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# Effect of Thiamine in Wine Fermentation with Yeast *Saccharomyces cerevisiae* ILS6

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**ABSTRACT**

The purpose of this study was to determine the effect of adding thiamine to wine fermentation with the yeast Saccharomyces cerevisiae ILS6. The research's design used a randomized block design (RBD with two factorials. Firstly, factorial is the variation of thiamine concentration 0.5 and 1% (w/v). Secondly, factorial is the length of fermentation time 7, 14, 21, 28, and 35 days. Each experiment was repeated two times to obtain 20 experimental sets. The variables observed were alcohol content, sensory quality (aroma, taste, and colour), and polyphenol compounds. The results showed that the addition of 0.5% w/v thiamine showed an alcohol content of 12.5%. Organoleptic test with panelists revealed that the quality of wine from the addition of 0.5% w/v thiamine with a fermentation time of 21 days. The taste, aroma, and color were better, with the best overall effectiveness test of 1.3. The polyphenol content analysis showed that some of the compounds produced galloyl-hexoxide, ferulic acid, chlorogenic acid, gallic acid, caffeic acid, catechins, epicatechin, punicalagin, and ellagic acid. Therefore, the results of the data analysis showed that the addition of thiamine had a positive effect on wine fermentation.

**Keywords:** Wine, Saccharomyces cerevisiae ILS6, Polyphenol, Organoleptic

## Introduction

Alcoholic fermentation is an essential step in winemaking. In the fermentation process, yeast converts sugar into alcohol and bio-transforms various compounds to produce the aroma and taste of wine (Tian et al., 2021), (Arvisenet et al., 2016). Wine quality is determined by the content of phenolic compounds and sensory quality (Saïdi & Giraud, 2020). These two aspects are the visual identity that wine shows. Its existence depends on the balance between the bitterness and sour taste of the wine (Saïdi & Giraud, 2020), (Han et al., 2019). Various efforts have been made to improve the quality of the wine. Those are (1) the use of superior Saccharomyces cerevisiae yeast, (2) using non-Saccharomyces cerevisiae yeast, either singly or in combination (a consortium with various types of yeast and Lactobacillus bacteria (BAL) in fermentation The increase in alcohol content during fermentation makes yeast experience stress (Ferreira et al., 2017). Studies on the addition of thiamine in indigenous yeasts are still relatively rare.

Therefore, alcoholic fermentation using local indigenous yeast continues to be carried out. The taste and aroma of wine can be optimum when the nutritional conditions for yeast are met (Isoton et al., 2021), and illnesses are suitable for its growth, such as sugar, minerals, and vitamins. Vitamins are needed as cofactors for enzymes involved in fermentation. One of these vitamins is thiamine.

In fermentation, thiamine (Vit B1) has two essential functions: an enzyme cofactor and can protect yeast from stress due to environmental changes. The increased alcohol content causes the yeast to experience stress (Labuschagne & Divol, 2021). Most yeast species can assimilate exogenous thiamine into cells and synthesize thiamine de novo (Labuschagne & Divol, 2021) (Delin & Lee, 1991). Several studies have shown that the mechanism and extent of thiamine accumulation depend on several factors, including the yeast species Saccharomyces cerevisiae, as well as current knowledge about (1) the intracellular function of thiamine, (2) the balance between and regulation of thiamine uptake and synthesis and (3) the many factors, which affect the availability and utilization of thiamine (Tita et al., 2011).

For the latter, particular emphasis is placed on the conditions that occur during the fermentation of wine. The adequate concentration of thiamine in wine must ensure successful fermentation is discussed, along with the effect of thiamine concentration on wine's fermentation kinetics and sensory properties. This knowledge can serve as a resource for optimizing thiamine concentrations for optimal industrial yeast applications (NIXON et al., 1991)

Yeast contributes to the taste of Wine in three main ways: it affects the ecology of the wine-making process, metabolism and enzymatic activity, and the organoleptic impact of individual species or combinations of species on wine taste. The distinctive taste of grapes is mainly a mixture of exile acetate, ethyl caproate, ethyl caprylate, isoamyl acetate, and 2-phenylethyl acetate (Ramírez et al., 2020). Various aromas and flavours arise depending on the characteristics of the type of yeast used and the thiamine concentration (Wolak et al., 2014).

The Wine produced in Singaraja is an alcoholic beverage made from local fermented grapes from the typical grape group in northern Bali, namely Alphonso LaValle. This type of grape has adapted very well in tropical areas such as Seririt and Banjar sub-districts, providing a fairly high income. Wine production involves yeast (yeast) which converts sugar into alcohol and carbon dioxide gas, Wine with local hybrid Saccharomyces cerevisiae yeast has been produced by craftsmen in Banjar Village and can produce Wine using local yeast. The aging process affects the aroma and taste of distinctive Wine (Tika et al., 2021).

Local hybrid Saccharomyces cerevisiae yeast in general treatment can produce wine with a content of 13%. (data not published). The aroma and taste of wine with various local ingredients can affect the quality of the wine produced. However, the problem still arises is the formation of deposits and granules in the wine-making process, so the resulting wine is not clear. However, the use of this local hybrid Saccharomyces cerevisiae yeast has not been revealed about the content of phenolic compounds and the quality when thiamine is added in the wine-making process because thiamine can control stress on yeast (Tika et al., 2021).

Therefore, using thiamine in this local hybrid Saccharomyces cerevisiae yeast in wine production makes it possible to maintain optimum fermentation during the fermentation and aging process. The critical point that needs to be revealed in this research is the ability of yeast to produce aroma compounds during fermentation with the addition of thiamine concentration and fermentation time. The purpose of this study was to determine the optimum concentration of thiamine and the length of fermentation time in the fermentation process to produce wine quality (phenolic compounds content and sensory quality) of wine in the yeast fermentation of Saccharomyces cerevisiae ILS6. The addition of thiamine is expected to support the synthesis of aroma compounds and distinctive wine flavours in local wines.

## METHODOLOGY

The material used is grapes (cultivar Isabella) which are dominantly grown in the grape plantations of Banjar village, Banjar Buleleng sub-district, Bali. Pure culture of dried yeast (Saccharomyces cerevisiae Local hybrid). This culture is stored in the Biochemistry Lab, FMIPA Undiksha. The media and chemicals used were granulated sugar (gulaku, PT Sweet Indo Lampung), aqua dest (maxLab), dextrose (Pudak), Potato Dextrose Broth (Himedia), Potato Dextrose Agar (PDA) (Himedia), ammonium phosphate (Smart Lab .), Sodium metabisulfite (Smart Lab) alcohol 95% (Rofa Laboratory Centre), phosphate buffer pH 7 and pH 4 (Eduscientia), NaOH 0.01 N (Himedia), citric acid (Sandi Mitra Kimia), PP indicator, solution nelson A and nelson B, Arsenomolybdate solution (Nitra Kimia) Sodium alginate (Smart Lab), and calcium chloride (Smart Lab).

The tools used in this research include alcohol hydrometer, digital scale (US Solid, Model Number: USS-DBS15-2), alcohol meter gas stove (Rinai), pan, washbasin, knife, Erlenmeyer (Pyrex), filter, funnel , measuring cup, pH meter (Istek), autoclave, test tube (Iwaki), petri dish (Pyrex), laminar flow, incubator, bent glass rod, glass bottle, volume pipette, gas chromatography, refractometer, alcohol meter (Istek), spectrophotometer, thermometer, dropper, volume pipette and refrigerator. Equipment GC-MS type Waters GCT (Waters, Etten-Leur, Netherlands) equipped with Agilent 6890 gas chromatography and Agilent 7683 autosampler (Agilent, Amstelveen, Netherlands). which is in the Bali Police Forensic Lab Denpasar.

**Experimental Design**

This study used a randomized block design (RAK) with a factorial experimental pattern consisting of two factors. Treatments were grouped based on the concentration of thiamine and without containing thiamine when making wine. The first factor is the concentration of thiamine, namely concentrations 0.5 and 1% (w/v), while the second factor is the variation in the length of fermentation time, namely 7; 14; 21; 28, and 35 days. The treatment obtained ten units of combination treatment. Each treatment was grouped into two based on the processing time so that 20 experimental units were obtained. The data obtained were analyzed by variance, and if there was an influence between treatments, it was continued with Duncan's test (Permanasari et al., 2010).

**Observed Parameters**

The parameters observed in this study aimed to determine the chemical properties, polyphenols, and sensory quality of wine from local grapes.

**Wine Making from Grapes**

Fresh grapes were sorted and weighed as much as 300 grams. The grapes were put in a topless glass and added 600 ml of water. Added 25% sugar (w/v), then the pH of the grape juice was adjusted to 4.0 (NaOH was used to increase pH, while citric acid for lowering the pH). Then the starter was added according to the 15%v/v treatment. Further, it was continued with the fermentation according to the treatment time; 7, 14, 21, 28, and 35 days. Then an aging process was carried out for one month to improve the taste and aroma, precipitating suspended solids, hydrolyzing pectin and protein so that the color becomes clear and the flavor will increase. Aging is the process of forming flavors composed of alcohol, aldehyde, ketone, and ester, which are volatile compounds. The aging process can last from several months to several years (Tika et al., 2021). After the aging process is complete, the wine is filtered and bottled. At the time of daily fermentation, the specific growth rate of the local hybrid Saccharomyces cerevisiae was calculated.

**Ethanol content**

The measurement of ethanol content was using gas chromatography model Aligent series 7890. A total of 1g sample was added with 0.5 ml of diethylene glycol dimethyl and added distilled water to the volumetric flask line (10 mL). It was shaken until homogeneous. After that, it is injected into a gas chromatography instrument; then the concentration is measured from the area of ethanol (Official, 2005); as a comparison also uses an alcohol hydrometer (alcohol meter).

**Sensory quality**

Ten trained panelists carried out sensor quality (color, aroma, taste, and overall acceptability) and effectiveness tests (Official, 2005). These panelists were appointed based on the recommendation of the SME wine company Amertha Nadi, trained and often involved in testing their wines.

**Content of Polyphenol Compounds**

Polyphenol analysis was carried out by adapting the method developed by Grün et al. (Grün et al., 2008), using GC-MS. Type Waters GCT (Waters, Etten-Leur, The Netherlands) Samples were analyzed on a mass spectrometer using three silica columns to separate phenolic acids: a VF-5 ms (30 m × 0.25 mm, df = 0.25 m), column VF-17 ht (30 m × 0.25 mm, df = 0.10 m) (both from Varian, Middelburg, The Netherlands), and the ZB-5 ms phenomenon (30 m × 0.25 mm; df = 0, 25 m) (Bester, Amstelveen, Netherlands). Helium is the carrier gas and is used at a flow rate of 1 ml/min. Sample (1 l) was injected into a liquid nitrogen-cooled CIS-4 injector (Gerstel, Mulheim an der Ruhr, Germany) operated in split mode at a split ratio of 20:1 (The injector used a temperature gradient of 55°C (holding for 0.05 min) to 300 C (holding for 5 min) at 8 C/s. The column oven temperature program is as follows: isothermal 0–1 min at 45 C, 1–6.5 min at 10 C/min to 100 C, 6,5–26.5 min at 7.5 C/min to 250 C., and 26.5–29 min at 10 C/min to 300 C., were held for 6 min. For plasma extracts, the final temperature was raised to 350 C and stayed at that temperature for 10 min before returning to initial conditions. The interface and source temperatures of the mass spectrometer were 250 C and 180 C, respectively. The mass spectra were recorded in EI mode from 6 to 30 min at a scan time of 0.3 sec., the time between scans 0.1 seconds, and scan range m/z 50–600.

## Results and Discussion

### Ethanol Level

The analysis's result of the wine produced with yeast Saccharomyces cerevisiae ILS6 and thiamine showed that treatment with thiamine had a very significant effect (P<0.01) on the alcohol content made and its interactions. The average value of the ethanol content of wine can be seen in Table 1.

**Table 1. Alcohol Concentration Against Fermentation Time**

| Weeks | Alcohol (%) | | |
|---|---|---|---|
| | WTh0.5 | WTh1.0 | Control |
| 1 | 11.2± 0.332a | 10.8± 0.232 a | 9.2± 0.234 |
| 2 | 12.3± 0.135b | 11.2± 0.532bc | 10.4± 0.246 |
| 3 | 12.5± 0.232c | 11.5± 0.137de | 11.4± 0.257 |
| 4 | 12.42± 0.234d | 9.23± 0.234 | 9.2± 0.253 |
| 5 | 11.43± 0.237 | 9.11± 0.332 | 8.34± 0.132 |

Note: Different letters behind the mean value indicate a significant difference (P<0.05), mg= week; WTh0.5 = wine fermented with the addition of 0.5% w/v thiamine, WTH1.0 = wine fermented with the addition of 1.0% w/v thiamine.

From the results contained in table 1, it appears that the lowest average value of ethanol content in the 1-week (7 days) fermentation treatment, namely the control is 9.2%, while the highest level is shown in the 3-week fermentation time (21 days) of 12, 5%.

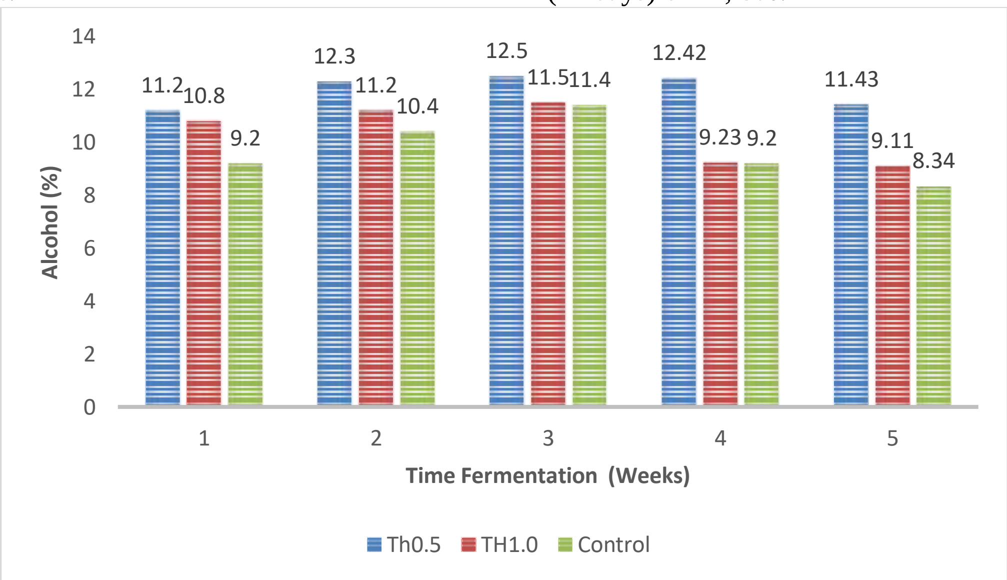


**Figure 2. The alcohol content in the fermentation of Saccharomyces cerevisiae ILS6 yeast**

The description of the increase and decrease can be seen in Figure 2. Yeast Saccharomyces cerevisiae ILS6 showed that the ethanol content had reached 11.2% in the early stages, compared to the control, which was 9.2%.

Higher alcohol content at a concentration of 0.5% w/v, the presence of thiamine in certain concentrations can increase alcohol levels compared to 1.0% levels. That is, the need for vitamins does not need a lot, but there needs to be. From these data, it can be explained that thiamine's function can be a stress reliever for yeast. However, if the concentration is higher, the function is not proportional to the increase in thiamine concentration.

Several enzymes require thiamine as a cofactor for the enzyme from the Saccharomyces cerevisiae ILS6 yeast, which breaks down sugar into alcohol. Fermentation is a process that refers to microorganisms that break down organic matter. It was to obtain the energy needed to stay alive. Additionally, fermentation makes organic compounds (alcohols and organic acids) and inorganic compounds (carbon dioxide and hydrogen), depending on the substance formed. This process is called alcoholic fermentation, lactic acid fermentation, amino acid fermentation, etc. On the other hand, yeast produces enzymes in fermenting flour process into glucose and carbon dioxide. During the fermentation process, alcohol is created, which gives the taste to the resulting product.

The presence of thiamine at a certain concentration level is significant to support yeast growth and ultimately ensure complete fermentation and production of optimized wine flavours compared to deodorizing compounds. In particular, the vitamin thiamine plays a vital cofactor role for several enzymes involved in various metabolic pathways, including those leading to the production of wine-relevant flavour compounds, and aids yeast survival through thiamine-dependent stress protection functions. Most yeast species can assimilate exogenous thiamine into cells and synthesize thiamine de novo. However, the mechanism and extent of thiamine accumulation depends on several factors (Labuschagne & Divol, 2021) Delayed thiamine uptake at higher concentrations for K. marxianus Y885 suggested differential regulation of thiamine uptake compared to S. cerevisiae EC1118. In addition, the different trends in metabolites produced between species suggest that thiamine concentrations impact carbon metabolism fluxes differently in these two yeasts, potentially influencing the properties of the final wine (Labuschagne & Divol, 2021).

**Wine Sensory Quality**

The wine's sensory quality can be determined through a sensory test or an organoleptic test. A sensory test is a test method that uses human senses as the main tool for measuring the acceptance of wine products fermented with the yeast Saccharomyces cerevisiae ILS6 with the addition of a thiamine activator. In this study, the test was carried out with an alcohol content of 11% and above. Therefore, there are 12 samples tested.

**Table 2. Wine Sensory Test Results**

| Exp | Exp | Color | Taste | Aroma | Total |
|---|---|---|---|---|---|
| WT0.5 -1 | WT0.5 -1 | 2.2 | 2.8 | 3.3 | 2.8 |
| WT0.5 -2 | WT0.5 -2 | 2.3 | 2.6 | 3.8 | 2.9 |
| WT0.5 -3 | WT0.5 -3 | 3.7 | 4.5 | 3.8 | 4.0 |
| WT0.5 -4 | WT0.5 -4 | 3.4 | 4.1 | 3.6 | 3.7 |
| WT0.5 -5 | WT0.5 -5 | 3.5 | 3.1 | 4.1 | 3.6 |
| WT1-2 | WT1-2 | 2.3 | 2.1 | 4.2 | 2.9 |
| WT1-3 | WT1-3 | 2.6 | 2.3 | 3.9 | 2.9 |
| WC-3 | WC-3 | 2.7 | 2.3 | 3.5 | 2.8 |

Notes; WT1 = wine that uses 1% w/v thiamine, and WC3 = is a control win at 3 weeks fermentation.


**Figure 3. Organoleptic test**

### Effectiveness Test

The effectiveness test aims to determine the best treatment in producing precursor components of wine taste from local Singaraja grapes (DeGarmo et al., 1984). The effectiveness test used the observed variables' values, namely alcohol, colour, flavour, aroma, and overall acceptance. The variable weight value is obtained after averaging the tabulation of all respondents' ranking results. The variable with the highest average value is given a weight of 1. In contrast, other variables' weight is obtained from the quotient of each variable's average—the average variable, which is given a weight of 1. Table 3 shows that the aroma variable has the highest value of 1, followed by other variables, namely, alcohol, taste, and color. Based on the result, the aroma has the highest score, maintaining that aroma has the most important role in determining the wine quality from the best grapes.

**Table 4. Results of Effectiveness Test Calculations to Determine the Best Treatment**

| Variable | Alcohol | Color | Taste | Aroma | Total | Ne |
|---|---|---|---|---|---|---|
| BV | 0.94 | 0.75 | 0.79 | 1.00 | 0.85 | 0.00 |
| WT0.5 -1 | 0.90 | 0.75 | 0.62 | 0.79 | 0.69 | 0.9 |
| WT0.5 -2 | 0.99 | 0.78 | 0.58 | 0.90 | 0.73 | 0.9 |
| WT0.5 -3 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.3 |
| WT0.5 -4 | 0.99 | 1.00 | 0.91 | 0.86 | 0.93 | 1.2 |
| WT0.5 -5 | 0.91 | 0.96 | 0.69 | 0.98 | 0.89 | 1.1 |
| WT1-2 | 0.90 | 0.77 | 0.47 | 1.00 | 0.72 | 0.9 |
| WT1-3 | 0.92 | 0.79 | 0.51 | 0.93 | 0.73 | 0.9 |
| WC-3 | 0.91 | 0.77 | 0.51 | 0.83 | 0.71 | 0.9 |

Note: Ne = effectiveness value, BV = weight value, WT1 = wine using 1% w/v thiamine, and WC3 = control win in 3 weeks fermentation

**Polyphenol Content in Wine**

The polyphenol content analysis results showed that some of the compounds produced galloyl-hexoxide, ferulic acid, chlorogenic acid, gallic acid, caffeic acid, catechins, epicatechin, punicalagin, and ellagic acid.

**Table 2. Polyphenol Fompounds in Sacharomyces Cerevicae Fermentation**

| Polyphenol compounds | The content of polyphenol compounds (% w/v) in wine | | |
|---|---|---|---|
| | WTh 0,5 | WTh1,0 | Control |
| Galloyl-Hexoxide | 243 | 235 | 213 |
| Ferulic Acid | 84 | 73 | 56 |
| Gallic Acid | 34 | 30 | 41 |
| Caffeic Acid | 56 | 62 | 43 |
| Catechins | 47 | 46 | 33 |
| Epicatechin | 635 | 592 | 442 |
| α -Punicalagin | 35 | 31 | 26 |
| Ellagic Acid. | 43 | 38 | 28 |

The most commonly encountered compounds are galloyl-hexoxide and Epicatechin. Epicatechin is the first compound ever researched to modulate both regulators of muscle growth increase in strength favourably. The expression of foli statin aids muscle hypertrophy by inhibiting the preventative effects of myostatin muscle growth.

In addition, the epicatechin content in wine fermented with Saccharomyces cerevisiae ILS6 has been added with Thiamine. The presence of epicatechin compounds is significant because these compounds can inhibit the growth of cancer cells, especially breast cancer. Additionally, Epicatechin is a phenolic compound with antioxidant activity in natural foods and beverages, such as cocoa and red wine. Epicatechin contains anticancer activity, revealing that Epicatechin can increase ROS and upregulation of pro-apoptotic proteins (Bad and Bax) was observed and about the molecular mechanism of (−)-Epicatechin to induce apoptosis (Pereyra-Vergara et al., 2020). Therefore, the presence of this compound is relatively abundant in local wine fermentation.

## Conclusion

This study showed that the addition of thiamine levels in the wine fermentation process from local grapes with the yeast Saccharomyces cerevisiae ILS6 positively affected the quality of the wine. It is for both aroma and taste, as the content of polyphenol compounds. Therefore, adding thiamine in the wine-making process is needed, since bioactive compounds' content, epicatechin, must be revealed for the human body's metabolism.

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