Effect of Storage and Ageing Time on Sensorial Quality and Composition of Wine Phenolic Compounds Improved with Saccharomyces Cerevisiae Local Hybrid

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Abstract. High quality wine can be produced by the perfect ageing process. The purpose of this study was to determine the effect of ageing process container on sensory quality and composition of phenolic compounds of wine fermented with local hybrid Saccharomyces cerevisiae. The research design used a randomized block design with two factorial designs. The first factor is the materials for storing during the ageing process, namely plastic bottles, glass bottles and pottery barrels (from Banyuning). The second factor is ageing time, namely 1, 2, 3, 4 and 5 months. The experiment was carried out twice over so that 30 experimental units were obtained. The variables measured were phenolic compounds and sensory quality (aroma and taste, color, and turbidity level). The composition of the phenolic compounds was determined by gas chromatography-mass spectrometer (GC-MS) and sensory quality test using 15 trained panelists. The results showed that the composition of the highest phenolic compound content was shown successively with containers: plastic bottles, glass and earthenware and the ageing time was 4 months. The content of phenolic compounds is Isoamyl alcohol (ISM), Isobutanol (ISB), n-propanol (PR), Acetaldehyde (ADE), 2,3-Butanediol (BUT), Acetone (ACE), while the best sensory quality is using pottery because it has better aroma and taste, lower turbidity level. Based on data analysis, it can be concluded that the length of time for the ageing process and the container during the ageing process has a significant effect on the quality and content of phenolic compounds in grape wine fermented with local hybrid Saccharomyces cerevisiae.

1. Introduction
Wine is a fermented drink. The process of aging wine is an important process to produce high-quality wine [1]. Traditionally, the process of aging wine uses oak barrels. At least 150 types of oak are used to improve the quality of wine [2]. Various attempts were made to produce high quality wines in the aging process, namely using oak wood fragments [3] and microoxygenation applications [4]. Treatment through increasing levels of lees (yeast waste) which is made in high concentrations [5] or the application of physical methods by replacing non-wooden containers [6]. Various modifications are still problematic, namely a long time being inefficient, unhygienic, yeast activity is not disturbed,
causing microbial damage to grow, so that the quality of wine decreases, and the esterification process is disturbed [7]. Another breakthrough, using materials that have an orthodoxy high, allows for rapid and homogeneous esterification, and resistance to other microbial contaminants [8]. This condition can use hybrid yeast types that have stable properties, namely nutrient deficiencies and high alcohol content between yeast Saccharomyces cerevisiae and microbes [9].

This strategy has continued to be developed in the last decade, but the results have not been satisfactory [10]. Yeast hybrid technology can be developed to produce wine, namely local hybrids of Saccharomyces cerevisiae [11] and has been applied to a structured fermentation method that can produce zero percent alcoholic wines [12]. Local hybrid Saccharomyces cerevisiae yeast in general medicine can produce wine with levels of 13% (unpublished data). Therefore, in this study it is necessary to know in research the ability of yeast to produce phenolic compounds and sensory qualities in the aging phase using various materials such as plastic bottles, glass bottles and pottery from Banyuning Buleleng Bali.

2. Materials and methods

The main material used in this study. Grapes from a people's garden in Banjar village, Buleleng, Bali. The grapes are black and ripe on the trees. The chemicals used are all in the proanalytic grade, 70% alcohol, glucose, citric acid, NaOH, and sodium metabisulfite, Nelson A's reagen, Nelson B's reagen, Arsenomolybdate solution, Ethanol, Methanol, and Phenolphthalein. The main tools are a spectrophotometer, digital scale (Mettler Toledo AB 204), autoclave, laminar flow, centrifuge, refrigerator, pH meter (Istek), injector, stove (Rinai), and a distiller and a set of glassware.

2.1. Experimental design

This research was conducted with a factorial randomized block design. The first factor is the storage materials for plastic bottles, glass bottles and local ground barrels (Banyuning). The second factor was storage time from 1, 2, 3, 4 and 5 weeks. The experiment was carried out in two repetitions so that 20 experimental units were obtained. The variables measured were phenolic compounds and sensory quality (aroma and taste, color, and turbidity level). The data obtained were analyzed for their diversity and continued with the Duncan test if the treatment had a significant effect (P <0.05).

2.2. Observed variables

The variables observed in this study were reducing sugar levels using the Nelson Somogyi method [13], ethanol content using the gas chromatography method [14], phenolic compounds using GC MS, and methanol content [14], total acid using the titration method, and the degree of acidity using a pH meter and a session test [15] by 15 trained panelists from UKM Amertha Nadi.

2.3. Experimental procedure

The minimum medium (MM) consists of: 1.34% basic yeast nitrogen without ammonium sulfate and amino acids (YNB, Difco, Detroit, USA) and 4% glucose supplied by Merck Farma Quimica (Barcelona, Spain). Wine Grapes should be treated with 0.4 gl 1 bentonite for 2 days at 48°C to enhance sedimentation prior to fermentation, and be inoculated with 5% a local hybrid Saccharomyces cerevisiae yeast preculture. Bottles with 250 ml MM of medium or wine should be covered with two layers of sterile gauze to avoid contamination, and fermentation carried out in duplicate at 18 °C for 20 hours.
2.4. Sequential fermentation

Mixed fermentation carried out with a sterile 250 ml aliquot should be maintained at 18°C using 106 ufc / ml of P. fermentans for 2, 3, 4, 6 or 8 days. After each of these time intervals, 106 ufc / ml of S. cerevisiae was added to form the yeast mixture, which was then maintained for a total of 20 days in all cases. All of this sequential fermentation was made in duplicate. The resulting wines were centrifuged and stored at 20.8°C, for a maximum of one month, for co-analysis.

2.5. Analysis using GC-MS

The volatile and alcohol compounds were analyzed, in triplicate, by direct injection of 1 microliter each of the final product into a gas chromatograph of Variant 3900 with a flame ionization detector (220°C) and CPWAX Column 57CB (50 0.25 mm). Both the injector and detector are operated at 220°C. The carrier gas N₂ (99.999%) at a flow rate of 36 ml / min. The injection is divided into 30 modes with an air flow of 300 ml / min. The column temperature was programmed from 40 °C for 2 minutes to 120 ° C at an increment rate of 3 minutes and 4 minutes at 120 ° C. The samples were analyzed in triplicate. The identification and quantification of compounds is carried out by comparing the retention times and concentrations with standard solutions used in the alcohol industry.

3. Result and Discussion

The aging process was carried out for 1, 2, 3, 4, 5 months, carried out on plastic bottles, glass bottles, and pottery from Banyuning. The best storage time for the aging process is 4 months, with a score of 4.75. The lowest sensory quality was found in pottery within 2 months of treatment (see Figure 1).

![Figure 1. The sensory quality of the length of time in the aging process.](image-url)

Storage in plastic bottles, during the aging process, shows that the sediment that occurs during the aging process does not stick to the bottle walls. The same thing happens with glass bottles, making the wine appear clear. The tightly closed plastic bottle allows for the formation of aroma due to the better esterification reaction of glass and pottery containers, while the storage of good quality pottery from Banyuning village takes 1 month, then decreases in the 2nd month then increases again at 3 months.
storage and remains stagnant in the fourth month. Thus, the use of pottery is still relevant for short storage periods.

The content of wine polyphenols after the fermentation process in the aging phase is used to see the continued activity that occurs during the aging process. Some polyphenol compounds in storage containers such as plastic bottles, glass bottles and barrels or pottery from Banyuning are shown in Table 1. The phenolic compounds are Isoamyl alcohol (ISM), Isobutanol (ISB), n-propanol (PR), acetaldehyde (ADE), 2,3 - Butanediol (TAPI), Acetone (ACE). The highest content is shown by plastic bottle containers. Storage in plastic bottles, in the aging process the sediment does not stick to the wall, and allows the wine to appear clear, and closes tightly, so the process of forming aroma for esterification is better than glass and earthenware containers.

**Table 1. Polyphenol compounds in Sacharomyces cerevicae fermentation**

| Polyphenol compounds | The content of polyphenol compounds (mg / L) in the aging process container |
|----------------------|--------------------------------------------------------------------------|
|                      | Plastic bottle | Glass bottle | Pottrey barell from Banyuning Village |
| Isoamyl alcohol (ISM) | 258           | 231          | 218                                 |
| Isobutanol (ISB)     | 78            | 67           | 58                                  |
| n-propanol (PR)      | 32            | 31           | 42                                  |
| Ethyl acetate (ETAC) | 54            | 65           | 43                                  |
| Acetaldehyde (ADE)   | 46            | 56           | 53                                  |
| 2,3 - Butanediol (BUT) | 630        | 562          | 541                                 |
| Aceton (ACE)         | 25            | 21           | 35                                  |

This study also shows that wines are made from grapes from community gardens in Buleleng Bali, indicating that the aging process affects the quality of taste, aroma and color, as well as the level of clarity of the wine. This justifies modern alcoholic beverages undergoing an aging process in which compounds extracted from the container are used, such as wood, which can affect the chemical composition of the wine. The porous container contributes to the overall character of the wine [16]. Therefore, in making wine from Buleleng Bali grapes, choosing a storage place during the aging process is very important, because it can accelerate the chemical changes in wine [17].

Furthermore, aging for 4 months shows the highest sensory quality, this shows that the curing time affects sensory properties (taste, color, taste and clarity) and physicochemical (alcohol content, color and turbidity level), thus giving a change in the aroma and taste of wine [18]. The increased chemical content of wines preserved in plastic bottles produces a smoky aroma like toast, while wines preserved in glass bottles show the aroma of vanilla and cocoa, while wines using Banyuning gerbah containers are like toast and appear clearer.

### 4. Conclusion

The aging process in wines made from Bali Buleleng grapes fermented with local hybrid yeast Saccharomyces cerevisiae, which uses different places, actually affects the sensory qualities and the wine's polyphenolic compounds. Storage containers with plastic bottles work best for a storage period of 4 months. However, storage with pottery from Banyuning village is suitable for short term storage. The results of this study recommend the possibility of other ingredients for storage, so as to improve the wine quality of the Buleleng Bali grapes.
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References

[1] Ugliano M 2013 Oxygen contribution to wine aroma evolution during bottle aging Journal of agricultural and food chemistry 61 (26) 6125-6136
[2] Dumitriu G D, Teodosiu C, Gabur I, Cotea V V, Peinado R A and de Lerma N L 2019 Evaluation of Aroma Compounds in the Process of Wine Ageing with Oak Chips Foods 8 (12) 662
[3] Tao Y, García J F and Sun D W 2014 Advances in wine aging technologies for enhancing wine quality and accelerating wine aging process Critical reviews in food science and nutrition 54 (6) 817-835
[4] Schmidtke L M, Clark A C and Scollary G R 2011 Micro-oxygenation of red wine: techniques, applications, and outcomes Critical reviews in food science and nutrition 51 (2) 115-131
[5] Loira I, Vejarano R, Morata A, Ricardo-da-Silva J M, Laureano O, González M C and Suárez-Lepe J A 2013 Effect of Saccharomyces strains on the quality of red wines aged on lees Food Chemistry 139 (1-4) 3783-3788
[6] Contreras A, Hidalgo C, Schmidt S, Henschke P A, Curtin C and Varela C 2015 The application of non-Saccharomyces yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content International journal of food microbiology 205 7-15
[7] Tika I N 2017 Widya Laksana Singaraja: Undiksha Press
[8] Goold H D, Kroukamp H, Williams T C, Paulsen I T, Varela C and Pretorius I S 2017 Yeast's balancing act between ethanol and glycerol production in low-alcohol wines Microbial biotechnology 10 (2) 264-278
[9] Shao Y and Lin A H M 2018 Improvement in the quantification of reducing sugars by miniaturizing the Somogyi-Nelson assay using a microtiter plate Food chemistry 240 898-903
[10] Cardenia V, Toschi T G, Scappini S, Rubino R C and Rodriguez-Estrada M T 2018 Development and validation of a Fast gas chromatography/mass spectrometry method for the determination of cannabinoids in Cannabis sativa L journal of food and drug analysis 26 (4) 1283-1292
[11] Volmer D A, Curbani L., Parker T A, Garcia J, Schultz L D and Borges E M 2017 Determination of titratable acidity in wine using potentiometric, conductometric, and photometric methods Journal of Chemical Education 94(9) 1296-1302
[16] Teodosiu C, Gabur I, Cotea V V, Peinado R A and López de Lerma N 2019 Evaluation of aroma compounds in the process of wine ageing with oak chips *Foods* 8 (12) 662

[17] Coppola F, Picariello L, Forino M, Moio L and Gambuti A 2021 Comparison of Three Accelerated Oxidation Tests Applied to Red Wines with Different Chemical Composition *Molecules* 26 (4) 815

[18] Liu Q, Weng P and Wu Z 2020 Quality and aroma characteristics of honey peach wines as influenced by different maturity *International Journal of Food Properties* 23 (1) 445-458