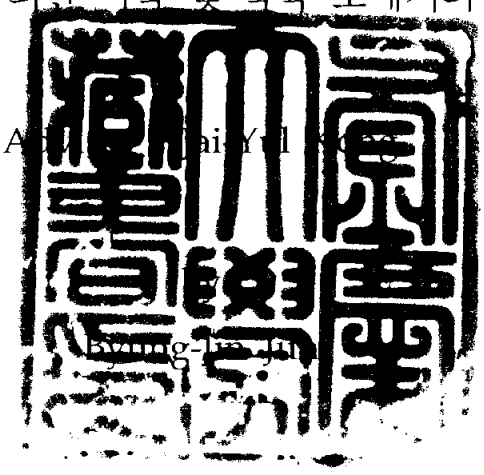
Effects of Rhamnolipid produced from *Pseudomonas aeruginosa* BYK-2 KCTC 18012P on the Quality of Surimi Gel and Pork Sausage

Pseudomonas aeruginosa BYK-2 KCTC 18012P로부터 생산된 rhamnolipid에 의한 어묵 및 축육 소세지의 물성개선



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Effects of Rhamnolipid produced from *Pseudomonas aeruginosa* BYK-2 KCTC 18012P on the Quality of Surimi Gel and Pork Sausage

A Dissertation

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ABSTRACT

An investigation showed the effects of pH and NaCl concentration on rhamnolipid's Emulsifying Activity Index (EAI), Emulsion Stability, Fat Binding Capacity (FBC) and textural properties of surimi gel and pork sausage containing rhamnolipid.

The EAI of rhamnolipid increased with the increasing of pH and decreasing of NaCl. The emulion stability of rhamnolipid was affected the NaCl regardless of pH. The FBC of rhamnolipid was 156.7% of the value at pH 7 and 2% NaCl compared to other emulsifiers. Rhamnolipid's FBC was showed pH-dependant and salt.

Alaska pollack surimi (RA grade) and pork were used for making heat-induced surimi gel and sausage, respectively. Textural properties and color were measured by the punch test and colorimeter, respectively. The screening of ingredients and optimum formulation were performed by the statistics program.

The whiteness of surimi gel containing rhamnolipid was significantly ($p < 0.05$) lower than that of the control. However, the breaking force and deformation of surimi gel were enhanced by rhamnolipid. Optimum concentration of rhamnolipid for surimi gel was 0.1 %. Rhamnolipid used for preparation of surimi gel was in the form of emulsion curd. The breaking force and deformation improved when the rhamnolipid emulsion was used. Optimum amounts of ingredients in processing pork sausage were 70% for pork ,10% for fat , 20% for water,1.6% for salt ,0.3% for sodium polyphosphate ,1.0% for soy protein concentrate and 0.1% for rhamnolipid. The pork sausage containing rhamnolipid did not improved in the breaking force but the deformation did. And the sausage containing rhamnolipid decreased in emulsifying activity by NaCl.

The results suggested that rhamnolipid could be used in surimi gel and pork sausage to improve textural properties.

I. INTRODUCTION

An emulsion is traditionally defined as a dispersion of droplets of one liquid into another, the two being immiscible[1]. Many products can exist as emulsions including some pharmaceuticals and cosmetics, coal, insecticides and crude oil[2, 3, 4, 5]. In foods, emulsions have a broad meaning encompassing systems where there may also be solids, gases and/or liquid crystals present (cake butter, ice cream, mayonnaise, etc.). Oil-in-water (O/W) emulsions (cream, dressings, etc.) are typically fluid and may contain a (partially) crystalline oil phase where as food-related water-in-oil (W/O) emulsions (butter, margarine, etc.) are typically solid-like[6].

Emulsifiers constitute an important class of industrial chemicals widely used in almost every sector of modern industry. During the last decade the demand for emulsifiers increased about 300% within the US chemical industry[7]. Current world wide production exceeds three million tonnes per annum (at an estimated value of US $ 4 billion) and is expected to rise to over four million tonnes by the end of the century[8, 9, 10]. About 54% of the total emulsifier output is utilized in household/laundry detergents with only 32% destined for industrial use. Most of the commercially available emulsifiers are chemical emulsifiers, mainly petroleum-derived. However, rapid advances in biotechnology and increased environmental awareness among consumers, combined with expected new legislation, has provided further impetus for serious consideration of biological emulsifiers as

possible alternatives to existing products[11].

Bioemulsifer are a structurally diverse group of surface active molecules synthesized by microorganisms. Bioemulsifiers have several advantages over the chemical emulsifiers, such as lower toxicity; higher biodegradability[12]; better environmental compatibility[13]; higher foaming [14]; hight selectivity and specific activity at extreme temperatures, pH, and salinity[15, 16]; and the ability to be synthesized from renewable feedstocks[17].

Bioemulsifiers can be classified into several groups based on their chemical structure: glycolipids, lipopeptides, lipopolysacchrides, phopholipid, and fatty acids/neutral lipids. Most known bioemulsifiers are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxy-aliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids, and sophorolipids.

On the other hand, surimi-based products have constituted traditional Japanese food for centuries. The Japanese produce many kinds of foods from surimi by adding different flavors and ingredients and the mixture in different ways. Products such as kamaboko, chikuwa, hanpen, and satsumaage are staples in the Japanese diet. More recently a new type of surimi based product was created which had the appearance and taste of shellfish. The new-generation product, the so-called shellfish analog, not only gained popularity in Japan and Korea but has also become well accepted by the Western world, being particularly successful in America. Production of these surimi-based products requires an understanding of the functional properties of surimi and other ingredients as raw materials, as

well as a knowledge of the processing equipment needed and the production principles concerning the effects of processing on the surimi-based formulation.

Previous papers had reported the characteristics of bioemulsifier produced from the marine bacterium *Pseudomonas aeruginosa* BYK-2 KCTC 18012P and the bioemulsifiers have been identified as rhamnolipid (Mw. 650) and rhamnolipid methyl ester (Mw. 664), respectively[18, 19, 20, 21].

The objective of this study was to investigate the effects of pH and NaCl on functional properties of rhamnolipid and the effects of textural properties of surimi gel and pork sausage.

II. MATERIALS & METHODS

Rhamnolipid was produced from *Pseudomonas aeruginosa* BYK-2 KCTC 10812P[22]. Alaska pollack surimi (RA grade) was obtained from SEONGJIN Co. (Nambumin, Pusan) and the Surimi was frozen at -20℃. Storage time of Surimi did not exceed 6 months before use. Pork was obtained from the M market (In-Pyeong, Tongyeong)

1. Emulsifying Activity Index (EAI) and Emulsion Stability

The Emulsifying activity index was evaluated according to the procedure described by Pearce & Kinsella[23]. 0.1% soy protein concentrate (SPC), whey protein concentrate (WPC), rhamnolipid and lecithin (LC) were initially prepared by dissolving the lyophilized material in water. The pH of

0.1% rhamnolipid and emulsifier solution (0, 0.5, 1.0, 1.5, 2.0% NaCl) were adjusted to 5, 6, 7, 8 and 9 by NaCl or HCl. The solutions were stored overnight at 4℃ to ensure good hydration or solubilization and then a good repeatability of the results.

The emulsions were then prepared by homogenizing 4 ml rhamnolipid and 16 g corn oil at 25℃ with an polytron homogenizer (Biospec products. INC. Switzerland) for 2 min at 8,000 rpm. Aliquots (1 mL) of the emulsions were diluted serially with 1 g/L SDS to give a final dilution of 1/100. The absorbance of the diluted emulsions was then determined in a 10-mm path length cuvette of 500 nm by a spectro-photometer (Kontron Instruments). The emulsifying activity index (EAI) was measured as initial absorbance, and emulsion stability was calculated by the equation:

$$Eumlion\ stability\ (min) = EAI \times T/(EAI - t_{10})$$

EAI was the turbidity at 0 min, t_{10} was the turbidity at 10 min after the homogenization, T = 10 min

2. Fat Binding Capacity (FBC)

Fat binding capacity (FBC) was determined using the procedure developed by Lin et al[24]. Samples weighing 0.5 g were placed in a tared 10 ml centrifuge tube and mixed a thoroughly with 5 ml corn oil for 1 min. They were left alone for 1 h at room temperature, mixing every 15 min for 5 seconds. After they were centrifuged at 3800 rpm for 25 min. Non-bounded oil was then removed by pouring off the supernatant liquid and standing the centrifuge tube with its opening facing downwards at an angle of inclination of 45° for 30 min.

The centrifuge tube and its contents were then weighed. The FBC was calculated using the following equation:

$$\text{FBC}\ (\%) = \frac{\text{mass of sample after fat binding (g)}}{\text{mass of sample (g)}} \times 100\ (\%)$$

3. Surimi gel and pork sausage preparation

3-1. Surimi gel preparation

The ingredients of surimi gel is shown in Table 1. The frozen surimi sample was prepared from Alaska Pollock, partially thawed at room temperature, and was cut into small pieces and chopped in a vacuum cutter (Stephan, model UMC 5 electronic, Deutch). reasonable amounts of added ingredients to the surimi gel were 6% of corn oil, 3% of soy protein concentrate, 7% ofr potato starch, and 0.1% of rhamnolipid. The moisture content of each formulation was adjusted to 78%. The paste was stuffed into polyvinylidene chloride film (Φ 2.0 cm × 18 cm). After the surimi gel was cooked at 90℃ for 15 min, it was immediately cooled at 4~5℃ for 15 min. All gels were removed from the tubes and stored overnight at 4 ℃ in polystyrene bags, prior to testing.

3-2. Pork sausage preparation

The ingredients of pork sausage were shown in Table 2. The process of making pork sausage was the same as that of surimi gel.

4. Measurement of textural properties and color

This was done by the method of Okada[25]. Test samples (2 cm in

Table 1. Composition of ingredients for the preparation of frozen Alaska pollack.

Ingredient	Contents (%)
Oil	6.0
Soy Protein Concentrate	3.0
Potato starch	3.0
Rhamnolipid	0.1
Salt	2.0

Table 2. Composition of ingredients for the preparation of pork sausage.

Ingredient	Absolute value (g)	Contents (%)
Pork	105.00	70.00
Fat	15.00	20.00
Ice	30.00	10.00
NaCl	2.40	1.60
Sodium polyphospahate	0.45	0.30
Soy protein concentrate	1.50	1.00
Potato starch	1.50	1.00
Rhamnolipid	0.15	0.10

length, 1.9 cm in diameter) were cut perpendicular to the long axis of each cylindrical gel. Breaking force (g) and deformation (mm) were measured by the punch test using a rheometer (Model CR-100D, Sun Scientific Co. Tokyo, Japan) equipped with a 5 mm spherical end plunger[26]. The plunger was placed over the center of the cell plate wells, and the sample was compressed at a crosshead speed of 60 mm/min. Five samples were analyzed for each treatment. Each sample was obtained with two replicates.

The CIE Lab color of the surimi gel and pork sausage were evaluated using a color meter (Model ZE 2000, Nippon Denshohoku, Tokyo, Japan). L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were measured and whiteness was calculated as described by L*-3b*[27].

5. Optimum formulation

To investigate the effects of rhamnolipid and emulsifiers on the textural properties of surimi gel and pork sausage, the most effective emulsifier was selected for using the JMP program[28]. Through screen design, effective rhamnolipid and emulsifiers for improving jelly strength were the selected factors in selection. Then, it was investigated fort the textural properties of surimi gel and pork sausage containing rhamnolipid and emulsifiers. (Table 3, 4, 5)

6. Statistical analysis

The data was subjected to analysis of the variance (ANOVA). Comparison of means was carried out by Turkey's HSD test[25]. Analysis

Table 3. Alaska pollack surimi gels containing rhamnolipid and emulsifiers formulation by experimental design.

Exp. No.	Surimi (g)	Oil (ml)	Ice/water (ml)	SPC (g)	WPC (g)	Rhamnolipid (g)	LC (g)	Salt (g)	Total (g)
1	132.0	1.2	62.8	0.0	0.0	0.0	0.0	4.0	200.0
2	130.8	1.2	63.7	0.2	0.2	0.0	0.0	4.0	200.0
3	130.8	1.2	63.7	0.0	0.2	0.0	0.2	4.0	200.0
4	130.8	1.2	63.7	0.2	0.0	0.0	0.2	4.0	200.0
5	130.8	1.2	63.7	0.0	0.2	0.2	0.0	4.0	200.0
6	130.8	1.2	63.7	0.2	0.0	0.2	0.0	4.0	200.0
7	130.8	1.2	63.7	0.0	0.0	0.2	0.2	4.0	200.0
8	129.5	1.2	64.5	0.2	0.2	0.2	0.2	4.0	200.0

SPC : Soy Protein Concentrate, WPC : Whey Protein Concentrate,
LC : Lecithin

Table 4. mulsion type of Alaska pollack surimi gel formulation by experimental design.

Surimi gel	Surimi (g)	Oil (ml)	Ice/water (ml)	potato starch (ml)	SPC (g)	Rhamnolipid (g)	Salt (g)	Total (g)
CNE	82.35	1.20	93.45	14.00	6.00	0.00	5.95	200
NE	81.61	1.20	94.00	14.00	6.00	0.20	5.98	200
CE	82.35	1.20	93.45	14.00	6.00	0.00	5.98	200
E	81.61	1.20	94.00	14.00	6.00	0.20	5.95	200

CNE : the Control of Not Emulsion-type surimi gel (not-added rhamnolipid)

NE : None Emulsion-type surimi gel (added rhamnolipid)

CE : the Control of Emulsion-type surimi gel (not-added rhamnolipid)

E : Emulsion-type surimi gel (added rhamnolipid)

Table 5. Pork sausage containing rhamnolipid on the pork, fat and water formulation by experimental design.

Exp. No.	Pork (g)	Fat (g)	Ice/water (ml)	total (g)	NaCl (g)	SPS (g)	SPC (g)	PS (g)	Rhamnolipid (g)
1	120	30	0	150	2.40	0.45	1.50	1.50	0.15
2	105	30	15	150	2.40	0.45	1.50	1.50	0.15
3	105	15	30	150	2.40	0.45	1.50	1.50	0.15
4	90	30	30	150	2.40	0.45	1.50	1.50	0.15
5	120	0	30	150	2.40	0.45	1.50	1.50	0.15
6	150	0	0	150	2.40	0.45	1.50	1.50	0.15
7	135	0	15	150	2.40	0.45	1.50	1.50	0.15
8	135	15	0	150	2.40	0.45	1.50	1.50	0.15

SPS : Sodium Polyphosphate, SPC : Soy Protein Concentrate, PS : Potato Starch

was performed using a JMP 5.0 program (SAS Institute. USA).

III. RESULTS AND DISCUSSION

1. Emulsifying Activity Index (EAI) and Emulsion Stability

The effectiveness of food proteins as emulsifiers is a commonly measured turbidity of an emulsion at a single wavelength and is expressed as EAI[29]. Table 6 indicates that there are significant differences among the samples at $P < 0.05$ for the EAI determined at pH 5 to pH 9 without NaCl. At all the emulsifier examined, the rhamnolipid showed a higher EAI than the other emulsifier. EAI of rhamnolipid and emulsifiers decreased with the increasing of NaCl (Fig. 1). The addition of NaCl greatly decreased the EAI of rhamnolipid and emulsifiers due to solubility reduction[30]. However, in the case of the EAI on pH, alkali condition improved the EAI more than did the acidic condition. Dependence of emulsifying activity on pH was expected, as it is known that emulsifying activity of a total protein, depends upon the hydrophilic-lipophilic balance, which is affected by pH[31]. Table 7 and Fig. 2 show the effects of the NaCl and pH on emulsion stability of rhamnolipid and emulsifiers. In all the cases, emulsion stability of rhamnolipid and emulsifiers significantly decreased with the increasing of NaCl but it was not affected by pH. De Feijter et al.[32] and Goff[33] suggested that during the emulsification process, it showed that competitive adsorption between different proteins

Table 6. Emulsifying Activity Index of rhamnolipid and emulsifiers without NaCl.

Sample	pH				
	5	6	7	8	9
SPC	ND	0.17 ± 0.00^{d}	0.36 ± 0.02^{d}	0.40 ± 0.04^{d}	0.39 ± 0.04^{c}
WPC	0.20 ± 0.00^{c}	0.65 ± 0.04^{c}	0.80 ± 0.00^{c}	0.50 ± 0.02^{c}	0.99 ± 0.06^{b}
Rhamolipid	1.35 ± 0.02^{a}	1.59 ± 0.02^{a}	1.64 ± 0.06^{a}	1.66 ± 0.02^{a}	1.62 ± 0.06^{a}
LC	1.10 ± 0.01^{b}	1.02 ± 0.11^{b}	1.01 ± 0.09^{b}	1.28 ± 0.03^{b}	1.55 ± 0.08^{a}

- $^{a-d}$ Means with different letters are significantly different ($P < 0.05$), and those with the same letter represent no significant difference ($P > 0.05$) within a subgroup of a column.
- Data are shown as mean ± SD.

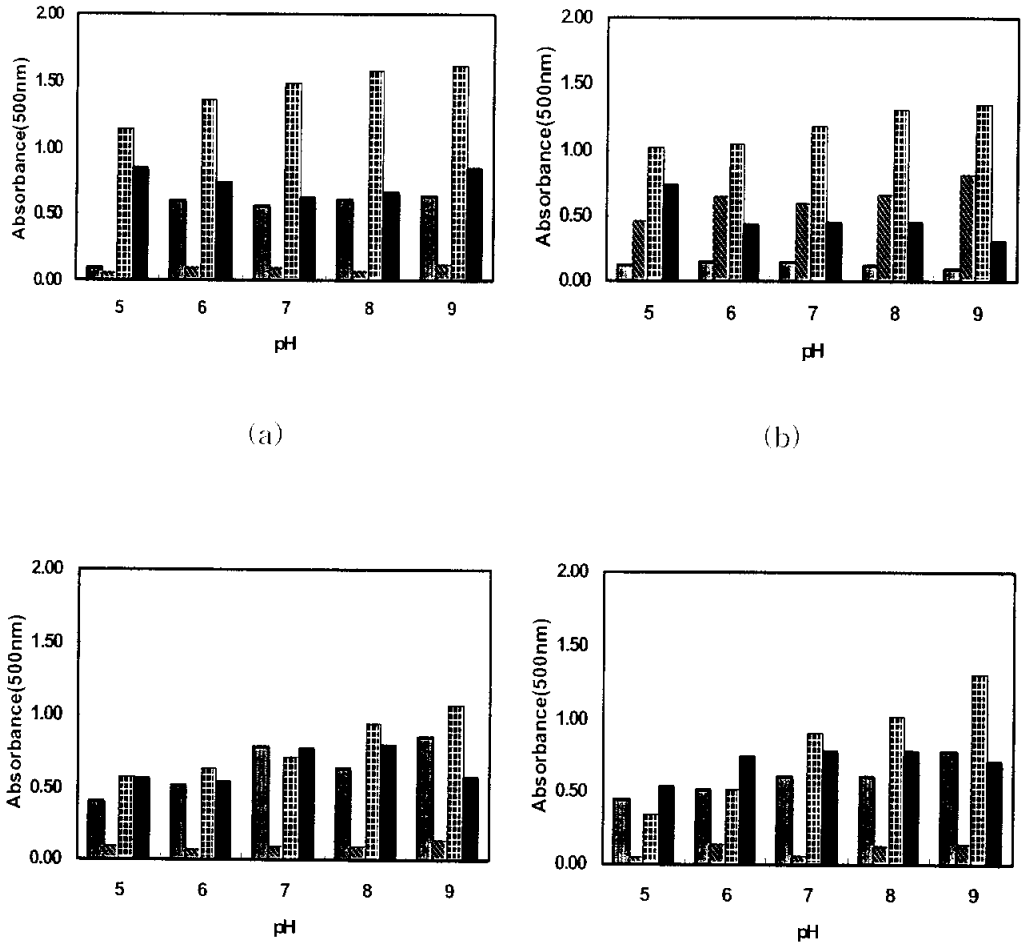


Fig. 1. Emulsifying Activity Index of rhamnolipid and emulsifiers by NaCl and pH.(a) 0.5 % NaCl, (b) 1.0 % NaCl, (c) 1.5 % NaCl, (d) 2.0 % NaCl (■ : SPC, ■ : WPC, ■ : Rhamnolipid, ■ : LC)

Table 7. Emulsion stability of rhamnolipid and emulsifiers without NaCl.

Sample	pH				
	5	6	7	8	9
SPC	ND	15.96±0.01^{b}	11.95±0.00^{b}	13.89±0.01^{b}	23.33±0.00^{b}
WPC	16.66±0.00^{ab}	13.90±0.00^{b}	13.72±0.00^{b}	18.44±0.00^{b}	19.75±0.00^{b}
Rhmnolipid	109.08±0.10^{a}	176.89±0.01^{a}	72.24±0.01^{a}	153.55±0.01^{a}	168.82±0.01^{a}
LC	26.53±0.01^{ab}	20.03±0.00^{b}	16.13±0.00^{b}	16.99±0.00^{b}	25.50±0.00^{b}

- $^{a\ b}$ Means with different letters are significantly different ($P < 0.05$), and those with the same letter represent no significant difference ($P > 0.05$) within a subgroup of a column.
- Data are shown as mean ± SD.

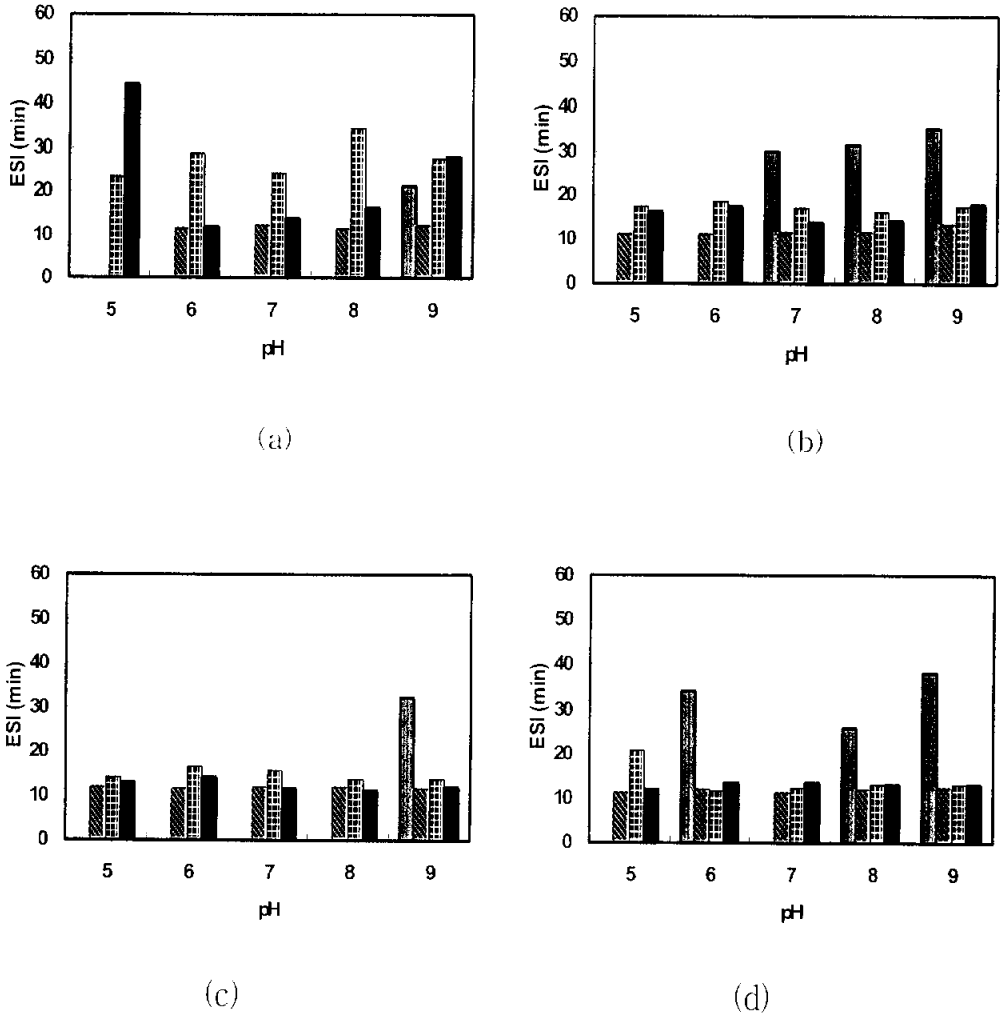
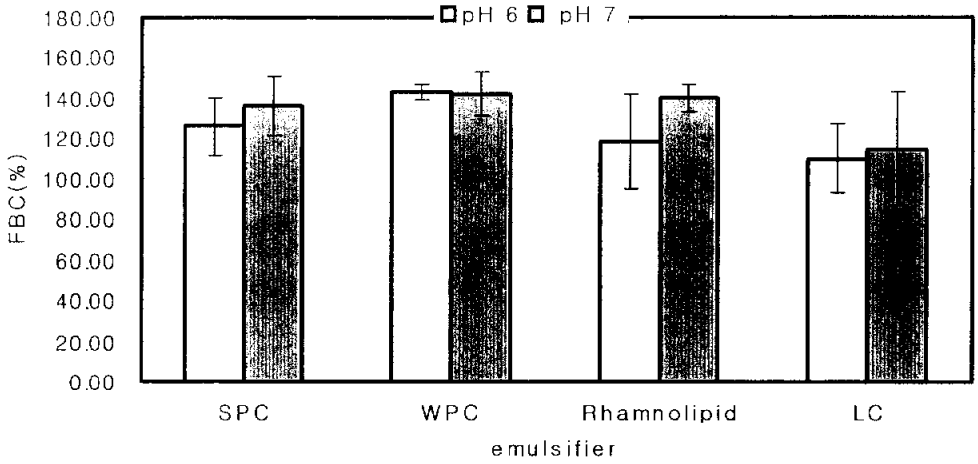


Fig. 2. Emulsion stability of rhamnolopid and emulsifiers by NaCl and pH.(a) 0.5 % NaCl, (b) 1.0 % NaCl, (c) 1.5 % NaCl, (d) 2.0 % NaCl (▬ : SPC, ▬ : WPC, ▬ : Rhamnolipid, ▬ : LC)

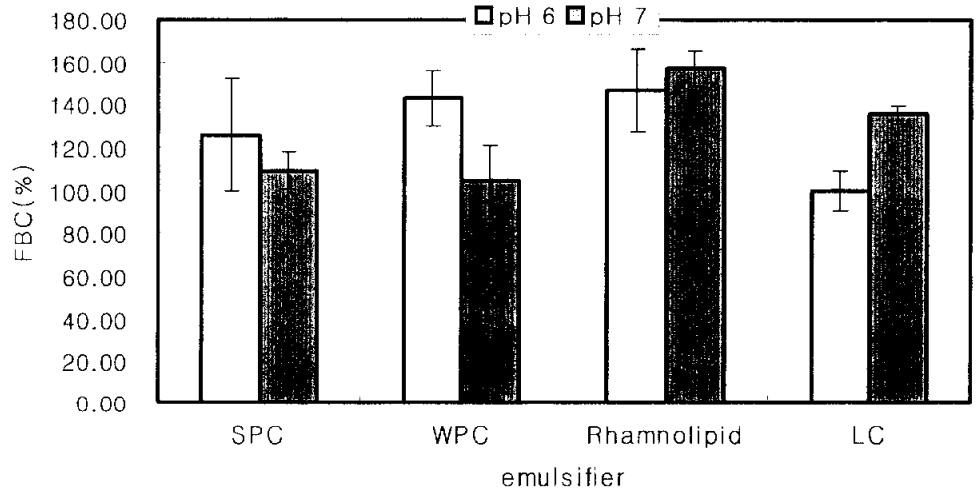
and between emulsifiers and proteins occurs at the emulsion interface where they compete for attachment thereby affecting emulsion stability during the emulsification. Israelachvili[34] suggested that given the environmental conditions in emulsions (i. e. water + oil + surface-active agent), this leads to a net association between the protein and fat crystals due to hydrophobic interactions. Besides, Hung and Zayas[35] suggested that various factors, including pH, droplet size, net charge, interfacial tension, viscosity and protein on formation, could affect the values of emulsion stability.

2. Fat Binding Capacity (FBC)

FBC is another functional property of some ingredients used in formulated food. Ingredients with a high FBC allowed the stabilization of high fat food products and emulsions[36]. In this study, the FBC was 109 ~ 136% for SPC, 104 ~ 143% for WPC, 118 ~ 156% for rhamnolipid and 99 ~ 135% for LC (Fig. 3). Rhamnolipid was observed as high in the FBC value compared with other emulsifiers and FBC of rhamnolipid was the best (156.67%) at 2% NaCl and pH 7. These result revealed that FBC of rhamnolipid increased with the increasing of pH and NaCl. The results revealed that rhamnolipid was affected by pH and NaCl. Kinsella[20] explained the mechanism of fat absorption as a physical entrapment of oil and several authors have related the fat binding capacity to the nonpolar side chains of the protein as well as to the different conformational features of the proteins. Our results suggested that the rhamnolipid had good fat binding capacity.



(a)



(b)

Fig. 3. Fat Binding Capacity of rhamnolipid and emulsifiers by (a) 0 % NaCl, pH 6 and pH 7 and (b) 2 % NaCl, pH 6 and pH 7.

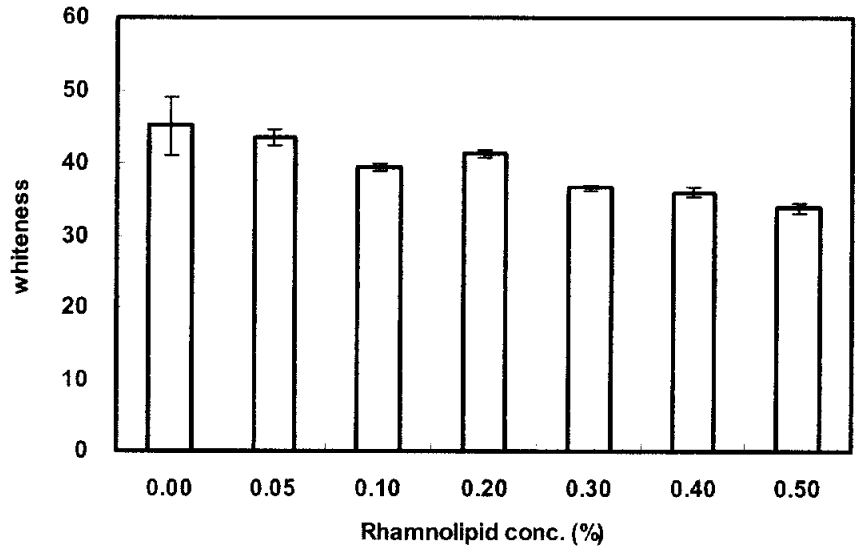
3. Effects of rhamnolipid of textural properties on the surimi gel

3-1. Optimum concentration of rhamnolipid on the surimi gel

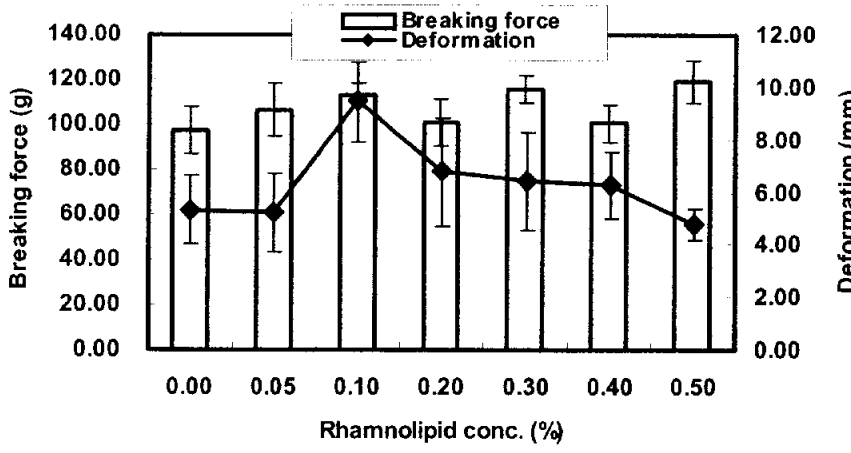
The effects of rhamnolipid concentration on textural properties of surimi gel was evaluated (Fig. 4). The whiteness of surimi gel decreased with the increasing of the rhamnolipid concentration due to the color of rhamnolipid. In general, both the breaking force and deformation of surimi gel were improved with the increasing of rhamnolipid concentration. Especially, when the addition of 0.1% rhamnolipid, deformation of surimi gel was higher than that of the others. In these result, we concluded that the optimal concentration of rhamnolipid added on surimi gel was 0.1%.

3-2. Determination of the effective factor on the surimi gel

The effects of rhamnolipid and emulsifiers on textural properties of surimi gels were evaluated by the punch test (Fig. 5). The surimi gel containing rhamnolipid led to a decrease in whiteness. The whiteness of surimi gel containing rhamnolipid led to a significant decrease due to the color of rhamnolipid. Except for surimi gel containing WPC and LC, the breaking force led to the increase with SPC and rhamnolipid. With the emulsifiers, surimi gel led to tan increase in deformation. Especially, the breaking force and deformation on surimi gel containing rhamnolipid led to more of a significant increase than that of the surimi gel containing other emulsifiers. In these results, we found that surimi gel containing rhamnolipid had more improved gel properties than that of the surimi gel containing SPC, WPC and LC.



(a)



(b)

Fig. 4. Effects of whiteness (a), breaking force and deformation (b) of Alaska pollack surimi gel under optimal concentration of rhamnolipid.

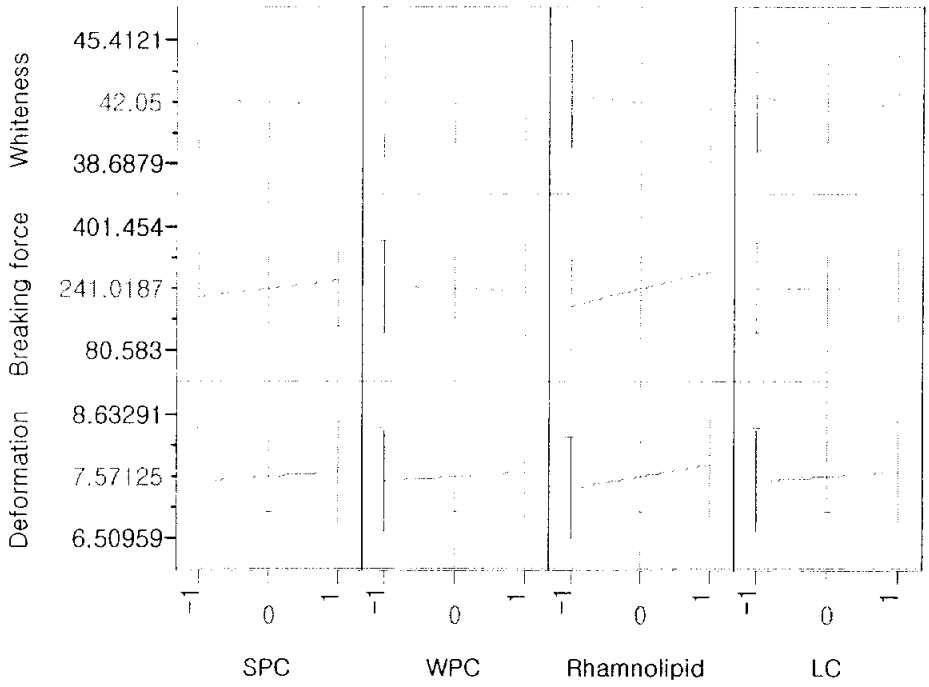


Fig. 5. Prediction Profiler of rhamnolipid and emulsifiers on whiteness, breaking force and deformation of Alaska pollack surimi gel.

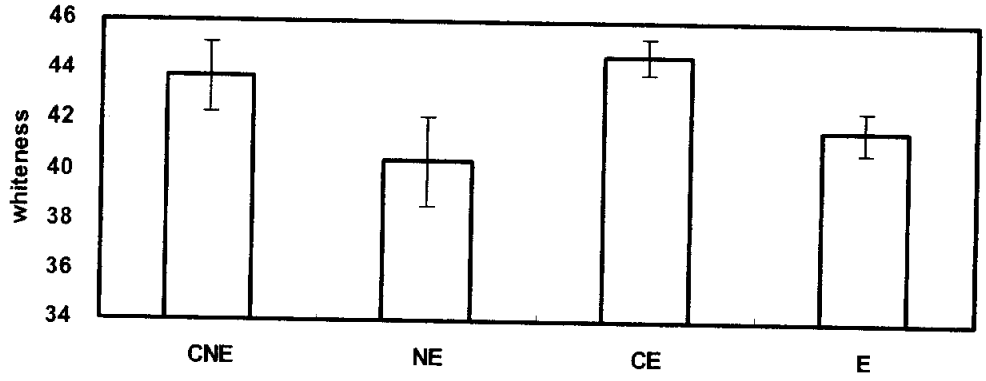
3-3. Textural properties of emulsion-type surimi gel

Surimi gel containing rhamnolipid prepared by using a combination of factors at the factory (Fig.6). As in the same aforementioned results, the effect of rhamnolipid on whiteness of surimi gel showed that was a lower value than that of surimi gel that had not rhamnolipid added due to the color of the rhamnolipid. However, the breaking force and deformation of NE and E had higher values than those of the CNE and CE. Morever, the textural properties of E showed a higher value than those of NE. Cho and Park[37] evaluated the functionality of soy protein isolate (SPI) and SPC in surimi seafood. In these result, it can be seen that the surimi gel containing rhamnolipid exhibit textural properties comparable to that of the other emulsifiers.

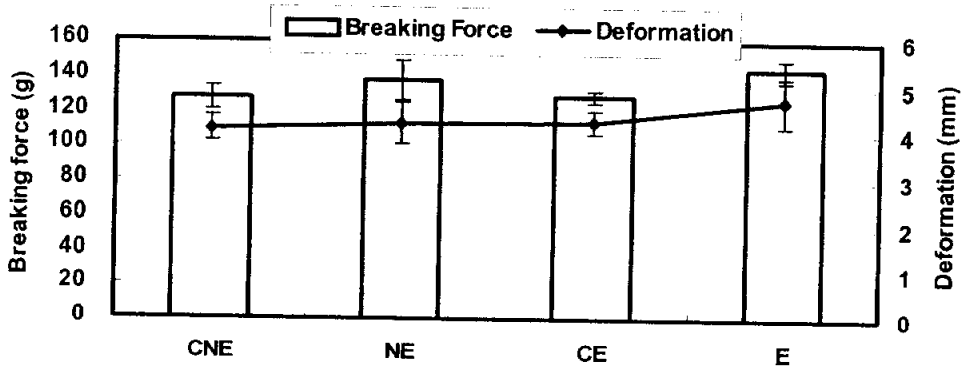
4. Effects of rhamnolipid of textural properties on the pork sausage

4-1. Optimal mixture ratio of ingredient on the pork sausage

The effects of the optimal mixture ratio of pork, fat and water on sausage containing rhamnolipid were evaluated (Table 8, Fig. 7). Except for the mixture of pork, fat and water, the ingredients added fixed contents. Ingredients in the processing of sausage were 1.6% of NaCl, 0.3% of SPS, 1.0% of SPC, 1.0% of PS and 0.1% of rhamnolipid. In the breaking force and deformation, the third sample had higher values than those of other samples. Especially, the deformation of the third sample significantly increased compared with other sausages. Theses results revealed that the



(a)



(b)

Fig. 6. Effects of whiteness (a), breaking force and deformation (b) of emulsion-type surimi gel containing rhamnolipid.

Table 8. Effects of the color value by mixture ratio of sausage containing rhamnolipid.

*Exp. No.	L	a	b
1	53.91±1.04^{c}	2.05±0.39^{a}	10.80±0.45^{ab}
2	53.80±1.02^{bc}	1.84±0.35^{a}	10.20±0.29^{bc}
3	52.61±0.78^{c}	1.60±0.39^{ab}	9.89±0.29^{c}
4	57.51±1.36^{a}	0.98±0.40^{bc}	10.36±0.30^{bc}
5	58.31±1.08^{a}	0.32±0.21^{cd}	11.24±0.38^{a}
6	55.32±0.80^{abc}	0.60±0.32^{cd}	11.42±0.24^{a}
7	56.44±0.66^{ab}	0.29±0.15^{d}	11.23±0.21^{a}
8	55.11±0.71^{abc}	1.43±0.29^{ab}	11.12±0.28^{a}

* 1. Pork : Fat : Water = 80% : 20% : 0%
2. Pork : Fat : Water = 70% : 20% : 10%
3. Pork : Fat : Water = 70% : 10% : 20%
4. Pork : Fat : Water = 60% : 20% : 30%
5. Pork : Fat : Water = 80% : 0% : 20%
6. Pork : Fat : Water = 100% : 0% : 0%
7. Pork : Fat : Water = 90% : 0%: 10%
8. Pork : Fat : Water = 90% : 10% : 0%

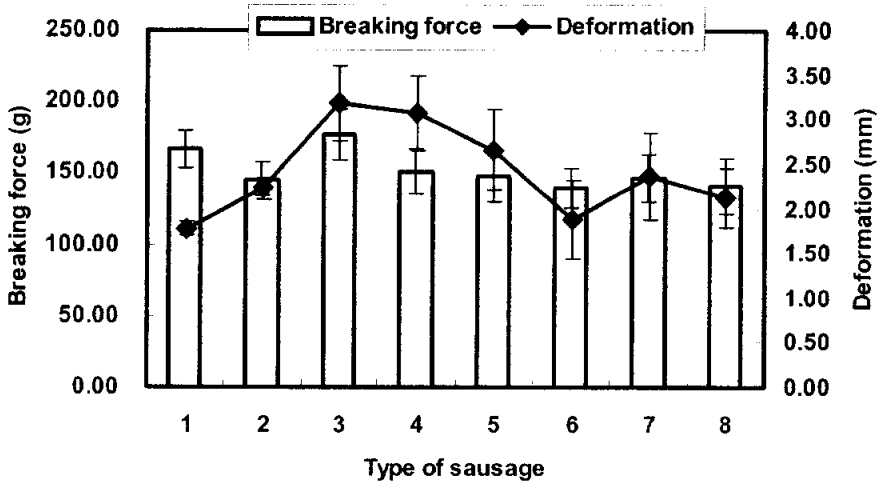


Fig. 7. Effects of the breaking force and deformation by mixture ratio of sausage containing rhamnolipid.

1. Pork : Fat : Water = 80% : 20% : 0%
2. Pork : Fat : Water = 70% : 20% : 10%
3. Pork : Fat : Water = 70% : 10% : 20%
4. Pork : Fat : Water = 60% : 20% : 20%
5. Pork : Fat : Water = 80% : 0% : 20%
6. Pork : Fat : Water = 100% : 0% : 0%
7. Pork : Fat : Water = 90% : 0%: 10%
8. Pork : Fat : Water = 90% : 10% : 0%

optimal mixture ratio of sausage was pork : fat : water = 70% : 10% : 20%.

4-2. Textural properties of emulsion-type pork sausage

Investigated were the effects of textural properties on emulsion-type sausage (Table 9, Fig. 8). All the sausages observed were similar in color value. The breaking force and deformation of pork sausage containing rhamnolipid was higher than those of pork sausage without rhamnolipid, and the breaking force of NE had a higher value compared to E. From the results, we found the NE exhibited textural properties comparable to that of the other sausage.

4-3. Textural properties of pork sausage containing rhamnolipid without NaCl

Chobert et al.[27] have reported that the emulsifying activity of protein gradually decreased due to the salting effect of NaCl. An investigation to find the effects of textural properties on pork sausage containing rhamnolipid without NaCl was conducted(Table 10, Fig. 9). The sausage with salt showed significantly ($P < 0.05$) lower breaking force than the sausage without salt, and the sausage of deformation increased a little with NaCl. Rhamnolipid decreased the emulsifying activity by the salt, the same as protein.

Table 9. Effect of the color value on emulsion-type sausage.

Type of sausage	L	a	b
CNE	59.50±0.45^{a}	0.108±0.32^{a}	10.95±0.36^{ab}
NE	58.61±0.45^{bc}	-0.36±0.37^{a}	10.70±0.51^{ab}
CE	59.15±0.42^{ab}	0.182±0.25^{a}	10.52±0.58^{b}
E	58.32±0.35^{c}	0.146±0.45^{a}	11.42±0.42^{a}

CNE : the Control of None Emulsion-type sausage (not-added rhamnolipid)

NE : None Emulsion-type sausage (added rhamnolipid)

CE : the Control of Emulsion-type sausage (not-added rhamnolipid)

E : Emulsion-type sausage (added rhamnolipid)

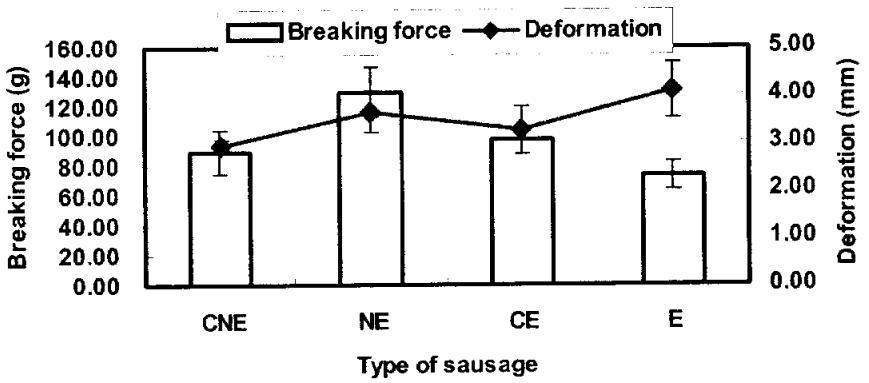


Fig. 8. Effects of the breaking force and deformation of emulsion-type sausage containing rhamnolipid.

CNE : the Control of None Emulsion-type sausage (not-added rhamnolipid)

NE : None Emulsion-type sausage (added rhamnolipid)

CE : the Control of Emulsion-type sausage (not-added rhamnolipid)

E : Emulsion-type sausage (added rhamnolipid)

Table 10. Effect of the color value on pork sausage containing rhamnolipid without NaCl.

*Exp. No.	L	a	b
1	55.24±0.69[b]	0.93±0.19[a]	10.58±0.41[a]
2	56.05±0.33[a]	0.69±0.53[a]	10.76±0.61[a]

*1. pork sausage containing rhamnolipid without NaCl

2. pork sausage containing rhamnolipid with NaCl.

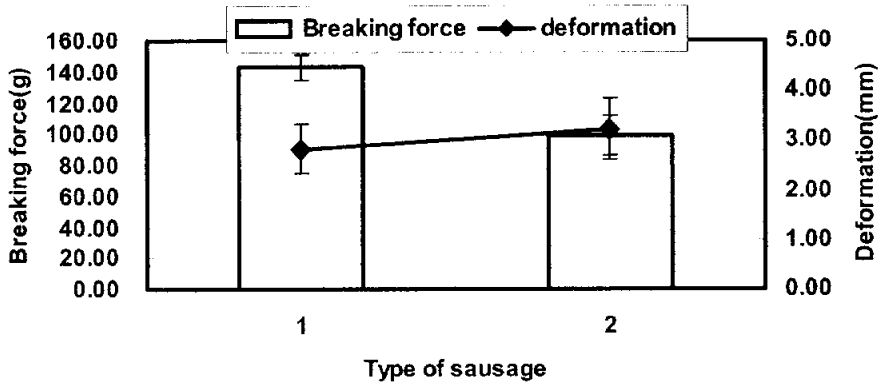


Fig. 9. Effects of the breaking force and deformation on pork sausage containing rhamnolipid without NaCl.

(1. pork sausage containing rhamnolipid without NaCl, 2. pork sausage containing rhamnolipid with NaCl).

IV. CONCLUSIONS

A view of improving the quality of surimi gel and pork sausage, the ingredient that was used as a bioemulsifier (rhamnolipid) was produced from *Pseudomonas aeruginosa* BYK-2 KCTC 18012P. As the funtional properties of a food ingredient, it was investigated for the Emulsifying Activity Index (EAI), Emulsion Stability (ESI), and Fat Binding Capacity (FBC) of rhamnolipid.

Rhamnolipid had higher EAI than other emulsifiers and decreased with the increasing of NaCl due to solubility reduction. In the case of EAI on pH, the alkali condition improved the EAI compared to the acidic condition. The ESI of rhamnolipid significantly decreased with the increasing of NaCl and those without were not affected by pH.

The rhamnolipid showed high FBC value compared with other emulsifiers and the FBC of rhamnolipid was the best (156.67 %) at 2 % NaCl concentrate and pH 7. The result showed that the FBC of rhamnolipid increased with the increasing of pH and NaCl .

In the surimi gel containing rhamnolipid, 0.1 % rhamnolipid was the optimum concentrate for surimi gel. The whiteness of surimi gel significantly ($p < 0.05$) decreased due to the color of rhamnolipid. However, rhamnolipid enhanced the breaking force and the deformation of surimi gel.

The optimal mixture ratio of ingredients in processing pork sausage were pork 70 %, fat 10 %, water 20 %, salt 1.6 %, sodium polyphosphate 0.3 %, soy protein concentrate 1.0 % and rhamnolipid 0.1 %. The deformation of pork sausage with rhamnolipid improved but, the breaking force was not. The sausage containing rhamnolipid with NaCl decreased in textural

properties because emulisifying activity decreased with NaCl.

These data show ingredients used in surimi can have a profitable effect. Except for the whiteness, surimi gel containing 0.1 % rhamnolipid enhanced the effect of breaking force and deformation. Especially, the results showed that value of deformation improved greatly. Therefore, we suggest that rhamnolipid could be used in surimi gel and sausage to improve textural properties.

V. 국문초록

해양 미생물 *Pseudomonas aeruginosa* BYK-2 KCTC 18012P로부터 생산된 생물유화제를 어묵 및 축육 소세지에 첨가하여 물성의 변화를 관찰하였다. 식품첨가제로써 pH 및 염 농도에 대한 생물 유화제의 유화능 및 유화안정성, 지방흡착력을 조사한 결과, 생물유화제의 유화능은 모든 염농도 및 pH에서 다른 유화제에 비해 높은 값을 나타내었고 pH가 증가할 수록 유화능이 증가하는 것으로 나타났다. 그러나 염농도에 대한 유화능은 농도가 증가할 수록 다른 유화제들과 마찬가지로 생물유화제도 활성이 현저히 감소하였다. 유화안정성에 있어서 생물유화제는 pH에 대해서는 큰 영향을 받지 않았으나 염농도가 증가할 수록 안정성이 감소하였다. 지방흡착력의 경우 다른 유화제에 비해서 대체로 높은 값을 나타내었으며 특히 2 % 염농도, pH 9에서 156.7 %로 가장 높은 값을 나타내었으며 염농도와 pH가 증가함에 따라 생물유화제의 FBC도 증가함을 알 수가 있었다.

어묵 및 축육 소세지에 0.1 % 생물유화제를 첨가한 경우 색택을 제외한 breaking force 및 deformation이 무첨가구에 비해서 훨씬 좋은 결과를 나타내었으며 다른 식품급 유화제에 비해서 더 좋은 물성을 나타내는 경향을 보였다. 이들 결과를 바탕으로 공장에서 사용하는 방법으로 생물유화제 및 부재료들을 emulsion curd의 형태로 만들어 어묵을 제조한 결과에서도 생물유화제는 어묵의 breaking force 및 deformation을 증가시켜 제품의 품질을 향상시킴을 알 수가 있었다.

생물유화제를 첨가한 축육 소세지를 제조하기 위한 축육 및 지방과 수분의 최적 비율은 각각 70 %, 10 %, 20 %였으며, 이를 바탕으로 유화형 소세지를 제조한 소세지의 경우 deformation은 무첨가구에 비해서 1. 5배정도 증가시켰

으나 breaking force는 오히려 감소를 시켰다. 그리나 축육 소세지의 제조에 있어 생물유화제가 식염과 함께 첨가될 경우 유화능이 붕괴되어 breaking force를 증가시키지 못하였다.

이로 미루어 보아 생물유화제는 어묵 및 축육 소제지의 deformation을 향상시킴으로써 제품의 품질향상에 기여할 수 있음을 알 수가 있었다.

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