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Development of Blue Mussel Hydrolysate as a Flavouring

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Abstract

The hydrolysis conditions of blue mussel were evaluated by response surface methodology(RSM) for the alkaline protease Optimase™ APL-440. Conditions favoring the highest degree of hydrolysis in blue mussel were pH 9.8, 58°C reaction temperature, 2.9 hrs reaction time, 46.8%(w/v) substrate concentration, and 0.34% (v/w) enzyme/substrate ratio. Levels of n-3 fatty acids, e.g. C18:3, C18:4, and C20:5, did not change after hydrolysis in blue mussel sample.

Key words: blue mussel, enzyme hydrolysis, flavouring

INTRODUCTION

Blue mussel, having a desirable and unique taste and aroma, is an important marine resource in Korea(1). Processing of blue mussel is limited mainly to dried and seasoned or canned products(2,3). In recent years processing utilization of blue mussel has declined due to decreasing harvesting yields associated with marine environmental contamination. Therefore, an alternative use of blue mussel, such as production of flavorants via enzymatic hydrolysis, for having a supplementary value may be a fundamental benefit to seafood industry.

General methods for production and application of natural blue mussel extract have been reported by Ochi(4). However, use of enzymatic hydrolysis of shellfish protein products is limited. Several studies have addressed the production of seafood flavorings by enzymatic hydrolysis (5,6). Rebeca et al.(7) reported about nutritional value on fish protein hydrolysates produced with bacterial proteases. However, little information is available on the enzymatic hydrolysis of shellfish protein. Only recently has research in this area focused on statistical approaches to the optimization of such processes(8).

The objective of this study was to set an optimal condition for production of a flavouring from blue mussel with alkaline protease Optimase™ APL-440.

MATERIALS AND METHODS

Materials

A fresh blue mussel(*Mytilus edulis*) was purchased from a fish market in Masan, Korea and transported on ice in polyethylene bag to the Flavor Lab., Changwon National University within 1 hr. The sample was refrigerated(4°C) until analysis. Before analysis, blue mussel was homogenized using a Waring blender(Waring Products Co., Winsted, USA). Proximate analysis of blue mussel such as moisture, protein, lipid, and ash was done by official methods(9). Optimase™ APL-440 was obtained from Solvay Enzymes, Inc.(Elkhart, USA).

Optimization of hydrolysis conditions

Five independent variables affecting hydrolysis of blue mussel, e.g. pH, temperature(T), reaction time(t), substrate concentration(S), and enzyme/substrate(E/S) ratio, were optimized by using response surface methodology(RSM) (10). Experimental design levels for each parameter were coded from -2 to +2 with 1 interval as described by Baek and Cadwallader(8)(Table 1). As shown in Table 2, the central composite design consisted of 36 experiments including 16 fractional factorial designs, 10 star points, and 10 central points. The equation of second order model was defined as described by Baek and Cadwallader(8).

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Table 1. Experimental design levels expressed in coded and natural units

Code unit	Independent variables				
	pH	Temp (°C)	Time (hr)	S[1] (%)	E/S[2] (%)
-2	8.0	40	0.5	15	0.1
-1	9.0	50	1.5	30	0.2
0	10.0	60	2.5	45	0.3
+1	11.0	70	3.5	60	0.4
+2	12.0	80	4.5	75	0.5

[1]Concentration(w/v) of blue mussel
[2]Ratio(v/w) of Optimase™ APL-440 to blue mussel

Table 2. Response of dependent variables to the hydrolysis conditions for Optimase™ APL-440

Run No.	Independent variables[1]					
	pH	Temp	time	S	E/S	DH(%)[2]
1	-1	-1	-1	-1	-1	33.25
2	1	-1	-1	-1	1	44.98
3	-1	1	-1	-1	1	42.93
4	1	1	-1	-1	-1	42.99
5	-1	-1	1	-1	1	47.12
6	1	-1	1	-1	-1	49.56
7	-1	1	1	-1	-1	46.61
8	1	1	1	-1	1	39.20
9	-1	-1	-1	1	1	38.18
10	1	-1	-1	1	-1	34.08
11	-1	1	-1	1	-1	54.15
12	1	1	-1	1	1	38.78
13	-1	-1	1	1	-1	53.69
14	1	-1	1	1	1	50.64
15	-1	1	1	1	1	47.36
16	1	1	1	1	-1	29.17
17	-2	0	0	0	0	48.59
18	2	0	0	0	0	26.64
19	0	-2	0	0	0	40.69
20	0	2	0	0	0	33.27
21	0	0	-2	0	0	36.59
22	0	0	2	0	0	58.36
23	0	0	0	-2	0	47.64
24	0	0	0	2	0	54.16
25	0	0	0	0	-2	44.68
26	0	0	0	0	2	69.27
27	0	0	0	0	0	46.70
28	0	0	0	0	0	45.53
29	0	0	0	0	0	52.31
30	0	0	0	0	0	50.62
31	0	0	0	0	0	49.24
32	0	0	0	0	0	48.44
33	0	0	0	0	0	47.05
34	0	0	0	0	0	51.74
35	0	0	0	0	0	50.67
36	0	0	0	0	0	53.12

[1]The codes of independent variables were same as represented in Table 1
[2]Degree of hydrolysis(%)

Enzymatic hydrolysis of blue mussel

Blue mussel homogenate(50g) plus distilled water (30ml) was hydrolyzed in a 100ml jacketed reaction vessel at each experiment of 36 central composite designs shown in Table 2. Further details about enzymatic hydrolysis have been discussed elsewhere(8,11). Degree of hydrolysis(DH) was also estimated as described by Baek and Cadwallader(8).

Statistical analysis

Response surface equations were obtained using the RSREG procedure of the Statistical Analysis System (SAS)(12).

Lipid analysis

Lipids were extracted from untreated(UBM) and hydrolyzed blue mussel(HBM) using the method of Bligh and Dyer(13). Extracted lipids were separated into neutral, glycolipid and phospholipids by silicic acid column chromatography(14). The procedures for saponification and methyl esterfication were the same as described by Cha and Park(15). One μl of each hexane extract was injected (split mode, 1:100) in an HP 5890A GC(Hewlett-Packard Co., Palo Alto, USA), equipped with a flame ionization detector(FID) and a fused silica open-tubular column (Supelcowax 2330, 30m×0.25mm i.d.×0.20μm film thickness; Supelco, Inc., Bellefont, USA). Oven temperature was programmed from 180°C to 220°C at 2°C/min with initial and final holding times of 14 min. Injector and detector temperatures were maintained at 230°C and 240°C, respectively. He was used as a carrier gas at a head pressure of 11 psi. FAMEs were identified by comparison with standards(Applied Science Lab., Deerfield, USA). Duplicate analysis were carried out on each sample.

RESULTS AND DISCUSSION

Optimization of hydrolysis condition

The proximate composition of fresh untreated blue mussel(UBM) was composed of 79.4% moisture, 7.9% protein, 2.1% lipid, and 2.0% ash. The pH was 5.78. Compared with other shellfish harvested in Korea, blue mussel was higher in protein content on a dry weight basis(1), and therefore was considered a good feedstock for production of hydrolysate.

Responses of dependent variables to hydrolysis conditions for Optimase™ APL-440 were calculated(Table 2). Estimated regression coefficients for each dependent variable were obtained using the RSREG procedure(12) to evaluate significant differences among the treatments (Table 3). Regression coefficients showed that pH($p<0.01$), reaction time(t)($p<0.01$) and enzyme/substrate (E/S)($p<0.05$) had linear effects on DH for blue mussel, and pH($p<0.01$) and temperature(T)($p<0.05$) had quadratic effects, respectively. However, interaction effects, having negative values, were significant between pH and T($p<0.05$), pH and substrate(S)($p<0.05$), and T and t($p<0.01$). The pH and t were considered the important linear variables affecting on DH with -3.24 and 3.23 values, respectively, and followed by E/S(value= 2.28). Here, the positive values of t and E/S implied that increasing values of reaction time and E/S ratio were contributed to increasing of DH. From the results of RSM, a model equation was estimated by multiple linear regression for DH as follows: $\%DH = 49.73 - 3.24pH + 3.23t + 2.28(E/S) - 3.29pH^2 - 3.44T^2 - 2.99pH \times T - 2.98pH \times S - 4.18T \times t$. Although the lack of fit was significant, which means no secondary order of regression equation, this model equation was considered adequate with its coefficient of determination ($R^2=0.844$) and significant F value($p<0.01$). However, stationary point by canonical analysis of response surface was saddle-shape, and showed a DH of only 40.12%. Therefore, ridge analysis was executed to obtain maximum hydrolysis condition near the center of original design. Using results of ridge analysis, optimal condition was set at 0.3 coded radius(52.8% of DH, S.E.=1.63) ridge point. Optimal hydrolysis conditions with Optimase™ APL-440 were established as pH 9.8, temperature of 58°C, reaction time of 2.9 hr, substrate concentration of 46.8%(w/v), and an enzyme/substrate ratio of 0.34%. Blue mussel hydrolysate was produced under these conditions, followed by heat treatment(121°C for 15min) which had the dual purposes of generating Maillard reaction flavor and enzyme inactivation.

Table 3. Model coefficients estimated by multiple linear regression for Optimase™ APL-440

Factor	Coefficient
Constant	49.73
Linear	
pH	-3.24[a]
T	-1.05
t	3.23[a]
S	0.53
(E/S)	2.28[b]
Quadratic	
pH^2	-3.29[a]
T^2	3.44[b]
t^2	0.81
S^2	0.04
$(E/S)^2$	1.56
Interactions	
pH×T	-2.99[b]
pH×t	1.16
pH×S	-2.98[b]
pH×(E/S)	1.86
T×t	4.18[a]
T×S	0.24
T×(E/S)	-0.93
t×S	-0.16
t×(E/S)	0.30
S×(E/S)	0.14
R^2	0.844
F	4.05
Probability of F	0.004*

Superscript letters are significantly different: [a]$p<0.01$, [b]$p<0.05$

*Probability > F

Fatty acid composition in blue mussel hydrolysate

Fatty acid(FA) compositions of HBM and UBM are shown in Table 4. The principal FAs of UBM were composed of 32.8% saturated FAs(SFAs), 17% monoene FAs(MUFAs), and 50.4% polyene FAs(PUFAs). The PUFAs of UBM were composed mainly of n-3 FAs, e.g. C18:3, C18:4, C20:5, and C22:6, and did not changed after hydrolysis. With regards to the FA composition of HBM, SFAs were thought to be contributed from neutral FA, while MUFAs and PUFAs were derived from glycolipid and phospholipid. Aside from SFAs, n-3 PUFAs were the major FAs in both HBM and UBM. However, these n-3 PUFAs were well known to be very unstable and decompose to form secondry reaction products that adversely affect flavor quality(16-18).

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Table 4. Fatty acid compositions in UBM and HBM produced by Optimase™ APL-440[1)] (Area %)

Fatty acid (FA)	UBM	HBM			
	Total FA	Total FA	NL[2)]	GL[3)]	PL[4)]
C14:0	3.8	4.2	4.9	4.1	3.6
C15:0	0.1	-[5)]	-	-	-
C16:0	18.2	19.6	36.8	26.2	21.5
C17:0	6.0	1.6	3.0	6.8	3.2
C18:0	4.1	4.1	15.2	-	6.2
C19:0	0.6	-	2.1	-	-
Saturated	32.8	29.5	62.0	37.1	34.5
C14:1	0.7	0.6	-	-	0.7
C16:1	6.9	5.8	-	5.8	4.8
C17:1	0.7	-	-	-	0.1
C18:1	3.1	4.0	19.7	6.6	4.4
C20:1	2.7	3.5	-	0.9	4.5
C22:1	2.3	2.5	-	-	-
C24:1	0.6	0.6	7.4	6.9	-
Monoene	17.0	17.0	27.1	21.6	15.4
C18:2	1.6	1.2	1.9	6.4	1.1
C18:3	0.8	0.9	-	0.9	0.5
C18:4	4.7	4.7	-	2.1	3.9
C20:2	0.5	0.4	-	-	-
C20:4	0.2	-	1.0	0.8	2.0
C20:5	16.6	17.7	5.3	13.3	17.0
C22:5	1.4	1.7	-	-	3.0
C22:6	24.6	16.7	2.8	19.2	22.5
Polyene	50.4	49.1	11.0	42.7	50.0

[1)]UBM, Untreated blue mussel; HBM, Enzyme hydrolyzed blue mussel with Optimase™ APL-440
[2)]Neutral lipid, [3)]Glycolipid, [4)]Phospholipid, [5)]Detected in trace amount

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