Food Sci. Biotechnol. Vol. 15, No. 1, pp. 77 ~ 81 (2006)

Food Science and Biotechnology

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Network Structure and Dextran Formation of *Jeungpyeon* Made with Yeast Starter

Young-Sook Hahn*, **Hae-Eun Lee, Ju-Yeon Park and Kyung-Ja Woo**[1]

Department of Food and Nutrition, Sung-shin Women's University, Seoul 136-742, Korea

[1]*Department of Food and Nutrition, In-ha University, Incheon 402-751, Korea*

Abstract The dextransucrase activity of microorganisms which were identified as contributing to the fermentation of *jeungpyeon* made with yeast was measured. The dextran generated during fermentation was quantified and the viscosity changes were measured. The mechanism of network structure formation was clarified by observing the inside of the network structure over the fermentation periods ranging from 1 to 7 hr using scanning electron microscopy (SEM). The pH of *jeungpyeon* batter decreased significantly as the fermentation proceeded, whereas the viscosity increased. The identified lactic acid bacteria (LAB) were *Leuconostoc mesenteroides* subsp. *mesenteroides, Pediococcus pentosaceus, Tetragenococcus halophilus,* and *Leuconostoc mesenteroides* subsp. *dextranicum*. The yeast was identified as *Saccharomyces cerevisiae* A/Tor. *Pretorien.* The dextransucrase extracted from those microorganisms showed high activity. On the other hand, the amount of dextran generated from the batter increased significantly beyond 2 hr of fermentation, and the viscosity increment showed a similar trend. The SEM photos showed that the most homogeneous fine network structure was observed in the batter fermented for 2 hr. Therefore, we assumed that the dextran that was generated by microorganisms during fermentation interacted with the components of the batter to increase the stability of the network structure.

Keywords: *jeungpyeon*, dextran, dextransucrase, yeast, lactic acid bacteria

Introduction

Jeungpyeon is a Korean traditional fermented rice cake, which is made with rice flour and rice wine. Recently, *jeungpyeon* has been prepared with yeast instead of wine in order to shorten the preparation time and to improve the quality (1). However, the resulting cake tastes a little sour and has a structure like spongy tissues similar to breads in western countries (2). For western breads, gluten, the major protein of wheat, contributes to the formation of spongy tissue structure by trapping air inside of the network structure. Nevertheless, although there is no gluten-like protein in rice, it is speculated that the spongy tissue structure of *jeungpyeon* which is similar to that of bread is caused by the interaction of amylose, proteins and other high-molecular weight polymeric compounds during fermentation (3).

Some products are made with rice using a process similar to that of *jeungpyeon* production. They include Idli (rice pudding) and Dosai (rice cake) in Indonesia (4, 5), Puto (rice cake) in Philippines (6), and yeast-leavened bread made with only rice for patients with an allergy to wheat. Mukherjee and others (7) studied the fermentation process of Idli and reported that *Leuconostoc mesenteroides* were isolated during batter fermentation, and that the microorganism produced carbon dioxide. Also, dextran, a high-molecular weight compound, is known to be generated from sugar by some microorganisms containing dextransucrase. Bean *et al.* (8) examined the effects of different treatments of rice flour on rice bread production. Kulp *et al.* (9) and Nishita *et al.* (10) studied the effects of adding various gums and surfactants to improve the good feeling of the final product, gas holding capacity and volume expansion. As reported in another study (11), during the formation of spongy tissue structure in rice fermented products, a stable network structure is formed as some ingredients are added to the rice materials or as compounds are produced during fermentation process. Kang and Kang (3) reported the production of high molecular weight polymer compounds during *jeungpyeon* fermentation. Shin and Woo (12) reported that the addition of beans improved the sponge tissue structure of *jeungpyeon.* Shin and Woo (13) separated dextran from *jeungpyeon.* Furthermore, another research paper reported that yeast and lactic acid bacteria (LAB) contributed to the formation of dextran (14).

The objectives of this study were to examine the activity of dextransucrase produced by microorganisms that contribute to the fermentation of *jeungpyeon* made with yeast instead of with rice wine for shortened preparation time, to quantify the dextran generated and to observe the network structure formed during fermentation. In addition, the contribution of those microorganisms on the network structure was examined by observing their morphological changes.

Materials and Methods

Materials and chemicals Polished rice (Il-poom variety) for *jeungpyeon* preparation was purchased from the Crops Laboratory of the Rural Development Administration. Refined sugar (CJ, Korea), salt with a NaCl purity over 88% (Hae-Pyo, Korea), a commercial compressed baker's yeast (Ottogi, Korea) and distilled water were used for *jeungpyeon* production. Potato dextrose agar (PDA: Difco Laboratories, Detroit, MI, USA), Biolog universal growth

*Corresponding author: Tel: 85-2-920-7210; Fax: 85-2-921-3197
E-mail: yshan@sungshin.ac.kr
Received August 19, 2005; accepted December 15, 2005

(BUG) and yeast (BUY) (Biolog Inc., Hayward, CA, USA) culture media and other chemical reagents (Sigma, St. Louis, MO, USA) were also used in this study.

Preparation of *jeungpyeon* The rice was washed with distilled water three times and soaked with water at 30°C for 1 hr. The proportion of other ingredients is presented in Table 1 and was set in accordance with the results of preliminary tests and other research (15).

The soaked rice was dehydrated on a sieve, mixed with the other ingredients without the yeast in accordance with preset conditions, and ground for 2 min in a mill (FM-808, Han-il, Korea). Yeast was then added to the mixture which was ground for a few more seconds to form a sticky paste batter. The batter was divided into segments of 30 g each, kept in paper cups (5 cm in diameter, 7.1 cm in height) covered with aluminum foil to prevent loss of moisture and fermented at 30°C for 2 hr (first fermentation). After the first fermentation, the batter was stirred to remove gas, and placed in a steamer for second fermentation at 60°C for 30 min to raise the batter. Finally, the batter was steamed for another 30 min at boiling temperature for cooking and then cooled at room temperature (Fig. 1).

Examination of the physical properties of *jeungpyeon* batter The viscosity of the batter was measured with a batter sample collected after the first fermentation at time intervals of 0, 1, 2, 3, 5, and 7 hr using a viscometer (B8M, Tokimec Inc., Tokyo, Japan). Distilled water (25 mL) was added to 30 g of each batter and homogenized with a stirrer. While the batter was fermented, the pH was measured using a pH meter (Mettler Toledo 345 pH meter, Mettler-Toledo, columbus, OH, USA).

Table 1. Formula for *jeungpyeon* preparation

Ingredients(g)				
Rice[1)]	Water	Salt	Sugar	Yeast
100	30	0.8	15	1

[1)]Rice soaked in water for 1 hr at 30°C.

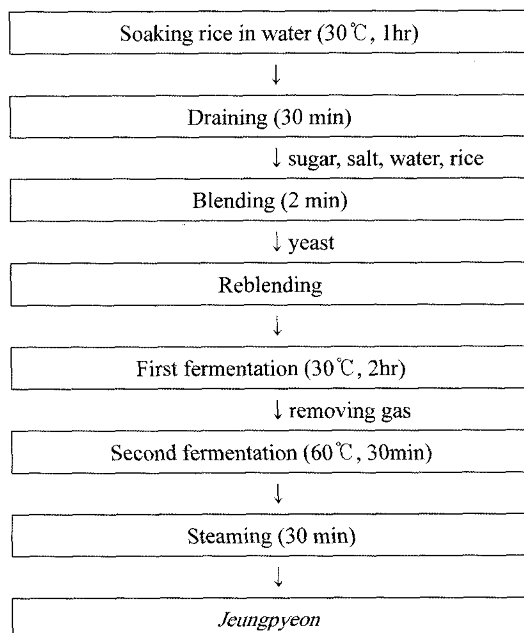

Fig. 1. Preparation process of *jeungpyeon.*

Isolation and identification of the microorganisms contributing to the fermentation *Jeungpyeon* batter samples were collected at 1, 2, 3, 5, and 7 hr during the first fermentation at 30°C and diluted down by a factor of 10^{-7} by the gradual dilution method. The lactic acid bacteria (LAB) were isolated in the phenylethyl alcohol sucrose agar (PES) medium agar (16) at 20°C. The yeasts were isolated from PDA medium at 30°C for 24 hr. The separated LAB were then cultured in the media made with 95% BUG agar and 5% sheep blood at 35°C for 48 hr. The isolated yeasts were also cultivated in the BUY agar medium at 30°C for 24 hr. MicroLog system (Biolog Inc.) was used for the identification of the isolated microorganisms.

The bacteria were suspended in GN/GP-IF (0.40% sodium chloride + 0.03% pluronic F-68 + 0.01% gellan gum), and a 15 μL sample was pipetted into microplates for the cell suspension. The yeasts from cultivated agar were suspended in sterile water, and a 100 μL sample was pipetted into microplates for the cell suspension. After the samples were sealed, the microplates were incubated for 24 hr, at 37°C for bacteria and 30°C for yeasts, and finally were read using the Microstation reader at 660 nm.

Measurement of dextransucrase activity The culture broths of *Saccharomyces cerevisiae* A/To. *pretorien* and LAB cultivated in PES medium for 24 hr were filtered and their dextransucrase activities were determined. Acetate buffer (3 M; pH 5.4) and 60 g of sucrose melted in 100 mL of distilled water were used as substrate. The culture solution was centrifuged for 10 min at 8,000×g, and its upper solution was utilized to measure the activity. Substrate solution (5 mL) and microbial solution (1 mL) were mixed into a test tube and reacted for 1 hr at 30°C, after which 0.04 N NaOH was added as the reaction inhibitor and protein precipitator. The solution was centrifuged for 10 min at 8,000×g and its upper solution was taken as the sample. The activity of microbial dextransucrase was measured with the following methodology: 100 μL of sample was placed into 150 μL of dinitrosalicylic acid (DNS) reagent in accordance with the DNS method (17, 18), vortexed carefully and left in a water bath at 80°C for 3 min. Then the sample was held in ice for 2 min after which 0.8 μL of distilled water was added. The reduced sugar (fructose) was quantified by measuring the absorbance at 520 nm using the fructose standard curve. Protein was determined by the Bradford method using standard protein BSA (19). The activity of enzyme was represented by the quantity of the enzyme that changed 1 mg of sucrose to dextran in 1 hr reaction at 30°C. It was equivalent to 0.52 mg of fructose generated in the reaction.

Extraction and measurement of dextran generated during fermentation The content of dextran in the

batter was determined in accordance with the Copper method (20, 21). Ethanol anhydride (40 mL) was added to 1 g of *jeungpyeon* batter that was left for 5 min to form precipitate. After centrifuging at 2,000×g for 10 min, the upper layer solution was discarded and then 40 mL of 80% ethanol was added to wash the precipitate. Then the sample was centrifuged at 2,000×*g* for 10 min, and the upper layer solution was discarded. The procedure was repeated twice. Distilled water was added to the precipitates to top up to 25 mL, 10 mL was removed and additions were made of 2 mL of 2.5 N NaOH reagents, 50 mL of Cu reagent, 2 mL of anhydrous sodium sulfate solution and 0.2 g of diatomaceous earth in 50 mL of distilled water. The sample was placed in a water bath at 100°C for 5 min, and then held at room temperature for 20 min. The precipitate was filtered through a sintered glass filter and loaded in the test tube. The test tube was washed with 10 mL of wash solution (10 mL of Cu reagent and 10 mL of 2.5 N NaOH reagents in 50 mL of distilled water).

Two milliliters of 2 N H_2SO_4 solution was poured on the precipitate with a funnel, and then the precipitate was depressurized to allow the acid solution to pass through. The process was repeated three times. The precipitate was washed with 2 mL of distilled water. The washed solution was topped up to 25 mL with distilled water from which 2 mL samples were taken out consecutively, and subjected to the phenol-H_2SO_4 test.

One milliliter of phenol solution and 10 mL of concentrated H_2SO_4 were mixed with the 2 mL-samples using a vortex mixer in the water bath at 20°C. The tube was placed in the water bath at 100°C for 2 min for reaction, and then cooled at room temperature for 30 min. The optical absorption was measured at 485 nm. To quantify the dextran, the standard curve was plotted with the standard dextran solution (MW: 77,000) of which the density was known. The dextran was determined by subtracting the initial values from the dextrans quantified from the standard curve.

Network structure of *jeungpyeon* by SEM To observe the inner structure of *jeungpyeon* formed during fermentation, 1 cm^3 of sample was cut from the center of the *jeungpyeon* cake. The sample was wrapped in vinyl wrapper, frozen at -80°C and then lyophilized for 24 hr using a freeze dryer (FD-3, Heto, Denmark). The freeze-dried sample was coated with gold using a gold iron coater (ID-2, EIKO Eng., Japan) and observed with scanning electron microscopy (SEM, JSM 5410LV, JEOL, Japan) at an acceleration voltage of 15 KV (2,000× and 10,000×).

Results and Discussion

Physical changes of *jeungpyeon* batter during fermentation Figure 2 shows the viscosity and pH of *jeungpyeon* batter over the fermentation time of 1, 2, 3, 5, and 7 hr. The pH of the initial batter was 5.59, but during the fermentation process the pH declined significantly to 5.16, 5.08, 4.99, and 4.74 at 1, 2, 3, and 7 hr of fermentation, respectively. It was speculated that the decline of pH during fermentation was caused by the production of organic acids, and the growth of LAB, yeast and some by-products generated during fermentation.

The viscosity of *jeungpyeon* batter increased significantly up to 2 hr, which was the first fermentation period. Despite a slight decrease at 3 hr, the viscosity was reasonably consistent throughout the fermentation period. In general, for most fermented foods, viscosity decreases with extended fermentation time due to the disintegration of high-molecular weight polymeric substances (3). However, the raised viscosity of *jeungpyeon* didn't decrease during fermentation, suggesting that high-molecular weight polymeric substances were generated over the fermentation period.

Separated and identified microorganisms from *jeungpyeon* batter Among the LAB isolated and identified from *jeungpyeon*, more than 85% was *L. mesenteroides* subsp. *mesenteroides*. The other identified LAB were *T. halophilus, P. pentosaceus, P. urinaeequi, L. mesenteroides* subsp. *dextranicum* and *L. lactis* subsp. *lactis*. In particular, *L. mesenteroides* subsp. *mesenteroides, P. pentosaceus, P. urinaeequi* and *T. halophilus* were identified at 1 and 2 hr of fermentation. At 3 hr of fermentation, *T. halophilus, P. pentosaceus, L. lactis* subsp. *lactis* and *S. suis* serogroup1/2 were identified. Then, after 5 hr of batter fermentation, *L. mesenteroides* subsp. *mesenteroides, T. halophilus* and *L. lactis* subsp. *lactis* were identified. *T. halophilus* and *L. lactis* subsp. *diacetylactis* were identified from the batter fermented for 7 hr. In addition, the yeast isolated from *jeungpyeon* was identified as the *S. cerevisiae* A/Tor. *pretorien*.

It is known that dextransucrase (α-D-1,6-glucan; 6-α-D-glucosyl transferase, EC 2.4.1.5), which is an extracellular enzyme formed by genus *Leuconostoc*, produces fructose and dextran from sucrose which is used as the substrate (22, 23). It could also be assumed that the viscosity increment during *jeungpyeon* fermentation was caused by the high-molecular weight polymeric compounds such as dextran that were formed by dextransucrase during fermentation. Therefore, to clarify the dextransucrase and dextran production during *jeungpyeon* fermentation, the activity of dextransucrase extracted from the identified LAB was examined, and the amount of dextran extracted from *jeungpyeon* batter was quantified. Furthermore, to measure the activity of dextransucrase extracted from the identified LAB from *jeungpyeon*, 14 strains of microorganisms with excellent colony forming capability were selected.

Dextransucrase activity Table 2 shows the activity of dextransucrase extracted from the LAB isolated from *jeungpyeon* batter. The dextransucrase activity of the 14 selected strains was very high. *L. mesenteroides* subsp. *mesenteroides* 2-9 and *T. halophilus* 7-2 had the highest specific activities at 28.5 DSU/mg and 27.0 DSU/mg, respectively. The specific activity of yeast JY-3 (*S. cerevisiae* A/Tor. *pretorien*) was 21.5 DSU/mg. A relatively high activity was also shown in *L. mesenteroides* subsp. *dextranicum* 5-13, *L. mesenteroides* subsp. *mesenteroides* 1-4, *L. mesenteroides* subsp. *mesenteroides* 2-13, *L. mesenteroides* subsp. *mesenteroides* 1-1 and *T. halophilus* 5-6.

Measurement of dextran changes during fermenta-

Table 2. Dextransucrase activity of selected isolates

Sample No.	Species ID	enzyme activity (DSU)[1]	Protein (mg)	Specific activity (DSU/mg protein)
1-1	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.54	0.04	13.5
1-3	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.55	0.06	9.17
1-4	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.55	0.04	13.75
1-6	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.57	0.06	9.5
2-8	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.53	0.05	10.6
2-9	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.57	0.02	28.5
2-13	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.55	0.04	13.75
3-11	*Pediococcus pentosaceus*	0.54	0.05	10.8
5-2	*Leuconostoc mesenteroides* subsp. *mesenteroides*	0.57	0.06	9.5
5-4	*Tetragenococcus halophilus*	0.56	0.07	8.0
5-6	*Tetragenococcus halophilus*	0.54	0.04	13.5
5-13	*Leuconostoc mesenteroides* subsp. *dextranicum*	0.87	0.06	14.5
7-2	*Tetragenococcus halophilus*	0.54	0.02	27.0
JY-3	*Saccharomyces cerevisiae* A/Tor. *pretorien*	0.86	0.04	21.5

[1]DSU (one dextransucrase unit : amount of enzyme producing 1mg fructose per 1 hr).

tion The amount of dextran extracted from *jeungpyeon* batter during fermentation was proportional to the viscosity profile of the batter (Fig. 2 and 3). The dextran extraction was 16.78 mg/g batter before fermentation. It increased to 28.43 and 36.65 mg/g batter after 1 and 2 hr of fermentation, respectively, but then decreased to 25.55 and 23.5 at 3 and 5 hr of fermentation, respectively, before finally increasing to 27.88 mg/g batter at 7 hr of fermentation. From the combined results of viscosity, dextransucrase of LAB and yeast and the changes of dextran, it was assumed that dextran was the major factor contributing to the physical properties of *jeungpyeon* and thereby determining the overall sensory quality of the rice cake.

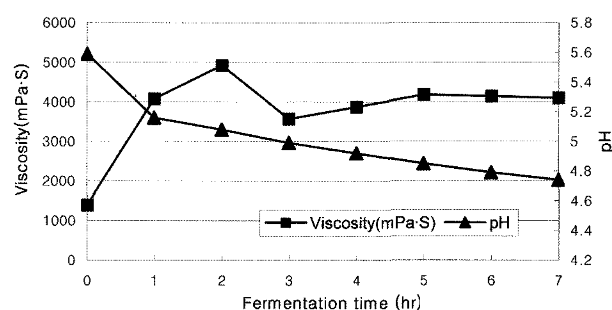

Fig. 2. Variation of viscosity and pH of *jeungpyeon* batter during fermentation.

Network structure of *jeungpyeon* by SEM analysis Figure 4 shows the SEM photos magnified at 2,000× of the network structure during the first fermentation period of *jeungpyeon*. As the fermentation proceeded, fine pores were observed trapping the produced gas bubbles which resulted in the formation of a network structure. The most stable network structure was formed at 2 hr fermentation. The SEM photos at 3 hr fermentation showed that a large quantity of dextran had been produced in the batter and remained inside the structure of *jeungpyeon*. However, after 5 hr of fermentation the air bubbles started to deform and the network structure began to break down due to excessive fermentation. The SEM photo in Fig. 5 shows a 10,000× magnification image of the network structure of *jeungpyeon* fermented for 2 hr. The microorganisms seen in the photo appeared to be yeast based on an estimation of their size, and most likely *S. cerevisiae* A/Tor. *pretorien*, the sole yeast isolated from *jeungpyeon*. It is known that the formation of dextran in bread enhances the softness, crumble texture and loaf volume of bread. Currently, *L. mesenteroides, S. cerevisiae, L. plantarum,* and *L. sanfrancisco* are utilized for dextran formation in bakeries without restrictions (14).

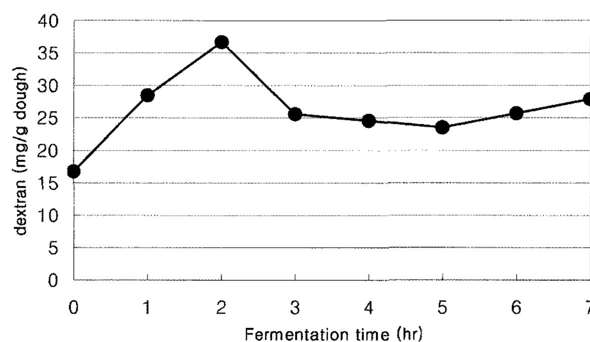

Fig. 3. Variation of dextran contents of *jeungpyeon* batter during fermentation time.

The results indicated that the network structure of *jeungpyeon* was produced primarily by the trapping of carbon dioxide during the batter fermentation and heating process, and that the network was stabilized by the dextran that was generated by microorganisms and remained inside the network structure.

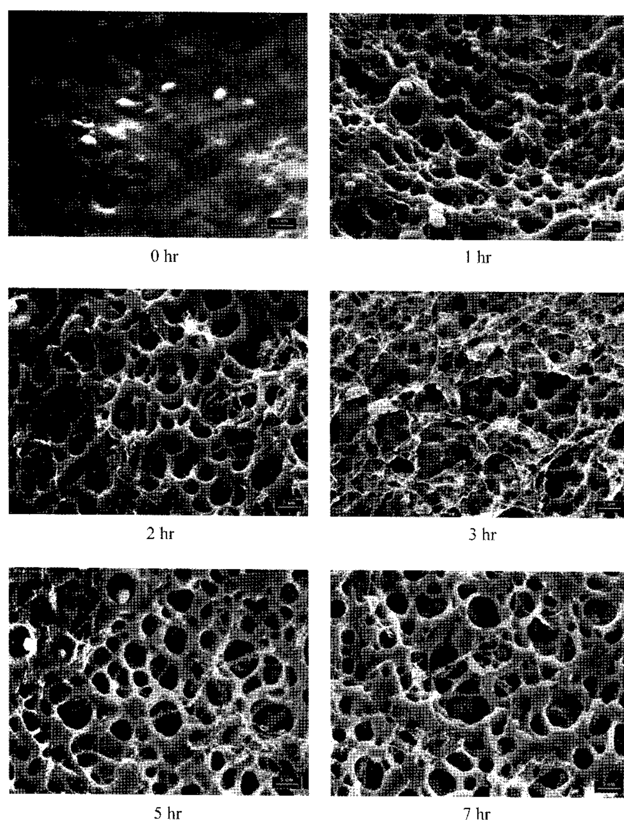

Fig. 4. Scanning electron microscopic images of *jeungpyeon* according to the fermentation time (2,000×).

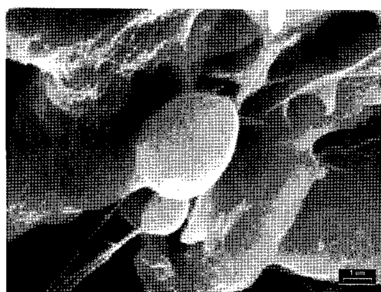

Fig. 5. Scanning electron microscopic images of the yeast in *jeungpyeon* structure (10,000×).

The stable structure of *jeungpyeon*, a Korean rice cake, was partially attributed to the dextran formed by the dextransucrases of some yeasts and LAB during the fermentation of the rice cake batter.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (R04-2001-000-00280-0)

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