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Development of Boiled-type Shrimp Flavor by Maillard Reaction and Sensory Evaluation

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Abstract

Boiled-type shrimp flavor was developed using Maillard reaction to reduce or mask fish odor or off-flavor in seafood. Model systems were created using enzymatic hydrolysate of shrimp and adding precursor compounds to increase flavor quality and stability. Amino acid precursors of cysteine and methionine and sugar precursors such as glucose, xylose, ribose and sucrose were tried and their flavor qualities were tested by sensory evaluation. After the optimal precursors were determined, the optimum reaction condition was investigated using pHs of 5, 6, 7, and 8 and reaction times of 1, 2 and 3 hours. The best precursors for boiled-type shrimp flavor were methionine and sucrose. The optimum reaction condition was pH 8.0 and a one hour reaction time.

Key words: boiled-type shrimp flavor, reaction flavor, Alcalase, Maillard reaction, sensory evaluation

INTRODUCTION

Flavor is defined as the comprehensive and complicated sensation of taste, smell, texture, stimulus, and thermal sensation, or as the materials of having these effects. Food flavoring is widely used with diverse food products, including soup sauces, fish paste products and other processed products. The first function of food flavor, as a required factor, is to improve food preference, to impose new image onto food, to improve value of food, and to create and develop new food products. The second function of flavor is to strengthen, reinforce, and standardize natural food flavor, to mask off-smell and processed smell of food products themselves and, finally, to strengthen the natural good flavor and to improve food preference. Therefore, flavor is a required factor in creating and improving food taste. Several flavor technologies for the revival of natural flavors and masking of off-smells have been used in order to satisfy the diverse demands of consumers (1).

Maillard reaction of non-enzymatic browning reaction is produced by heating during cooking and baking. Complicated and mixed flavor compounds are involved in the Maillard reaction. Processed flavor or reaction flavor that results from natural processing methods can be created by heat treatment of the precursors that exist as natural components (2). This technology has been widely used for processing meat aroma and savory aroma in flavor the industry (3,4). Many papers on reaction flavors of reaction precursors have been reported. Mainly, these papers discuss the model systems of reaction precursors to help our understanding of the formation mechanism of flavor compounds. Some papers investigated the processing of reaction flavor. Jang et al. (5) processed reaction flavor with enzymatic hydrolysate of dark muscle of tuna and reaction precursors. Kim and Kim (6) developed meat flavor extract by using non-enzymatic browning reaction of reaction precursors. Kim and Beak (7) developed baked beef flavor by using the technology of reaction flavor. However, few papers studied reaction flavor with enzymatic hydrolysate of seafood and precursors.

The main objective of this study was to optimize the precursors and reaction conditions of the Maillard reaction that would produce the best shrimp flavors, using enzymatic hydrolysate of shrimp to enhance the seafood flavor and to mask fishy odor and off-flavor that are released from shrimp itself. Another objective of this study was to determine the best precursors for developing the boiled-type shrimp flavor.

MATERIALS AND METHODS

Materials

The shrimps used for this study were the spotted shrimp (*Trachysalambria curvirostis*), caught near Korean seas.

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Table 1. Experimental design upon the addition of precursors for boiled-type shrimp flavor

	Ingredient (g)	A	B	C	D	E	F
Addition of amino acid	Cysteine·HCl	0.3	−	0.15	0.3	−	0.15
	Methionine	−	0.3	0.15	−	0.3	0.15
	Water	50.0	50.0	50.0	−	−	−
	Shrimp hydrolysate	−	−	−	50.0	50.0	50.0
	Glucose	0.3	0.3	0.3	0.3	0.3	0.3
	Total	50.6	50.6	50.6	50.6	50.6	50.6

	Ingredient (g)	A	B	C	D	E	F	G	H
Addition of reducing sugar	Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	Glucose	0.3	−	−	−	0.3	−	−	−
	Xylose	−	0.3	−	−	−	0.3	−	−
	Ribose	−	−	0.3	−	−	−	0.3	−
	Sucrose	−	−	−	0.3	−	−	−	0.3
	Water	50.0	50.0	50.0	50.0	−	−	−	−
	Shrimp hydrolysate	−	−	−	−	50.0	50.0	50.0	50.0
	Total	50.6	50.6	50.6	50.6	50.6	50.6	50.6	50.6

Their prices were relatively cheap. Their length and weight were 5～6 cm and 3～4 g, respectively. The fresh shrimp were purchased from a local market and freeze-dried until time for the experiments.

Procedure of enzymatic hydrolysate

Enzymatic hydrolysate of the shrimp was made by a slightly modified method from Oh (8). Six grams of the spotted shrimps were chopped and the chopped samples were put into 40 mL distilled water. The autolytic enzyme of the shrimp solution was inactivated by heating at 85°C for 15 min. Its pH was adjusted to the certain values with 2M HCl or NaOH. The 40 μL commercial protease of Alcalase 2.4LFG (Novozymes Korea Ltd., Seoul, Korea) that was kept at 5°C was added. Then, the sample solution was hydrolyzed for 30 min. The hydrolyzed shrimp solution was inactivated by heating at 85°C for 15 min. The solution was centrifuged at 4,000 rpm for 40 min. The supernatant was used for the reaction flavor.

Preparation of the mixture of reaction precursors

The mixing ratio of cysteine·HCl and methionine for boiled-type reaction flavor is shown in Table 1. Their effects on the shrimp's reaction flavor were compared by sensory evaluation. For each step of sensory evaluation, the best precursors of amino acid and sugar which have the highest total sensory score and the closest boiled shrimp flavor were chosen. The reaction condition of model systems for determining precursors was 100°C reaction temperature of water boiling point and a 1:1 ratio of amino acid and sugar. The reaction time and pH for model systems were 2 hr and pH 7.0, respectively. The reaction solution was distilled water or enzymatic hydrolysate.

Processing condition

The processing conditions of the precursors determined for boiled shrimp flavor from model systems were investigated by Table 2. The reaction pHs were pH 5.0, 6.0, 7.0, and 8.0. The optimal pH was chosen by sensory evaluation. At the determined pH, the reaction times tested were 1 hr, 2 hr, and 3 hr.

Sensory evaluation

Sensory evaluation was carried out to investigate the best reaction conditions for boiled shrimp flavor. The sensory panelists were 9 students of Pukyong National University. The sensory time was mainly carried out at about 4 pm in order to increase sensory sensitivity. Each sample was provided in a transparent container (5.0 cm inside diameter×7.0 cm height). Each panel directly smelled the sample and then tested it again with a smelling blotter. Its evaluation was 9 grade method: 1 point is very weak, 5 points is normal, 9 points is very strong. The statistical analysis of these data was done by apply-

Table 2. Experimental design for the optimum reaction condition of boiled-type shrimp flavor

Ingredient (g)	Reaction pH				Reaction time (hrs)		
	5.0	6.0	7.0	8.0	1	2	3
Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Shrimp hydrolysate	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Total	50.6	50.6	50.6	50.6	50.6	50.6	50.6

ing a randomized complete block design (9). Their multiple comparisons were carried out with Duncan method at the significance level of α=0.05 (10).

RESULTS AND DISCUSSION

Reaction flavor for boiled-type shrimp flavor can be made by adding the precursors of amino acid and sugar which are substrates for Maillard reaction. Also, in seafood type reaction flavor, sulfur-containing compounds are important, just as in meat type reaction flavor (11). To increase these sulfur-containing compounds, cysteine·HCl and methionine were investigated as the amino acid precursors. For sugar precursors, glucose, xylose, ribose, and sucrose were investigated. The temporary reaction condition was 2 hr at 100°C.

Determination of amino acid precursors

The quality of the reaction flavors upon the addition of cysteine·HCl and methionine into model system and shrimp hydrolysate was tested by sensory evaluation and their results were shown in Table 3.

In the sensory results for the model systems of reaction precursors, the reaction flavor of cysteine·HCl precursor showed the very strong sulfuric pungent score of 7.6 points, and the reaction flavor of methionine precursor got the high score of 6.7 points in boiled potato smell. Cysteine·HCl, as sulfur-containing amino acid, is reported to produce mercapto compounds by Strecker degradation in Maillard reaction. These mercapto compounds have a pungent aroma and are reported to help the formation of meat flavor (12,13). Methionine produces methional by Strecker degradation and methional is known to be produced by boiling potatoes and vegetables (12).

In the results of flavor quality of reaction flavors upon the addition of cysteine·HCl and methionine into the enzymatic hydrolysate of spotted shrimp, the sensory scores were 4.4 points for sulfuric pungent and 4.7 points for total preference. The sensory scores for the reaction flavor of adding methionine were 3.0 and 6.7 points in boiled potato smell and total preference, respectively. In the case of adding the 1:1 ratio of cysteine·HCl and methionine, the sensory score of total preference was 5.8. Therefore, reaction flavor of seafood type scored higher sensory points with the addition of methionine compared to the addition of cysteine·HCl, showing a different result from the reaction flavor of meat type. Methionine has been shown to be the preferred amino acid precursor to make boiled-type shrimp flavor.

Table 3. Sensory results of boiled-type shrimp flavor upon the addition of amino acid precursors

Category	Model system[1)]					
	1	2	3	4	5	6
Total preference	1.9[d2)]	6.2[ab]	3.1[cd]	4.7[bc]	6.7[a]	5.8[b]
Boiled potato	1.2[b]	6.7[a]	2.4[b]	2.3[b]	3.0[b]	2.1[b]
Boiled vegetable	1.0[b]	3.7[a]	2.0[ab]	1.9[ab]	2.6[a]	1.8[b]
Sulfuric pungent	7.6[a]	2.1[d]	6.3[ab]	4.4[bc]	2.1[d]	3.7[c]
Boiled shrimp	1.1[c]	3.4[b]	1.3[c]	4.6[b]	5.9[a]	5.8[a]
Grilled shrimp	1.2[c]	4.1[b]	1.2[c]	5.2[ab]	7.2[a]	5.9[a]
Balance of flavor	2.0[c]	5.6[ab]	2.4[c]	4.9[b]	6.8[a]	5.8[a]

[1)]1: 0.3 g cysteine+0.3 g glucose+50 mL water, 2: 0.3 g methionine+0.3 g glucose+50 mL water, 3: 0.15 g cysteine +0.15 g methionine+0.3 g glucose+50 mL water, 4: 0.3 g cysteine+0.3 g glucose+50 mL enzymatic hydrolysate of shrimp, 5: 0.3 g methionine+0.3 g glucose+50 mL enzymatic hydrolysate of shrimp, 6: 0.15 g cysteine+0.15 g methionine+0.3 g glucose+50 mL enzymatic hydrolysate of shrimp.

[2)]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 Duncan's multiple range test.

Determination of sugar precursors

The quality of reaction flavors upon the addition of glucose of hexose, xylose and ribose of pentose, sucrose of disaccharide into the model system and shrimp hydrolysate was tested by sensory evaluation and the results are shown in Table 4. In the sensory results for the model systems of reaction precursors, the reaction flavors of adding glucose, xylose, ribose and sucrose with methionine were 2.1, 3.9, 3.6 and 3.5 points, respectively, for total preference. The total preference results of reaction flavors upon the addition of sugars with methionine into enzymatic hydrolysate of spotted shrimp, were 5.5, 6.0, 6.3 and 7.6 points for glucose, xylose, ribose and sucrose, respectively. When sucrose was used as a sugar precursor, the total balance of aroma was better than that of ribose. Therefore, sucrose was determined to be the preferred sugar precursor for making boiled-type shrimp flavor.

Determination of optimum processing condition

The quality of reaction flavor mainly depends on the precursors and reaction conditions. Among several factors of reaction conditions, pH and time are known to be important. Mottram and Whitfield (14) reported that the pH in meat was an important factor on the formation of sulfur-containing compounds and pyrazine compounds. To decide the optimum pH for boiled-type shrimp flavor, pHs of 5, 6, 7, and 8 were investigated under the reaction conditions of 100°C and 2 hr. Then, at the optimal pH, the optimum reaction time was chosen by sensory evaluation at the reaction hours of 1, 2 and 3 hours.

Determination of reaction pH

The quality of reaction flavors that were made at sev-

Table 4. Sensory results of boiled-type shrimp flavor upon the addition of reducing sugar precursors

Category	Model system[1)]							
	1	2	3	4	5	6	7	8
Total preference	$2.3^{d2)}$	3.1^{cd}	3.6^{bcd}	3.5^{c}	5.5^{abc}	6.0^{abc}	6.3^{ab}	7.6^{a}
Boiled potato	5.3^{ab}	5.8^{a}	6.0^{a}	6.0^{a}	1.9^{c}	1.6^{c}	2.4^{bc}	1.6^{c}
Boiled vegetable	3.8^{ab}	4.6^{a}	4.8^{a}	4.6^{a}	1.6^{b}	1.5^{b}	1.6^{b}	1.5^{b}
Sulfuric pungent	1.8^{a}	2.0^{a}	2.6^{a}	2.8^{a}	1.1^{c}	1.9^{a}	1.3^{a}	1.3^{a}
Boiled shrimp	1.9^{b}	1.9^{b}	1.9^{b}	1.9^{b}	5.5^{a}	5.1^{a}	5.5^{a}	7.0^{a}
Grilled shrimp	1.6^{c}	1.8^{c}	2.0^{c}	2.3^{c}	5.0^{b}	5.8^{ab}	6.4^{ab}	7.6^{a}
Fishy smell	1.4^{a}	1.5^{a}	1.8^{a}	1.8^{a}	2.8^{a}	2.8^{a}	2.8^{a}	2.4^{a}
Balance of flavor	3.0^{b}	4.0^{ab}	3.8^{ab}	4.3^{ab}	5.4^{ab}	5.9^{ab}	6.0^{ab}	7.4^{a}

[1)]1: 0.3 g methionine + 0.3 g glucose + 50 mL water, 2: 0.3 g methionine + 0.3 g xylose + 50 mL water, 3: 0.3 g methionine + 0.3 g ribose + 50 mL water, 4: 0.3 g methionine + 0.3 g sucrose + 50 mL water, 5: 0.3 g methionine + 0.3 g glucose + 50 mL enzymatic hydrolysate of shrimp, 6: 0.3 g methionine + 0.3 g xylose + 50 mL enzymatic hydrolysate of shrimp, 7: 0.3 g methionine + 0.3 g ribose + 50 mL enzymatic hydrolysate of shrimp, 8: 0.3 g methionine + 0.3 g sucrose + 50 mL enzymatic hydrolysate of shrimp.

[2)]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 Duncan's multiple range test.

eral reaction pHs was evaluated and the sensory results are shown in Table 5. In the sensory results of reaction flavors for boiled-type shrimp flavor, the sensory scores in total preference were 1.7, 2.0, 5.6 and 7.0 at reaction pH 5, 6, 7, and 8, respectively. These results show a big difference between acidic pHs (pH 5 and 6) and neutral or alkali pHs (pH 7 and 8). This big difference is thought to be due to the difference of intermediates, which are formed differently from the change of pH, so reaction pH is important for Maillard reaction and flavor formation. Mottram and Whitfield (14) reported that the types of volatile compounds formed depended on reaction pH in a meat-like Maillard system, showing that the reaction system at a neutral pH produced more sulfur-containing compounds and pyrazine compounds than that at acidic pH. The reaction flavors of model systems at pH 7.0 and pH 8.0 showed higher scores in total preference. The model system of pH 8.0 had a little better quality of less off-flavor in chemical flavor. Therefore, the optimum reaction pH for boiled-type shrimp flavors was thought to be pH 8.0.

Table 5. Sensory results of boiled-type shrimp flavor on reaction pH

Category	Reaction pH of model systems			
	pH 5	pH 6	pH 7	pH 8
Total preference	$1.7^{b1)}$	2.0^{b}	5.6^{a}	7.0^{a}
Boiled potato	1.4^{b}	2.2^{ab}	2.8^{a}	2.3^{ab}
Boiled vegetable	1.7^{a}	1.7^{a}	1.4^{a}	1.7^{a}
Sulfuric pungent	3.8^{a}	2.7^{ab}	2.1^{b}	2.0^{b}
Boiled shrimp	2.1^{b}	2.8^{b}	5.1^{a}	6.4^{a}
Grilled shrimp	2.3^{b}	3.2^{b}	6.1^{a}	7.4^{a}
Fishy smell	3.6^{a}	3.9^{a}	2.6^{a}	2.1^{a}
Chemical flavor	5.9^{a}	3.8^{ab}	3.6^{ab}	3.0^{b}
Soy sauce flavor	6.9^{a}	5.0^{a}	2.4^{b}	2.9^{b}
Balance of flavor	1.7^{b}	2.0^{b}	5.6^{a}	6.7^{a}

[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 Duncan's multiple range test.

Determination of reaction time

The quality of reaction flavors that were made at several reaction times was evaluated and the sensory results are shown in Table 6. In the sensory results of reaction flavors for boiled-type shrimp flavors, the sensory scores in total preference were 6.8, 6.8 and 5.9 points at reaction times of 1, 2 and 3 hr, respectively. There were no significant differences between 1 and 3 hr, so the optimum reaction time was determined to be 1 hr for boiled-type shrimp flavor. Also, as the reaction time of boiled-type shrimp flavor was increased, its flavor was changed to grilled-type shrimp flavor. Ko et al. (13) reported that browning materials and pyrazine compounds were increased as reaction time was increased. Based

Table 6. Sensory results of boiled-type shrimp flavor[1)] on reaction time

Category	Reaction time		
	1 hr	2 hr	3 hr
Total preference	$6.8^{a2)}$	6.8^{a}	5.9^{a}
Boiled potato	2.4^{b}	2.9^{ab}	4.4^{a}
Boiled vegetable	2.5^{a}	2.9^{a}	3.3^{a}
Sulfuric pungent	3.9^{a}	3.4^{a}	2.6^{a}
Boiled shrimp	6.6^{a}	5.3^{a}	5.0^{a}
Grilled shrimp	2.8^{a}	4.6^{a}	4.6^{a}
Fishy smell	4.5^{a}	3.5^{a}	3.4^{a}
Chemical flavor	4.9^{a}	3.0^{a}	2.9^{a}
Soy sauce flavor	3.8^{a}	3.6^{a}	3.9^{a}
Balance of flavor	6.4^{a}	6.6^{a}	5.9^{a}

[1)]The reaction pH was pH 8.0 of the optimum condition.

[2)]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 Duncan's multiple range test.

on this report, the change of boiled-type shrimp flavor to grilled-type shrimp flavor is thought to be due to the formation of pyrazine compounds of nutty aroma.

CONCLUSION

Boiled-type shrimp flavor was made at the optimum conditions of pH 8.0 and 100°C for 1 reaction time with the precursors methionine and sucrose. The flavor profile was completely different as the pH changed. The changes of the volatile compounds need to be investigated to explain the flavor changes. This boiled-type shrimp flavor can be used for seafood sauces, surimi and other related products. Also, grilled-type shrimp flavor can be made with different precursors and reaction conditions. With the data of grilled-type shrimp flavor, boiled-type shrimp flavor can be easily controlled.

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