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Optimization of Reaction Flavors Preparation Using Antarctic Krill(*Euphausia superba*)

by

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남극크릴(*Euphausia superba*)을 이용한 반응향 제조의 최적화

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김예주

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요 약

남극크릴(*Euphausia superba*)은 이미 오래 전부터 새로운 단백질원 및 식품 소재로 각광받아왔다. 아홉 가지의 필수아미노산을 함유하고, 불포화지방산 중에서도 EPA, DHA 및 올레인산의 함량이 매우 높아 인체에 매우 유익한 식량자원이 될 수 있지만 크릴의 식용화 기술에 대해서는 개발이 다소 부족한 실정이다. 따라서 본 연구는 Plackett-Burman design을 통해 반응전구체들의 배합비를 최적화하여 크릴 반응향을 제조하고 이를 토대로 남극크릴을 이용한 식량자원개발의 기초자료를 제공하는데 연구의 목적을 두고 있다.

Plackett-Burman design을 통해 12가지의 크릴 반응향을 제조하였고, 이의 흡광도값을 통계처리를 하여 갈변반응에 대한 반응 전구체들의 영향력이 cysteine < thiamine = glucose < fructose = galactose = methionine < ribose = xylose < tryptophan < lecithin = glucosamine의

순으로 나타나는 것을 확인하였다. 그리고 관능검사를 실시하였을 때 xylose, fructose, ribose, tryptophan, glucosamine과 크릴 가수분해물을 배합하여 제조한 11번 반응향이 전체적인 기호도가 가장 높은 것으로 나타났다. 반응향의 생성 메커니즘을 연구하기 위해 휘발성 향기 성분을 측정하여 총 51종의 향기성분을 분리·동정하였고 aldehyde류 11종, aliphatic compounds 10종, sulfur-containing compounds 2종, furan류 3종, ketone류 5종, acid류 4종, ester류 2종, alcohol류 2종 등으로 구성되어있으며, 첨가되는 전구체의 종류에 따라 향기 성분이 다르게 생성되는 것을 확인하였다.

본 연구를 토대로 반응향 개발기술은 크릴뿐만 아니라 다양한 분야에서 응용될 수 있을 것이며, 이 연구에서 개발된 반응향은 향미제 및 증미제로서 식품에 이용될 수 있을 것으로 기대된다.

Introduction

Development of new food resources is required to meet the needs of the expanding world population that caused by the industrial revolution and the development of medicines (Boserup, 1981; Mun, 2014). As the population of the world increases, many countries are concerned about insufficiency of food resources and search for new future sources of food. Krill have been recognized as one of the food resources of the future since long before. First, Russia has tried to develop krill-based foods since 1961. Poland, Japan, West Germany, France, Chile and Korea have also attempted to commercialize krill as food ingredients (Kim *et al.*, 2000).

Krill are zooplankton of the order *Euphausiacea*, and are small, shrimplike crustaceans, but are unrelated to shrimp (Gigliotti *et al.,* 2008). Krill occur worldwide in all oceans, and about 85 species of krill have been recorded (Nicol and Endo, 1999). The Antarctic krill (*Euphausia superba*) which belong to krill are commonly found in the Antarctic waters of the Southern ocean. A recent estimate of total biomass of krill is around 500 million tonnes (MT). Actual catch of krill is only 150,000 tonnes as opposed to total allowable catch quotas, which amount to nearly 3.5 MT (Vital Choice Wild

Seafood & Organics, 2008). Therefore, krill are abundant species, so they are promising future food resources (Kim and Kim, 1999). Krill contain 79.1% moisture, 13.1% protein, 4.0% lipids, 2.7% ash, chitin and chitosan (Lee, 1999). The amino acid composition of krill protein indicated that all nine essential amino acids of krill protein were present in sufficient quantities to meet FAO/WHO/UNU requirements for adults (Chen *et al*., under review). In addition to being a promising source of high quality protein, krill also offer advantage of having high amounts of omega-3 polyunsaturated fatty acids (ω-3 PUFAs). It was reported that omega-3 PUFAs accounted for ～19% of total fatty acids in krill (Kolakowska *et al*., 1994). Of the omega-3 PUFAs, eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) that have been linked to a wide-range of health benefits to human were particularly abundant (Chen and Jaczynski, 2007), and krill oil also contains various potent antioxidants, including vitamins E, A and D, astaxanthin and anthoxanthin providing 48 times higher antioxidant potency than fish oil in terms of oxygen radical absorbance capacity values (Farooqui, 2009); thus, krill appear to be a promising valuable food resources for human. Some studies have investigated the food potential of krill and have reported on paste (Lee *et al*., 1985), oil (Zhu *et al*., 2008), surimi from krill (Suzuki and Shibata, 1990); however, more studies are needed to find ways to enhance flavor of food product by using krill flavor.

Reaction flavor is a kind of process flavor which is formed by heating food ingredients and enzymatic reaction (Desai and Schowalter, 2014). It found in thermally treated foodstuffs such as meat, chocolate, coffee, caramel, popcorn and bread (Taylor and Linforth, 2010). Reaction flavor is known as an important factor in food processing for commercial application and is an important research area because instant convenience foods are increasingly popular due to changes in modern lifestyles (Baek *et al*., 2008). Several types of reaction flavor can result from reaction conditions of Maillard reaction, lipid oxidation and other reactions. Therefore, precursors are important factors for food flavor processing.

Plackett–Burman designs are experimental designs presented in 1946 by Robin L. Plackett and J. P. Burman (Plackett and Burman, 1946). The Plackett-Burman designs are fractional two-level multifactor designs of 2k with n = k+1 design points which should be a multiple of 4. It is kind of screening design to identify the factors having large effects worthy of further experimentation (Song, 2009). Independent variables (factors) can be designed from 3 upto 47 for 4 to 48 observations on the response (Minitab, 2014). Plackett-Burman design is very useful for economically detecting large main effects among various independent variables through small-scale experiment during short time.

Table 1. Plackett–Burman design for 4 runs and 3 two-level factors

Run	Independent variables		
	X_1	X_2	X_3
1	+	+	+
2	+	-	-
3	-	+	-
4	-	-	+

The example for Plackett-Burman design is shown in Table 1. The above 4 rows lead to the fitting of the linear model (1) with the 3 factor values defined as X_1, X_2, X_3 and with 4 observations on the response.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + e \quad (1)$$

The "-" sign corresponds to the minimum value and the "+" sign does to the maximum value of the input variable range. As the matrix forms of Plackett-Burman design are already designed such as Table 1, it is less cumbrous that to use the matrix forms according to the number of variables. Therefore, Plackett-Burman design is applied to several areas of food, pharmacology, biology, chemical engineering, mechanical engineering, safety engineering and others.

Our objectives were to develop reaction flavor using Antarctic krill by optimizing mixing ratio of precursors for the Maillard reaction depending on Plackett-Burman design and to contribute to the advancement of krill utilization as food resource.

Materials and Methods

1. Materials

Fresh krill obtained from Dongwon (Gimhae, Korea) was freeze-dried to make krill powder and then the powder was stored at –40℃. Alcalase 2.4L (Novo, Denmark), used for enzymatic hydrolysis of krill meat, was stored at around 4℃ until used. Glucose, xylose, fructose, L-cysteine hydrochloride and thiamine were purchased from Junsei Chemical Co. (Tokyo, Japan). L-Methionine and L-tryptophan were purchased from Sigma-Aldrich Co. (St.Louis, USA), D-Glucosamine was purchased from MNtech Co. (Cheongwon, Korea), and D-galactose from Avocado Research Chemicals Ltd. (Leysham, Lancester, UK).

2. Methods

2.1. Processing of krill hydrolysate

Twenty grams of krill powder were combined with 300ml distilled water and the enzyme of krill was deactivated by heating for 20 min at 95℃. Temperature of the deactivated solution were reduced to 65~68℃ by placing the solution at room temperature. The solution was fermented with

300㎕ commercial alcalase for 40 min, and then, it was boiled at 95℃ to deactivate the alcalase. Krill shell in solution was filtered by a 95-mesh filter, after which the filtered solution was centrifuged at 3,000 rpm for 30 min to gain the supernatant liquid as krill hydrolysate.

2.2. Reaction condition and mixing ratio of precursors according to Plackett-Burman design

For optimizing the Maillard reaction in krill hydrolysate with precursors, eleven independent variables and twelve experimental runs were selected according to Plackett-Burman design (Table 2). Five sugars of glucose, xylose, fructose, ribose, galactose and three amino acids of methionine, cysteine and tryptophan were independent variables. Also, thiamine, lecithin and glucosamine were used for the reaction flavor. All eleven precursors were made in 0.1M solutions. The symbol of "+1" was 5ml of the precursors and that of "–1" was 5ml of distilled water. Krill hydrolysate was made in 0.3M according to molecular weight of valine and its 5ml was added. Final volume was 60ml which was controlled at pH 7 by using 0.1M HCl and 0.1M NaOH. This mixture was reacted at 120℃ for 2 hrs in a dry oven. Twelve reaction flavors were prepared through those processes.

Table 2. Plackett-Burman design for optimization of reaction flavor

Runs[1)]	Factors										
	Glu	**Xyl**	**Fru**	**Rib**	**Gal**	**Met**	**Cys**	**Try**	**Thi**	**Lec**	**Gluc-osa-mine**
1	1	1	1	1	1	1	1	1	1	1	1
2	-1	1	-1	1	1	1	-1	-1	-1	1	-1
3	-1	-1	1	-1	1	1	1	-1	-1	-1	1
4	1	-1	-1	1	-1	1	1	1	-1	-1	-1
5	-1	1	-1	-1	1	-1	1	1	1	-1	-1
6	-1	-1	1	-1	-1	1	-1	1	1	1	-1
7	-1	-1	-1	1	-1	-1	1	-1	1	1	1
8	1	-1	-1	-1	1	-1	-1	1	-1	1	1
9	1	1	-1	-1	-1	1	-1	-1	1	-1	1
10	1	1	1	-1	-1	-1	1	-1	-1	1	-1
11	-1	1	1	1	-1	-1	-1	1	-1	-1	1
12	1	-1	1	1	1	-1	-1	-1	1	-1	-1

[1)] The number of experimental condition by Plackett-Burman design.

2.3. Measurement of browning degree of reaction flavor

The browning degree of Maillard reaction was determined by recording the absorbance of solutions of reaction flavors. Each of the twelve samples was transferred into a cuvette for measuring absorbance. Then, it was measured three times at 420 nm by using a spectrophotometer (UV-1800, Shimadzu Co., Japan). The results were calculated as an average.

2.4. Sensory evaluation

For sensory evaluation, ten students of Pukyong National University were selected, and trained to evaluate reaction flavors with evaluation sheets. The solutions of reaction flavors were heated at 60℃ to have strong smells, and they were placed in test tubes to provide to panels. The samples were evaluated by using a 9-point hedonic scale method graded from 1 to 9 (no flavor to very strong flavor). Total preference was also performed by grading from 1 to 9 (worst to best).

2.5. Headspace volatile compounds

2.5.1. Extraction of headspace volatile compounds

Fifteen milliliter of reaction solution was put into a 250ml screw ambor bottle. It was heated in a dry oven at 50℃ for 30 min, and then, cooled at room temperature for 30 min.

2.5.2. GC-MSD analysis

Analysis of volatile compounds of reaction flavors was performed by automatic thermal desorption (ATD) coupled with gas chromatography (GC)/mass-selective detector (MSD), using a Perkin-Elmer ATD 400 (Perkin-Elmer, Waltham, MA, USA) and a Shimadzu QP-2010 plus GC fitted with a MSD (Shimadzu, Japan). The headspace volatile compounds of reaction solution were absorbed on Tenax-TA (Supelco, Bellefonte, PA, USA) by using a vaccum pump (VPC-10, Shimadzu, Japan) and a mass flow controller (Shimadzu, Japan). Thermal desorption of the sampling tubes was carried out at 350℃ for 4 min (primary desorption), during which time the eluted volatile compounds were swept from the tube maintained at -30℃. After primary desorption, the cold trap was rapidly heated from -30℃ to 350℃ (secondary desorption) and then maintained at this temperature for 1 min. The column was a AT-1 from Alltech (Portland, ME, USA), 60m × 0.32mm with 1.0μm film thickness. Oven temperature was kept at 30℃ for 10 min, increasing to 120℃ at a rate of 8℃/min and holding for 10 min, then increasing to 180℃ at a rate of 12℃/min and holding for 7 min, finally increasing to 230℃ at a rate of 15℃/min and holding for 10 min. Injector temperature was 230℃, while the detector temperature was 250℃. Helium (99.9999%) was used as the carrier gas, and a 20~350m/z mass range was recorded. (Table3)

Identification of compounds was based on comparisons of their mass spectra with those recorded in the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and Wiley (Hoboken, NJ, USA) database. Quantitative analysis of each identified volatile compounds (expressed as area percentage) was carried out by peak area normalization measurement.

2.6. Statistical analysis

2.6.1. Comparisons of precursors' effects on Maillard reaction

Three dimensional graphs were drawn by using Minitab (Version R16, Minitab Inc., Pennsylvania, USA) to compare the effect of each precursors with another on browning degree.

2.6.2. Statistical analysis on results of sensory evaluation

The results of sensory evaluation were subjected to analysis of variance (ANOVA) ($p<0.05$) by using SAS software (Ver. 9.3, Statistical Analysis System, U.S.A), and means were separated by using LSD multiple range test ($\alpha=0.05$).

Table 3. Operating conditions of headspace analysis

Parameters		Conditions
ATD 400	Primary tube type	Tenax-TA
	Cold trap type	Tenax-TA 20 mg
	1st Desorption	350℃ - 4 min
	2nd Cryo temp.	-30℃
	2nd Desorption	350℃ - 1 min
	Desorb flow	50.2 mL/min
	Inlet split	No
	Outlet split	11.5 mL/min
GC-MS	Oven temp.	35℃ - 10 min 8℃/min-120℃ - 10 min 12℃/min-80℃ - 7 min 15℃/min-230℃ - 10 min
	Column	AT1-60m×0.32mm×1.0μm
	Interface temp.	230℃
	Mass range	20~350 m/z
	Column pressure	15.9psi
	MS Det. temp.	250℃
	Carrier gas	Nitrogen (99.9999%)
	Mass filter type	Quadrupole

Results and discussion

3.1. Browning degree of reaction flavors

Absorbance increases with the browning degree of Maillard reaction (Ryu *et al*., 2003). Therefore, twelve reaction flavors were measured to know degree of browning, and the results are shown in Table 4. The range of absorbance is from 0.065 to 0.650. Degree of browning of sample 11 was high because it shows high absorbance (0.650).

3.2. Precursors' effects on Maillard reaction

Three dimensional graphs were drawn to compare the effect of each precursor with another on browning degree of reaction flavors. Some graphs which show remarkable results are shown in Fig. 1, and others are in Appendix. In these graphs, independent variables were precursors, and dependent variables were absorbance. As shown in the graph, effects of two precursors on Maillard reaction were compared. Absorbance of cysteine and thiamine were compared in Fig. 1-a. The value is as going to '-1', the amounts of cysteine and thiamine are decreased, and '1' is the opposite. The absorbance of cysteine was higher than thiamine's one, so thiamine was more effective than cysteine on Maillard reaction. Effect of thiamine was the

Table 4. Absorbance of reaction flavor

	1	2	3	4	5	6	7	8	9	10	11	12
Abs	0.457	0.506	0.172	0.069	0.072	0.397	0.277	0.476	0.214	0.144	0.650	0.085

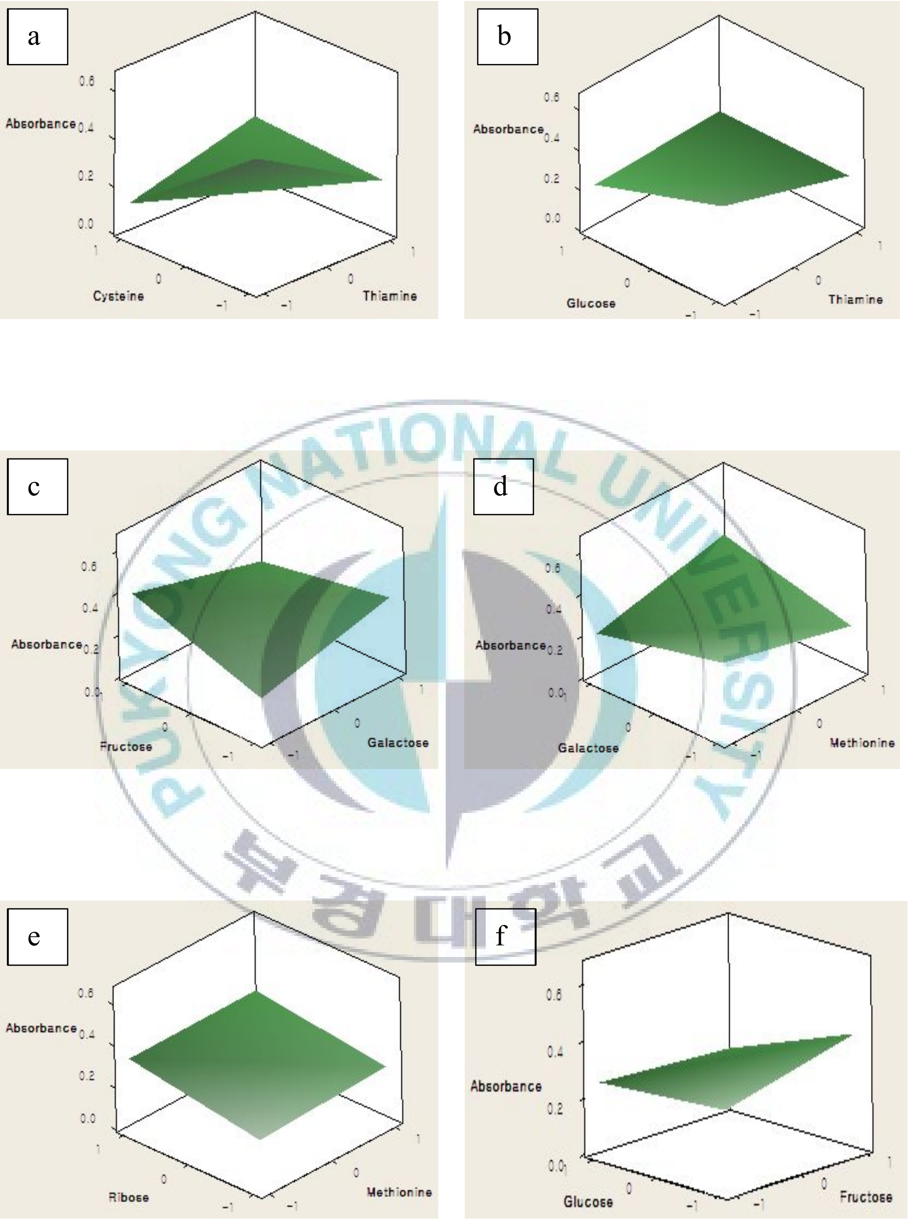

Fig. 1. Relationship of Maillard reaction effects on the browning degree between two compounds among sugars, amino acids, glucosamine and lecithin.

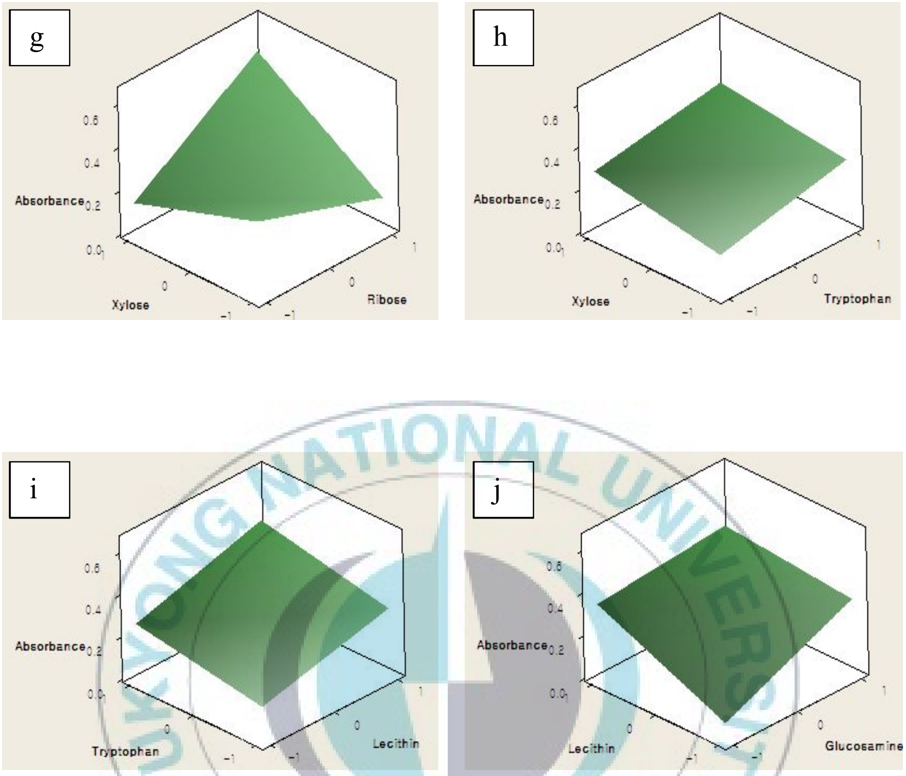

Fig. 1. (Continued).

same as glucose (Fig. 1-b), and fructose, galactose and methionine was higher than fructose (Fig. 1-c, d, e). Ribose and xylose had larger influences than methionine (Fig. 1-f, g), but tryptophan had higher influence than those (Fig. 1-h). Lecithin and glucosamine had the greatest influence among precursors (Fig. 1-i, j). In the compared results of 55 graphs, finally the order of influence on the Maillard reaction were cysteine < thiamine = glucose < fructose = galactose = methionine = ribose = xylose < tryptophan < lecithin = glucosamine.

3.3. Results of sensory evaluation

The results of sensory evaluation for the twelve reaction flavors were analyzed by using ANOVA and multiple comparisons. Significant differences were observed from the results and were shown in Table 5. In the case of ‘fishy smell’, there was no significant difference. The scores of ‘burnt flavor’ were lower than those of other evaluations. Sample 5 had the highest score for fishy smell, and Sample 9 was evaluated as having a strong meat flavor. Sample 10 had the highest score for boiled egg smell. Sample 11 which was made with Maillard reaction of xylose, fructose, ribose, tryptophan, glucosamine and krill hydrolysate got the highest score for total preference. The range of score of total preference on reaction flavors is

Table 5. Results of sensory evaluation of twelve kinds of reaction flavor

Sample	Shrimp flavor	Meat flavor	Burnt flavor	Fishy smell	Boiled egg smell	Total preference
1	1.9±0.2^{bc}1)	3.1±1.2^{a}	2.7±0.7^{a}	4.0±2.8^{a}	4.4±3.0^{bc}	4.2±2.0^{abc}
2	3.7±1.1^{ab}	2.7±0.8^{ab}	2.0±1.4^{ab}	3.7±2.4^{a}	2.5±1.6^{cd}	4.2±1.6^{abc}
3	2.5±0.5^{bc}	1.1±0.3^{b}	1.7±0.9^{ab}	3.9±1.2^{a}	5.9±2.7^{ab}	2.5±0.7^{c}
4	1.7±0.2^{c}	2.0±1.0^{ab}	1.6±1.9^{ab}	3.9±1.1^{a}	5.8±3.2^{ab}	3.2±1.5^{abc}
5	1.9±0.3^{bc}	1.8±0.3^{ab}	1.4±1.3^{ab}	4.2±2.7^{a}	4.5±2.8^{bc}	2.7±0.8^{bc}
6	3.7±1.6^{ab}	3.1±1.2^{a}	1.4±0.8^{ab}	2.8±1.0^{a}	1.8±1.0^{d}	4.3±2.2^{abc}
7	2.4±1.2^{bc}	1.5±0.8^{ab}	1.7±1.9^{ab}	3.9±1.7^{a}	4.6±2.1^{bc}	3.0±1.2^{abc}
8	4.4±1.3^{a}	2.3±1.1^{ab}	1.5±1.1^{ab}	4.2±2.7^{a}	1.5±0.8^{d}	3.7±1.3^{abc}
9	4.5±2.0^{a}	3.2±1.0^{a}	1.4±1.3^{ab}	3.2±1.9^{a}	2.6±1.3^{cd}	4.5±2.5^{abc}
10	2.0±0.5^{bc}	2.4±1.3^{ab}	1.3±0.7^{ab}	3.2±1.6^{a}	7.4±2.2^{a}	3.0±1.6^{abc}
11	3.7±1.8^{ab}	2.0±1.3^{ab}	1.1±0.3^{b}	3.4±1.6^{a}	1.9±0.4^{d}	5.0±2.4^{a}
12	3.4±1.4^{abc}	2.4±1.1^{ab}	1.9±1.9^{ab}	3.5±1.4^{a}	1.4±0.7^{d}	4.7±2.3^{ab}

1) The different superscript letters within the same row for the average values mean significantly different at the $p < 0.05$ level of significance as determined by LSD test.

2.5~5.0 by natural fishy smell of krill. It is required to study on reaction flavor with precursors which can reduce the fishy smell to increase the degrees of preference in future.

3.4. Headspace volatile compounds of reaction flavors

Sample 5 (fishy smell), 9 (shrimp flavor and meaty flavor), and 10 (boiled egg smell) which were evaluated as having strong particular flavor were selected to analyze the headspace volatile compounds. Sample 11 was also analyzed by GC-MSD because it got the highest score for total preference. Total ion chromatograms of headspace volatile compounds in sample 5, 9, 10, 11 are shown in Fig. 2, and their identified volatile compounds are shown in Table 6.

A total of fifty-one volatile compounds were identified from four reaction flavors, and there were many aldehydes followed by other aliphatic compounds (Fig. 3). In sample 5, four aldehydes, five aliphatic compounds, one sulfur-containing compound, three furans and one alcohol were identified. Among them, acetaldehyde, hexane and ethanol are predominantly detected from krill hydrolysate (Choi, 2014). Especially, ethanol was abundantly detected. Benzothiazole, a sulfur-containing aromatic compound, was considered, which would be generated from

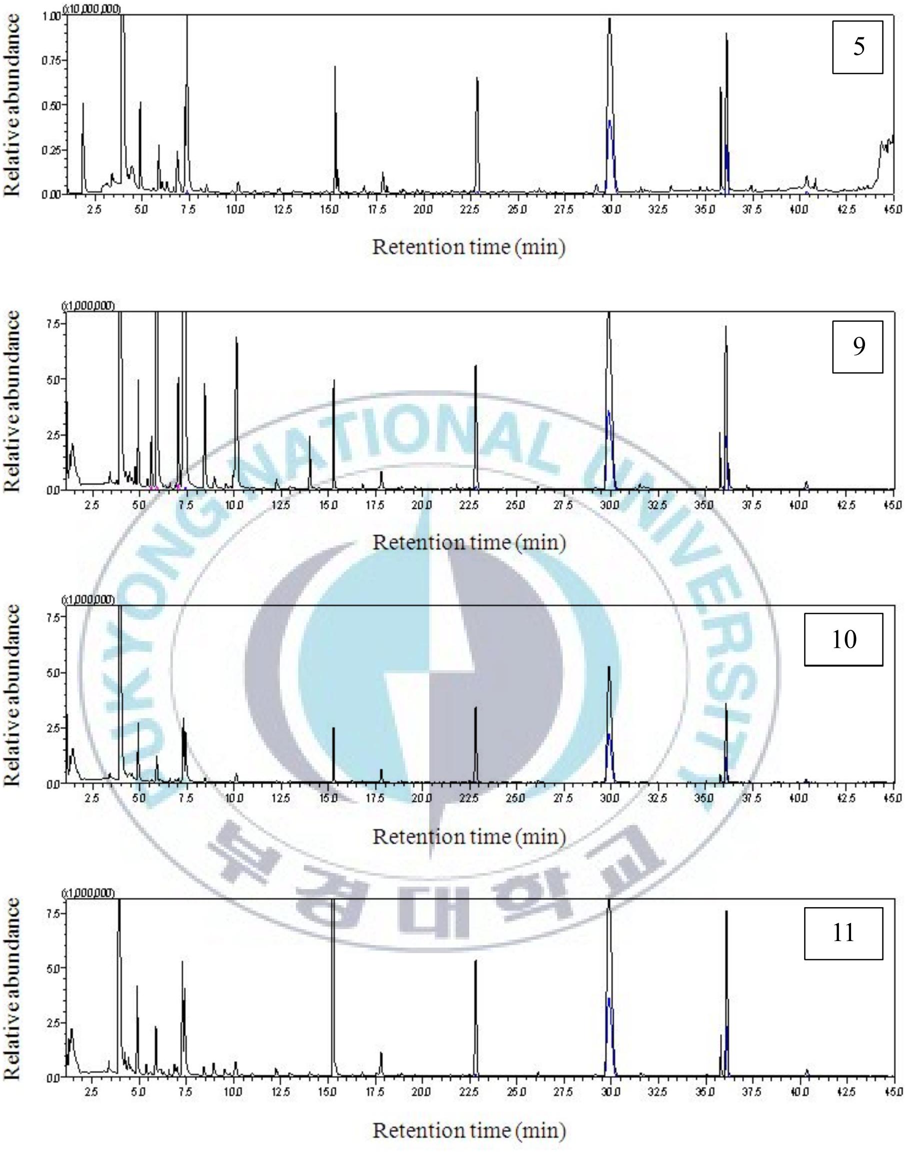

Fig. 2. Total ion chromatograms of sample 5, 9, 10, 11.

Table 6. Headspace volatile compounds identified from sample 5, 9, 10, 11 of reaction products

Compounds	RT1)	RI2)	Ratio of peak area (%)3)			
			5	9	10	11
Aldehydes (11)4)						
Acetaldehyde	3.40	418[a] 5)	0.52	0.78	0.54	0.79
Benzaldehyde	21.46	943[a]	0.25	0.55		
Nonanal	26.13	1079[a]	0.31		0.23	0.28
Decanal	31.54	1186[a]	0.32			
2-Hydroxybenzaldehyde	24.21	957[T] 6)		0.52		
Pentanal	16.28	687[a]				
2-Methylpropanal	5.38	647[b] 7)		0.33		0.57
Formaldehyde	3.12	<600[T]			0.11	
Hexanal	16.26	772[a]			0.18	
3-Methylbutanal	8.95	647[b]		0.61		1.03
2-Methylbutanal	9.55	645[c] 8)		0.47		0.61
Sub total			1.40	3.26	1.06	3.28
Aliphatic compounds (10)						
3-Methylpentane	6.32	<600[T]	0.82			
Hexane	6.87	600[a]	3.17		0.26	0.72
2-Methylhexane	10.44	639[a]				
3-Methylhexane	11.00	641[a]				
Heptane	12.32	700[a]	0.32			0.24
6-Methyloctadecane	25.02	967[T]	0.30		0.09	0.09
Toluene	15.27	756[a]	5.86	7.30	5.70	31.97
Dodecane	35.06	1200[a]			0.10	
Tridecane	39.91	1300[a]			0.06	
Nonane	19.92	900[a]		0.26		
Sub total			10.47	7.56	6.21	33.02

Table 6. (continued)

Compounds	RT[1)]	RI[2)]	Ratio of peak area (%)[3)]			
			5	9	10	11
Sulfur-containing compounds (2)						
Benzothiazole	33.14	1223[a]	0.42			
Dimethyl disulfide	14.01	725[a]		2.31		0.34
Sub total			0.42	2.31		0.34
Furans (3)						
2-Methylfuran	7.03	603[b]	0.74			
2-Ethylfuran	12.24	700[b]	0.25	0.85	0.19	0.44
Furan	4.44	<600[b]		2.11		0.77
Sub total			0.99	2.96	0.19	1.21
Ketones (5)						
2-Hexanone	4.26	789[b]				
1,1-Dimethoxy-3-butanone	6.13	<600[T]				
3-Hydroxy-2-pentanone	4.45	<600[T]			0.63	
2-Propanone	4.24	<600[T]		1.93		0.62
2-Butanone	6.49	<600[T]		1.10		
Sub total			0.00	3.03	0.63	0.62
Acids (4)						
4-Acetylbutyric acid	18.88	740[T]			0.15	0.15
Acetic acid	7.62	710[a]		1.06		
Propanoic acid	13.26	651[a]		0.83		
Butanoic acid	16.78	729[T]		1.18		
Sub total			0.00	3.07	0.15	0.15
Esters (2)						
Formic acid, ethyl ester	3.48	<600[b]			0.08	
Acetic acid, ethyl ester	7.44	614[b]		1.40	5.08	5.75
Sub total			0.00	1.40	5.16	5.75

Table 6. (continued)

Compounds	RT1)	RI2)	Ratio of peak area (%)3)			
			5	**9**	**10**	**11**
Alcohols (2)						
Ethyl alcohol	3.95	649^{a}	69.05	70.62	85.74	53.54
2-ethyl-1-Butanol	10.99	680^{T}				0.18
Subtotal			69.05	70.62	85.74	53.72
Others (12)						
2-(1,1-Dimethylethyl)-phenol	34.69	1195^{T}	0.15			
Ethyl acetate	7.38	602^{a}	12.79			
1-Methoxy-2-propyl acetate	18.87	740^{T}	0.26	0.47		
Triacetin	35.79	1306^{c}	3.93	4.35	0.39	1.62
Benzene	9.80	648^{a}				
2,3-Dicyanopropionamide	14.01	712^{T}				
Methoxyethene	6.30	$<600^{T}$			0.08	
4-(Bromomethyl)cyclohexene	16.65	728^{T}			0.08	
2-(2-butoxyethoxy)-Ethanol acetate	36.36	1226^{T}		0.97		
Methylpyrazine	17.58	811^{a}				0.30
Ethyl ether	4.57	515^{a}	0.53		0.30	
Subtotal			17.66	5.79	0.85	1.92

1), 2) RT and RI stand for retention time and Kovat's retention index, respectively.

3) Identified volatile components are showed in relative ratio thereby total peak is 100 percent.

4) Numbers in the parenthesis represent the number of volatile compounds in each class.

5) Superscript of "T" means "tentatively identified with by only matching MS spectrum with reference one".

6), 7), 8) The references are a: flavornet; b: the NIST webBook; c: Vcf

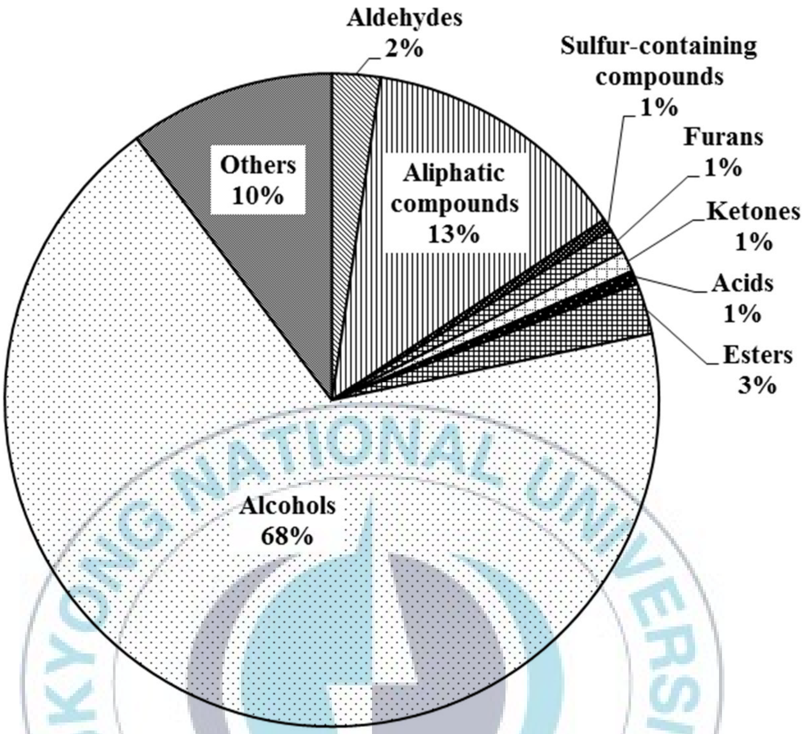

Fig. 3. Composition of volatile compounds of sample 5, 9, 10, 11.

cysteine and thiamine, and can be made from Strecker degradation (Li *et al*., 2012). Moreover, a little amount of furans from Maillard reaction were detected (Fors, 1983). Decanal, which is reported to produce a fishy smell (Varlet *et al*., 2006), is considered to effect on results of sensory evaluation despite of small amounts. In sample 9, six aldehydes, two aliphatic compounds, one sulfur-containing compound, two furans, two ketones, three acids and one ester were identified. In those compounds, there are the volatile compounds detected in krill hydrolysate. Because methionine and thiamine were added to sample 9, dimethyl disulfide was identified. This compound was previously identified in fish, meat and milk (Herbert and Shewan, 1976; Jensen *et al*. 2002; Zahir, 2014). Among volatile compounds of sample 10, there were five aldehydes, four aliphatic compounds, one sulfur-containing compound, two furans, one ketone, one acid and two alcohols. Some volatile compounds from krill hydrolysate were detected from sample 10. It can be predicted that lecithin, which was precursor of sample 10, affects the boiled egg smell. Besides, sulfur-containing compounds were considered to be produced by cysteine. Among 2-methylbutanal and 3-methylbutanal was reported that are produced by Maillard reaction through Strecker degradation during heating (Siegmund and Murkovic, 2004). Finally, in sample 11, four aldehydes, five aliphatic compounds, one furan, one ketone, one acid, two esters and one alcohol were identified. It also had volatile compounds, which is in krill hydrolysate, and ethanol was abundantly detected. This compound is known to contribute to the characteristic flavor of fruits and vegetables (Hui *et al*., 2010; Maarse, 1991), and makes sweety flavor (Mian N, 2014). Therefore, it is considered to have an effect on degrees of preference of sample 11.

Conclusion

Antarctic krill is considered as a future food resource of protein for essential amino acids, minerals and polyunsaturated fatty acids, so it has been studied to be used as various types of food in the world. The objectives of this study were to develop the reaction flavor using Antarctic krill, to investigate effects of precursors for Maillard reaction and to study the formation mechanism of volatile compounds depending on the reaction precursors. Twelve reaction flavors were carried out by using krill hydrolysate and eleven precursors. Their products were evaluated by their absorbance, sensory evaluation and headspace volatile compounds analysis. The precursors of reaction flavor effected on Maillard reaction according to order in 'cysteine < thiamine = glucose < fructose = galactose = methionine < ribose = xylose < tryptophan < lecithin = glucosamine'. By sensory evaluation, the best recipe of reaction flavor was xylose, fructose, ribose, tryptophan, glucosamine and krill hydrolysate. Among total fifty-one compounds identified from the reaction flavor, there were eleven aldehydes, ten aliphatic compounds, two sulfur-containing compounds, three furans, five ketones, four acids, two esters, two alcohols which are thought to be closely related with flavor qualities.

It is required to the study on the improvement of fish smell in reaction flavor in the future, and application of reaction flavor as a flavoring agent to food products.

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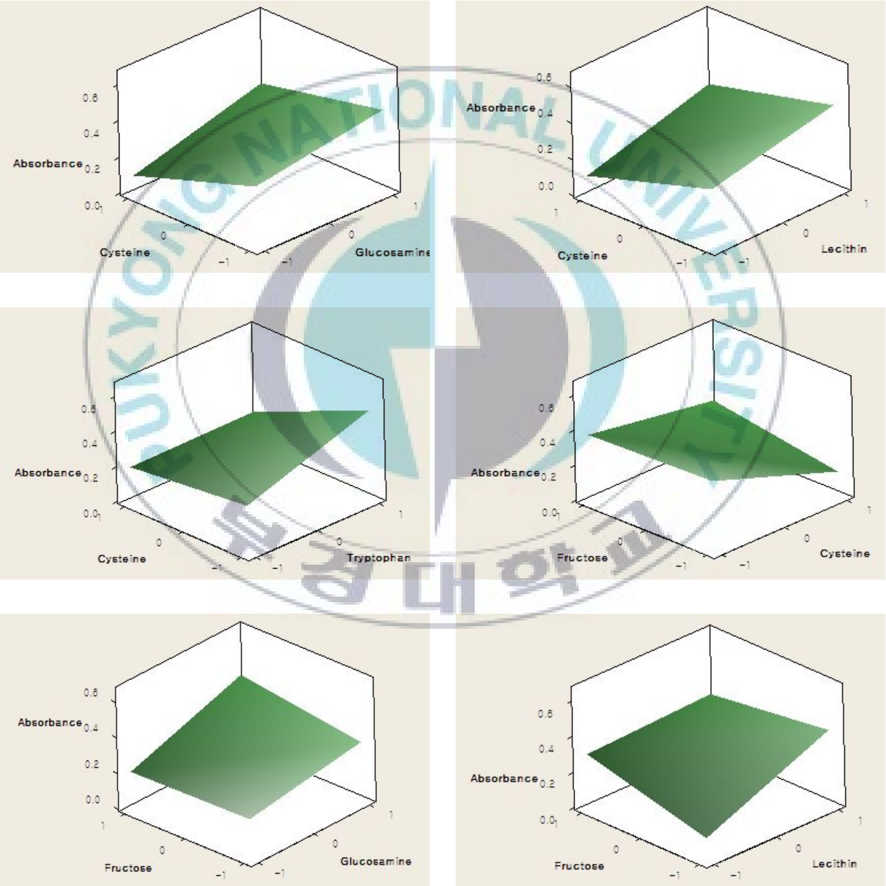
Appendix

Appendix 1. Additional relationship of Maillard reaction effects on the browning degree between two compounds among sugars, amino acids, glucosamine and lecithin



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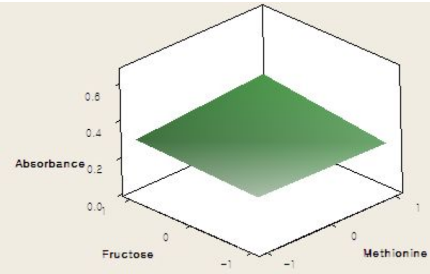

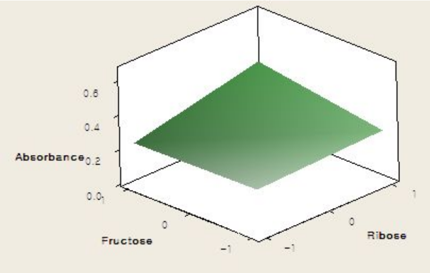

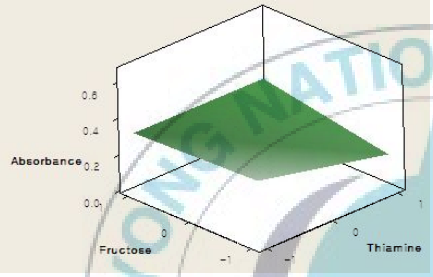

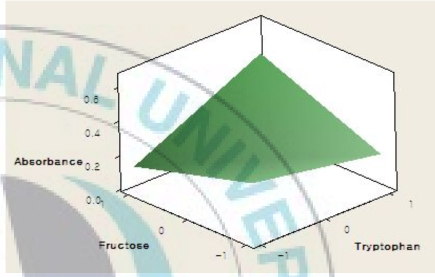

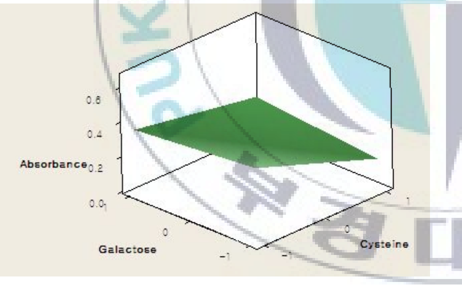

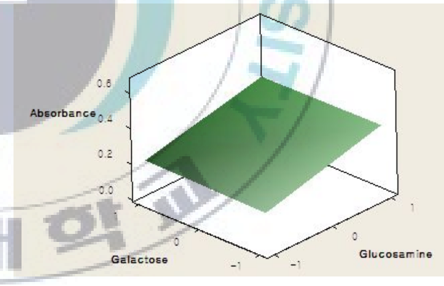

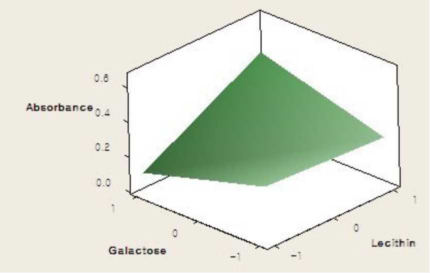

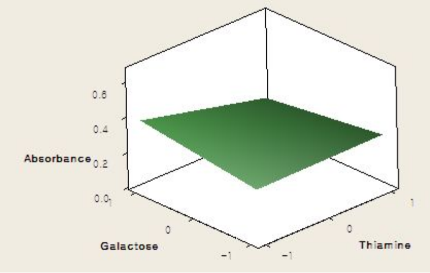

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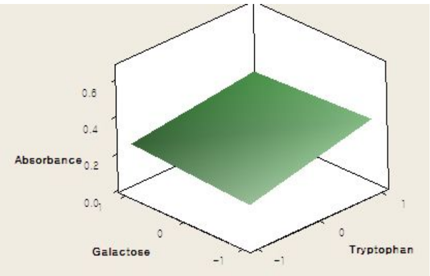

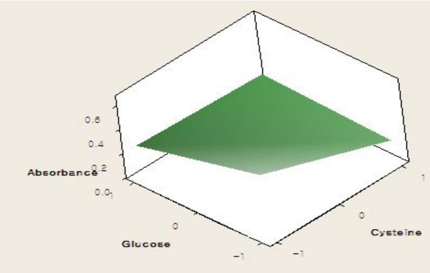

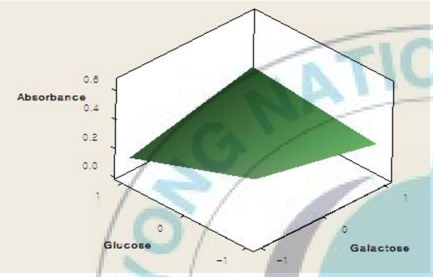

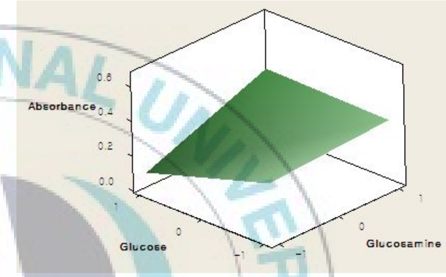

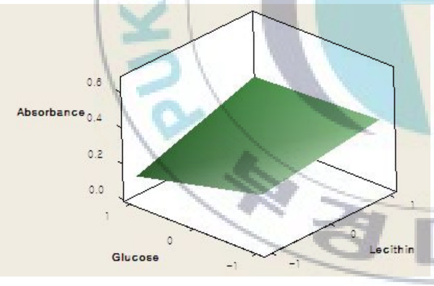

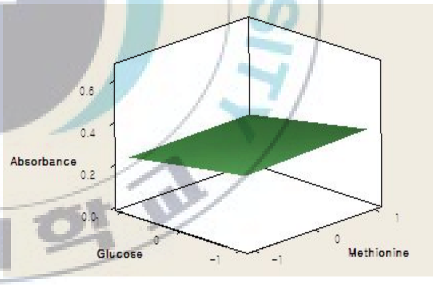

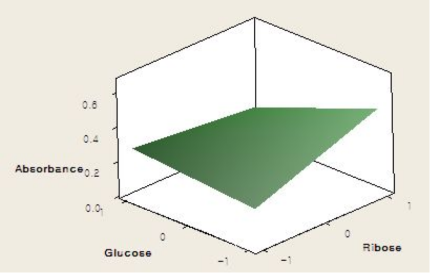

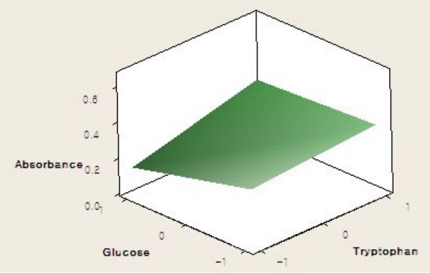

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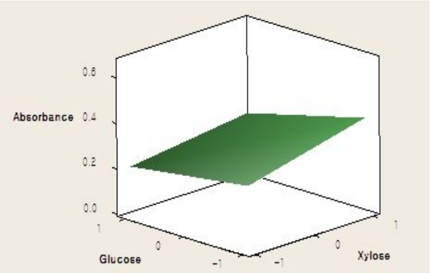

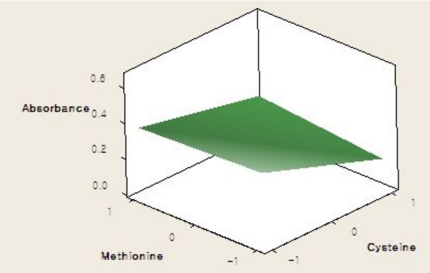

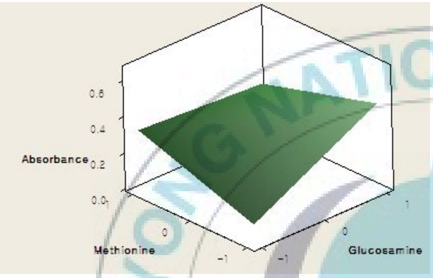

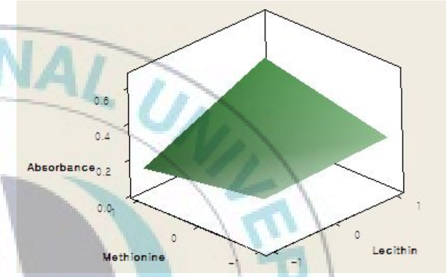

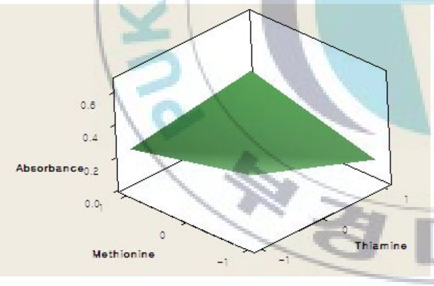

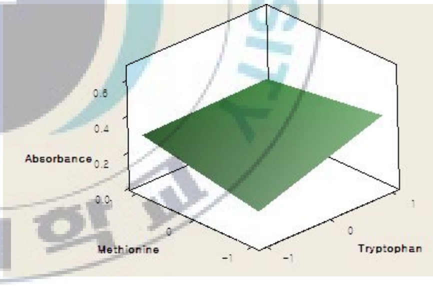

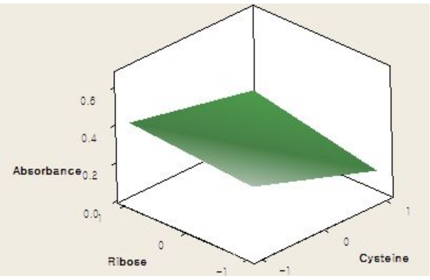

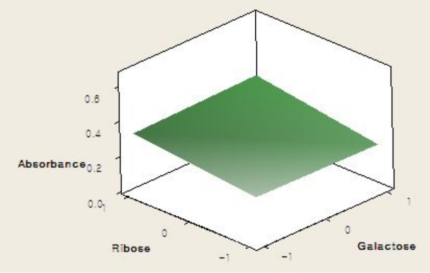

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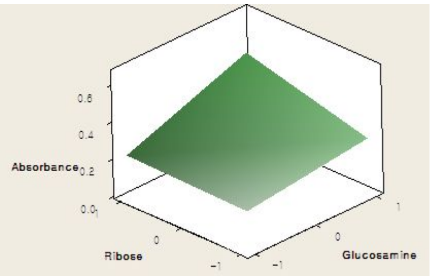

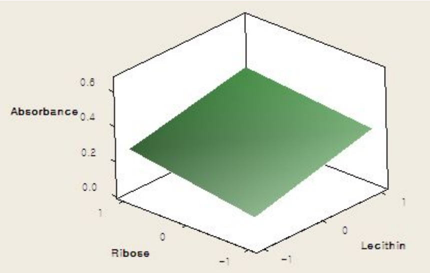

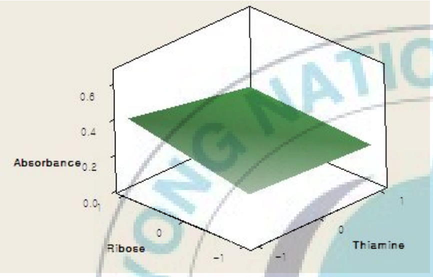

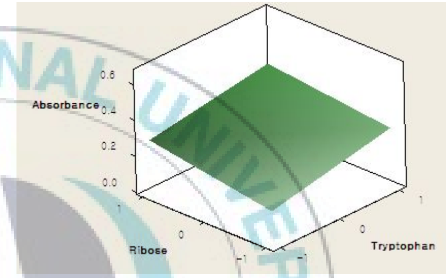

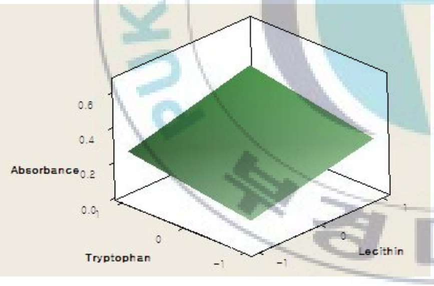

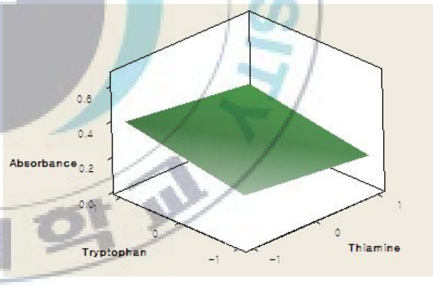

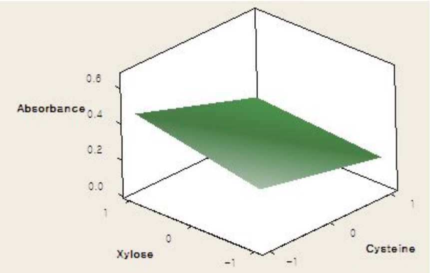

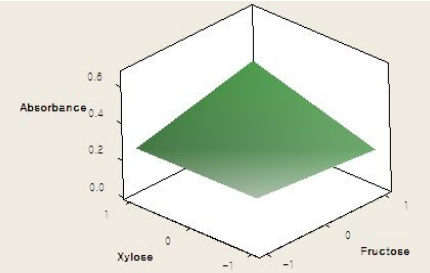

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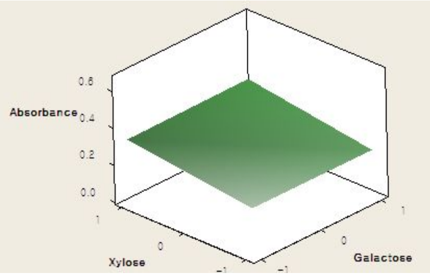

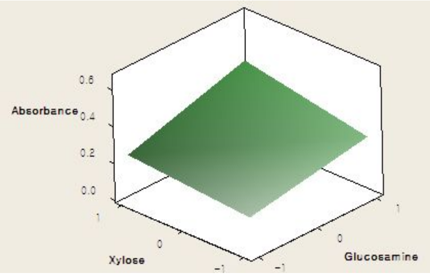

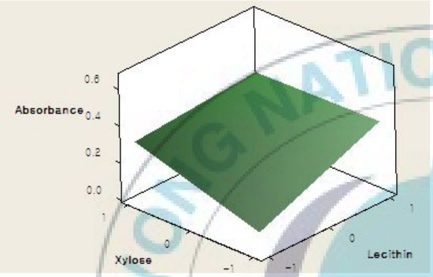

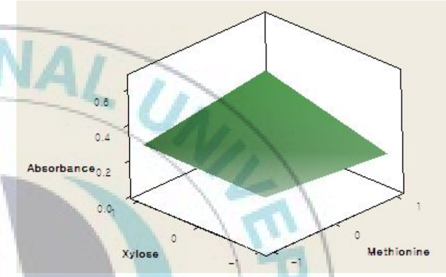

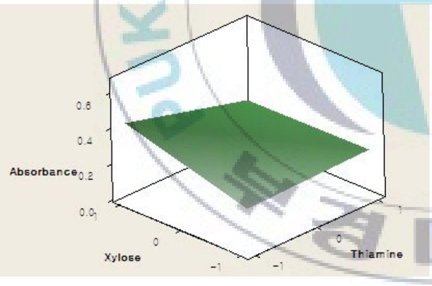

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가장 먼저 저를 무사히 졸업으로 이끌어주시고, 실수를 하여도 웃음으로 넘겨주시던 자상한 저의 지도교수님인 이양봉 교수님께 진심으로 감사의 인사를 올립니다. 부경대학교라는 새로운 환경에서 실험실 생활을 한번도 해보지 못한 저에게 교수님께서는 너그러이 대해주시고, 서두르지 않고 차근차근 가르침을 주셔서 저는 부담감을 덜고 빠르게 적응할 수 있었습니다. 정말 감사합니다. 또한 논문을 감수해주시고 조언을 아끼지 않으신 김선봉 교수님과 양지영 교수님께 진심으로 감사를 드립니다. 그리고 많은 가르침으로 이끌어 주신 조영제 교수님, 전병수 교수님, 안동현 교수님, 김영목 교수님께도 깊은 감사를 드립니다.

함께 석사과정을 지내오면서 뒤에서 묵묵히 도와주었던 올해 서른살이지만 나이가 믿기지 않는 진용이오빠, 비밀을 털어놓고 이야기할 수 있어서 좋았고 앞으로도 쭉 연락하고 싶은 미경이, 동갑이지만 아들 같아서 옷, 머리 등을 봐주었던 창민이, 성공한 끼쟁이 종봉이, 사기 그만 당하길 바라고 곧 토익이 특기가 될 익식이, 같

은 생일이면서 내 스토커인 은주, 댄싱머신이면서 자기 닮은 개 키우는 소희, 석사가 꿈이면서 인턴을 잘할 것 같은 현희, 할 말 다 해서 맘에 드는 패션피플 다희, 석사과정 중에 친해진 외국인 친구 Shanika와 Ritchie, 이제 석사를 시작할 수경이와 지은이 파이팅, 2년간의 룸메이트이자 많이 도와주고 챙겨주었으며, 친언니 같고 벌써 보고 싶은 효정이언니, 항상 웃으면서 맞이 해주는 식품화학 실험실의 연이언니와 수빈이, 은희, 학회 준비를 하면서 부쩍 친해진 현진이, 그리고 졸업한 생화학실험실 선배님들 모두에게도 그동안 도움을 많이 받아 고마운 마음을 전하고 싶습니다.

마지막으로 오빠 같은 우리 동생 신성아 누나 많이 챙겨줘서 고맙고 상담 많이 해줄 테니 얼른 연애하길 바란다. 그리고 애교 없는 딸이지만 많이 사랑해주고 버팀목이 되어주신 부모님, 부끄러워서 직접 말은 못했었지만 마음은 항상 감사하고 사랑합니다. 꼭 성공해서 부모님의 자부심이 되겠습니다.