

소나무 (*Pinus koraiensis*) 추출물과 결합 된 막걸리 발효의 특징

Characteristic of Makgeolli and Pine (*Pinus koraiensis*) Extract Fermentation

데스티아니 수페노, 권순홍, 정성원, 권순구, 박종민, 김종순, 최원식*

Destiani Supeno, Kwo Soon Hong, Chung Sung Won, Kwon Soon Goo,
Park Jong Min, Kim Jong Soon, Choi Won Sik*

〈Abstract〉

In this research, the improvement of Korean rice wine (makgeolli) with pine (*Pinus koraiensis*) extract addition was evaluated due to the increase in alcoholic Korean traditional beverage. Makgeolli fermentation was prepared using Korean rice and nuruk (traditional starter) supplemented by pine needle (MPN) and pine sprout (MPS) extract. The average of initial pH level for MPN was 3.95 and MPS was 4.55, the average of initial sugar content for MPN was 0.4% and MPS was 0.3%. The sugar content and pH level behavior were investigated every 24h during fermentation period. The observation of microbial colony was done at days 8 of fermentation period with three time sample dilution. Afterward, the physical appearance of fermentation solution and microbial development were investigated in the final of fermentation period. The number of yeast and LAB (402 x10³ CFU/mL) in MPN was greater than the yeast and LAB count in MPS (224 x10³ CFU/mL). The pH level obtained by addition pine sprout have value of R2 higher than addition of pine needles (leaf), the sugar content (%) behaviour was opposite with pH level behaviour.

Keywords : Fermentation, makgeolli, pinetree, rice wine, alcoholic beverage

* 최원식, 바이오 산업 기계 공학과, 부산대학교
경상남도 밀양시 삼랑진읍 삼랑진로 1268-50
우편번호 50463
E-mail: choi@pusan.ac.kr Tel:+82-55-350-5425

* Corresponding Author, Professor, Dept. of Bio-Industrial
Machinery Eng., Pusan Natl. Univ
Gyeongsangnam-do Miryang-si Sangnamjin-eub
Sangnamjin-ro 1268-50, 50463 REPUBLIC OF KOREA

1. Introduction

Red pine needles and sprout have long been used as a nourishing tonic drug in Korean traditional medicines and are frequently used to brew tea in Korea, the previous research shows that pine needles extract has supported the role that antioxidants, including several compounds, play in the prevention of anti-aging and several chronic diseases such as cardiovascular disease, cancer, diabetes, and anti hypertension [1][2]. They play crucial roles in traditional herbal remedies and are under investigation for their antibacterial and other pharmaceutical effects [3]. In this research we combine the Korean traditional rice wine (makgeolli), makgeolli is a unique traditional turbid rice wine in Korea, which in the past was mostly consumed by farmers [4]. Unlike other alcoholic beverages, makgeolli is highly nutritious and functional because it contains proteins, sugars, vitamins, bio-active compounds, and various organic acids [5][6].

This study was investigate the fermentation characteristic of makgeolli fermentation supplemented by pine needle (MPN) and pine sprout (MPS) extract based on pH level, sugar content (%) during fermentation period, final microbial development using microbial plate count method and final physical appearance.

2. Material and methods

2.1 Sampling preparation

Fresh pine sprouts or pine needles were selected and harvested from Korean pine trees (*Pinus koraiensis*) in the middle of May to late June at Miryang city. 500 g of fresh pine needles and sprout was washed with tap water. Then, boil the pine needles and pine lower separately using 1000ml of tap water.

After cooling, the extract solution for each sample was mixed with cooked rice 554g (before cooking, rice was soaked in the clean water for 3hours), 3g of yeast and 200g of nuruk as starter, then storage in the incubator at 28° C for 8days. Table 1. shows the ingredient and initial condition for makgeolli supplemented by pine needles (MPN) and pine sprout (MPS) extract fermentation. Figure 1. shows the sample preparation pine needle and pine sprout extraction. For MPN, the amount of pine

Table 1. The experimental ingredient and initial pH level and sugar content (%)

Parameters	Pine Needle (MPN)	Pine Sprout (MPS)
Sample (g)	447	390
Rice (g)	554	554
Nuruk (g)	200	200
Water (ml)	1000	1000
Initial brix (%)	0.4	0.3
Initial pH	3.95	4.55

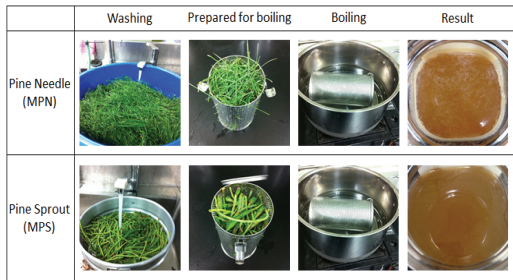


Fig. 1 Sample preparation for pine needle (MPN) and pine sprout (MPS) extract

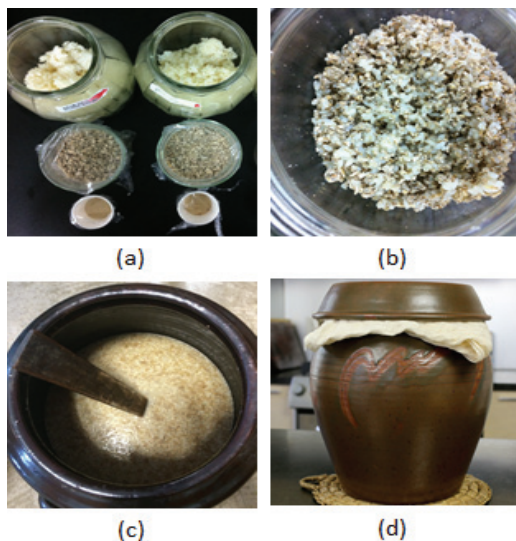


Fig. 2 Sample preparation for makgeolli with pine needle (MPN) and pine sprout (MPS) extract, (a) the ingredient: boiled rice, nuruk, and EM, (b) mix up the dry ingredient for each sample, (c) mix up dry ingredient with MPN and MPS extract, (d) fermented at 28°C for 8 day

needle was 447g and for MPS, the amount of pine sprout was 390g. The different amount of samples caused due to the difficulty to get the pine sprout than pine needles. Therefore, the making of sample preparation for

makgeolli for each sample can be seen in Figure 2 as a follow. The traditional makgeolli starter, named nuruk was added in to the sample solution in the first stage of makgeolli fermentation.

2.2 Instrument analysis

The pH level measurement was done using pH-meter (SATO, Japan). The device used is pH meter type sk-620PH, this device can measure the pH with a value of 0-14. Sugar content in this research was measured using a refractometer type: Master-53M, capacity: 0.0-53%, ATAGO, Japan. Refractometer was designed to measure the refractive index of a solution. The sugar content scale based on sucrose (sugar) and water solution.

The number of microbial in the colony were determining using plate counting analysis. A colony-forming unit (CFU) is a unit used tod estimate the number of viable bacteria of fungal cells in a sample. Agar liquid were prepared by 8g of nutrient broth and 18g of MRS agar powder, stir the ingredient with 1000ml of distillation water in the Erlenmeyer then cover with aluminum foil. Sterilize the agar in the autoclave at 121° C for 15 minute. After the agar liquid at 50° C or change into warm, then pour the MRS agar liquid into circle petri dish. Moreover, keep the MRS agar plates in the refrigerator for 24 hour, furthermore sterilize the chamber and MRS media for 15 minute. The samples was prepared with 1-3 dilution



Fig. 3 Digital refractometer



Fig. 4 Digital pH meter

(x1, x10, x100, x1000) and the plating triplicates of each dilution, the average data of triplicates data was used for analysis. Figure 5 shows the analysis instrument and agar plate method to analysis the research data. After plating, agars plate contain with spreaded colonies culture were keep in the incubator at 28° C for 48 hours until the colonies were growth. The counting process was done manually.

The colony form unit was calculated using following formula:

$$CFU = \frac{a \times b}{c}$$

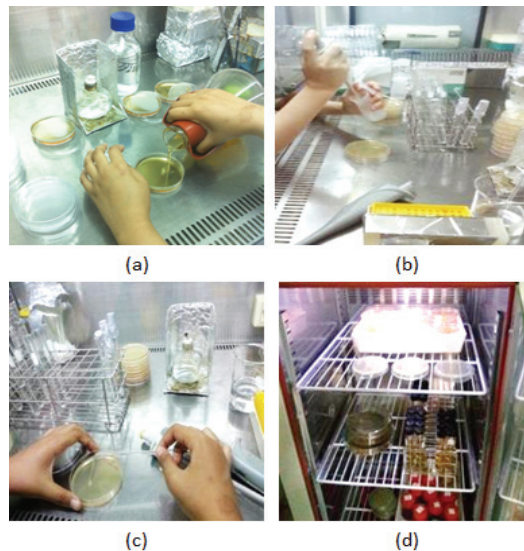


Fig. 5 Microbial colony form unit (CFU) analysis preparation, (a) medium preparation (MRS type), (b) dilution preparation for sample solution, (c) agar plate with sample cultivation, (d) cultivated the agar plate with sample cultivation in the incubator at 28°C for 1 days

where, CFU was used to determine the number of viable bacterial cells, for convenience the result for the results are given as CFU/ml (colony-forming units per millimeter) because the sample was liquids, a is the number of colonies, b is the dilution factor and c is volume of culture plate.

3. Result and discussion

The behavior of sugar content development during the fermentation period can be seen in the Figure 6. It shows that the pH

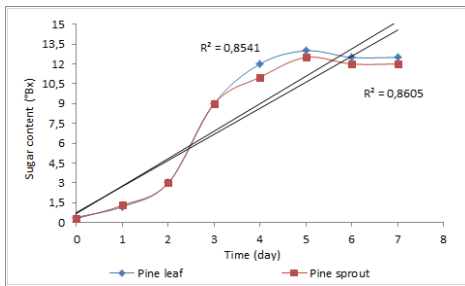


Fig. 6 The behavior of sugar content (%) development during fermentation periods

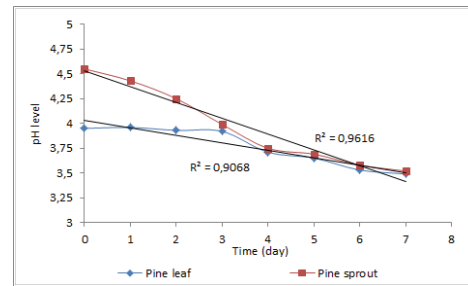


Fig. 7 The behavior of pH development during fermentation periods

obtained by MPS have value of R2 higher than MPN, the sugar content (%) behavior was opposite with pH level behavior during fermentation period (Figure 7). R2 shows the goodness of fit of the trend-line to the data, a value of 1 is a perfect fit. The number of R2 indicates the proportion of the variance in the dependents variable that is predictable from the independent variables. Based on the initial data, the positive values of R2 was occur due to the fitting linear functions to

data.

After 8 days of fermentation process, the starter (*nuruk*) allowing the growth of various wild microorganisms such as fungi, yeast, and lactic acid bacteria (LAB) [7]. The MRS media was shown to be effective in allowing the growth of yeast and LAB (Figure 8,9). Total viable counts of LAB and yeast were obtained as shown in Figure 6. It is noted that the number of yeast and LAB (402×10^3 CFU/mL) in MPN was greater

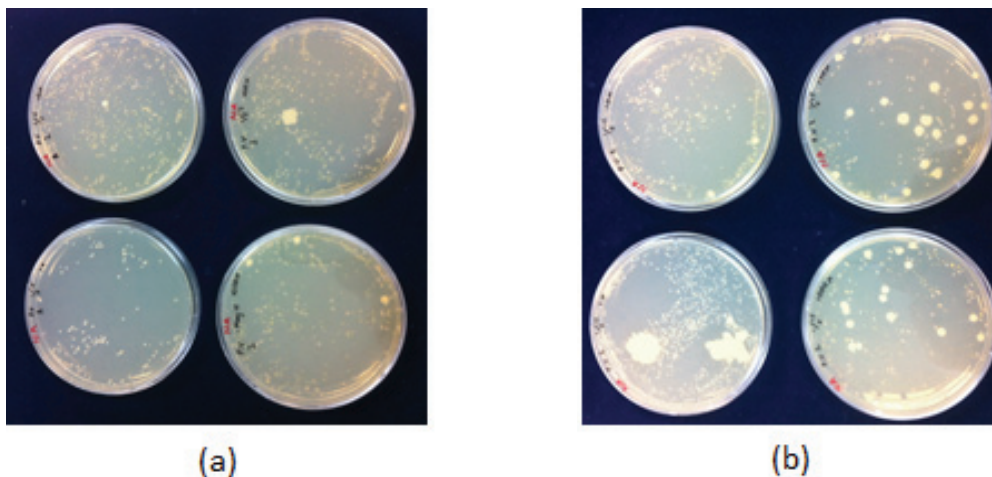


Fig. 8 Microbial counter plate, (a) MPN (makgeolli pine needles), and (b) MPS (makgeolli pine sprout)

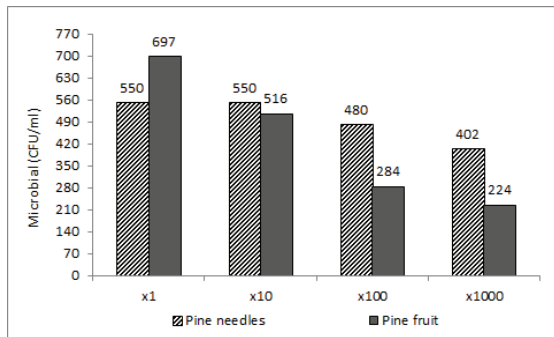


Fig. 9 The microbial number based on the agar plate counting method

than the yeast and LAB count in MPS (224×10^3 CFU/mL). The previous research [8] shown that the most predominant yeast species was *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in LAB.

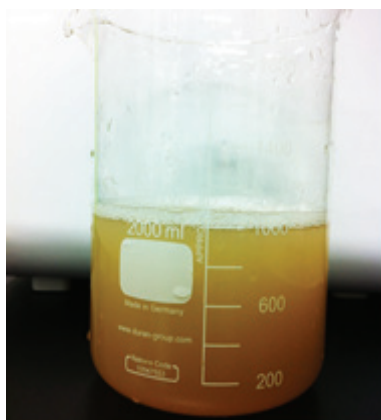
First treatment is makgeolli fermentation supplemented pine needle (MPN) and second treatment was addition with pine sprout (MPS). Figure 10 shows the korean rice (makgeolli) afer 8 days of fermentation period

after filtering. The picture shows the similarity of makgeolli addition pine needle (MPN) and pine sprout (MPS), the liquid color was golden bright color and have a deep sweet aroma. Moreover, the original makgeolli liquid color was bright yellowish white color with sweet aroma and strong alcoholic aroma.

4. Conclusion

From this experimental result we can conclude that :

- 1) The pH level obtained by addition pine sprout have value of R2 higher than addition of pine needles (MPN), the sugar content (%) behaviour was opposite with pH level behaviour.
- 2) The pH and Brix level in makgeolli addition with extract of pine needles and



(a)



(b)

Fig. 10 The fermentation solution result after filtering after 8 days, (a) MPN (makgeolli pine needles), and (b) MPS (makgeolli pine sprout)

pine sprout development in the early fermentation stage influences the changes in LAB bacteria, and yeast in second period of fermentation.

3) The number of yeast and LAB (402 x10³ CFU/mL) in MPN was greater than the yeast and LAB count in MPS (224 x10³ CFU/mL).

4) The makgeolli addition pine needle and pine sprout extract was not look different, the liquid color was golden bright color and have a deep sweet aroma.

[6] Lee S. R. "Korean fermentative food". Ewha Woman's University Press, Seoul, Korea. pp.205, 1986.

(접수: 2017.10.23. 수정: 2017.11.21. 게재확정: 2017.11.28.)

Reference

- [1] Jung, M. J., H. Y. Chung, J. H. Choi, and J. S. Choi. "Antioxidant principles from the needles of red pine, *Pinus densiflora*". *Phytother. Res.* Vol.17, pp 1064-1068, 2003.
- [2] Yen, G. C., P. D. Duh, D.W. Huang, C. L. Hsu, and T. Y. Fu, "Protective effect of pine (*Pinus morrisonicola* Hay.) needle on LDL oxidation and its anti-inflammatory action by modulation of iNOS and COX-2 expression in LPS-stimulated RAW 264.7 macrophages". *Food Chem. Toxicol.* Vol. 46, pp. 175-185, 2008.
- [3] G. Y. Park, Dilli P. P., I. D. Hwang, Giri R. T., Y. Yang, and H. S. Cheong, "Production of fermented needle extracts from red pine and their functional characterization". *Biotechnol. Bioprocess Eng.* Vol.13, pp 256-261, 2008.
- [4] Yang J. Y. and Lee K. H., "Shelf-life and microbiological study if *Sansung takju*". *Korean J. Food. Sci. Technol.* Vol.28, pp. 779-785, 1996.
- [5] Kim I. H., Min B. Y. and Lee S. K., "Effect of lysozyme and glycine on the *makgeolli* brewing process". *J. Korean Agric. Chem. Soc.* Vol.18, pp. 1678-1682, 1990.