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Alcohol Fermentation Characteristics of the Korean Native Mulberry (*Morus* spp.)

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Abstract

This study was carried out to investigate the fermentation characteristics and optimum conditions for alcohol fermentation of the Korean native mulberry. The yeast strains of *S. kluyveri* DJ97, La parisienne (*Saccharomyces cerevisiae*, Netherlands) and Enoferm (*Saccharomyces cerevisiae*, Denmark) produced higher alcohol concentrations than other strains, and further study was therefore performed with these three species. The optimum additional water content for maximizing alcohol concentration was 250% (v/w). The alcohol concentrations were rapidly increased in the first 4 days under the optimum conditions and reached 13.8% for *S. kluyveri* DJ97, 14.0% for La parisienne and 14.0% for Enoferm, respectively. Residual sugar concentration was decreased steadily from the beginning of fermentation until 5 days, after which it maintained a constant level. The pH was decreased steadily in the log phase during further maturation. However, the pH underwent a slight decrease after 4 days and then was stabilized during further maturation. Methanol concentrations for the three species used were analyzed after 60 days maturation and were lower than the levels regulated by the food standard. Fusel oils such as *n*-propanol, *iso*-butanol, and *iso*-amyl-alcohol were produced as by-products with the highest production from Enoferm and the lowest from *S. kluyveri* DJ97.

Key words: *Morus*, mulberry, alcohol, wine, fermentation

INTRODUCTION

The Larch tree is classified in the family *Moraceae* of the genus *Morus*. The fruit of this tree, called mulberry, is a mature fruit picked when black or dark red in May and June. The dried fruit has been used as both food and medicine ingredients, and in oriental medicine it is used for dizziness, tinnitus and thirst (1, 2). This fruit from the *Moraceae* family is safely used with no toxic effects to prevent hypertension due to its effectiveness in removing unwanted hotness from the body (3). Mulberry, prepared as a juice or wine for ingestion, protects the internal organs and cleans the ear and eye (4). Mulberry extract is drawing attention as a functional food, not only due to various physiologic activities such as anti-diabetic, anti-inflammatory, and anti-hyperlipemia effects, but also due to the presence of physiologically active substances with anti-oxidative and anti-hepatoxicity activities including flavonoids, stilbenes, prenylflavonoids and coumarin (2,5). It also contains abundant anthocyanin with cyanidine-3-glucoside and cyanidine-3-rutinoside as the major components (6). Many researchers are interested in anthocyanin as a natural coloring agent without harmful effects on the body and as a functional food with the recent results accrediting this color with various physiologic activities for preventing aging, treating retinal dysfunction, improving vision, and having anti-oxidative activity (7,8). Mulberries are usually processed to make jam, jelly, syrup and juice since they are difficult to store because of their high moisture contents. They are also used as a natural red color in the food and cosmetic industries (5,9,10). Nowadays, the silk industry in Korea is focused only on the production of silkworms and *Cordyceps*, with a consequent decrease in the amount of mulberry fruit production. With possible production levels of more than 1,000 kg of mulberry per 10 acre, farmers could increase their profit if the local community more fully utilized these local resources (9). Thus, research is needed to develop means of producing additional mulberry products to increase mulberry consumption and produce more high-value added products. While various researchers have investigated alcohol

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fermentation utilizing domestic fruits (11-15), this study was conducted to investigate the alcohol fermentation characteristics of mulberry wine using various strains.

MATERIALS AND METHODS

Materials

Mulberries were harvested during June of 2005 near Jungeup City, Chollabuk province and frozen at -40°C until used.

Microorganisms

Various yeast strains were compared for mulberry wine production. *Saccharomyces kluyveri* DJ97 (KCTC 8842P), *Saccharomyces cerevisiae* JK99, *Zigosaccharomyces cerevisiae* Wine, *Saccharomyces cerevisiae* OMK, *Saccharomyces cerevisiae* No. 9, and *Saccharomyces cerevisiae* GRJ were obtained from the department of fermentation at Keimyung university. The commercially available dried yeasts, La parisienne (*Saccharomyces cerevisiae*, Netherlands), La parisienne-H (*Saccharomyces cerevisiae*, Netherlands), Fermivin (*Saccharomyces cerevisiae*, France), and Enoferm (*Saccharomyces cerevisiae*, Denmark) were also used. Microorganisms were cultured in YPD agar medium consisting of (units: g L^{-1} distilled water): yeast extract, 10.0; peptone, 20.0; glucose, 20.0; and agar, 20.0 at 30°C for 24 hr, and kept at 4°C until used.

Starter culturing

For culturing of the starter, the water content was adjusted to 100% (v/w) using 100 g of mulberry homogenate. Sugar was added to 24°Brix and the mixture was sterilized at 121°C for 15 min. Then, one platinum inoculation loop of yeast was added to 10 mL of the sterilized mixture, which was cultured in a shaking incubator (HB-201SL, Hanbaek Co., Korea) at 30°C for 24 hr.

Screening for strains

In order to screen for optimum yeasts for mulberry wine fermentation, wines made with ten different strains were evaluated for alcohol concentration. Microorganism for inoculation were added into the mulberry homogenate at 5% (v/v). The mixture was incubated in a shaking incubator at 25°C with 50 rpm for 60 hr. The supernatant was obtained by filtering the mixture with a filter paper (Whatman No. 3) using a circulating aspirator (Sibata WJ-15, Japan) and analyzed for alcohol content.

Additional water content

Various concentrations of water content, 0~300% (v/w) were used to make the culture medium containing 24°Brix of sugar concentration. The starter culture was cultured in a shaking incubator (HB-201SL, Samsung Co., Korea) at 25°C with 50 rpm for 60 hr. Supernatant was prepared by filtering the sample through a paper filter (Whatman No. 3) using a circulating aspirator (Sibata WJ-15, Japan) for analyzing the wine characteristics.

Component changes during fermentation

After adding 150% (v/w) of water into 100 g of mulberry, the sugar concentration was adjusted to 24°Brix, and 5% (v/v) of starter. The mixture was cultured in a shaking incubator (HB-201SL, Hanbaek Co., Korea) at 25°C with 50 rpm for 5 days. After fermentation, the sample was filtered through a filter paper (Whatman No. 3) using a circulating aspirator (Sibata WJ-15, Japan) and matured for 60 days for analyzing the wine component changes.

Component analysis

Alcohol and sugar analysis : After 100 mL of fermented sample was collected and distilled, it was adjusted to 15°C using the Gay Lussac Table (16) for alcohol concentration analysis. The sugar concentration was measured using a digital refractometer (PR-101, Japan).

pH and titratable acidity : The pH was measured using a pH meter (Metrohm 691, Switzerland) at room temperature. Titratable acidity was measured with a 20 mL sample by titrating the sample with 0.1 N NaOH until the pH reached 8.2 and converting the amount based on acetic acid.

Alcohol measurement : Different types of alcohol were evaluated according to the National Tax Service Liquor Analysis Regulation (17) using a gas chromatograph (GC-14B, Shimadzu Co., Japan). Anhydrous alcohol (99.9%) was used as the standard reference. The analytical conditions were as follows: HP-FFAP (30 m × 0.32 mm × 0.25 μm), carrier gas: He, detector: FID, injection temp.: 200°C, detector temp.: 230°C and injection volume: 2 μL.

RESULTS AND DISCUSSION

Screening of strains

Fermentation characteristics are shown in Figs. 1 and 2. Among the strains of *S. kluyveri*, *Zigosa. cerevisiae*, and *S. cerevisiae* produced the highest alcohol contents at 12.7% with *S. kluyveri* DJ97. Among the commercially available dry yeasts, the alcohol contents in wines prepared with La parisienne, La parisienne-H and Enoferm were 13.6, 13.3 and 13.7%, respectively. Although Fermivin produced the highest ethanol content, it had an unacceptable taste and odor. Therefore, *S. kluyveri* DJ97, La parisienne, and Enoferm were selected for further study.

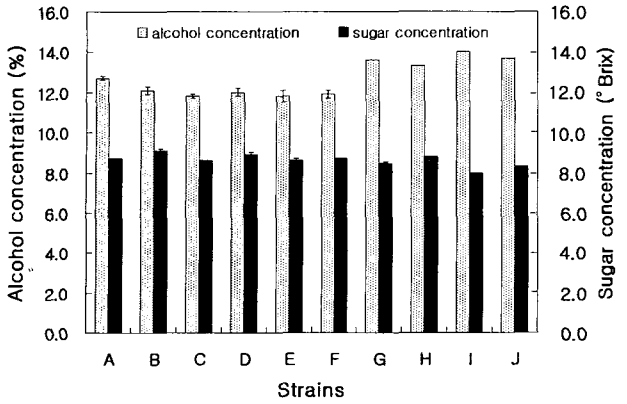

Fig. 1. Comparison of alcohol and sugar concentrations in alcohol fermentation with different yeasts.
A: *S. kluyveri* DJ97 (KCTC 8842P), B: *S. cerevisiae* JK99, C: *Zigosa. cerevisiae* Wine, D: *S. cerevisiae* OMK, E: *S. cerevisiae* No. 9, F: *S. cerevisiae* GRJ, G: La parisienne (*S. cerevisiae*, Netherlands), H: La parisienne-H (*S. cerevisiae*, Netherlands), I: Fermivin (*S. cerevisiae*, France), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

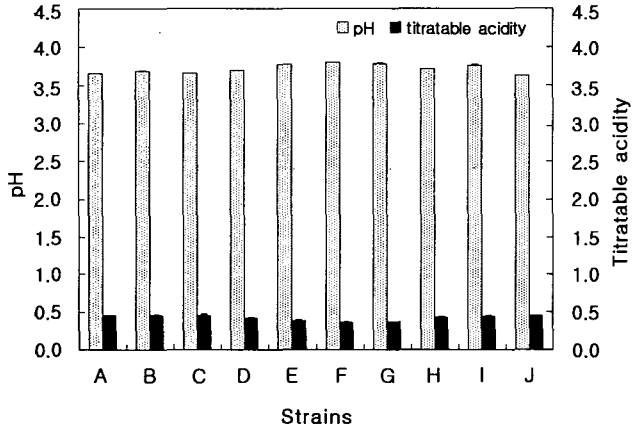

Fig. 2. Comparison of pH and titratable acidity in alcohol fermentation with different yeasts.
Refer to Fig. 1 for letters. Values are mean±SD (n=3).

Additional water content

In order to evaluate the effect of water content on fermentation, media were prepared with the additional water amount ranging from 0 to 300% (v/w) and fermentation was performed at 25°C for 60 hr. The supernatant obtained by filtration was used for the evaluation of alcohol, residual sugar, pH, and titratable acidity as shown in Figs. 3~6. The alcohol content was highest at the additional water amount of 150%, with alcohol levels of 13.3, 13.7 and 13.7% in wines prepared with *S. kluyveri* DJ97, La parisienne and Enoferm, respectively. Alcohol concentration decreased when the additional amount water exceeded 150%. The residual sugar concentration was decreased slightly between 100 to 150%, increased significantly when the additional

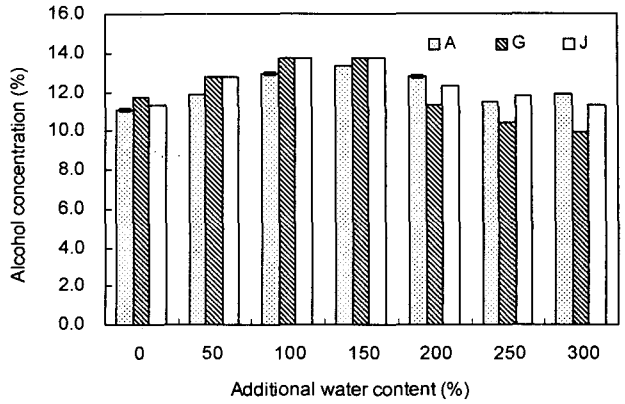

Fig. 3. Effect of additional water content on alcohol concentration (%) in alcohol fermented by (A) *S. kluyveri* DJ97, (G) La Parisienne and (J) Enoferm.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

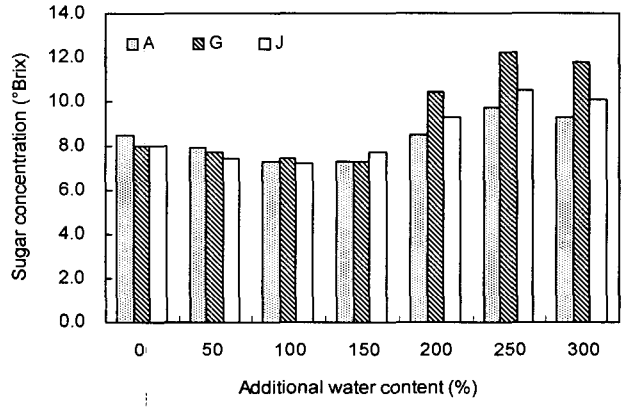

Fig. 4. Effect of additional water content on sugar concentration (°Brix) in alcohol fermented by (A) *S. kluyveri* DJ97, (G) La Parisienne and (J) Enoferm.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

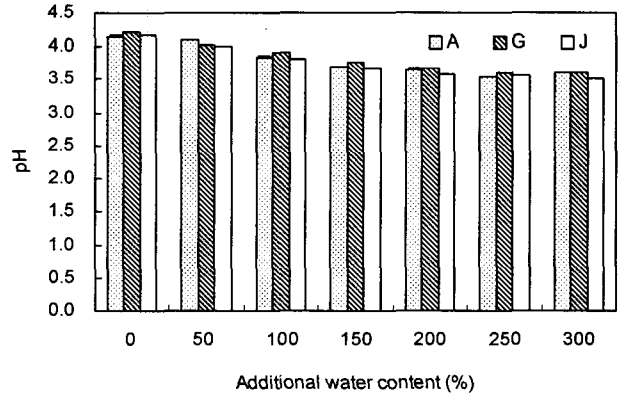

Fig. 5. Effect of additional water content on pH in alcohol fermented by (A) *S. kluyveri* DJ97, (G) La Parisienne and (J) Enoferm.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

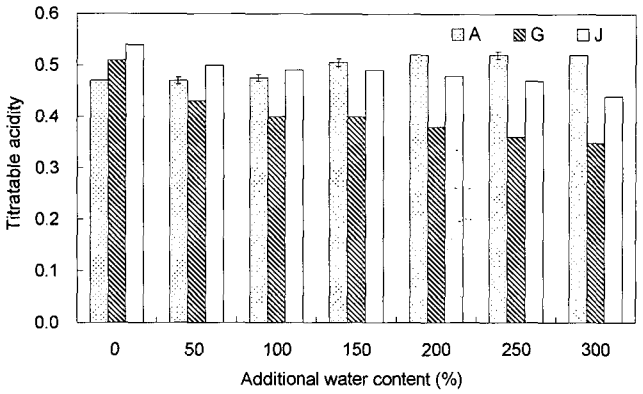

Fig. 6. Effect of additional water content on titratable acidity in alcohol fermented by (A) *S. kluyveri* DJ97, (G) La Parisienne and (J) Enoferm.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

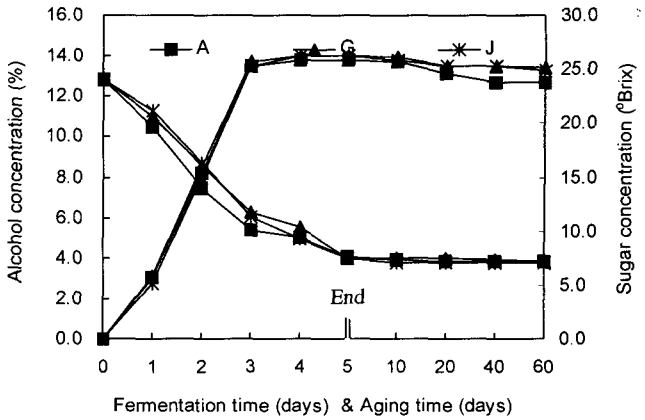

Fig. 7. Changes in alcohol and sugar concentration during alcohol fermentation and aging.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

water amount was 200%, and decreased again when the additional water amount was 300%. With increasing water amount, the pH was increased from 3.54 to 4.16, 3.60 to 4.21 and 3.50 to 4.18, while the titratable acidity was 0.47 to 0.52, 0.35 to 0.51 and 0.44 to 0.54, for *S. kluyveri* DJ97, La parisienne and Enoferm respectively. Thus, the water concentration had no affect on wine fermented with *S. kluyveri* DJ97. However, the alcohol content was decreased with increasing water content in wine fermented with La parisienne and Enoferm. These results confirmed the optimum water content of 150% in mulberry wine production, with ethanol concentration being inhibited in media with higher water contents. Similarly, Jung et al. (11) reported that the alcohol concentration was increased at water amount up to 200%.

Changes in mulberry wine components during alcohol fermentation and aging

Changes in alcohol and sugar concentration: The effects of aging on alcohol and sugar concentration during mulberry wine fermentation are shown in Fig. 7. The alcohol concentration was increased significantly for the first 3 days of fermentation and peaked on the 4th day of fermentation at 13.8%, 14.0% and 14.0% for *S. kluyveri* DJ97, La parisienne and Enoferm, respectively. The kinetic curve for alcohol leveled off after 4 days of fermentation with La parisienne and Enoferm. Alcohol concentration was stable after the 4th day of fermentation in wine fermented with *S. kluyveri* DJ97 and then decreased slightly from the 20th day of aging. Jung et al. (9) reported that the alcohol concentration in mulberry wine fermented at 25°C for 10 days was 11.2%, probably due to the different culture medium used for fermentation in their study. Sugar concentration was decreased steadily throughout fermentation and until stabilization occurred on the 5th day of fermentation. The results indicated that the optimum period of alcohol production was 3~4 days of fermentation, and that alcohol concentration did not decrease significantly during fermentation.

Changes in pH and titratable acidity: The changes in pH and titratable acidity during mulberry wine fermentation and aging are shown in Fig. 8. In the initial stage of fermentation, pH was 4.56 and on 4th day of fermentation, decreased to 3.62, 3.67 and 3.66 for *S. kluyveri* DJ97, La parisienne and Enoferm, respectively. After 20 days, the pH increased slightly and then maintained a steady state. In the early stage of fermentation, titratable acidity was 0.15, but peaked by the 4th day of fermentation at 0.49, 0.44 and 0.47 in wines fermented with *S. kluyveri* DJ97, La parisienne and Enoferm, re-

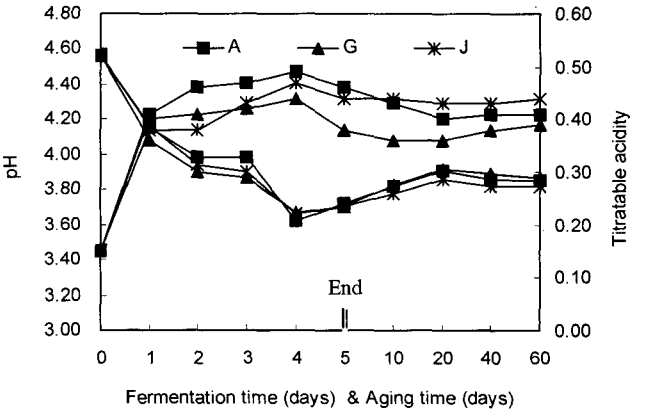

Fig. 8. Changes in pH and titratable acidity during alcohol fermentation and aging.
A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark). Values are mean±SD (n=3).

Table 1. Fusel oils of aged mulberry wine during 60 days (ppm)

Components	Concentration of fusel oils		
	A	G	J
Acetaldehyde	144	24	92
Methanol	179	127	155
Ethanol	129,371	134,011	129,539
n-Propanol	71	61	174
iso-Butanol	281	325	301
iso-Amylalcohol	110	123	88
n-Amylalcohol	355	448	369

A: *S. kluyveri* DJ97 (KCTC 8842P), G: La parisienne (*S. cerevisiae*, Netherlands), J: Enoferm (*S. cerevisiae*, Denmark).

spectively. It was then slightly decreased up to 20 days of fermentation, but subsequently increased again after 20 days, although these changes were not significant. Although pH and titratable acidity changed significantly during fermentation and maturation, no acidification occurred. Lee et al. (18) reported that the titratable acidity by the 50th day of fermentation in wines prepared with different grape varieties ranged over 0.24~0.6 and that pH was maintained between 3.21 ~3.47 after 5 days of fermentation. This study showed that the titratable acidity after 40~60 days of fermentation was 0.38~0.44, and that the pH was 3.20~3.92 after 5 days of fermentation, which were slightly higher values than those reported by Lee et al. (18).

Alcohol components concentration: The concentration of seven major alcohol components, i.e., acetaldehyde, methanol, ethanol, *n*-propanol, *iso*-butanol, *iso*-amyl-alcohol and *n*-amyl-alcohol, were analyzed in wine produced at optimum conditions using different strains during 60 days (Table 1). The highest concentration of acetaldehyde and methanol was produced in wine fermented with *S. kluyveri* DJ97, followed by Enoferm and La parisienne. However, the methanol concentration did not exceed the regulatory maximum level of less than 1,000 ppm (19) in wine so that it did not exceed the standard level in mulberry wine with any of the 3 different strains. The fusel oil contents, including *n*-propanol, *iso*-butanol, and *iso*-amyl-alcohol, were the highest in wine fermented with Enoferm, followed by La parisienne and *S. kluyveri* DJ97. These fusel oils, which are produced through the reduction of aldehyde caused by the deamination and decarboxylation occurring during alcohol fermentation by yeast, are a major factor in deciding the alcohol product quality (20). Thus, the quality of mulberry wine fermented with La parisienne or Enoferm was better than that of wine fermented with *S. kluyveri* DJ97.

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