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Rapid Enzymatic Fermentation of Anchovy Sauce by Protease

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Abstract

We evaluated the possibility of rapid fermentation of anchovy sauce using a commercial protease. The fermentation characteristics were monitored by response surface analysis. The content of total nitrogen was high (around 1%) with fermentation at 51.7~57.5°C after 10.2~16.4 hours, but rapidly decreased at higher temperatures (60°C or over), while the R^2 of polynomial equation was 0.9185 ($p<0.05$). The amino acid content rapidly decreased to approximately 600 mg% and less at high temperature (60°C and over), and the R^2 was 0.9578 ($p<0.01$). The free amino acids were affected more by fermentation time when fermentation temperature was lower, and the R^2 for total free amino acids was 0.8496 ($p<0.10$). The R^2 for sweet free amino acids was 0.9144 ($p<0.05$). According to the results of this study, the optimal conditions for anchovy sauce fermentation were predicted to be 52.5~56.9°C and 13.3~16.4 hours, and the predicted values and actual values of each response variable were similar to each other when the fermentation was performed at a random point within the optimal range. Also, the comparison of the quality between the quick anchovy sauce and sauces currently on the market showed that the content of sweet amino acids was higher in the former than in the latter.

Key words: anchovy sauce, response surface methodology (RSM), protease

INTRODUCTION

Jeotkal, salted and fermented seafood sauce, is a Korean traditional seafood sauce having peculiar palatable taste and flavor produced by the decomposition of the bodies of salted anchovy, sandlance, and sardine by the enzymatic digestion resulting from autolysis and halophilic bacteria (1,2). Korean families traditionally produced *jeotkals* on a small scale, but nowadays products manufactured in factories are on the increase (3). Most of the salted-fermented anchovy sold at stores depend on natural fermentation, a process in which salted anchovies are poured into fermenters and fermented for at least six months in dugout or underground storage spaces (4). Such natural methods may induce contamination or production of harmful compounds, and it is difficult to produce uniform-quality products because of the difficulty in process control. The popularity of other fermented products, like *Kimchi*, has increased the interest in developing safe and sanitary fermentation methods for those products as well as for *jeotkals*. There is currently much interest in developing new and improved varieties of *jeotkals* as well as for developing better methods of producing existing products.

Quick methods for producing *jeotkals* include use of *koji*, mycelia immobilization, and addition of enzymes or natural additives, but the products lack in taste and flavor when compared to those by produced by autolysis (5,6). However, rapid fermentation satisfies basic factors including sanitation and safety and reduces the turnover of production cost and equipment cost for facilities, and therefore, the method is expected to be the mainstream for *jeotkal* production in the near future. Protease use, in particular, is easy to maintain quality control and low in process cost when compared to *koji* or natural additives in mass production, and may be put to practical use when sensory evaluation can be used to confirm quality. Domestic studies on rapid fermentation using enzymes have included studies by Kim (7), Han et al. (8), Bae & Choi (9), Kim et al. (10) and fermentation conditions of *jeotkal*; Choi et al. (11) reported on the peptide characteristics of quick anchovy sauce, but few studies have reported on various proteases. In a previous study, the authors confirmed that thermoase, a kind of alkali protease with superior in proteolytic activity, is useful in preparing quick red pepper soy paste (12).

In this study, the authors used response surface analysis to investigate the optimal fermentation conditions for making high-quality anchovy sauce.

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MATERIALS AND METHODS

Materials

Quick-frozen anchovies (*Engrulis japonicus*, 6.0~10.0 cm) caught in off Jeju-do, South Korea, April 2001 were stored at -30°C until used. Thermoase (origin: *Bacillus thermoproteolyticus*), a kind of endopeptidase produced by Daiwa kasei Co. (Japan) was used as fermenter.

Preparing anchovy sauce by central composite design

Quick anchovy sauce was prepared using a central composite design (13,14) and a SAS program was used for response surface analysis. The conditions of the sauce fermentation included the temperature (X_1) and the time (X_2), and were coded into five stages (-2, -1, 0, 1, and 2) to be divided into 10 selected blocks. Meanwhile, total nitrogen content (Y_1), amino nitrogen content (Y_2), and total free amino acid content (Y_3), and sweet free amino acid content (Y_4) were considered response variables (Y_n) associated with the quality characteristics of anchovy sauce. The amounts of water and thermoase added were equally applied to all the blocks, based on the results for the yield and degree of hydrolysis in the pilot study. The anchovy paste (1 kg) had a 50% (w/w) moisture content, 0.3% (w/w) of thermoase, and was spun in a water bath at 150 rpm during fermentation. The fermented solution was primarily filtered by filter paper (Whatman No. 1) to remove the residue, refined salt (20%) was added, and the solution was centrifuged at 5000 rpm for 20 minutes. The supernatant liquid was used as samples for this study.

Analysis of general compounds

Total nitrogen was analyzed by a micro-Kjeldahl method (15), while amino nitrogen was measured by the Formol method (16).

Analysis of free amino acid

After 5 mL of sample was added to 50 mL of 75% ethanol, each sample was extracted in a water bath for one hour, filtered, and the residue was added to 75% ethanol and extracted two more times. The extracted liquid was vacuum concentrated, added by 30 mL of 25% trichloroacetic acid (TCA), and centrifuged at 15,000 rpm for 20 minutes to remove the residue. The supernatant liquid was then added to 30 mL of ethylether to remove the TCA, vacuum concentrated, dissolved in pH 2.2 sodium citrate buffer, and then analyzed using an amino acid analyzer (LKB 4150, alpha autoanalyzer, Ultrapac 11 cation exchange resin) (7).

RESULTS AND DISCUSSION

Changes in total nitrogen and amino nitrogen

Table 1 shows the contents of total nitrogen and amino nitrogen of the anchovy sauce fermented by thermoase on the market according to central composite design, while Table 2 reveals the polynomial equation of each variable based on the fermentation conditions. The R^2 for total nitrogen content was high (0.9185), and the

Table 1. Experimental data for the contents of total nitrogen, amino nitrogen, total free amino acid and sweet amino acid

Exp No.	Fermentation conditions		Physicochemical properties			
	Temp. (°C)	Time (hr.)	Total nitrogen (%)	Amino nitrogen (mg%)	Total free amino acid (mg%)	Sweet amino acid[1)] (mg%)
1	60 (1)	8 (-1)	0.82	629.16	3165.47	596.65
2	60 (1)	16 (1)	0.90	717.22	3643.98	748.46
3	50 (-1)	8 (-1)	0.88	696.78	2691.41	665.54
4	50 (-1)	16 (1)	0.95	791.56	3894.80	873.70
5	55 (0)	12 (0)	1.12	761.32	3977.42	848.93
6	55 (0)	12 (0)	1.14	711.62	3505.00	888.01
7	65 (2)	12 (0)	0.70	631.26	3026.56	611.66
8	45 (-2)	12 (0)	0.75	725.48	2759.94	633.14
9	55 (0)	20 (2)	0.96	806.12	3485.72	758.39
10	55 (0)	4 (-2)	0.81	613.76	2497.80	547.87

[1)]Sweet amino acid means glycine, alanine, threonine, serine and proline.

Table 2. Polynomial equations calculated by RSM program for anchovy sauce

Response	Polynomial equation[1)]	R^2	Pro>F
Total nitrogen	$Y=-10.8129+0.4154X_1+0.0858X_2-0.0038X_1^2-0.0001X_1X_2-0.0030X_2^2$	0.9185	0.0267
Amino nitrogen	$Y=-917.4133+57.3268X_1+25.6823X_2-0.5621X_1^2-0.0840X_1X_2-0.3849X_2^2$	0.9578	0.0075
Total free amino acid	$Y=-26577+938.2426X_1+640.1971X_2-7.8355X_1^2-5.6860X_1X_2-10.9465X_2^2$	0.8496	0.0845
Sweet amino acid[2)]	$Y=-7223.3514+265.1114X_1+132.3324X_2-2.3692X_1^2-0.7044X_1X_2-3.2217X_2^2$	0.9144	0.0294

[1)]X_1: Temperature (°C), X_2: Time (hr).
[2)]Sweet amino acid means glycine, alanine, threonine, serine and proline.

significance was 0.0267 which was statistically significant at the 5% level. As shown in Fig. 1, total nitrogen content of anchovy sauce was significantly affected by fermentation temperature (F-ratio=13.41, $p<0.05$), while the content was high (around 1%) at 51.7~57.5°C and 10.2~16.4 hours, but was rapidly decreased when the temperature was 60°C or over. The R^2 for amino nitrogen content was 0.9578 ($p<0.01$), and because the F-ratio of fermentation temperature was 21.18 ($p<0.01$), the content was affected more by fermentation time than by temperature (F-ratio 9.54, $p<0.05$). The amino nitrogen was production was maximum at 51.34°C fermentation temperature and 19.44 hours fermentation time; but rapidly decreased to 600 mg% and less when the temperature was 60°C or over, similar to total nitrogen. According to the standards of quality for anchovy sauce (17), the total nitrogen should be at least 1% (at least 0.5% when the sauce is seasoned) and the amino nitrogen should be at least 600 mg% (at least 300 mg% when the sauce is seasoned). Other studies have reported that conventional anchovy sauce is superior in quality when the total nitrogen is around 2% and the amino nitrogen is at least 800 mg%, while most commercially available anchovy sauce is lower in quality with a total nitrogen of 0.70~1.87% and amino nitrogen of 338.6~662.2 mg% (4,18). The anchovy sauce fermented by thermoase in this study satisfied the standards of quality for the short term when compared to quick anchovy sauce fermented by *koji* (2) or fig (5). The total nitrogen and amino nitrogen contents were similar to those of products currently on the market.

Changes in free amino acid

Amino acids have characteristic flavors; glycine, alanine, serine, threonine, and proline are sweet while arginine, methionine, valine, leucine, isoleucine, and phenylalanine are bitter (19). Such characteristics of amino acid affect the tastes of foods, and it is reported that differences in palatability are the result of both the total content and composition of free amino acids contained in products (18,20). In this study, not only total and amino nitrogen were used as objective quality indicators of anchovy sauce, but also the total content of free amino acids (Y_3) and sweet amino acid (Y_4) were used as response variables for determining the optimal conditions for fermentation. Sensory evaluation was also taken into account.

As seen in Table 2, the R^2 for the total content of free amino acids was 0.8496, approved with a 10% in significance level. The total content of free amino acids was affected more by fermentation time when the fermentation temperature was lower, and the maximum value was expected to be 3827.47 mg% at 54.4°C and 15.2 hours (Fig. 2). The response surface for the content of sweet amino acids among free amino acids including alanine showed the form of maximum point, and the optimal fermentation condition was at 53.6°C and 15.00 hours (the maximum value was 873.84 mg%). The R^2 for sweet amino acids was 0.9144, approved within 5% in significance level. Both total free amino acids and sweet amino acids were remarkably affected by time, and the composition of the free amino acids of quick anchovy sauce was similar to that reported by Park (21).

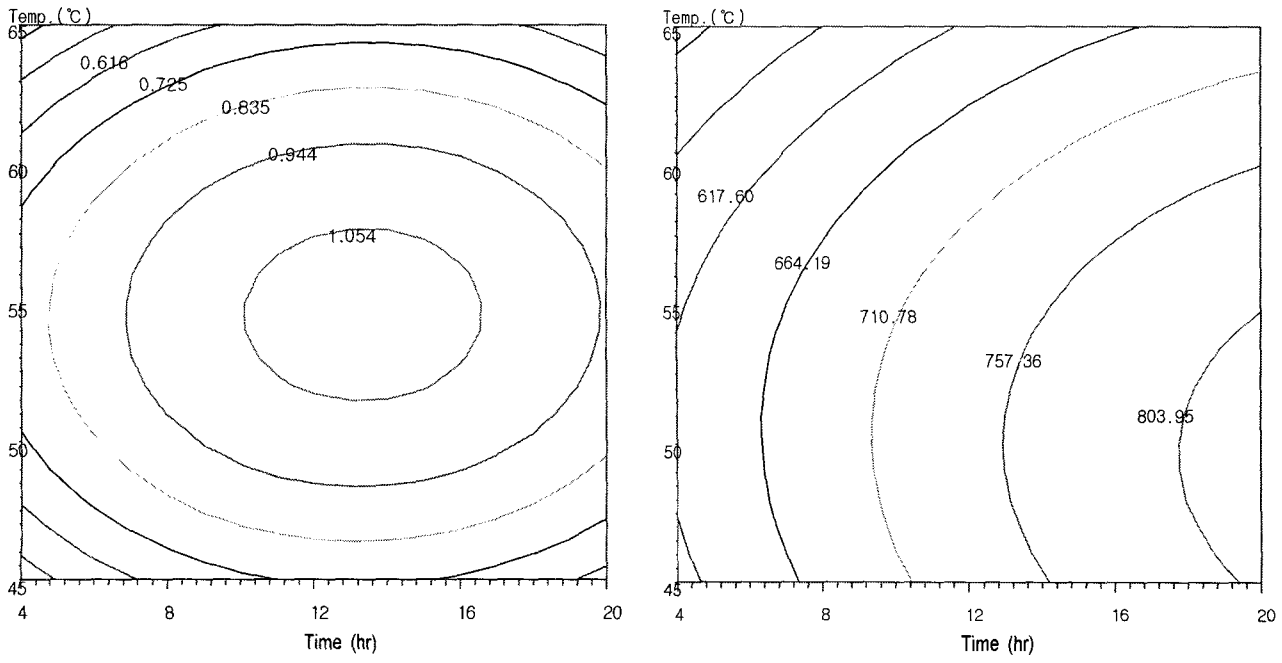

Fig. 1. Contour maps of total nitrogen (left) and amino nitrogen (right) in anchovy sauce.

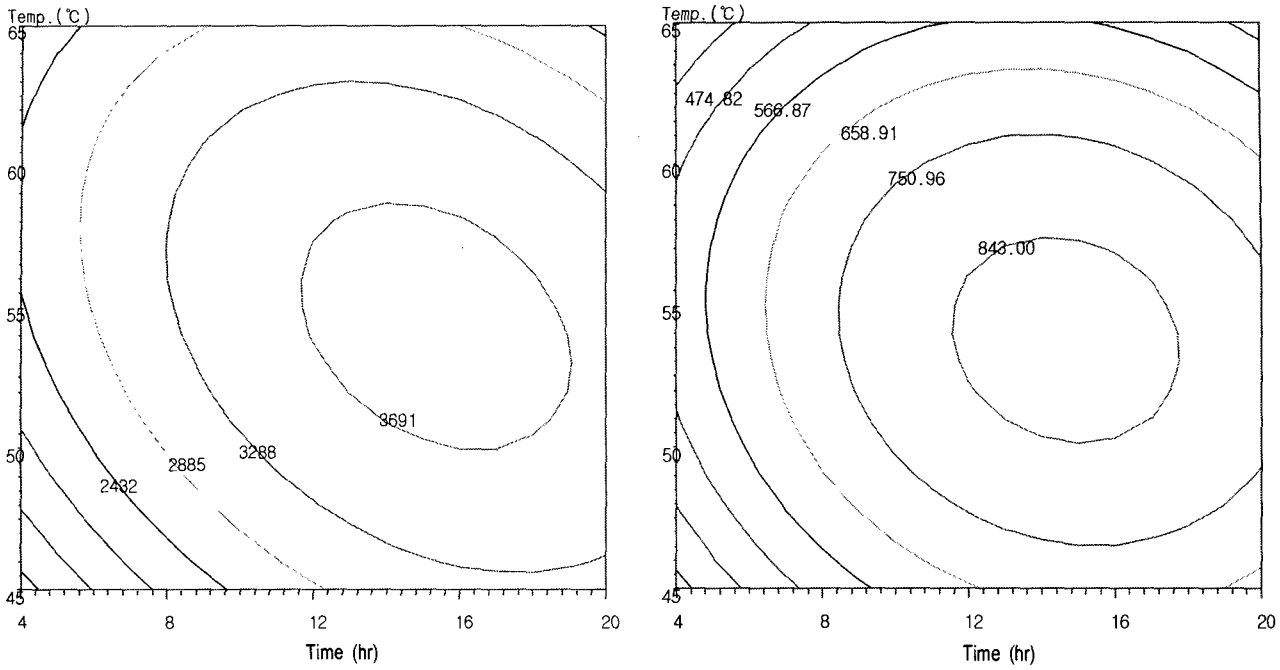

Fig. 2. Contour maps of total amino acid (left) and sweet amino acid (right) in anchovy sauce.

Optimization of the conditions for fermentation

In order to optimize the conditions for fermentation of quick anchovy sauce, the range showing superior quality was calculated by superimposing of the contour map of each dependent variable for fermentation temperature and time (Fig. 3). As seen in Table 3, the predicted optimal conditions for fermentation were 52.5~56.9°C for temperature and 13.3~16.4 hours for time. In order to test the predicted level from this study, the fermentation condition was set at 56°C for 16 hours, the random optimal point within the optimal range. As seen in Table 4, the actual values and the predicted values were similar, since the analyzed quality characteristics of the anchovy sauce fermented under the specified conditions closely matched with the predicted values.

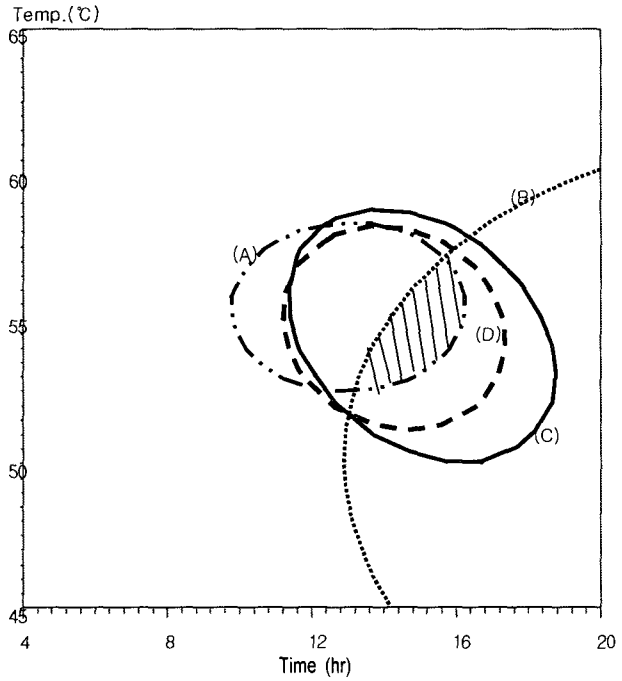

Fig. 3. Superimposed contour map for optimization of total nitrogen (A), amino nitrogen (B), total amino acid (C), sweet amino acid (D) of anchovy sauce.

Table 3. The optimum range of fermentation conditions for response variables by superimposing of contour maps for anchovy sauce

	Fermentation temp. (°C)	Fermentation time (hr)
Optimum ranges	52.5~56.9	13.3~16.4

Quality comparison with commercial products

Table 5 shows the results of the comparison between the quick anchovy sauce fermented at a random optimal

Table 4. Predicted and experimental values of response variables for anchovy sauce at a given condition within the range of optimum fermentation conditions

Response variables[1)]	Predicted values	Experimental values
Total nitrogen (Y_1)	1.05	1.06
Amino nitrogen (Y_2)	767.26	780.81
Total amino acid (Y_3)	3738.65	3806.11
Sweet amino acid[2)] (Y_4)	854.50	826.36

[1)]Total nitrogen (%), amino nitrogen (mg%), total amino acid (mg%), sweet amino acid (mg%).
[2)]Sweet amino acid means glycine, alanine, threonine, serine and proline.

Table 5. Comparison of quality properties of anchovy sauce

Quality properties	Anchovy sauce		
	I[1)]	II[2)]	III[2)]
Total nitrogen (%)	1.06±0.10	1.13±0.18	1.01±0.16
Amino nitrogen (mg%)	780.81±47.54[b3)]	792.02±39.08[b]	911.12±68.80[a]
Total amino acid (mg%)	3806.11±196.38[b]	4117.84±287.12[b]	4582.43±261.77[a]
Sweet amino acid[4)] (mg%)	826.36±58.72[a]	709.71±32.01[b]	754.98±46.26[ab]

[1)]Anchovy sauce made at a given condition within the range of optimum fermentation conditions.
[2)]Commercial products.
[3)]Values with different superscripts in the same rows are significantly different ($p<0.05$).
[4)]Sweet amino acid means glycine, alanine, threonine, serine and proline.

point (56°C, 16 hours) within the optimal conditions and the two kinds of the sauces on the market. The total nitrogen was higher in the on-the-market sauce (II) than in the quick sauce (I), but there was no significance difference. The amino acids were varied according to the kinds of sauces (780.81~911.12 mg%). While total amino acids were higher in the on-the-market sauce (II, III) than in the quick sauce, sweet amino acids were highest in the quick sauce (826.36 mg%), which might affect the taste of anchovy sauce. Therefore, the results of this study suggest that the quick fermentation method may be successful for production of anchovy sauce by using thermoase, and that further studies are needed to focus on the characteristics of materials, sensory quality, and preservation in order for the results of this study to be used commercially.

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