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Effectiveness of banana additions for completion of stuck and sluggish fermentation of blueberry wine

Seung-Ho Seo [1], Chang-Su Na [1], Dae-Hwan Youn, Seon-A Yoo, Seong-Eun Park, Hong-Seok Son*

School of Oriental Medicine, Dongshin University, Naju, Jeonnam, 520-714, Republic of Korea

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ABSTRACT

In this study, we investigated the effect of bananas on improving of sluggish blueberry wine fermentation. The alcoholic fermentation was carried out with yeast extract, diammonium phosphate, or 2%, 4%, and 6% banana. The number of viable yeast cells gradually decreased during fermentation and the population stabilized at approximately 10^4 cfu/ml by the end of fermentation. The average ethanol production rate was 1.32–1.68 times faster according to the addition of banana during 20 days of fermentation. The sugar consumption rate (°Brix/day) was increased 1.30, 1.49 and 1.63-fold by 2%, 4% and 6% of banana addition, respectively. The wines supplemented with 4% and 6% banana contained 61.7 μg/l and 60.8 μg/l of ethyl carbamate, respectively, which was higher than that with 2% banana (54.5 μg/l). Thus, we concluded that 2% banana can be used as a nutritional supplement for yeast to resolve stuck and sluggish blueberry wine fermentation.

1. Introduction

A stuck fermentation is one in which fermentation has ceased prematurely or the rate of fermentation is considered too low for practical purposes, thus leaving a higher residual sugar content than desired in the wines at the end of fermentation (Bisson, 1999). The principal mechanisms involved in stuck and sluggish fermentations have been elucidated: nitrogen deficiency (Bely, Sablayrolles, & Barre, 1990); thiamine depletion (Bataillon, Rico, Sablayrolles, Salmon, & Barre, 1996); lack of oxygen (Sablayrolles, Dubois, Manginot, Roustan, & Barre, 1996); presence of toxic fatty acids, especially octanoic and decanoic acids (Lafon-Lafourcade, Geneix, & Ribereau-Gayon, 1984); killer toxins, pesticides, and high residual fructose (Berthels, Cordero Otero, Bauer, Thevelein, & Pretorius, 2004).

Many studies have been conducted to identify solutions for problematic fermentations such as the addition of yeast extract (Taillandier, Ramon Portugal, Fuster, & Strehaiano, 2007), oxygen and diammonium phosphate (Blateyron & Sablayrolles, 2001; Sablayrolles et al., 1996), or active carbon and commercial yeast cell walls (Carrau, Neirotti, & Gioia, 1993). However, once fermentation gets stuck at any stage of its progress, it is difficult to complete fermentation even if the efforts explained above were added to the problematic fermentation. Stuck fermentations directly decrease productivity and may reduce wine quality. Indeed, the resulting wines, which contain high amounts of residual sugar, are particularly susceptible to microbial spoilage (Maisonnave, Sanchez, Moine, Dequin, & Galeote, 2013). Despite many improvements in the winemaking processes, both stuck and sluggish fermentations have been major problems in winemaking, resulting in large losses in the wine industry.

The exact cause of decline of the fermentation rate in blueberry wines is not yet understood, but stuck fermentation is frequently observed during the fermentation of blueberry wine. While it takes approximately 7–10 days to complete alcoholic fermentation in grape wines, it takes more than 3 weeks to the end of fermentation in blueberry wines. In our previous study (Seo, Yoo, Park, & Son, 2014), the fermentation rate of blueberry wine decreased in proportion to the amount of water added prior to fermentation, suggesting that nutrient deficiency may be a cause of the fermentation problem, especially with a shortage of assimilated nitrogen. We then investigated the effect of various yeast nutrients on stuck and sluggish fermentation of blueberry wine, and we accidentally found that bananas could be used as a nutritional supplement for yeast to solve stuck and sluggish blueberry wine fermentation.

* Corresponding author.
E-mail address: hsson@dsu.ac.kr (H.-S. Son).
[1] These authors contributed equally to this work.

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The aim of the present work is to describe the effects of bananas on improving sluggish fermentation in blueberry wine. We report here the statistical consumption rate of glucose and fructose in must during alcohol fermentation and investigate the kinetic aspects related to their conversion into ethanol in order to estimate the possible causes of stuck and sluggish fermentation in blueberry wines.

2. Materials and methods

2.1. 1. Blueberry wine making

The 'Northland' cultivar of highbush blueberry (*Vaccinium corymbosum* L.) harvested in June 2013 was obtained from Eumseongblueberryone (Eumseong, Korea). The total soluble solids (°Brix) of blueberry were measured using a digital refractometer (PR-32, Atago, Tokyo, Japan) with temperature compensation and showed 11.4°Brix. The pH of the blueberry determined with a pH meter (pH-250L, ISTEK, Seoul, Korea) was 2.94, and total acidity as tartaric acid was 1.33%, which was determined by titration to pH 8.3 with 0.1 N NaOH. The blueberry was crushed manually, and the same amounts of water were added to the must. Sugar was added to adjust the must to 24°Brix. After the addition of 100 ppm of $K_2S_2O_5$ to the must, *Saccharomyces bayanus* Lalvin EC1118 (Lalvin, Montreal, Canada) was used for alcoholic fermentation. Dried yeasts were first activated in yeast malt (YM) broth (3 g/l yeast extract, 10 g/l peptone, 3.0 g/l malt extract, and 10 g/l dextrose, natural pH) for 48 h to obtain a final cell count of 2×10^7 cfu/ml. The must was distributed into 18 2-L glass bottles, producing six batches for each of the 5 supplements and control. After the addition of yeast extract (300 mg/l), diammonium phosphate (DAP, 300 mg/l), and 2%, 4%, and 6% banana (w/v), the alcoholic fermentation was carried out in 18 glass bottles at 25 °C for 38 days. Bananas were purchased at a fully ripe stage from a local market in Naju (Korea).

2.2. Yeasts analysis

One milliliter of blueberry must was aseptically transferred to a conical tube and diluted serially with 9 ml of sterilized saline water (0.85% NaCl). The yeast count was determined by growing yeast in YM agar (Difco, Sparks, MD, USA) and by incubating at 30 °C for 48 h. Tests were carried out in duplicate, and the results were expressed as log cfu/ml.

2.3. Sugar analysis

All standards and must samples were filtered through a 0.45 μm PTFE membrane filter prior to HPLC analysis. The levels of glucose, fructose, and sucrose were analyzed using a liquid chromatography (LC) apparatus (LC-10Avp, Shimadzu, Kyoto, Japan) equipped with a TSKgel amide-80 column (250 × 4.6 mm, 5 μm; Tosho, Japan) and a refractive index (RID) detector. The column and detector were adjusted to a temperature of 35 °C. Twenty microliters of blueberry musts and standards were injected into the column. Elution was carried out at a flow rate of 1.0 ml/min with 75% acetonitrile as the mobile phase.

2.4. Ethanol analysis

The ethanol concentrations in the samples were analyzed using a gas chromatography (GC-17A, Shimadzu, Kyoto, Japan) with a flame ionization detector. A WAX-10 capillary column (length 30 m × 0.25 mm ID, film thickness 0.25 μm; Supelco, USA) was used with helium as the carrier gas at a flow rate of 1.0 ml/min. The injector temperature was 250 °C, and the detector temperature was 280 °C with a split ratio of 1:20. The GC oven temperature was first set to 50 °C for 5 min and then increased to 250 °C at a rate of 10 °C/min. The sample injection volume was 1 μl and all of the chemical measurements were repeated 3 times with the average values reported.

2.5. Ethyl carbamate analysis

GC–MS was carried out with an Agilent 6890 GC-5973MS. Substances were separated on a DB-5 MS column (length 30 m × 0.25 mm ID, film thickness 5 μm; Stabilwax, USA). The temperature program was: 50 °C hold for 4 min, 5 °C/min up to 120 °C, hold for 0 min, 15 °C/min up to 300 °C, and then hold for 5 min. The injector port and mass spectrometer interface line were heated to 200 °C and 250 °C, respectively. The carrier gas was helium with a constant flow rate of 1.0 ml/min and splitless injections were made with a volume of 1 μl. The primary electron ionization (EI) mass spectra and the product spectra of the analytes were recorded in full-scan mode (m/z 62.1) to determine the retention times and characteristic mass fragments. For quantification, the peak area ratios of the analytes to the internal standard (buthyl carbamate) were calculated as a function of the concentration of the substances.

2.6. Statistical analysis

Statistical analyses were performed using the SPSS version 14.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). ANOVA and Duncan's multiple range tests were applied to the data to determine significant differences, and a value of $p<0.05$ was considered statistically significant.

2.7. Chemicals

For the analyses, all chemical reagents were of analytical grade. Standards of glucose, fructose, sucrose, ethanol, and ethyl carbamate were purchased from Sigma (St. Louis, MO, USA).

3. Results

3.1. Influence of nutrients on yeast growth

We compared the effect of yeast nutrients (yeast extract and DAP) and different banana concentrations (2, 4, and 6%) on the growth of commercial wine yeast during fermentation. Each batch was inoculated with 1% volume of a yeast culture in YM broth, with a final cell count of 2×10^7 cfu/ml. Fig. 1 shows the growth kinetics of viable yeast cells at different yeast nutrients during alcoholic fermentation. Blueberry musts contained about 10^4~10^6 cfu/ml on the first day of fermentation and the concentration remained steady for about 10 days. After that time, the number of viable yeast cells in the musts began to decrease, and the populations reached little more than 10^4 cfu/ml in all tested samples after 22 days of fermentation. Although similar, the population growth pattern displayed by yeast growth in blueberry must without yeast nutrients (control) showed several variations from the tested samples. The number of viable cells gradually decreased starting from the first day of fermentation, and the population stabilized at approximately 5×10^3 cfu/ml after 22 days of fermentation, indicating that the early addition of yeast nutrients (DAP and yeast extract) and banana affected the evolution of yeast during fermentation. In all cases, the yeast viability was greater than the control starting from the 12th day of fermentation. Nevertheless, the specific growth rate did not vary according to different yeast nutrients added prior to fermentation.