NH_3$  has been distilled
8. Titrate  $H_3BO_3$  receiving solution with standard 0.1 M HCl solution to first trace of pink
9. Record ml HCl to at least nearest 0.05 ml
10. Repeat procedure the step 2-9 for making a blank, calculate results as follows:

$$\text{Nitrogen (\%)} = \frac{1.4007 \times (V_s - V_b) \times M}{W}$$

Where  $V_s$  and  $V_b$  = ml HCl titrant used for test portion and blank, respectively

M = molarity of HCl solution

W = weight of sample (g)

Multiply percent nitrogen by factor 6.25 to calculate percent protein

**E2. Fat content determination (Soxhlet extraction method) (AOAC. 2000)**

1. Dry the sample at  $102^\circ C$  for 2 hrs. to remove moisture and cool in a desiccator
2. Rinse all glassware with petroleum spirit, drain in an oven at  $102^\circ C$  for 30 min. and cool in desiccator
3. Accurately weigh 5 g of sample into the thimble
4. Insert thimble in a Soxhlet extractor
5. Put about 90 ml of petroleum spirit into the flask

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6. Heat the solvent in the flask until it boils. Adjust the heat source so that solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second
7. Continue the extraction for 2 hrs.
8. Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent
9. Place the flask in an oven at 102°C and dry the contents until a constant weight is reached
10. Cool the flask in a desiccator and weigh the flask and contents

$$\text{Crude fat (\%)} = \frac{(W_2 - W_1) \times 100}{S}$$

Where  $W_1$  = weight of empty flask (g)

$W_2$  = weight of flask and extracted fat (g)

$S$  = weight of sample (g)

### E3. Moisture content determination (AOAC. 2000)

1. Dry the empty aluminium can and lid in an oven at 105°C for 3 hrs. cool in desiccator. Weigh the empty aluminium can and lid
2. Accurately weight 3 g of sample into the aluminium can and spread the sample
3. Dry the sample in an oven at 105°C for 1-2 hr.
4. Cool in desiccators and weight the aluminium can and dried sample
5. Repeat procedure the step 3-4 until the weight is stable

$$\text{Moistent (\%)} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Where  $W_1$  = weight of sample before drying (g)

$W_2$  = weight of sample after drying (g)

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