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## Research of preparing seafood flavor applying maillard reaction products derived from the hydrolysate of mussel juice

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### ABSTRACT

Mussel juice, the byproduct of the mussel processing. Due to the limitation of traditional mussel processing, the residual mussel juice cannot be fully utilized. Meanwhile, the relevant optimizations about the mussel juice have not been reported yet. In this study, the optimum technology of mussel flavor product by using hydrolysates from mussel juice were investigated. Response surface methodology (RSM) was applied to define the thermal reaction conditions for improving the sensory evaluation of Maillard reaction products (MRPs), which was rare been used to preparing mussel juice seasoning in the published information. The contents of amino acids and volatile compounds in MRPs which prepared under the optimum conditions were determined by HPLC and GC-MS, respectively. Results illustrate that 17 kinds of amino acids were both detected in MRPs and original. Whereas, total amino acids and essential amino acids in MRPs revealed an increase by 314.95% and 423.62% respectively. Totally 52 volatile compounds were described by mass spectrum (MS) and relevant literature. Moreover, the pyrazines was accounted for 37.49% of total volatile compounds and made a great contribution to forming seafood flavor with caramel aroma. In conclusion, the investigations not only promote the deep processing and utilization of mussel byproduct, but also provide theory basis for developing mussel seasonings.

### KEYWORDS

Mussel juice; Enzymatic hydrolysate; Maillard reaction products; Volatile components.



## INTRODUCTION

Mussel, which belong to the family *Mytidea* (*Mollusca*, *Lanellibranchia*) are distributed all over the world, especially occurring in Japan, China, and Korea. It has been reported that mussels contain rich protein, essential amino acids, taurine, DHA, EPA and other unsaturated fat<sup>[1]</sup>. In the past few years, the enzymatic hydrolysates from mussel meat protein have aroused concern because of their significant biological activities, such as anti-cancer activity, reinforcing immunity function<sup>[2]</sup>, antihypertensive function<sup>[3]</sup> and antimicrobial activity<sup>[4]</sup>. Obviously, improving the utilization of protein resource from marine shellfish have become a research hotpot nowadays.

Although considerable attention have been paid to acquire the bioactive proteins and peptides from mussels, the available literature shows that fewer studies have been done with aspect to the by-products further processing from mussel than oyster and scallop<sup>[5-6]</sup>. On the other hand, traditional mussel processing was limited by the immature processing technique and mechanization. Thus, during industrial processing for meat, there are residual amount of boiled mussel juice that can't be utilized enough. Some studies have shown that mussel juice contain 5.36% protein content other than water-soluble nutrients and taurine<sup>[7]</sup>, but the applications about these mussel juice have not been reported yet. Therefore, taking advantage of these waste mussel juice effectively not only solves the environmental pollution, but also is of great significance to the high value-added commodity producing.

The Maillard reaction(MR) is also known as non-enzymatic browning reaction. It refers to the reaction between carbonyl groups of reducing sugars and amino groups from amino acids, peptides, or proteins<sup>[8]</sup>. Maillard reaction products(MRPs), which are naturally produced in foodstuff during thermal producing by reducing the sugars that interact with available amino acids, modify important food properties such as color, flavor and stability<sup>[9]</sup>. The flavor of MRPs mainly depend on different kinds of reducing sugar and amino acid in Maillard<sup>[10]</sup>, such as producing meat aroma synthesis<sup>[11]</sup> or barbecue shrimp essence<sup>[12]</sup>. Moreover, there are some research on processing fish by-products to produce seasoning by Maillard reaction abroad<sup>[13-14]</sup>. Thus it can be seen that Maillard reaction is already widely used in condiment market, but this method has rare been used to preparing mussel juice seasoning in the published information.

In order to provide a new raw material which can be developed as seafood condiment derived from mussel by-products, the overall objective of this work was to investigate the effect of amount of reducing sugar, amino acid, initial pH and times on the Maillard reaction, and optimize the condition from hydrolysates of mussel juice by RSM for the attainment of MRPs with good sensory and flavor properties.

## MATERIAL AND METHODOLOGY

### Material and chemicals

The mussel juice was supplied by Hainan Yedao (Group) Co., Ltd (Haikou, China). Papain (activity of 15.0 units/mg solid) was obtained from Jieli Bio-chemical Product Company(Guangxi, China). D-xylose and D-glucose were supplied by Guoyao Group Chemical Co., Ltd (Beijing, China). L-arginine and L-lysine were purchased from Xiehe Bio-chemical Product Co.(Shanghai, China) and Huaheng Bio-Technology Co.(Jizhou, China) respectively. All other reagents used in this study were analytical grade quality.

### Preparation of hydrolysed mussel juice and deodorized hydrolysates

Mussel juice (40g) was mixed with distilled water as a ratio of 1:1.5 (w/v) and homogenized with ultra-turrax<sup>®</sup> (Huanyu Science Instrument Co., Jiangsu, China) for 3 min, the mixture was adjusted to pH 6.5 with 1M NaOH, then the papain was added to the mixture with a concentration 7.6mg/g and held at 50.5°C in a water bath (Jintan Automation Instrument Co. Ltd., Jiangsu, China) for 4 hours determined in preliminary study as an optimum condition<sup>[15]</sup>. After hydrolysis reaction solution, the papain was inactivated at 95°C for 10min, cooled naturally to room temperature (25°C). The soluble fraction was clarified by centrifugation (Xiangyi Instrument Co. Ltd., Changsha, China ) at 4200 rpm for 15min to remove impurities and enzyme residues.

Hydrolysed mussel juice was then deodorized by using united method according to the previous experiments.

Yeast activation treatment, the dry yeast (1g) and D-glucose (0.5g) were dissolved in 10ml distilled water at pH 7.0, 40°C for 25 min. Subsequently, the hydrolysates was added with yeast liquor/hydrolysed mussel juice dosage of 3% by weight, then the mixture were performed at 30°C for 60min. Next, adding the active carbon granule (ACG) to the treated hydrolysates with a dosage of 2% by weight simultaneously, it was reacted at 55°C for 50min by ultrasonic oscillator (Xian'ou Instrument Product Co. Ltd., Nanjing, China). After being treated by yeast and ACG, the mixture was filtered through a filter paper as the substrate of Maillard reaction. The remaining was stored at 4°C before Maillard reaction.

### Sensory evaluation and verification

Sensory evaluations for seafood-like flavor were performed in the MR of *Pneumatophorus Japonicus* head according to the method<sup>[16]</sup>. The resultants were in covered beaker (80ml) at room temperature (20±1°C) and presented to 7 panelists who conducted a taste and sniff test. The panelists were demanded to evaluate the scores of seafood-like aroma compared to natural seafood products. A top sensory score of 10 indicated that the sample could not be differentiated from a natural seafood flavor product<sup>[17]</sup>. The sensory test were performed in triplicate.

### Measurement of browning degree

The degree of browning of the MRPs were measured according to the method<sup>[18]</sup>. Appropriate dilution (5-fold) was made by distilling water and the absorbance was measured at 420nm by a spectrophotometer (Puxi General Instrument Co. Ltd., Beijing, China) and the data were shown as mean values of the three determinations.

### Maillard reaction and experimental design

Based on the previous study, the solution was as the following formulation: MRPs model mixture were prepared in the mussel juice hydrolysates (100g) which has been processed through above method, then reducing sugar 3.5g (D-glucose,D-xylose,2:1), amino acids 3.0g (L-lysine,L-arginine,1:2) and cysteine hydrochloride 0.2g were diluted into 0.2M phosphate buffer at pH7.5, heating in beaker on electric heating board (Jiangdong Precision Instrument Company, Jiangsu, China) at 120°C for 60min. But, for sensory evaluation, the initial experiments showed that the influential factors by significance test were amount of reducing sugar( $X_1$ ), amount of amino acids( $X_2$ ), reaction time( $X_3$ ), initial pH( $X_4$ ), so RSM Box-Behnken design was used to estimate the four factors above.

MR was carried out with 4 influence factors and 3 categorical factors by the Design Expert software (version8.0.5, Stat-Ease Inc., Minneapolis, USA). The experimental designs of the coded (X) and actual levels of variables were shown in Table 1. The two responses (Y) were delicate flavor score and browning degree ( $A_{420nm}$ ).

**TABLE 1. Numeric and categorical factors of RSM**

Code levels	$X_1$ /Reducing sugar amount (%)	$X_2$ /Amino acids amount(%)	$X_3$ /Times (min)	$X_4$ /Initial pH
1	3.0	2.5	50	7.0
0	3.5	3.0	60	7.5
1	4.0	3.5	70	8.0

\*percentage of amount of reducing sugar and amino acids were defined as by weight of MRPs.

### Determination of amino acid composition

Under the optimal conditions, the amount of amino acids of MRPs was quantified by high-performance liquid chromatography (HPLC) on a AccQ-Tag amino acid analysis column (15cm length  $\times$  3.9mm i.d., Waters) coupled with a 2695-2475 system (Waters Corporation, Milford, MA) were detected at 248nm, 37°C. The sample was then eluted with two solvents (A: 10% acetate-phosphate buffer solution in ultrapure water, B: 60% acetonitrile in ultrapure water) at a flow rate of 1.0ml/min.

### Identification and quantification of the volatile content

As in the above condition, the ether soluble volatile components in MRPs (10g) was extracted with 15ml of aether by intermittent extraction 60min (each ultrasonic 15min, 5min), using ultrasonic oscillator. The organic extracts were dehydrated over anhydrous magnesium sulphate for 12h, and then dried the aether to obtain a final sample of 0.2ml.

Qualitative and quantitative identification of volatile components were analysed by a HP 6890N gas chromatography-5973 mass selective detector (GC-MS) (Hewlett-Packard, Palo Alto, CA,USA) equipped with a DB-5MS capillary column (30m length  $\times$  0.25mm i.d., Agilent). Helium was the carrier gas at settled flow rate of 0.3ml/min, no split. The GC oven temperature was initially at 40°C for 2min, and ramped at 5°C/min to 60°C, then raised to 100°C at a rate of 3°C/min, post run at 18°C/min, 240°C for 6min at last. Injector and detection temperature were 250°C and 240°C respectively. The electron-impact (EI) mass spectra were generated at 70eV, an ion source temperature of 200°C with m/z scanning range from 20 to 500 amu. For mass spectrum (MS), tentative identifications were compared with those in the NIST98 MS database.

## RESULT AND DISCUSS

### Optimization on flavor scores of mrps by response surface methodology (RSM)

RSM analysis of the values are carried out by using an analysis of variance (ANOVA), while the results of Maillard condition design and the ANOVA of flavor score were given in Table 2 and Table 3 respectively. The models present high R-square ( $R^2$ ) and low coefficients of variation (CV) for flavor score. These results indicate a good precision and reliability for the study. F value and Prob>F value which define the significance of the coefficient are listed in Table 3. Then the regression equation of the fitted model is calculated as :

TABLE 2. Design program and experimental results of RSM

Nr.	Reducing sugar amount(%)	Amino acids amount(%)	Times (min)	Initial pH	Average score of delicate flavor	Absorbance of 420nm
1	3.5	3.5	60	7.0	4	0.616
2	3.5	2.5	60	8.0	2	0.815
3	3.5	3.5	70	7.5	2	0.874
4	3.5	3.0	70	7.0	4	0.725
5	3.5	3.0	70	8.0	5	0.781
6	4.0	2.5	60	7.5	3	0.603
7	3.5	3.0	60	7.5	5	0.774
8	3.0	3.0	70	7.5	6	0.720
9	3.5	2.5	60	7.0	6	0.807
10	3.0	3.0	50	7.5	8	0.806
11	3.5	3.0	60	7.5	6	0.849
12	3.0	3.5	60	7.5	5	0.711
13	3.0	3.0	60	7.0	9	0.752
14	3.5	3.5	50	7.5	6	0.595
15	3.5	3.0	50	7.0	7	0.743
16	4.0	3.0	70	7.5	5	0.822
17	3.5	2.5	50	7.5	4	0.480
18	4.0	3.0	50	7.5	4	0.802
19	3.0	3.0	60	8.0	2	0.853
20	3.0	2.5	60	7.5	7	0.725
21	3.5	3.0	50	8.0	2	0.860
22	3.5	3.0	60	7.5	6	0.686
23	4.0	3.0	60	7.0	3	0.540
24	4.0	3.0	60	8.0	6	0.786
25	3.5	2.5	70	7.5	5	0.647
26	3.5	3.5	60	8.0	3	0.579
27	4.0	3.5	60	7.5	5	0.824
28	3.5	3.0	60	7.5	6	0.708
29	3.5	3.0	60	7.5	6	0.794

$$Y=5.80-0.92X_1-0.17X_2-0.33X_3-1.08X_4+1.00X_1X_2+0.75X_1X_3+2.50X_1X_4-1.25X_2X_3+0.75X_2X_4+1.50X_3X_4+0.27X_1^2-1.11X_2^2-0.36X_3^2-0.98X_4^2(1)$$

TABLE 3. Variance analysis of flavor score experiment

Variance source	Sum of squares	df <sup>a</sup>	Mean square	F-value	P-value Prob>F	
Model	89.14	14	6.37	25.11	<0.0001	significant
X <sub>1</sub>	10.08	1	10.08	39.77	<0.0001	
X <sub>2</sub>	0.33	1	0.33	1.31	0.2708	
X <sub>3</sub>	1.33	1	1.33	5.26	0.0378	
X <sub>4</sub>	14.08	1	14.08	55.54	<0.0001	
X <sub>1</sub> X <sub>2</sub>	4.00	1	4.00	15.77	0.0014	
X <sub>1</sub> X <sub>3</sub>	2.25	1	2.25	8.87	0.0100	
X <sub>1</sub> X <sub>4</sub>	25.00	1	25.00	98.59	<0.0001	
X <sub>2</sub> X <sub>3</sub>	6.25	1	6.25	24.65	0.0002	
X <sub>2</sub> X <sub>4</sub>	2.25	1	2.25	8.87	0.0100	
X <sub>3</sub> X <sub>4</sub>	9.00	1	9.00	35.49	<0.0001	
X <sub>1</sub> <sup>2</sup>	0.46	1	0.46	1.82	0.1988	
X <sub>2</sub> <sup>2</sup>	7.97	1	7.97	31.42	<0.0001	
X <sub>3</sub> <sup>2</sup>	0.83	1	0.83	3.28	0.0914	
X <sub>4</sub> <sup>2</sup>	6.27	1	6.27	24.73	0.0002	
Residual	3.55	14	0.25			
Lack of fit	2.75	10	0.27	1.38	0.4067	not significant
Pure Error	0.80	4	0.20			
Cor Total	92.69	28				

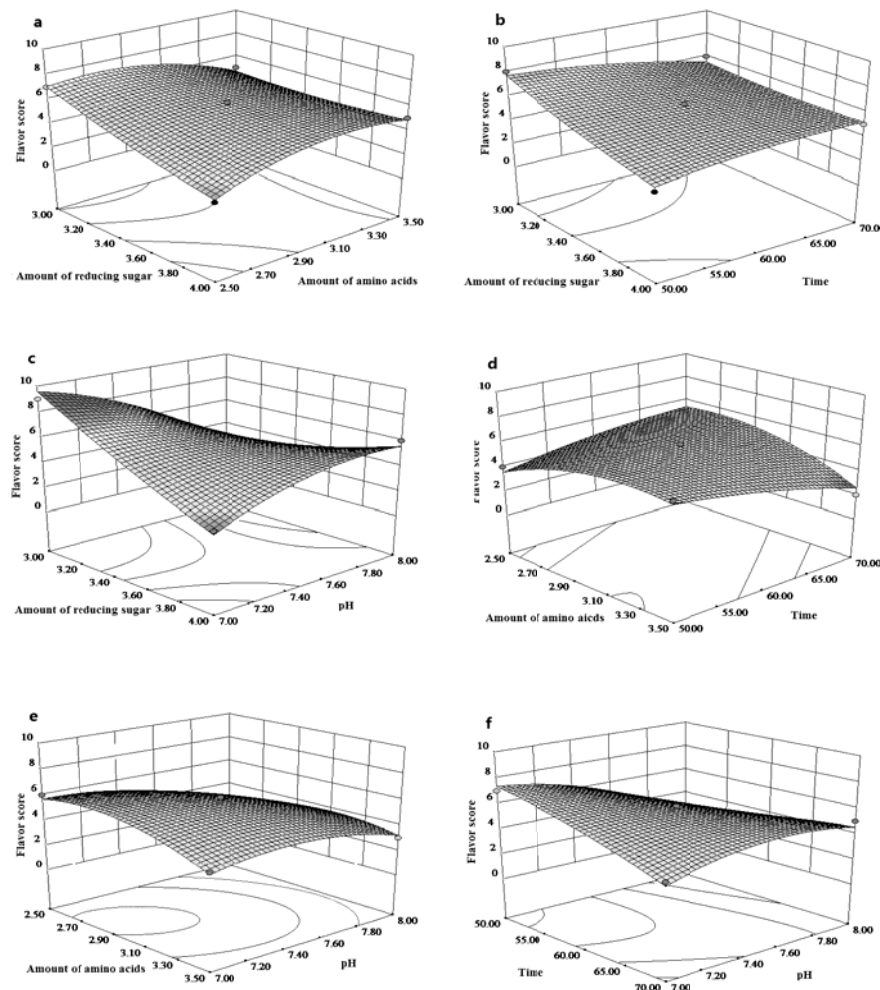
<sup>a</sup> df is degree of freedom. (R<sup>2</sup>= 0.962, CV=10.28)

The RSM describes the optimal processing parameters for response as:  $X_1$  (amount of reducing sugar)-3.0%,  $X_2$  (amount of amino acids)-2.8%,  $X_3$  (time)-50min and  $X_4$  (initial pH)-7.0. The highest flavor score of 9.85 obtained from the optimum conditions in this experiment was verified to be higher than any previous condition tested. As shown in Table 3, the flavor score has a positive linear effect with amount of reducing sugar, initial pH ( $p < 0.01$ ) and time ( $p < 0.05$ ), but no significant effects on amount of amino acids ( $p > 0.05$ ). The interaction effects and quadratic terms of the three factors are found to be significant considering flavor score as well. It can be observed that initial pH ( $X_4$ ) has the most effect on the flavor generation, amount of reducing sugar ( $X_1$ ) and time ( $X_3$ ) both increase seafood-like flavor moderately. Then little effect has been caused by amount of amino acids ( $X_2$ ) according to the linear coefficient of equation (1). So, high level of pH in a suitable range is beneficial to forming the delicate flavor of MRPs in the Maillard reaction. In brief, this study finding illustrates that RSM is a feasible analytic method to investigate optimal conditions for seafood aroma condiment production.

**Interaction effect analysis of 4 influence factors**

The optimum technology are determined to improve MRPs sensory evaluation with high score and delicate flavor. The average score of delicate flavor can be optimized from the main and interactive effects factors in figures (Fig.1(a)-(f)).

Fig.1(a) shows the dependence of flavor score with amount of reducing sugar and amino acids at a fixed time and pH. Obviously, the sensory score decreased gently with the increase reducing sugar amount. It also can be seen from Fig.1(a) that the score had a rising trend originally then decreased slightly with the increasing amino acids amount at a constant time and initial pH. The variation of flavor score with reducing sugar amount and time at a constant amino acids and pH is presented in Fig.1(b). It shows that under the optimum amino acids amount and pH condition, the flavor score decreased slightly as reducing sugar amount increased, while decreased linearly with time extended, respectively. Fig.1(c) shows the interactive effect of reducing sugar amount and pH on flavor score, which amino acids amount and heating time were at a fixed level. It is evident that the score is in the lowest under 2 situations:(1) with high amount of reducing sugar and low pH, and (2) low reducing sugar amount and high pH. The time against amino acids amount analysis in Fig.1(d). It indicates the score of sense increased at the first stage and decreased afterwards with long reaction time at a constant level of other three factors, the same consequence can be concluded for the variable of amino acids amount at a fixed condition of the other factors.



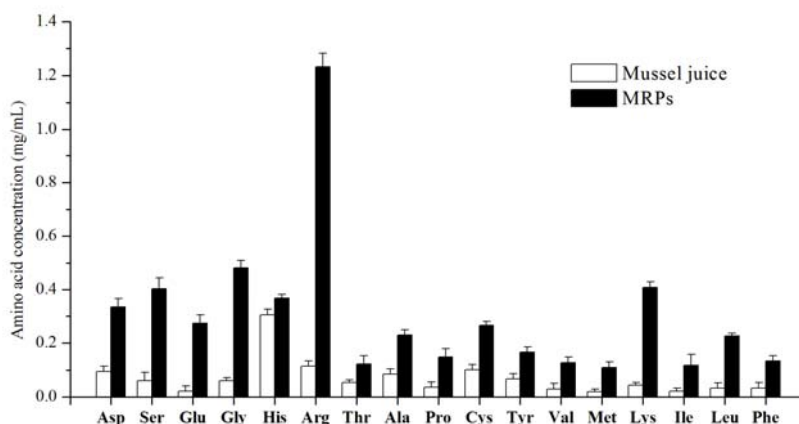
**Figure 1(a)-(f). 3D surfaces of interaction effect of amount of reducing sugar ( $X_1$ ), amount of amino acids ( $X_2$ ), time ( $X_3$ ) and initial pH ( $X_4$ ) on average score of seafood flavor**

Fig.1(e) presents variation of the flavor score with amino acids amount and initial pH at a given level of the other factors. As amino acids amount and pH were both curvilinear in nature on the surface, the score increased slightly and then decreased quickly from pH7.0 to pH8.0 at a fixed amount of amino acids, while at a fixed pH level, the flavor score gently increased at the beginning and decreased slowly with increased amino acids dosage. The interactive effect of time and initial pH is shown in Fig.1(f) at last. From the Fig.1(f) we can found that, the optimal conditions for delicate flavor product formation were with low pH and short time. When the time is short and pH increased the sensory score decreased, whereas the score increases with an long time at a low pH.

According to the analysis of the interaction effects among the four factors above, we demonstrated that a relatively low pH and reducing sugar amount around 3.0% (m/m) can provide delicate flavor to produce seafood condiment. This research results is similar to a previous report where low initial pH are beneficial to produce meat-like MRPs via Maillard reaction<sup>[17]</sup>.

### Analysis of amino acid component of mrps

The content and composition of amino acids play an important role in seafood condiment, which are used to evaluate the nutrition. Fig.2 shows the amino acids component at the optimal conditions in the Maillard reaction objectively. It's easy to see that, 17 kinds of amino acid were detected both in original mussel juice and the MRPs. While, the amount of total amino acids in MRPs has a significant increase comparing with the original mussel juice. In addition, researchers have reported that some amino acids, which were found rich in the MRPs derived from mussel juice hydrolysates, such as Leu, Met, Gly, etc., presented a strong seafood flavor<sup>[19]</sup>. The amount of essential amino acid and Arg in the MRPs were accounting for 22.08% and 23.75% of total amino acids, respectively. Especially, the Arg can not only control cell degradation, but also enhance our body immunity<sup>[20]</sup>. Therefore, our further work will focus on developing new seafood sauce with healthy function using the mussel juice.



**Figure 2. The content of amino acids in mussel juice and MRPs (under the optimal conditions of MR) derived from mussel juice hydrolysates.**

### Analysis of volatile compositions in the maillard reaction

The volatile components of MRPs extracted by ether were detected using GC-MS and analyzed both qualitatively and quantitatively. Table 4 lists the volatile components determined, their relative peak areas, and retention time identified on the DB-5MS column and in the literatures<sup>[21-22]</sup>. There are 52 volatile components mainly assorted into 7 chemical categories: (1)5 hydrocarbons, (2)5 alcohols were described as delicious smooth flavor formed with carbonyl compound<sup>[23]</sup>, (3)9 ketones, (4)3 carboxylic acid, (5)4 esters presented with fragrant and sweet aroma<sup>[21]</sup>, (6)14 pyrazine, (7)8 heterocyclic compounds, 1 aldehydes and 3 other compounds like disulfide, acetyl pyrrole and hydroxyl- methylacetamide.

Among the 52 volatile compounds identified, the contents of pyrazines were most 37.49% with a rate of total components. Ito and Mori<sup>[24]</sup> had investigated that the formation of pyrazines were effected by sugar types and pH level. In this study, we could see that 2-butyl-3,5-dimethyl-pyrazine reached 11.45% as the most abundant component in the pyrazines from table 4. The production of pyrazines in the MRPs may be the reason why the flavor had a relative high sensory score, because of caramel and nutty odor generated. This results also concurs with a precious reported research<sup>[17]</sup>, which pyrazines cannot be detected in the low pH on the other side. Ketones were the second most abundant class in the volatile components identified. According to Cha<sup>[25]</sup> and others' finding, ketones generation is maybe the result of degradation and thermal oxidation by the unsaturated fatty acid (UFA). We obtained 2,5-dimethyl-4-hydroxy-3(2H)-furanone only 0.44% amount, however, it had a positive function on the strawberry and other fruit flavor formation<sup>[21]</sup>. In addition, ketenes were the lipid oxide during thermally reacted. Although such as 6,10-dimethyl-5,9-diene-2-ketone and 1-tetradeceneketone were detected in the volatile, which presented meat-like and cream odor<sup>[26]</sup>, it had nothing significant increase on the seafood flavor of MRPs.

Among the volatile alcohol components, (E)-9-hexadecene-1-ol plays a key role in plant aroma formation accompanied mushroom flavor<sup>[27]</sup>. These aroma are beneficial to dispel the stench leading to a better sensory evaluation at the same time. Esters have ester group in the ring structure, which come from dehydration by carboxylic acid and alcohol, the flavor generated by the esters volatile is similar to the alcohols more or less. In particular, some esters such as dimethyl phthalate and 2-methylhexanoate showed the light fruit odor. Both carboxylic acids and aldehydes amount were only less than 6% , but these compounds are important for the particular flavor formation. For example, the propionic acid also had an acidity, cheese taste and pentanal presented subtly sweet aroma, respectively. Heterocyclic compounds which are involved in sulfur, nitrogen and oxygen groups were identified, such as 2-acetyl-3-aminothiophene, 2-propenyl-2-furan and others. In Lee's<sup>[28]</sup> research, these compounds presented low threshold value, but indicated important sensory characteristic. In general, peculiar seafood flavor of MRPs consist of the whole volatile compounds.

**TABLE 4. Volatile components of MRPs derived from hydrolysed mussel juice at 120°C**

Nr	RT/min	Compound	RC/%	Nr	RT/min	Compound	RC/%
1	7.65	mercaptopyruvic acid	0.14	27	20.47	valerenic acid	3.38
2	8.56	disulfide	0.35	28	20.56	3-methyl azobenzene	3.35
3	9.01	acetyl pyrrole	0.38	29	20.64	2-benzodiazepine-1-formamide	3.12
4	11.38	2-heptanone	0.33	30	20.74	isothiazoline	2.58
5	12.34	3,5-dimethyloctane	1.75	31	20.89	thiazolone	9.35
6	12.63	pyrazine	0.22	32	21.33	2-acetyl-3-aminothiophene	2.20
7	13.41	Styrene	0.35	33	21.46	2-methyl-5-(1-propenyl)-pyrazine	2.16
8	14.33	2,6-dimethylpyrazine	0.18	34	21.91	2-methyl hexanoate	4.07
9	14.39	2-methylpyrazine	0.35	35	22.00	1-(2-butoxyethoxy)-ethanol	1.56
10	14.45	1-hydroxy-2-acetone	0.27	36	22.10	hydroxy-methylacetamide	2.08
11	17.48	3-ethyl-2,5-diethyl-pyrazine	8.27	37	22.30	2-methyl-5,6,7,8-tetrahydropyrrole	1.80
12	17.86	2-ethylpyrazine	1.72	38	22.37	6,10-dimethyl-5,9-diene-2-ketone	2.36
13	18.17	pentanal	0.22	39	22.70	2,5,5,8-tetramethylpyrrole	2.65
14	18.53	dimethyl phthalate	0.19	40	22.93	phenylmethyl ethanol	1.58
15	18.64	Carbamic acid 2-dimethyl-aminoethyl	0.48	41	23.11	2-tridecanone	1.18
16	18.66	methylene benzene	0.57	42	23.86	phenethyl ethanol	2.95
17	18.70	3-octanone	1.14	43	24.36	1,3-dimethyl-1,3-butadiene	0.83
18	18.94	propionic acid	2.10	44	24.72	1-tetradecenone	0.39
19	19.02	2,3,5-trimethyl-6-methyl-pyrazine	1.53	45	24.87	octacosane	0.62
20	19.28	2,5-dimethyl-3-(2-methylpropyl)-pyrazine	1.65	46	24.95	2,5-dimethyl-4-hydroxy-3(2H)-furanone	0.44
21	19.46	2-methyl-6-(2-propenyl)-pyrazine	1.72	47	26.32	2-butyl-3,5-dimethyl-pyrazine	11.45
22	19.98	2-isoamyl-6-methyl-pyrazine	2.15	48	26.44	2,5-dimethyl-3-(1-propenyl)-pyrazine	0.57
23	20.13	2,6,10-trimethyl pentadecane	0.55	49	28.01	(E)-9-hexadecene-1-ol	0.27
24	20.28	1-(5-methyl-2-pyrazinyl)-ethanol	2.03	50	28.28	2-propenyl-2-furan	0.33
25	20.33	2,5-dimethyl-3-(3-methylbutyl)-pyrazine	2.05	51	29.34	heptadecanone	3.92
26	20.40	2-acetyl-3-methylpyrazine	3.28	52	30.64	isopropyl palmitate	0.34

\*Compounds were identified by comparison with the mass spectra in the electron impact mode (MS). Nr. indicate the kinds of volatile compounds; RT means retention time and RC means relative content calculated using the relative peak areas by the normalization method.

## CONCLUSIONS

Delicate flavor of MRPs derived from hydrolysate of mussel juice can be prepared under the optimal conditions, which included a reducing sugar amount of 3.0%, an amino acids amount of 2.8%, a heating time of 50min and an initial pH

of 7.0. It seems that we have preliminary studied the relationship between influencing factors and the sensory evaluation. Amino acids and 52 volatile compounds formed from Maillard reaction were already identified both qualitatively and quantitatively by HPLC and GC-MS, respectively. Our study aims at developing and improving the commercial value of mussel by-product. Importantly, we have obtained data that suggests a method to produce mussel products for the seafood condiment material processing.

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