



Optimizing Boiling Condition for the Preparation of Fish Extracts

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The optimum boiling condition was determined for fish extracts by response surface model. Model equations were designed with effect of time (T) and the amount of added water (W) on the level of released free amino acid. Based on the high (>0.9) coefficient of determination and low (<0.01) level of significant, those model was approved to be significant. The added water amount of higher regression coefficient (β_2), showed a greater influence on releasing free amino acids than boiling time. The optimum boiling times are 6 hours for crucian carp, 5 hours for bastard halibut, 7 hours for loach and 5 hours for jacobever. The ratio of added water to sample 1 (v/w) could be applied to all fish samples at $100 \pm 2^\circ\text{C}$.

Key words: fish extracts, optimum boiling condition, central composite design (CCD)

Introduction

The interest in nutraceuticals is very strong in the Korean health food market, and fish extracts play a bigger role than the other functional foods. Those extracts are conventionally processed at boiling temperature for over 10 hours. Fresh water fishes such as crucian carp, eel and snakehead are most frequently used in traditional fish extracts processing, and they have been served to undernourished persons for increasing the dietary intake of important nutrients. Recently, health food manufacturers designed an autoclave type extractor to improve the functionality of fish extracts processed at a traditional recipe. But some of the technical problems associated with those modified fish extracts processed at high temperature in autoclave were the risk of damage to protein (Fujimaki et al., 1980; Friedman et al., 1981; Abe et al., 1981; Banga et al., 1992) and organoleptic deterioration due to non-enzymatic browning (Ryu et al., 1998). Therefore, the studies on optimization of sensory and nutritional qualities for the processing should be need to minimize lessening nutritive value. Such protein hydrolyzates type fish extracts would be acceptable to a targeted

population. The objective of this study was to optimize the processing condition for boiled fish extracts based on the Korean conventional fish extracts recipe.

Materials and Methods

Materials

Live loach (*Misgurnus anguillicaudatus*), crucian carp (*Carassius carassius*), bastard halibut (*Paralichthys olivaseus*), and jacobever (*Sebastes ineronif*) were purchased from a local market in Pusan, Korea. All of fishes were eviscerated and scaled. Loach skins were scrubbed in 5% salt water to separate foreign bodies. Each fish sample was cut into proper blocks (3 cm×3 cm×2 cm) and the rubbed off loaches were used in experiments for hydrolysis. One kilogram of fish sample blocks that was put into a cotton cloth pocket (50 cm×50 cm) was placed in a 5L volume of round bottom flask with water condensor to minimize added water loss during processing on heating mantle. Filtered extracts through cheese cloth were freeze-dried for further experiments.

Boiling conditions for preparation of fish extracts

Boiling conditions were established on the basis

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of Central Composite Design (CCD) (Park, 1995). While boiling temperature (T) was fixed at $100 \pm 2^\circ\text{C}$ from Korean conventional fish extracts recipe, time (T) and the amount of added water (W) were selected as independent variables as shown in Table 1.

Optimization of boiling conditions

After boiling each fish sample, free amino acid content was measured as dependent variable. Parameters for boiling were chosen as the independent variables.

The coded values were obtained by the following formula:

$$x = \frac{(X - X^0)}{\Delta X}$$

where x is the coded value; X is the corresponding natural value; X^0 is the natural value in the center of the domain; and ΔX is the increment of X corresponding to one unit of x .

For each factor, five levels were given and a second order model was proposed. Data was analyzed by multiple regression to fit the following second order equation:

$$y = \beta_0 + \sum_{i=1}^2 \beta_i x_i + \sum_{i=1}^2 \beta_{ii} x_i^2 + \beta_{12} x_1 x_2$$

where β_0 , β_i , β_{ii} , and β_{12} represent regression coefficients of the model, and x_i and x_j represent the independent variables in coded values. The condition of boiling, which produced maximum free amino acid content was found from response surface equations.

Response surface equations were obtained using the RSREG procedure of the Statistical Analysis

Table 1. Independent variables and experimental design levels expressed in coded and natural units for boiling

Code units		Independent variables	
x_1	x_2	Time (hr)	W (ratio*)
-1	-1	5	1.25
-1	1	5	2.75
1	-1	11	1.25
1	1	11	2.75
0	0	8	2
0	0	8	2
0	0	8	2
0	1.414	8	3.0605
0	-1.414	8	0.9395
1.414	0	12.242	2
-1.414	0	3.758	2

*the ratio of added water to sample

System (SAS Institute, Inc., 1994) program.

Measurement of free amino acid content

To check the degree of released free amino acids, total free amino acid content was determined on 95 % ethanol deproteinized samples of 75°C water extracts from freeze dried samples (70 mesh) using the procedure of Ryu et al. (1988) modified from OPDA method (Church et al., 1983). Results of total free amino acid content were expressed as DL-lysine equivalent.

Results and Discussion

The contents of free amino acid in fish extracts by boiling with the experimental conditions established by central composite design (CCD) were shown in Table 2. The optimum condition was examined by regression and cononical analysis of SAS program. The regression constant and

Table 2. Responses of dependent variables to the boiling conditions for Crucian carp, Bastard halibut, Loach, Jacopever

Run No.	Independent variables		Dependent variables			
	T	W	Total Free Amino Acid (mg/ml)*			
			Crucian carp	Bastard halibut	Loach	Jacopever
1	-1	-1	1.7875	1.254	1.3842	1.4117
2	-1	1	1.111	0.649	0.6315	0.7240
3	1	-1	2.167	1.386	1.4236	1.4181
4	1	1	0.924	0.715	0.7386	0.9080
5	0	0	1.463	0.88	0.8082	1.0820
6	0	0	1.287	0.792	0.9969	1.0463
7	0	0	1.3475	0.737	0.9007	1.0930
8	0	1.414	1.2045	0.55	0.6232	0.6727
9	0	-1.414	2.6345	1.408	1.5454	1.5436
10	1.414	0	1.562	0.902	0.8751	1.0344
11	-1.414	0	1.738	0.792	0.8751	0.9401

*determined by OPDA method and described as equivalent of DL-lysine

significance for each variables by multiple regression were presented in Table 3.

The response surface equation for boiling of crucian carp on the relationship between two independent variables such as time (T, x_1), ratio of added water to sample (W, x_2) and dependent variables such as free amino acid content was as follows:

$$y = 1.37 - 0.01x_1 - 0.7x_2 + 0.14x_1^2 + 0.41x_2^2 - 0.28x_1x_2$$

The coefficient of determination (R_2) and level of significance for this model were 0.9161 and 0.01 respectively. So those results could confirm the significance of this model. The variable having the greater influence on boiling was the ratio of added water ($\beta_2 = -0.7$) of the largest regression constant, and the ratio showed linear effect and pure

Table 3. Model coefficients a estimated by multiple linear regression for boiling of Crucian carp, Bastard halibut, Loach, Jacopever

Factor	Coefficients			
	Crucian carp	Bastard halibut	Loach	Jacopever
Constant	1.37**	0.80***	0.90***	1.07***
Linear				
T	-0.01	0.06	0.03	0.06
W	-0.70*	-0.44***	-0.48***	-0.43***
Quadratic				
T ²	0.14	0.09	0.01	0.01
W ²	0.41*	0.22	0.21**	0.07
Crossproduct				
T×W	-0.28	-0.03	0.03	0.09
R ²	0.9161	0.9700	0.9702	0.9861
Probability	0.0101	0.0008	0.0008	0.0001

*model on which x_1 =boiling time (T), x_2 =The amount of added water ratio to sample weight (W) is

$$y = \beta_0 + \sum_{i=1}^2 \beta_i x_i + \sum_{i=1}^2 \beta_{ii} x_i^2 + \beta_{12} x_1 x_2$$

*significant at 0.10 level

**significant at 0.05 level

***significant at 0.01 level

Table 4. Optimum conditions of boiling for fish extracts at $100 \pm 2^\circ\text{C}$

Sample	Independent value	
	Time (hr)	Water (ratio*)
Crucian carp	6	1
Bastard halibut	5	1
Loach	7	1
Jacopever	5	1

*the ratio of added water to sample

secondary effect. Because regression constant was a negative number, free amino acid content was increased with decreasing added water.

On the other hand, the model equation of response surface analysis for bastard halibut was as follows:

$$y = 0.8 + 0.06x_1 - 0.44x_2 + 0.09x_1^2 + 0.22x_2^2 - 0.03x_1x_2$$

The coefficient of determination (R_2) and level of significance for this model were 0.9700 and 0.0008, respectively. Accordingly, response surface equation for bastard halibut was very significant. When bastard halibut were cooked, the ratio of added water to sample revealed as a more important variable than boiling time, and showed linear effect and pure secondary effect. Perhaps free amino acid content could increased with decreasing added water on the basis of negative number of regression constant ($\beta_2 = -0.44$).

In a regression result for loach, correlation equation of surface response model was as follows:

$$y = 0.90 + 0.03x_1 - 0.48x_2 + 0.01x_1^2 + 0.21x_2^2 - 0.03x_1x_2$$

The coefficient of determination (R_2) and level of significance for this model were 0.9702 and 0.0008, respectively. The amount of added water ($\beta_2 = -0.48$) was also more effective variables than boiling time as the same tendency as mentioned above.

Finally in the case of jacopever, the coefficient of determination (R_2) and level of significance for this model were 0.9861 and 0.0001, respectively and showed the highest accuracy among four fishes used in extracts processing. Response surface model of jacopever was as follows:

$$y = 1.07 + 0.06x_1 - 0.43x_2 + 0.01x_1^2 + 0.07x_2^2 - 0.09x_1x_2$$

When jacopever was cooked, added water ratio ($\beta_2 = -0.43$) was a variable that had the greater influence on boiling than boiling time. Because the regression constant value was a negative to added water ratio, free amino acid content would be increased with decreasing added water ratio.

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