

Artificial Botrytization Improves Fermentation Performance of Persimmon Wine

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Abstract

While sucrose chaptalization is commonly used in persimmon wine production to achieve adequate fermentable sugar levels, the fermentation suitability of a persimmon substrate modified by artificial botrytization has not been adequately investigated. In this study, persimmon must obtained from *Botrytis cinerea*-treated fruit and a sucrose-chaptalized control were fermented using the same yeast (*Saccharomyces cerevisiae* Fermivin) and temperature (20 °C) conditions. The noble rot-treated group displayed gradual changes in appearance during storage and faster alcohol production and reducing sugar depletion during fermentation compared with the control. Despite lower initial soluble solids (21.50 °Brix) compared with the control (23.00 °Brix), the noble rot-treated group achieved a higher final alcohol content (11.60% vs. 8.80%) within a shorter fermentation period (9 vs. 16 days). These findings suggest that artificial botrytization may serve as a distinct pre-fermentation treatment in persimmon wine production compared with simple sucrose chaptalization. However, further studies are needed to elucidate the compositional and metabolic factors underlying these differences.

Keywords: artificial botrytization; *Botrytis cinerea*; persimmon wine; fermentation performance; noble rot; sucrose chaptalization

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is a fruit rich in sugars, organic acids, and phenolic compounds with considerable potential as a raw material for fermentation-based processing as well as fresh consumption. Previous studies on persimmon wine have reported changes in sugars, organic acids, phenolics, and quality characteristics during fermentation, supporting persimmon's suitability as a fermentation substrate [1,2]. However, its fermentation suitability for winemaking can vary substantially depending on cultivar, maturity, soluble solids level, and pretreatment conditions, making substrate modification an important consideration for stable fermentation [3,4]. In particular, the balance between fermentable sugar content and other physicochemical factors, including acidity, tannin concentration, and nutrient availability, plays a critical role in determining whether persimmon must can support complete and efficient alcoholic fermentation [4,5]. When the sugar content is insufficient, the resulting wine may have a low alcohol content and reduced microbial stability, whereas excessively high tannin levels can impair yeast performance and affect sensory quality. Astringent persimmon cultivars, which contain high levels of soluble tannins, may present additional challenges during fermentation because these



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compounds can interact with yeast cells and influence fermentation progression [6]. In fruit wine production, soluble solids are commonly used as a practical indicator of fermentable substrate availability. However, soluble solids alone do not fully represent the fermentation suitability of fruit must, because yeast performance is also influenced by acidity, nitrogen availability, mineral composition, phenolic compounds, and the physical state of the fruit tissue [7,8]. This consideration is particularly relevant for persimmon, the composition of which may vary markedly depending on cultivar, maturity, and postharvest handling [6,9]. Therefore, evaluating persimmon must solely on the basis of soluble solids may overlook other substrate factors that contribute to fermentation performance.

Sucrose chaptalization is widely used to increase fermentable sugar levels in fruit wine production, but it raises soluble solids without improving other substrate characteristics that influence fermentation behavior, such as acidity, nitrogen availability, and nutrient composition [7,8]. Comparisons with pretreatments that modify the pre-fermentation substrate environment through mechanisms other than sugar addition may provide useful insight into whether fermentation performance is governed by substrate quality as well as sugar quantity. Understanding these relationships is important not only for improving persimmon wine production but also for advancing general knowledge of how pre-fermentation substrate characteristics influence yeast fermentation performance in non-grape fruit wine systems. Postharvest treatments that involve dehydration or biological modification of fruit have received increasing attention as strategies to improve fermentation substrate quality, as they can alter the physical and chemical properties of the raw material before fermentation [9,10]. Such treatments may concentrate sugars and other solutes, modify cell wall structure, and change the availability of nutrients that support yeast growth.

Among possible pretreatments, noble rot caused by *Botrytis cinerea* is known to accompany moisture loss and compositional changes in fruit, thereby altering the physicochemical properties of the raw material during storage and potentially affecting subsequent fermentation behavior [11–13]. These compositional changes arise from the enzymatic activity of *B. cinerea*, which secretes pectinases, laccases, and other hydrolytic enzymes that degrade plant cell walls and modify the chemical environment of the fruit tissue [8,9]. In grape-based winemaking, botrytization has been associated with enhanced sugar concentration, organic acid modification, and the formation of distinctive aroma compounds [10,13]. However, the application of artificial botrytization to non-grape fruits, particularly persimmon, has received limited attention despite its potential relevance for improving fermentation suitability. Although drying and other pretreatments have been reported to influence persimmon wine's fermentation characteristics and final quality [4,9], direct comparisons between an artificially botrytized persimmon substrate and a sucrose-chaptalized control remain limited. Given that persimmon is a non-grape fruit with a distinct compositional profile, examining whether noble rot can serve as an effective pre-fermentation treatment in this system may broaden the applicability of biological pretreatment approaches in fruit winemaking.

To our knowledge, this is the first study to directly compare the fermentation suitability of artificially botrytized persimmon substrate with a sucrose-chaptalized control under standardized fermentation conditions. Therefore, this study compared the fermentation suitability of artificially botrytized persimmon must with that of a sucrose-chaptalized control. Changes in soluble solids during storage and in sugar content, pH, alcohol content, and viable cell count during fermentation were monitored to determine whether artificial botrytization can serve as a pre-fermentation treatment distinct from simple sucrose chaptalization.

2. Materials and Methods

2.1. Materials and Microbial Strains

Cheongdo Bansi persimmon (*D. kaki* Thunb.) was used as the raw material and stored at 4 °C until use. Commercial *Saccharomyces cerevisiae* Fermivin (DSM Food Specialties, Delft, The Netherlands) was used as the fermentation yeast. A starter culture was prepared by incubating the yeast in YPD broth (1.0% yeast extract, 2.0% peptone, 2.0% dextrose) supplemented with 5% persimmon juice at 30 °C and 150 rpm for 24 h.

2.2. Artificial Botrytization Treatment

The *B. cinerea* strain used in this study (obtained from the Microbial Engineering Laboratory, School of Food Science and Technology, Kyungpook National University, Daegu, Republic of Korea) was cultivated on PDA (0.4% potato infusion, 2.0% dextrose, 1.5% agar) at 25 °C for 10 days. For spore harvesting, 5 mL of a sterile 0.1% Tween 80 solution was dispensed onto the plate; the mycelium was dislodged with a spreader; and the suspension was vortexed to release spores. The suspension was filtered through a syringe fitted with sterile gauze and washed twice by centrifugation at $10,000 \times g$ for 10–15 min. The final spore concentration was determined by counting with a hemocytometer after staining with lactophenol blue, adjusted to 2×10^6 spores/mL in 20% glycerol, and the suspension was stored at −70 °C.

For artificial botrytization, micro-perforations were formed on the persimmon skin surface using a dermaroller, followed by spray inoculation with the *B. cinerea* spore suspension. Inoculated persimmons were initially incubated at 25 °C for 2–3 days to allow fungal colonization and then stored at 4 °C. Relative humidity during the initial incubation period, the precise duration of subsequent cold storage, and the extent of dermaroller-induced skin perforation were not systematically recorded as controlled experimental variables in the present study; these parameters will be explicitly standardized and reported in future work.

2.3. Persimmon Wine Production and Fermentation Conditions

After washing, destemming, and crushing raw persimmon fruit, potassium metabisulfite (200 ppm) was added to prepare the fermentation substrate. For the control, food-grade sucrose was added to adjust the initial soluble solids concentration to 23.00 °Brix. For the treatment group, artificially botrytized persimmon was used as the fermentation substrate without chaptalization. Each must sample was inoculated with an *S. cerevisiae* Fermivin culture corresponding to 5% of the persimmon juice volume, and fermentation was conducted at 20 °C. Fermentation was terminated when the alcohol content plateaued and gas production became negligible; the wine was then filtered through sterile cheesecloth to remove particulate matter and stored at 4 °C. Fermentation volume, vessel type, headspace conditions, and closure type were likewise not systematically recorded as controlled experimental variables in the present study and will be standardized and reported in future work.

2.4. Fermentation Analyses

At each sampling point, the fermentation broth was centrifuged, and the supernatant was analyzed for soluble solids, pH, reducing sugar, and alcohol content. Soluble solids were measured in °Brix using a refractometer (ATAGO N-1E, Tokyo, Japan), and pH was assessed with a pH meter (Mettler Toledo Co., Model 340, Schwerzenbach, Switzerland). Reducing sugar was quantified by the 3,5-dinitrosalicylic acid (DNS) method [14]. Briefly, 1.0 mL of the sample was mixed with 1.0 mL of DNS reagent, heated at 95 °C for 5 min, and diluted with 7.0 mL of distilled water; the absorbance was measured at 550 nm using a spectrophotometer (Shimadzu Co., UV-1601, Kyoto, Japan), and concentrations were

calculated from a glucose standard curve. Alcohol content was determined by diluting 25 mL of supernatant with 75 mL of distilled water, collecting approximately 70 mL of distillate, making up to 100 mL with distilled water, and reading with an alcoholmeter. Values were corrected for temperature and dilution factor using the Gay-Lussac table and expressed as % (v/v) [15,16].

2.5. Viable Cell Count

Viable yeast counts during fermentation were determined by the spread plate method. Serially diluted samples were spread onto agar plates, incubated at 30 °C for 48 h, and enumerated; results are expressed as log CFU/mL [17].

2.6. Statistical Analysis

All experiments were conducted in triplicate, and results are expressed as mean $\pm$ SD. Statistical analyses were performed using GraphPad Prism 10 (Insightful Science, San Diego, CA, USA). Statistical comparisons between the two treatments were performed independently at each sampling time point. Significant differences were assessed by one-way ANOVA followed by Tukey's multiple comparison test at $p < 0.05$. The time-course profiles are presented as descriptive representations of fermentation dynamics, while formal statistical comparisons are reported for the summary parameters.

3. Results and Discussion

3.1. Artificial Botrytization Process and Fermentation Profiles

Figure 1 presents the overall experimental workflow, including *B. cinerea* inoculum preparation, artificial botrytization of persimmon fruit, and fermentation. During post-inoculation storage, the botrytized persimmons exhibited gradual changes in appearance, indicating progressive modification of the raw material. Previous studies have shown that noble rot caused by *B. cinerea* is accompanied by moisture loss and compositional changes that may alter the physicochemical environment of the resulting fermentation substrate [11–13]. The progressive browning and softening observed in the botrytized persimmons are consistent with the enzymatic degradation patterns reported for *B. cinerea* infection in other fruit systems [11,12], and these observations indicate that *B. cinerea* remained metabolically active during the storage period and continued to modify the persimmon tissue throughout the storage period. The extent of these changes likely depended on storage duration and the degree of fungal colonization, both of which may influence the final properties of the fermentation substrate. The appearance changes observed in the present study also suggest that botrytization modified the persimmon substrate's physicochemical state, providing a basis for interpreting the subsequent differences in fermentation behavior. It should be noted, however, that visual observation alone cannot provide quantitative confirmation of fungal colonization intensity or enzymatic activity; microbiological quantification of *B. cinerea* development will be necessary in future studies to establish a rigorous relationship between colonization degree and fermentation outcomes.

As shown in Figure 2, the two treatments display distinct fermentation patterns. The noble rot-treated group showed faster increases in alcohol content and decreases in reducing sugar than the sucrose-chaptalized control. Differences in pH trends were also evident, with the former maintaining a more stable acidic range throughout fermentation. Because pH is an important factor affecting yeast activity, organic acid balance, and microbial stability during fruit fermentation [7,18], these differences may be regarded as meaningful indicators of variation in the two treatments' fermentation environments. A stable acidic pH can help maintain the proton motive force across the yeast cell membrane, which is essential for nutrient uptake and ethanol excretion during active fermentation [7]. In

contrast, the sucrose-chaptalized control exhibited a relatively higher initial pH and greater pH fluctuation during fermentation, conditions that may have been less conducive to sustained yeast activity. Maintaining a favorable pH range throughout fermentation is particularly important for non-grape fruit substrates, where the buffering capacity and acid composition can differ substantially from those of grape must. Collectively, these results suggest that the botrytized persimmon must provided a more favorable fermentation environment than the control [4,7,19].

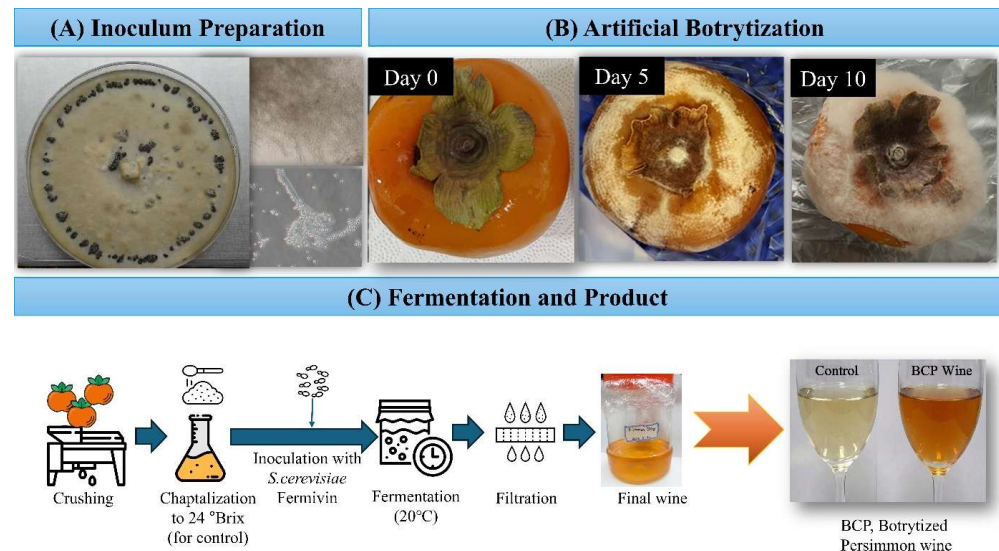


Figure 1. Schematic overview of artificial botrytization and persimmon wine production. (A) Preparation of *B. cinerea* inoculum. (B) Artificial botrytization of persimmon fruit during storage, showing representative appearance changes after inoculation. (C) Fermentation process and final products from sucrose-chaptalized control and noble rot-treated persimmon.

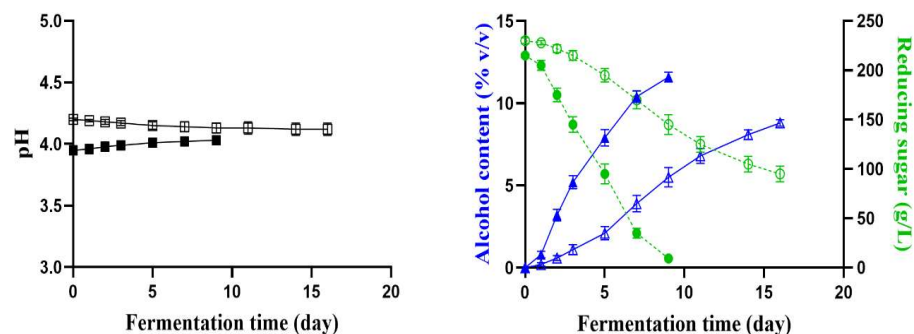


Figure 2. Changes in pH, alcohol content, and reducing sugar during fermentation of noble rot-treated persimmon must and sucrose-chaptalized control. The left panel depicts pH changes (black squares); the right panel depicts alcohol content (blue triangles, left axis) and reducing sugar (green circles, right axis). Filled symbols represent the noble rot-treated persimmon must; open symbols represent the sucrose-chaptalized control. Values are expressed as mean $\pm$ SD, $n = 3$.

3.2. Fermentation Characteristics and Implications for Fermentation Suitability

These trends are consistently supported by the data in Table 1. Despite lower initial soluble solids than the control, 21.50 °Brix versus 23.00 °Brix, the noble rot-treated group achieved a higher final alcohol content, 11.60% versus 8.80%, within a shorter fermentation period, 9 versus 16 days. It also showed lower final soluble solids, 10.50 °Brix versus 14.50 °Brix, and a higher maximum viable cell count, 8.11 log CFU/mL versus 7.85 log CFU/mL. These findings indicate that the observed differences in fermentation behavior cannot be attributed solely to initial sugar levels [5,7,8]. The present results

show that a higher initial soluble solids content did not necessarily lead to more efficient fermentation. Although the sucrose-chaptalized control had a higher initial °Brix value, it exhibited slower reducing sugar depletion, a longer fermentation period, and a lower final alcohol content than the noble rot-treated group. This discrepancy suggests that fermentation performance was not governed solely by the amount of added sugar but was also affected by the overall quality of the must composition. Comparable observations have been reported in other fruit wine systems, where fermentation rate was governed by substrate-related factors rather than by sugar concentration alone [7,19]. It should also be noted that the two treatments differed in initial soluble solids content (21.50 °Brix for the noble rot-treated must vs. 23.00 °Brix for the chaptalized control), which represents a confounding factor in the current experimental design. Inclusion of an additional control consisting of non-botrytized persimmon must, adjusted to the same soluble solids as the noble rot-treated group, would allow a more rigorous isolation of the biological effects of botrytization and is planned for future studies. The fact that the noble rot-treated group consumed a larger proportion of its initial sugars and converted them more efficiently into ethanol suggests that the overall fermentation environment, including nutrient availability and pH conditions, may have been more supportive of yeast metabolism in the botrytized must. Furthermore, the higher maximum viable cell count observed in the noble rot-treated group is consistent with the possibility that the botrytized substrate provided a substrate environment more supportive of yeast growth; however, this interpretation requires direct confirmation through nutritional analysis of the must. This interpretation is further supported by the shorter fermentation period observed in the noble rot-treated group, as faster attainment of maximum cell density generally correlates with better nutrient availability in the fermentation medium.

Table 1. Initial and final fermentation characteristics of noble rot-treated persimmon must and sucrose-chaptalized control.

Sample	Soluble Solids (°Brix)		pH		Final Alcohol [% (v/v)]	Fermentation Period (Day)	Max. Viable Cell Count (log CFU/mL)
	Initial	Final	Initial	Final			
Sucrose-chaptalized	23.00 ± 0.30 ^a	14.50 ± 1.00 ^a	4.20 ± 0.03 ^a	4.12 ± 0.05 ^a	8.80 ± 0.20 ^b	16	7.85 ± 0.12 ^b
Noble rot-treated	21.50 ± 0.20 ^b	10.50 ± 0.10 ^b	3.95 ± 0.01 ^b	4.03 ± 0.02 ^b	11.60 ± 0.30 ^a	9	8.11 ± 0.05 ^a

Values are expressed as mean ± SD; *n* = 3. Different letters within the same column indicate significant differences at *p* < 0.05.

The lower fermentation efficiency of the control, despite its higher initial sugar level, suggests that sucrose chaptalization effectively increases soluble solids but does not necessarily improve the overall quality of the fermentation substrate environment. In contrast, the botrytization process may have modified the physicochemical state of the fruit tissue and the composition of low-molecular-weight constituents, potentially contributing to the observed differences in fermentation rate and final alcohol yield [11,12]. Sucrose is a disaccharide that requires hydrolysis by yeast invertase before fermentation can proceed, and this additional metabolic step may have contributed to the slower fermentation onset observed in the control [8,20]. Moreover, the addition of exogenous sucrose does not alter the intrinsic composition of the persimmon must in terms of organic acids, amino acids, or mineral content, all of which are important factors influencing yeast fermentation performance [7]. As a result, although the chaptalized control had a higher initial sugar concentration, it may have lacked the additional nutritional and physicochemical advantages that the botrytized substrate appears to have provided. This may explain why the control fermented more slowly and reached a lower final alcohol content despite starting from a higher soluble solids level. Previous studies on grape fruits have reported that *B. cinerea* infection induces the release of amino acids, vitamins, and other micronutrients from fruit

tissue, which can improve yeast nutrition during fermentation [11,13]. In this context, the more efficient sugar depletion and higher final alcohol content observed in the noble rot-treated group support the hypothesis that fermentation performance depends on not only sugar quantity but also substrate quality prior to fermentation. This distinction between sugar quantity and substrate quality may be especially relevant for persimmon, the intrinsic composition of which differs markedly from that of conventional grape must. Moreover, the compositional changes induced during botrytization may be related to the pH stability observed during fermentation, which is partly consistent with the noble rot-treated group's fermentation profile [13,18]. The formation of organic acids during noble rot, as reported in grape-based studies, may hypothetically enhance the buffering capacity of the substrate and contribute to a more stable pH environment during fermentation [13,21]; however, because organic acid composition was not directly measured in this study, this remains a proposed explanation rather than an evidence-based conclusion.

Studies on grape-based noble rot have similarly reported that botrytized and non-botrytized must exhibit distinct compositional and fermentation characteristics [13]. A broadly comparable directional trend was also observed for the persimmon substrate in the present study. However, because persimmon and grape differ substantially in fruit composition and *B. cinerea* infection patterns, the specific mechanisms by which botrytization affects persimmon substrate cannot be directly equated with those reported for grape. Persimmon contains higher levels of soluble tannins and a different organic acid profile compared with grape [3,6], which may lead to distinct interactions with *B. cinerea* during the noble rot process. Therefore, while the general direction of the effects observed in this study is consistent with established knowledge from grape-based noble rot research, the specific magnitude and mechanistic basis of these effects in persimmon remain to be determined through targeted compositional analyses. Overall, the faster alcohol production and reducing sugar depletion observed in the noble rot-treated group suggest that artificial botrytization serves as a biological pretreatment distinct from simple sucrose chaptalization in persimmon wine production. Nevertheless, the specific compositional changes and metabolic factors underlying these differences were not directly elucidated in this study. A limitation of this study is that the specific compositional factors responsible for the improved fermentation performance of the noble rot-treated persimmon must were not directly identified. In particular, changes in organic acids, yeast-assimilable nitrogen, amino acids, aroma-related compounds, and *B. cinerea*-associated metabolites were not quantified [7,12]. Therefore, the proposed interpretation should be regarded as a fermentation phenotype-based explanation rather than a direct demonstration of the underlying metabolic mechanism. This distinction is important because the present study clearly establishes a difference in fermentation behavior between the two treatments but does not yet identify which compositional variables caused that difference. Thus, further investigation of organic acids, nitrogen sources, aroma-related compounds, and associated metabolic changes is warranted. In addition, monitoring the progression of *B. cinerea* colonization during storage and correlating it with specific compositional changes in the persimmon substrate would provide valuable information for optimizing the artificial botrytization process for non-grape fruit wine applications.

4. Conclusions

Artificially botrytized persimmon must exhibited faster fermentation and achieved a higher final alcohol content than the sucrose-chaptalized control despite lower initial soluble solids. The noble rot-treated group also maintained a more stable acidic pH and reached a higher maximum viable cell count during fermentation. The more efficient sugar-to-ethanol conversion, higher viable cell count, and more stable pH profile observed in the

noble rot-treated group collectively suggest that artificial botrytization may modify the pre-fermentation substrate environment in ways that are more favorable for yeast activity. In contrast, sucrose chaptalization, while effective in raising the initial sugar content, did not produce comparable improvements in fermentation efficiency under the conditions tested. These findings suggest that the observed differences in fermentation behavior cannot be explained by sugar level alone and that artificial botrytization functions as a distinct biological pretreatment in persimmon wine production. Further studies on organic acids, nitrogen sources, aroma-related compounds, and associated metabolic changes are needed to clarify the compositional basis of these effects. In particular, a detailed characterization of the compositional changes that occur in persimmon tissue during *B. cinerea* colonization would provide a mechanistic basis for the fermentation differences observed in this study. Given the growing interest in diversifying wine production beyond traditional grape varieties, understanding how biological pretreatments such as artificial botrytization affect fermentation substrate quality represents a relevant and increasingly important area for further scientific research and practical application. The present findings provide a preliminary but meaningful basis for exploring artificial botrytization as a viable pretreatment option in persimmon wine production and potentially in other non-grape fruit wine systems. However, it should be noted that no sensory evaluation, aroma profiling, or color assessment of the final wines was performed in the present study; improved fermentation kinetics do not necessarily translate into improved sensory quality, and comprehensive quality evaluation is essential for future work. Additionally, the long-term stability, aging potential, and quality evolution of persimmon wine produced by artificial botrytization during maturation represent important areas for subsequent investigation.

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