Food Sci. Biotechnol. Vol. 14, No. 5, pp. 576 ~ 580 (2005)

Food Science and Biotechnology

Isolation of Higher Alcohol-Producing Yeast as the Flavor Components and Determination of Optimal Culture Conditions

Dong-Jin Kwon* and Wang June Kim[1]

Department of Food Science, Wonju National College, Wonju 220-840, Korea
[1]Korea Food Research Institute, Seongnam 463-420, Korea

Abstract Ten yeast strains affecting *doenjang* flavor were isolated from soybean fermented foods (traditional *meju* and *doenjang*), among which *Zygosaccharomyces* sp. Y-2-5, showing excellent growth, glucose consumption, pH, and flavor production, was selected. Higher alcohols produced by *Zygosaccharomyces* sp. Y-2-5 related to flavor were 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, and 3.3-dimethyl-2-butanol. Optimal culture conditions for *Zygosaccharomyces* sp. Y-2-5 were 10% (w/v) NaCl, pH 4.0, 3.0% (w/v) glucose concentration, and inoculation time day 0 or 15 *doenjang* fermentation.

Key words: yeast, *Zygosaccharomyces*, higher alcohols, optimal culture condition

Introduction

According to the environmental conditions of each country, many different kinds of traditional fermented foods are available. In Korea, there are many traditional soybean fermented foods including soy sauce, *doenjang*, and *kochujang*, which are Korea's oldest prepared seasoning. These foods have played an important role as supplements of protein when animal proteins were in short supply. They supply nutrients such as essential amino acids and essential fatty acids (1). In addition, these soybean-fermented foods have several functional properties such as antimutagenicity, anticancer, biodefensive power, and control of high blood pressure (2-8).

The factors which determined the taste and flavor of soybean fermented foods are raw materials, microorganisms, and manufacturing process, among which microorganisms, yeasts in particular, play an important role on the taste and flavor of soybean-fermented foods.

Although *Bacillus* sp. is responsible for the production of major flavors of *doenjang* (9, 10), Lee *et al.* reported that the mixture of *Saccharomyces rouxii, Torulopsis versatilis,* and *Torulopsis etchellsii* added to the manufacture of *kochujang* improved the flavor and taste of *kochujang* (11)

The addition of *Zygosaccharomyces rouxii* mutant to Japanese *doenjang* produced higher alcohols, which improved the taste and flavor of *doenjang* (12, 13). Thus, major studies on the taste and flavor of soybean fermented foods have been focused on the comparison of the flavor components of *doenjang* and soy sauce with ones produced by microorganisms from those foods. However, no research has been performed on the improvement of the flavor components during manufacturing of soybean fermented foods with higher alcohols produced by adding yeasts.

*Corresponding author: Tel: 82-33-760-8452; Fax: 82-33-760-8450
E-mail: kdj6001@wonju.ac.kr
Received March 3, 2005; accepted August 1, 2005

To improve the flavor of *doenjang*, this study focused on isolating and establishing optimal culture conditions of yeasts producing higher alcohols as the flavor enhancing components.

Materials and Methods

Strains and medium Ten yeasts were isolated from soybean fermented foods, such as traditional *meju* and *doenjang*. The screening criterions were the growth, glucose consumption, pH, and the production of flavor components in YM broth (13). YM broth consisting of 10.0% (w/v) NaCl, 5% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract (pH was adjusted to 6.0-7.0 before steam-sterilization) was prepared, and the yeasts were incubated at 30°C for 3 days.

Determination of optimal culture conditions for yeast Optimal culture conditions for yeast growth were determined in YM broth with different concentrations of sugar and NaCl, and pH. The effects of glucose and NaCl concentrations on the growth of yeasts were examined at 30°C for 3 days using YM broth containing 1, 3, 5, and 9 % (w/v) glucose and 8 to 18% NaCl, respectively. The influence of pH on yeasts growth was determined by incubation at 30°C for 3 days with pH of YM broth adjusted from 3.0 to 6.0.

Determination on inoculation time of yeast The inoculation time of yeast for improvement of the taste and flavor of *doenjang* was determined during *doenjang* manufacture. The selected yeast was inoculated at days 0, 15, and 30 of 60-day *doenjang* fermentation. Viable cell count, reducing sugar, amino-type nitrogen, and flavor components of *doenjang* were measured, and the sensory evaluation was performed during fermentation for 60 days.

Preparation of *doenjang* Domestic soybeans were washed, soaked in tap water for 12 hr, and steamed for 4 hr. *Koji* was prepared by inoculating *Aspergillus oryzae* to

steamed soybeans and incubating at room temperature for 44 hr. *Doenjang* was prepared by adding salt and water to *koji*. NaCl concentration of *doenjang* was 10% (w/w). The number of yeasts was 1.0×10^5 cfu/g of *doenjang*.

Analysis of flavor components The flavor components, higher alcohols including 1-propanol, produced by the yeast in soy sauce medium (raw soy sauce, 100.0 mL/L; glucose, 100 g/L; NaCl, 10 g/L; pH 6-7; 25°C) incubated at 30°C for 3 days were measured. Ten grams of the sample mixed with nitrogen and 2-octanol, and internal standard were placed in 50 mL serum bottle, and the flavor components were extracted at 50°C for 30 min. To analyzed the flavor components, headspace gas in serum bottle was measured by gas chromatography (6890 series, Hewlett Packard, USA) under the following conditions: 12 % FFAP pack column, i.d. 2 mm × 2 m; injector temperature, 250°C; detector temperature, 280°C; temperature program, 50-125°C (2.5°C/min); carrier gas, He (99.99%); injection volume, 1 mL.

Analytical procedure The growth of yeasts in YM broth cultured at 30°C for 3 days was measured at 550 nm using a spectrophotometer (Model No. 8453, Hewlett Packard, USA). Glucose consumption of yeasts was measured by Somogyi method (14), and the pH of culture solution was determined using a pH meter (Model No. 8417, Hanna, Singapore). Viable cell counts of yeast were determined by plating the yeasts on a potato dextrose agar (Difco Labaratory, MI, USA) (15). The yeasts were tentatively identified by using an API kit 32C (API bioMerieux, France) based on their physiological characteristics.

Sensory evaluation For sensory evaluation of *doenjang*, 10 panel members performed a 9-point sensory test in terms of color, flavor, taste, and overall acceptance. The panel members were instructed on the objective of the experiment, and the samples were graded as like very much (9 point), average (5 point), and dislike very much (1 point).

Statistical analysis Data were analyzed by ANOVA using SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA). Differences among samples were analyzed using Duncan's multiple range test at 5% significance level (16).

Results and Discussion

Selection of yeast To select the yeast that produced higher alcohols including 1-propanol, 10 yeasts were isolated from soybean fermented foods, such as traditional *meju* and *doenjang*, and cultivated in YM broth at 30°C for 3 days. The pH of YM broth, growth and glucose consumption of yeasts are shown in Table 1. The pH was maintained between 4.16-4.40 at day 1 and decreased rapidly to 3.72-3.80 at day 3. The strains that produced acids were Y-4, Y-2-5, and Y-3-8. Significant differences in the growth of yeasts in YM broth containing high sugar and NaCl concentrations were observed among the strains. At day 1, strains Y-9-3 and Y-2-5 showed the lowest and highest growths, respectively. At day 3, the growths of strains Y-2-5 and Y-3-8, which showed the highest growth, were 2.72 and 2.71, respectively. To prevent post-fermentation of *doenjang* during storage, the glucose consumption of yeasts was measured. Among the 10 yeast strains, strains Y-4, Y-2-5, Y-23-2, and Y-23-5 consumed more than 50% glucose. Based on these results, strains Y-2-5 and Y-3-8 were selected.

The flavor components produced by strains Y-2-5 and Y-3-8 in soy sauce medium at 30°C for 3 days are shown in Table 2. Production of higher alcohols by strain Y-2-5 was 1.2 fold higher than that of strain Y-3-8. The higher alcohols produced by strains Y-2-5 and Y-3-8 were ethanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, ethyl propionate, 3.3-dimethyl-2-butanol, and ethyl acetate, among which higher alcohols related to the flavor of *doenjang* were 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, and 3.3-dimethyl-2-butanol. These results are similar to the results of Aoki *et al.* (12) and Nakamura (13). They reported that higher alcohols, such as *n*-propanol, *n*-butanol, iso-butanol, *n*-amyl alcohol, and iso-amyl alcohol, were produced by the mutant of *Z. rouxii* during *doenjang* manufacture. Therefore, strain Y-2-5, producing higher alcohols as the flavor components, was selected for use in the *doenjang* manufacture to improve the flavor.

Identification and optimal culture conditions of yeast

Table 1. Changes of pH, growth and glucose consumption of isolated yeasts in YM broth

Parameters	pH			Growth (Absorbance at 550 nm)			Glucose consumption (%)		
Time (days)	1	2	3	1	2	3	1	2	3
Y-4	4.27	3.87	3.72	0.56	2.02	2.40	47.0	53.0	59.0
Y-67	4.18	3.90	3.80	1.00	2.28	2.50	32.3	46.1	49.8
Y-2-5	4.16	3.91	3.78	1.08	2.46	2.72	41.0	42.4	52.1
Y-3-8	4.24	3.91	3.77	0.89	2.41	2.71	43.8	48.4	52.5
Y-8-7	4.38	4.07	3.87	0.31	2.01	2.55	41.9	43.8	46.5
Y-8-8	4.32	3.99	3.82	0.47	2.07	2.62	46.1	47.9	48.4
Y-9-3	4.40	4.01	3.88	0.24	1.78	2.35	41.9	41.0	45.2
Y-9-5	4.38	4.04	3.90	0.31	1.96	2.48	44.2	46.1	49.3
Y-23-2	4.32	3.96	3.84	0.43	2.30	2.65	44.2	47.0	52.5
Y-23-5	4.31	4.00	3.86	0.49	2.01	2.55	45.2	44.7	50.2

Table 2. Flavor components of yeasts incubated in soy sauce medium (Unit : area)

Parameters	Y-3-8	Y-2-5
Ethanol	1698.9±452.8	2049.3±28.40
2-Propanol	2.59±0.79	3.42±1.45
1-Propanol	4.40±1.07	5.38±0.61
Ethyl acetate	4.53±1.40	4.41±0.14
2-Methyl-1-propanol	32.93±9.92	37.54±0.33
1-Butanol	1.87±0.40	2.37±0.65
Ethyl-propionate	0.81±0.33	0.85±0.12
Unknown	88.74±27.22	95.79±0.92
3.3-Dimethyl-2-butanol	32.74±10.09	33.63±0.32
Total	1867.51	2232.69

Strain Y-2-5 was tentatively identified as *Zygosaccharomyces* sp. using the API kit. To obtain the optimal culture conditions of *Zygosaccharomyces* sp. Y-2-5, the growth of yeast in YM broth with different concentrations of sugar and NaCl, and pH was measured, and the results are shown in Table 3. *Zygosaccharomyces* sp. Y-2-5 showed poor growth in YM broth containing over 14% NaCl. On the other hand, the growth of yeast within 8-12% NaCl was good. Considering that the average NaCl concentration of a commercial *doenjang* is 10% (w/v), the optimal NaCl concentration of *Zygosaccharomyces* sp. Y-2-5 was determined to be 10%. The growth of *Zygosaccharomyces* sp. Y-2-5 in YM broth at pH 4.0 was the highest. Because the average pH of the commercial *doenjang* is mild acidic, the optimal pH was determined as 4.0. The growth of *Zygosaccharomyces* sp. Y-2-5 was the highest at 3% glucose concentration. Therefore, the optimal culture condition of *Zygosaccharomyces* sp. Y-2-5 was determined as 10% NaCl, 3.0% glucose concentration, and pH 4.0.

Table 3. Changes of growth on NaCl concentration, pH , and glucose concentration Of selected *Zygosaccharomyces* sp. Y-2-5 during the incubation for determining of the optimal culture conditions

Parameters	Conc.	Incubation times (days)		
		1	2	3
NaCl concentrations (%, w/v)	8.0	0.050	1.535	2.781
	10.0	0.043	1.094	2.638
	12.0	0.036	0.841	2.467
	14.0	0.032	0.522	2.018
	16.0	0.010	0.478	2.036
	18.0	0.011	0.188	0.915
pH	3.0	0.018	0.409	1.246
	3.5	0.074	1.518	2.481
	4.0	0.438	2.375	2.872
	4.5	0.287	1.933	2.715
	5.0	0.129	1.510	2.638
	5.5	0.110	1.501	2.673
	6.0	0.113	1.312	2.623
Glucose concentrations (%, w/v)	1.0	1.030	2.643	2.854
	3.0	1.342	2.585	3.021
	5.0	1.157	2.497	2.900
	7.0	1.070	2.512	2.884
	9.0	1.119	2.509	2.788

Determination of inoculation time To determine the optimal addition time of yeast, *Zygosaccharomyces* sp. Y-2-5 was inoculated at days 0, 15, and the 30, and the *doenjang* was fermented for 60 days. The yeast cell count, amino-type nitrogen, flavor components, and sensory evaluation of *doenjang* during fermentation are follows.

Figure 1 shows that the number of *Zygosaccharomyces* sp. Y-2-5 slowly increased up to day 20, decreased slowly up to day 40, then rapidly decreased thereafter. In the day 15-inoculated sample, which showed the highest growth rate, the number of cells rapidly increased up to day 20 then decreased slowly. Therefore, day 15 was selected as the optimal inoculation time of the yeast into *doenjang* during fermentation. Amino-type nitrogen of *doenjang* as an index of *doenjang* fermentation is shown in Fig. 2. The amino-type nitrogens of all samples showed an increasing trend during the course of fermentation, rapidly increasing up to day 20, then slowly thereafter. Maximum amount of amino nitrogen was produced from *Zygosaccharomyces* sp. Y-2-5-inoculated sample at day 15. Considering the optimal amino-type nitrogen amount of *doenjang* was more than 160 mg%, the ideal fermentation time was days 20-30.

The flavor components of *doenjang* with different addition time of *Zygosaccharomyces* sp. Y-2-5 are shown in Table 4. Methanol, ethanol, 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3,3-dimethyl-1-butanol, and one unknown peak were detected in all treated samples.

Approximately 1.4 fold higher amount of alcohols were produced from *Zygosaccharomyces* sp. Y-2-5 added at day 0 than the control, whereas day 15 sample and the control showed almost the same amounts. On the other hand, the production of higher alcohol from the batch with 30 day-added strain was lower than that of the control, and the

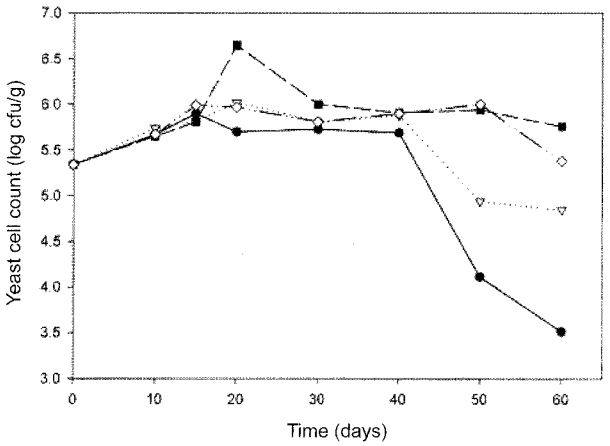

Fig. 1. Changes of yeast cell count in *doenjang* during fermentation. –●–: *Doenjang* A, *Doenjang* fermented without *Zygosaccharomyces* sp. Y-2-5 for 60 days fermentation (control), –▽–: *Doenjang* B; *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 0 of 60-day fermentation, – ■ –: *Doenjang* C; *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 15 of 60-day fermentation, –◇–: *Doenjang* D, *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 30 of 60-day fermentation.

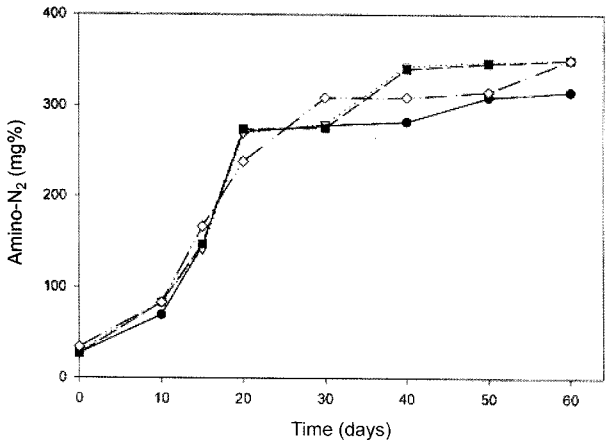

Fig. 2. Changes of amino-N_2 in *doenjang* during fermentation. – ● –: *Doenjang* A, *Doenjang* fermented without *Zygosaccharomyces* sp. Y-2-5 for 60 days fermentation (control). –▽–: *Doenjang* B, *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 0 of 60-day fermentation. – ■ –: *Doenjang* C, *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 15 of 60-days fermentation. –◇–: *Doenjang* D, *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 30 of 60-day fermentation.

effect of added yeast strain could not be detected. From these data, the most effective addition time of yeast was determined to be day 0, alcohols produced thereby contributing to the extension of shelf life of *doenjang*. Large amount of amino-type nitrogen was produced from the samples inoculated at days 0 and 15, and day 30 sample showed lower amount of amino-type nitrogen than the control, indications that inoculation of yeast during mid-fermentation is not desirable in terms of amino-nitrogen production.

The sensory evaluation of *doenjang* prepared by different methods is shown in Table 5. Generally, inoculation of yeast resulted in higher scores than the control. The average taste score for the control was 5.75, and the samples prepared by inoculation of yeast at days 0 and 15 showed higher, and those prepared at day 30 lower scores than the control. No significant difference ($p<0.05$) in flavor was observed between inoculated samples and the controls. The flavor score of the control was 4.92, lower than those of the samples inoculated with *Zygosaccharomyces* sp. Y-2-5 at days 0 and 15. Similar results were observed in the color and overall acceptance.

Considering the production of alcohols, sensory evaluation, and the growth of yeast strains, the most effective time for the addition of yeast was either day 0 or 15.

References

1. Kwon DJ. Comparison of characteristics of *koji* manufactured with *Bacillus subtilis* B-4 and *Aspergillus oryzae* F-5. Korean J. Food Sci. Technol. 34: 873-878 (2002)
2. Chung KS, Yoon KD, Kwon DJ, Hong SS, Choi SY. Cytotoxicity testing of fermented soybean products with various tumor cells using MTT assay. Korean J. Appl. Microbiol. Biotechnol. 25: 477-482 (1997)
3. Yoon KD, Kwon DJ, Hong SS, Kim SI, Chung KS. Inhibitory effect of soybean and fermented soybean products in the chemically induced mutagenesis. Korean J. Appl. Microbiol. Technol. 24: 525-528 (1996)

Table 4. Changes of flavor compounds in *doenjang* with different yeast inoculation time (Unit: Area)

Samples	*Doenjang*-A[1] (Control)	*Doenjang*-B[1]	*Doenjang*-C[1]	*Doenjang*-D[1]
Ethanol	74.90±5.74 (1.00)	116.06±5.12 (1.50)	78.86±16.42 (1.05)	57.56±29.58 (0.76)
1-Propanol	6.26±0.58 (1.00)	8.70±0.44 (1.38)	7.80±0.98 (1.24)	3.60±2.00 (0.57)
Ethyl acetate	0.63±0.15 (1.00)	0.77±0.06 (1.22)	0.64±0.06 (1.00)	0.17±0.06 (0.26)
2-Methyl-1-propanol	1.60±0.36 (1.00)	1.87±0.06 (1.16)	1.78±0.17 (1.11)	0.30±0.17 (0.19)
Unknown	10.50±1.20 (1.00)	9.40±0.44 (0.90)	5.73±1.04 (0.55)	4.30±2.38 (0.41)
3,3-Dimethyl-2-butanol	3.06±0.45 (1.00)	3.36±0.15 (1.10)	3.15±0.26 (1.03)	1.07±0.59 (0.35)

[1]*Doenjang* A; *Doenjang* fermented without *Zygosaccharomyces* sp. Y-2-5 during 60 days fermentation (control).
Doenjang B; *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 0 of 60-day fermentation.
Doenjang C; *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 15 of 60-day fermentation.
Doenjang D; *Doenjang* added with *Zygosaccharomyces* sp. Y-2-5 at day 30 of 60-day fermentation.
[2]The number in parentheses represented relative ratio to peak area of flavor components produced by *doenjang* without yeast.

Table 5. Sensory evaluation of *doenjang* by hedonic scale[1]

Parameters	*Doenjang*-A[2] (Control)	*Doenjang-B*[2]	*Doenjang-C*[2]	*Doenjang-D*[2]
Taste	5.75±1.66	5.92±2.50	5.83±2.08	5.17±1.75
Flavor	4.92±1.83[a]	6.33±1.61[a]	6.07±1.85[a]	4.08±2.02[b]
Color	5.33±2.23	6.25±1.71	6.33±1.37	5.41±1.73
Overall acceptability	6.08±1.98	6.50±1.78	6.67±1.78	5.42±1.51

[1]Each value represents the mean±SD of 10 observations using hedonic scale of 1 (dislike very much) to 9 (like very much).
[2]*Doenjang* A, B, C, and D referred to Table 4.
[ab]Means in row followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha=0.05$).

4. Cheigh HS, Lee JS, Moon GS, Park KY. Antioxidative activity of browning products fractionated from fermented soybean sauce. J. Korean Soc. Food Nutr. 22: 565-569 (1993)
5. Park KY, Moon SH, Baik HS, Cheigh HS. Antimutagenic effect of *Doenjang* (Korean fermented soy paste) toward aflatoxin. J. Korean Soc. Food Nutr. 19: 156-162 (1990)
6. Hong SS, Chung KS, Yoon KD, Cho YJ. Antimutagenic effect of solvent extracts of Korean fermented soybean products. Foods Biotechnol. 5: 263-267 (1996)
7. Atsuko I, Yuji T, Yukio K. Physiological of miso. Jpn. J. Brewing Soc. 89: 869-872 (1994)
8. Naohoko Y. Antioxidative activity of *Miso*. Jpn. J. Brewing Soc. 87: 721-725 (1992)
9. Park HK, Gil B, Kim JK. Characteristic flavor compounds of commercial soybean paste. Food Sci. Biotechnol. 12: 607-611 (2003)
10. Song JY, Ahn CW, Kim JK. Flavor components produced by microorganism during fermentation of korean ordinary soybean paste. Korean J. Appl. Microbiol. Bioeng. 12: 147-152 (1984)
11. Lee TS, Yang KJ, Park YJ, Yu JH. Studies on the brewing of Kochujang (red pepper paste) with the addition of mixed cultures of yeast strains. Korean J. Food Sci. Technol. 12: 313-323 (1980)
12. Aoki T, Uchida K. Amino acids-uptake deficient mutants of *Zygosaccharomyces rouxii* with altered production of higher alcohols. Agric. Biol. Chem. 55: 2893-2894 (1991)
13. Nakamura M. Isolation of mutants of miso yeast, *Zygosaccharomyces rouxii* with high productivity of C3 to C5 alcohols. J. Jpn. Soc. Food Sci. Technol. 37: 981-983 (1990)
14. Somogyi M. Notes on sugar determination. J. Biol. Chem. 195: 19-21 (1952)
15. Difco laboratories. Difco Manual. 10th (ed.). pp. 679-689. Michigan, USA (1984)
16. SAS Institute, Inc., SAS user's Guide. Statistical Analysis System Institute, Cary. NC, USA (1998)