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	$34.27^{c}\pm0.15$	$17.02^{a}\pm0.21$	$34.67^{a}\pm0.16$	$14.04^{a}\pm0.05$
SJ 5	$37.83^{a}\pm0.15$	$14.70^{c}\pm0.12$	$33.82^{b}\pm0.24$	$13.65^{b}\pm0.02$
ST 2	$36.68^{b}\pm0.09$	$15.80^{b}\pm0.19$	$33.57^{c}\pm0.21$	$13.95^{a}\pm0.04$

$^{a-c}$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
	L	a	b	(% w/w)
CM 60	88.03^{a}$\pm$0.44	-2.01^{b}$\pm$0.02	13.47^{a}$\pm$0.35	10.20^{a}$\pm$0.28
SJ 5	86.88^{b}$\pm$0.49	-1.56^{a}$\pm$0.25	12.77^{b}$\pm$0.29	9.45^{c}$\pm$0.23
ST 2	87.91^{a}$\pm$0.41	-2.27^{c}$\pm$0.13	13.50^{a}$\pm$0.25	9.59^{b}$\pm$0.03

$^{a\text{-}c}$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^{c}$\pm$0.53	29.51^{a}$\pm$1.91	5.95^{a}$\pm$0.38
SJ 5	60.34^{a}$\pm$0.75	28.60^{a}$\pm$1.33	5.87^{a}$\pm$0.42
ST 2	58.40^{b}$\pm$1.24	30.58^{a}$\pm$2.42	5.83^{a}$\pm$0.28

$^{a\text{-}c}$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.

Table 4.4 Correlation coefficients of soybean varieties on quality of soymilk and tofu powder

	Solid content	Protein of tofu	Protein of soymilk	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[A]	Emulsion activity[B]	Emulsion stability[B]	Yield (%w/w)
CM 60	$4.69^{a}\pm0.16$	$1.59^{a}\pm0.06$	$0.47^{a}\pm0.03$	$42.74^{a}\pm3.00$
SJ 5	$2.74^{c}\pm0.14$	$1.62^{a}\pm0.05$	$0.44^{ab}\pm0.04$	$41.04^{ab}\pm2.71$
ST 2	$3.41^{b}\pm0.26$	$1.62^{a}\pm0.11$	$0.41^{b}\pm0.02$	$39.94^{b}\pm1.12$

[a-c]Different letters within same column are significantly different at $p<0.05$, [A]absorbance at 260 nm (OD_{260} x dilution factor) and [B]absorbance at 500 nm (OD_{500} x 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). $CaCl_2$ 3.0% and $MgCl_2$ 3.0% were usually used for firm tofu with fully removed whey and $MgSO_4$ 2.2% and $CaSO_4$ 2.2% were normally used for hard tofu with partially removed whey (Liu, 1997). $CaCl_2$ and $MgCl_2$ were quick in coagulating than $CaSO_4$ and $MgSO_4$. 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 $CaCl_2$ and $CaSO_4$ 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 $MgCl_2$ and $MgSO_4$ 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 $MgSO_4$ was significant higher than the other coagulants. $MgSO_4$ gave the good emulsion activity and stability it was selected to the next experiment.

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

Coagulants	Solubility^A	Emulsion activity^B	Emulsion stability^B	Yield (% w/w)
3.0% $CaCl_2$	$4.42^a \pm 0.14$	$1.58^{ab} \pm 0.13$	$0.40^b \pm 0.02$	$43.05^a \pm 0.69$
2.2% $CaSO_4$	$4.17^a \pm 0.14$	$1.41^b \pm 0.10$	$0.39^c \pm 0.04$	$41.67^{ab} \pm 0.55$
3.0% $MgCl_2$	$3.74^b \pm 0.44$	$1.70^a \pm 0.03$	$0.30^d \pm 0.02$	$41.96^{ab} \pm 0.16$
2.2% $MgSO_4$	$3.68^b \pm 0.56$	$1.62^{ab} \pm 0.11$	$0.49^a \pm 0.04$	$40.31^b \pm 0.16$

$^{a-c}$Different letters within same column are significantly different at $p<0.05$, ^{A}absorbance at 260 nm (OD_{260} x dilution factor) and ^{B}absorbance at 500 nm (OD_{500} x 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^A	Emulsion activity^B	Emulsion stability^B	Moisture (%)
Tray drying	$3.56^b \pm 0.16$	$1.70^a \pm 0.12$	$0.40^b \pm 0.02$	$3.43^b \pm 0.41$
Drum drying	$3.15^c \pm 0.03$	$1.46^b \pm 0.14$	$0.58^a \pm 0.05$	$4.07^a \pm 0.50$
Freeze drying	$3.87^a \pm 0.07$	$1.65^a \pm 0.12$	$0.28^c \pm 0.01$	$0.68^c \pm 0.02$

$^{a-c}$Different letters within same column are significantly different at $p<0.05$, ^{A}absorbance at 260 nm (OD_{260} x dilution factor) and ^{B}absorbance at 500 nm (OD_{500} x 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 capacity (%)	Emulsion stability	
		TEF (%)	Fat loss (%)
Control (0)	$90.90^{c}\pm8.10$	$1.12^{a}\pm0.03$	$1.18^{a}\pm0.02$
20	$92.01^{b}\pm5.80$	$1.16^{a}\pm0.02$	$1.06^{b}\pm0.05$
40	$92.38^{b}\pm5.02$	$0.97^{b}\pm0.07$	$0.97^{c}\pm0.01$
60	$93.40^{a}\pm7.11$	$0.86^{c}\pm0.07$	$0.65^{d}\pm0.02$
80	$93.68^{a}\pm1.50$	$0.69^{d}\pm0.05$	$0.33^{e}\pm0.03$

$^{a-e}$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 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[e]±143.96	0.925[a]±0.05
20	3675.23[d]±219.81	0.932[a]±0.01
40	4908.85[c]±441.63	0.906[a]±0.06
60	6350.90[b]±393.79	0.901[a]±0.04
80	7100.82[a]±598.53	0.942[a]±0.02

[a-e]Different letters within same column are significantly different ($p<0.05$)

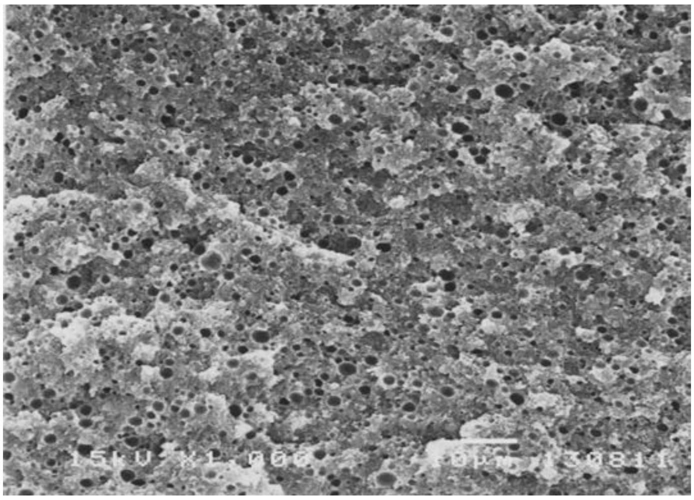
4.2.3 Effect of tofu powder on microstructure of surimi emulsion gel

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.

A



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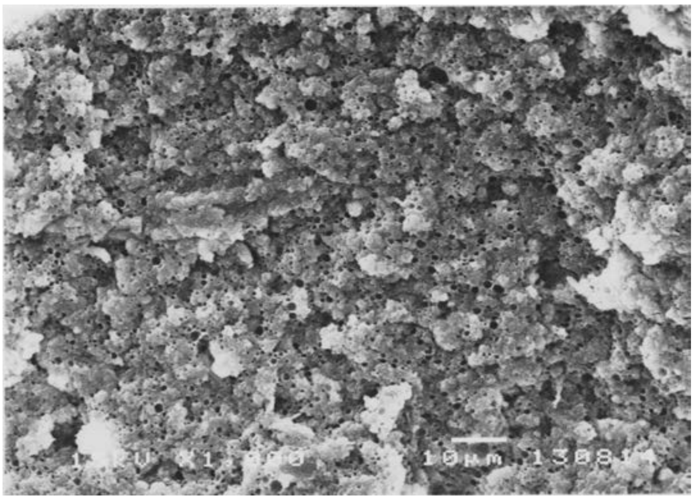

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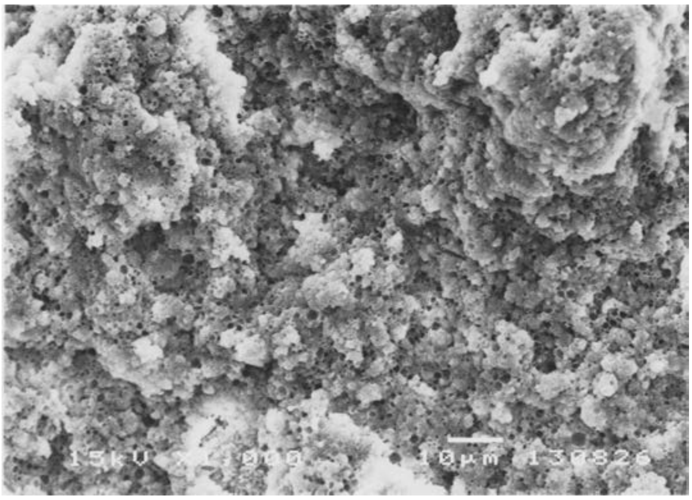


Figure 4.1 Scanning electron micrographs of surimi emulsion gel formulated with tofu powder at various concentrations (A) 0% (Control), (B) 40% and (C) 80% tofu power

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 capacity (%)	Emulsion stability	
		TEF (%)	Fat loss (%)
0	$93.68^{c}\pm1.10$	$0.69^{a}\pm0.03$	$0.33^{a}\pm0.03$
0.25	$93.62^{c}\pm2.70$	$0.52^{b}\pm0.04$	$0.30^{a}\pm0.01$
0.50	$94.38^{b}\pm1.40$	$0.38^{c}\pm0.03$	$0.22^{b}\pm0.01$
0.75	$95.47^{a}\pm2.30$	$0.29^{d}\pm0.05$	$0.19^{b}\pm0.03$

$^{a-d}$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 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

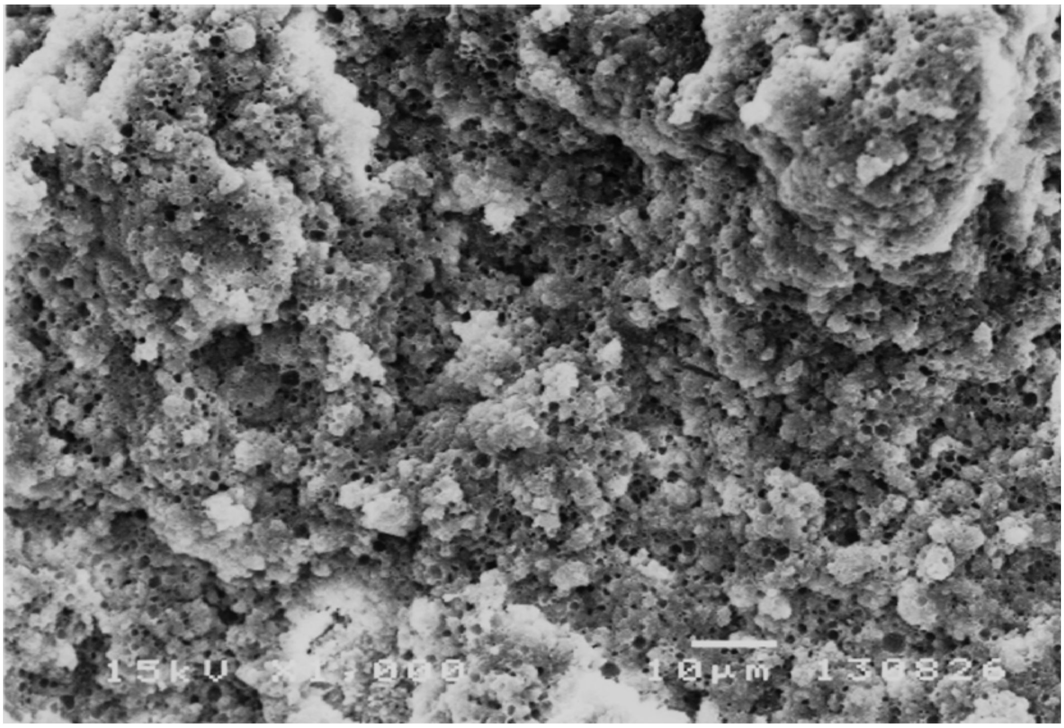
Carrageenan (%)	Hardness (g.force)	Springiness
0	7113.56$^{c}\underline{+}$537.00	0.940$^{a}\underline{+}$0.016
0.25	8298.01$^{b}\underline{+}$414.41	0.908$^{b}\underline{+}$0.022
0.50	8313.36$^{b}\underline{+}$350.80	0.915$^{b}\underline{+}$0.025
0.75	8841.38$^{a}\underline{+}$414.75	0.907$^{b}\underline{+}$0.016

$^{a-c}$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).

A



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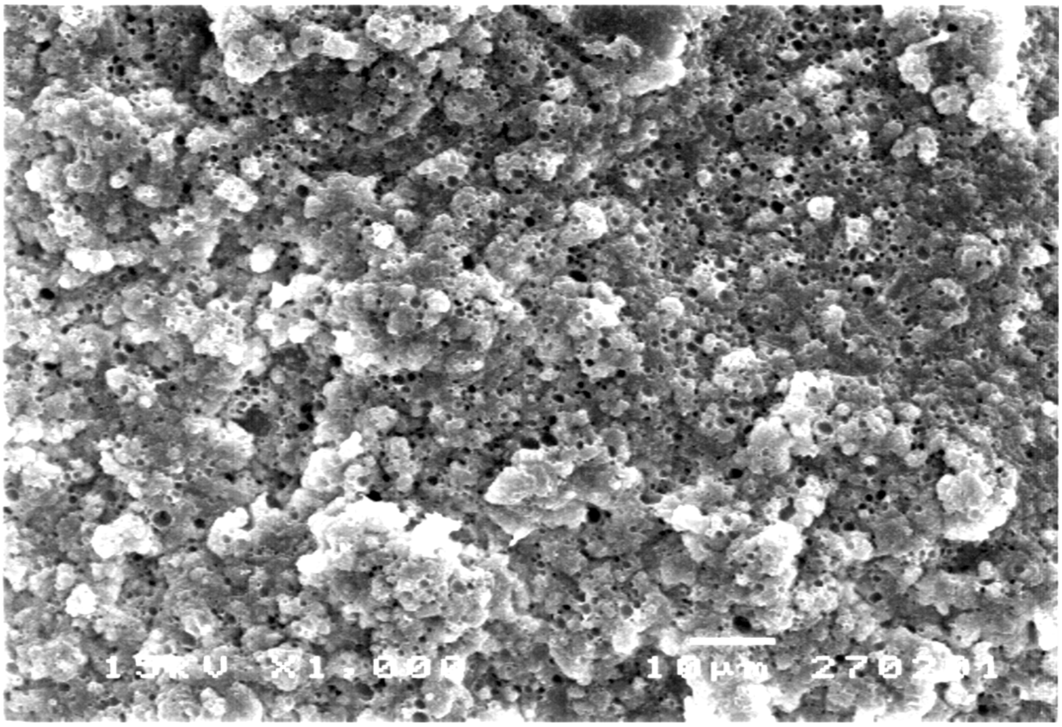

Figure 4.2 Scanning electron micrographs of surimi emulsion gel formulated with 80% tofu powder and carrageenan at various concentrations (A) 0%, (B) 0.25%, (C) 0.5% and (D) 0.75%

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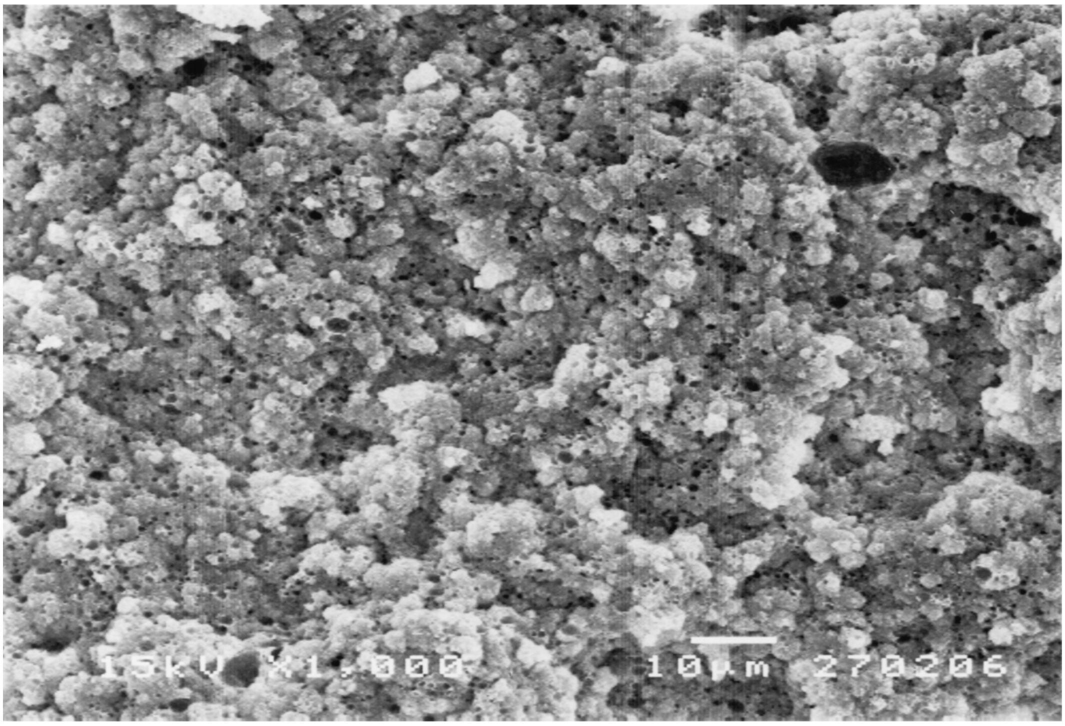

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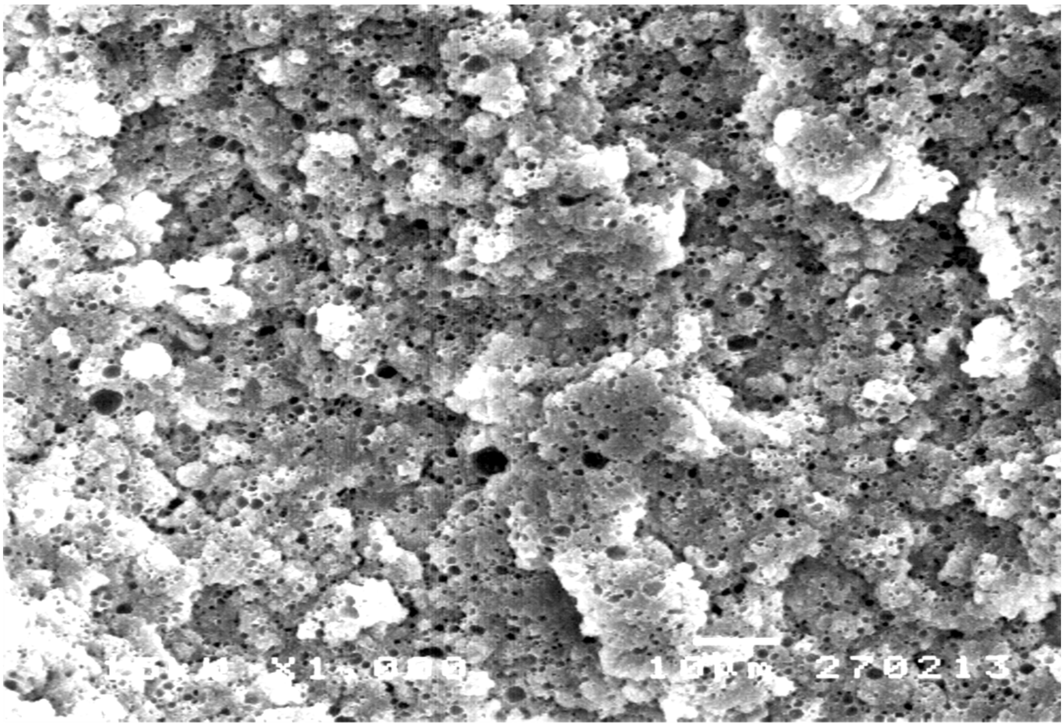

Figure 4.2(CONT.) Scanning electron micrographs of surimi emulsion gel formulated with 80% tofu powder and carrageenan at various concentrations (A) 0%, (B) 0.25%, (C) 0.5% and (D) 0.75%

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

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

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^{ns} (Hedonic)	Flavor (Hedonic)	Taste^{ns} (Hedonic)	Texture (Hedonic)	Acceptability (Hedonic)	Tofu flavor (QDA)	Fish Flavor^{ns} (QDA)	Firmness (QDA)	Springiness (QDA)	Juiciness (QDA)	Hardness (TPA)	Springiness^{ns} (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^{ns}	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^{ns}	0.02	3.37^{ns}	241.50***	8.92*	0.13	90.30***	27.99***	24.62**	44.77***	2.43
Carrageenan	1	0.04	0.20^{ns}	0.22	126.43***	71.89***	4.87^{ns}	0.47	15.41***	9.20*	118.15***	85.46***	0.00
Interaction	1	0.15	0.32^{ns}	0.012	15.14**	0.88^{ns}	1.03^{ns}	0.37	106.23***	13.68**	3.66^{ns}	228.40***	0.084
Residual	8												
Error													
Lack of fit	3	0.81	0.11^{ns}	0.88	1.70^{ns}	4.16^{ns}	5.35^{ns}	75.22***	2.85^{ns}	3.78^{ns}	2.43^{ns}	2227.51^{ns}	1.20
Pure error	5												
R^2(%)	-	14.60	87.32	18.09	97.15	98.77	97.94	30.96	98.72	98.69	99.25	99.81	53.54

^{ns}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 in 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 form 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					
	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}
Flavor (Hedonic)	5.19054	0.19543***	-0.13480ns	-0.00738ns	0.21647^{ns}	-0.01917^{ns}
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^{ns}
Tofu flavor (QDA)	9.07093	-0.27357***	0.55716*	0.00751*	-0.79882^{ns}	-0.02583^{ns}
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^{ns}
Hardness (TPA)	5556.68840	-28.8957***	2072.26974***	5.26260***	1047.02176***	-120.05008***

^{ns}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 hindering flavor perception (Ventanas et al., 2010) which may cause increased the sensory score in flavor acceptable.

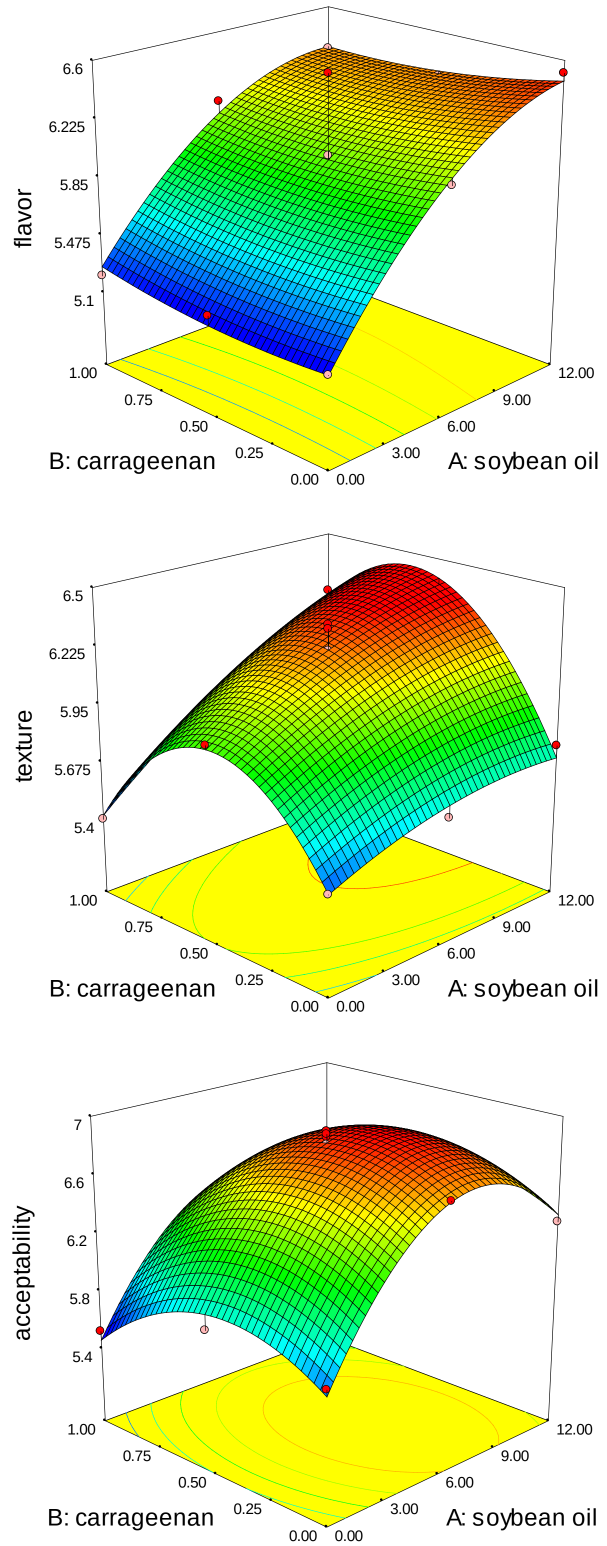

Figure 4.3 Response surface plot of the effect of amount of soybean oil and carrageenan concentration on preference test (Hedonic scale)

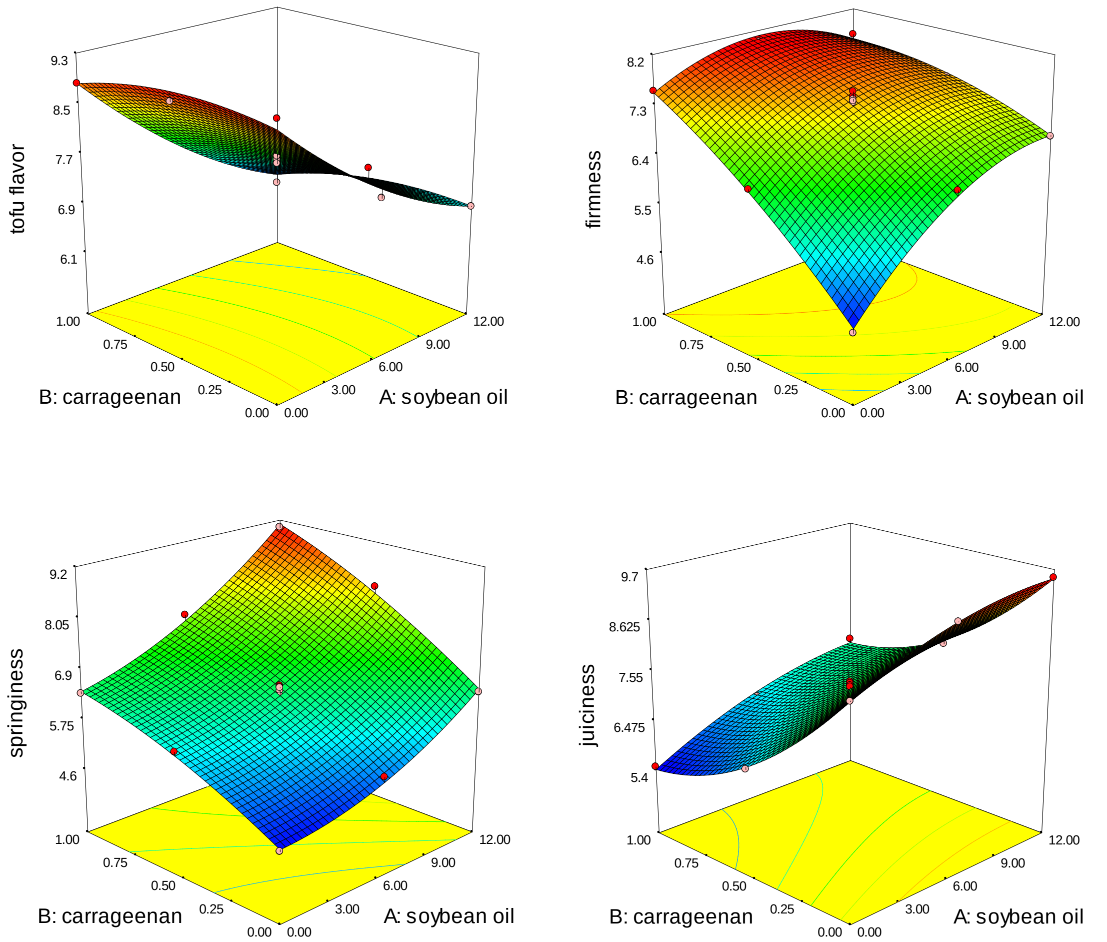

Figure 4.4 Response surface plot of the effect of amount of soybean oil and carrageenan concentration on QDA

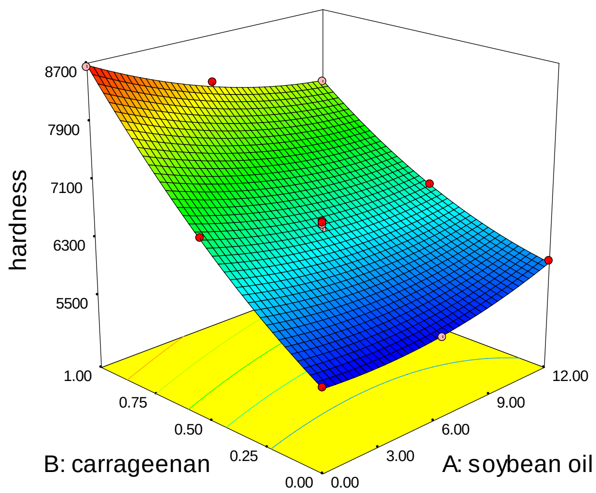

Figure 4.5 Response surface plot of the effect of amount of soybean oil and carrageenan concentration on TPA

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 (β_2= 2072.26) and quadratic (β_{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 spinginess 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 ($p<0.05$) (Table 4.18). Thus, the model can be used to optimize the basic emulsion gel with tofu powder formulation.

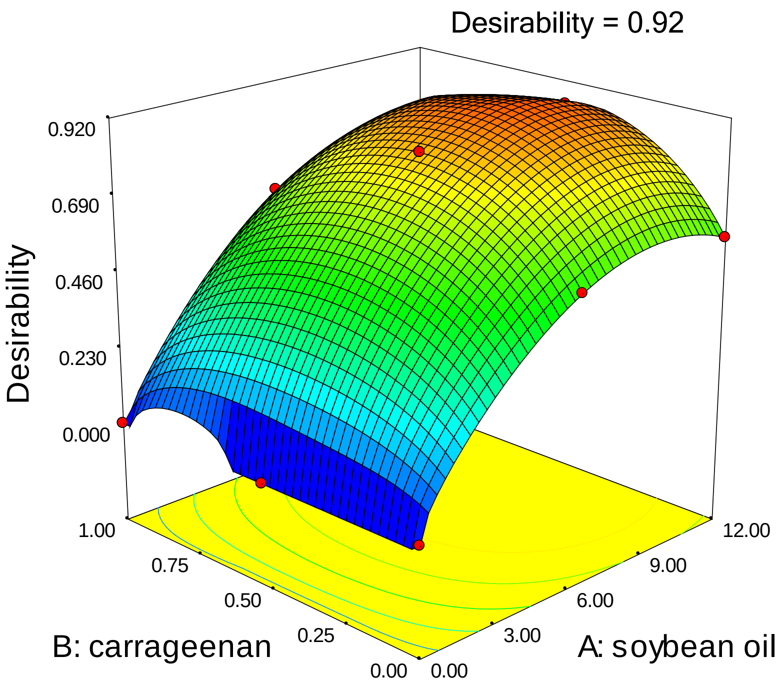

Figure 4.6 Desirability of the response variable optimize formulation of the surimi tofu emulsion gel

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