

# Chemical and Sensory Properties of Spontaneous Fermented Red Rice Vinegar with Fermentation Time Variation

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**Abstract:** Red rice is a variety of Indonesian rice that contains anthocyanins, which are antioxidant compounds that can neutralize free radicals. Red rice is used as another valuable food source, specifically for vinegar. The fermentation duration determines the quality of vinegar in terms of chemical and sensory characteristics. The purpose of this study was to examine the effect of fermentation time on the chemical properties and sensory attributes of red rice vinegar made by spontaneous fermentation. Red rice vinegar was produced through a spontaneous fermentation process at 30- and 60-day fermentation periods. The pH, alcohol content, acetic acid, antioxidant activity, and total phenolic content were measured. Red rice vinegar produced after 30 and 60 days of fermentation had an acetic acid concentration of  $73.02 \pm 4.91$  mg GAE/G. The alcohol content differed significantly between 30 and 60 day fermentation treatments ( $p < 0.05$ ). The fermentation time has no effect on the sensory qualities (aroma, taste, and colour) of the product. The yeast aroma, slightly sweet, bitter flavour, and brownish yellow colour were the best treatments based on sensory evaluation.

**Keywords:** Chemical, fermentation time, red rice, sensory properties, vinegar, spontaneous fermentation.

## 1. Introduction

Red rice is a variety of rice cultivated in Indonesia. The red colour of this rice originates from aleurone, which contains genes that produce anthocyanin, a pigment that imparts a red colour while also functioning as an antioxidant. Quality red rice is characterised by the grain's surface being coated in a dark red colour and remaining intact (Deng et al., 2013). Rice's colour is associated with antioxidant activity, particularly anthocyanins (Sompong et al., 2011). Red rice contains antioxidants, as indicated by an IC50 value ranging from 85.69 to 290.54 ppm (Pangerang, 2022). Red rice contains various bioactive compounds such as polyphenols and vitamins, including vitamin E, which serves as an antioxidant. This component is what makes red rice popular as a functional food. Red rice exhibits the highest antioxidant activity (95.05%) compared with white rice and brown rice (Azis et al., 2015). Currently, red rice is being investigated as a functional meal. One study on rice crackers utilized red rice (*Oryza nivara*) flour and *Moringa oleifera* leaf powder as a functional food (Malibun Fais Bintang, Husain Syam, 2019), and also milk with the main ingredient of red rice, revealing the potential of red rice milk as a functional beverage with antioxidants (Wijaya & Romulo, 2021). One example of a processed rice product that can become a functional food is vinegar.

Vinegar is an organic sour solution derived from sugar-rich substances like apples, grapes, rice, and black sticky rice that undergo fermentation (Kim et al., 2021). Vinegar is commonly employed as a spice, flavouring, preservative, and product for daily usage (Hidalgo et al., 2010). Vinegar is a liquid fermented product containing at least 4% acetic acid. The process of

producing vinegar is commonly referred to as "vinegaring." There are many types of raw materials that may be used to produce vinegar, including fruits (e.g., apples, grapes, dates, figs, plums, cherries, persimmons, etc.), grains (e.g., rice, sorghum, barley, malt, wheat, corn, rye), cane sugar, honey, coconut, roots and tubers (e.g., sweet potato), and other materials containing fermentable or hydrolyzable carbohydrates (Kandyliis, 2019). Vinegar fermentation consists of two stages: anaerobic fermentation, where sugar is converted into ethanol, and aerobic fermentation, where ethanol is transformed into acetic acid. Vinegar fermentation is typically carried out by combining the yeast *Saccharomyces cerevisiae* and *Acetobacter acetii* (Yuan et al., 2017).

Vinegar fermentation can occur spontaneously and be initiated by back-slopping or starters in both small- and large-scale operations (Ghosh et al., 2012). Spontaneous fermentation occurs naturally, without the use of any starter, using microorganisms that grow inherently in the medium and conditioning the medium such that specific microbes responsible for fermentation can develop adequately (Antoniewicz et al., 2021). The benefits of spontaneous fermentation include high economic value and easy, affordable processing (Jayus et al., 2020). The duration of the fermentation process influences the physical and chemical characteristics of kombucha made from *Sargassum* sp, affecting its overall quality in terms of both chemical and sensory properties (Pratiwi & Aryawati, 2012). The characteristics and quality of vinegar products are affected by various raw materials, microbial environments, and fermentation techniques. These factors influence the dynamic changes in chemical properties (e.g., pH, total acid, amino acid nitrogen, reducing sugar, acetic acid, and free amino acids) and sensory properties (including aroma and taste) of vinegar (Gong et al.,

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2021). Rice vinegar products have not become as popular as expected. There are currently no red rice vinegar products in Indonesia, and the objective of this study is to examine the effect of fermentation time on the chemical properties and sensory aspects of red rice vinegar produced by spontaneous fermentation.

## 2. Materials and Methods

### Materials and Reagents

N790 brand red rice, Aek Sibundong variety, was collected from Malang Regency, along with mineral water, distilled water, phenolphthalein indicator, 0.1 N NaOH, DPPH (Diphenyl Picrylhydrazyl), ascorbic acid, methanol, gallic acid, Folin Ciocalteu, and Na<sub>2</sub>CO<sub>3</sub>.

### Rice Preparation

Milled red rice was produced by placing 200 grams of washed red rice and 840 ml of water in a glass jar, soaking at room temperature (27°C–28°C) overnight. Next, the water was separated, and the rice was ground (semi-coarse) using a blender. Cooked red rice was prepared by washing 400 grams of red rice three times in running water, then placing it in a pan and adding 1.2 L of water. The red rice was cooked at 80°C for 15 min until the water was reduced.

### Vinegar Fermentation Process

Ground rice and cooked red rice were mixed in a sterile glass jar, with 560 ml of water added and stirred well. The jar was covered with a cloth and fermented for 30 and 60 days at room temperature. The mixture was stirred once a week during the fermentation process. The vinegar was strained using cheesecloth once fermentation was complete. The filtered liquid was fermented again at room temperature for 30 days. Next, the vinegar was filtered with several layers of filter cloth.

### pH and Alcohol Analysis

pH measurements were carried out with a pH meter (HP 9000) (Laily et al., 2019). A mixture of 25 ml of red rice vinegar and 100 ml of distilled water was placed into a distillation flask. The mixture was distilled until the distillate yield was exactly 25 ml. The pycnometer, whose weight was known, was filled with the distillate mixture and weighed at a specific temperature, such as 28°C. The weight of the distillate was calculated from the weight of the pycnometer containing the sample minus the weight of the empty pycnometer. Alcohol content was determined using a conversion table for the specific gravity of alcohol and its temperature (Ernawangtyas & Yudhayanti, 2017).

### Acetic Acid Analysis

Acetic acid level analysis followed the method of Laily et al. (2019). Ten milliliters of red rice vinegar were diluted to 100 ml and placed in an Erlenmeyer flask. A 25 ml sample was taken and placed in an Erlenmeyer flask, followed by the addition of 2–3 drops of phenolphthalein indicator. The sample was titrated with 0.1 N NaOH until the color changed to pink. The acetic acid

content was calculated by multiplying the percentage of total acid by the acetic acid molecular weight 60, and the formula for calculating acetic acid is:

$$\% \text{ Acetic acid} = \frac{(\text{titrated volume NaOH (mL)} \times N \text{ NaOH} \times \text{Greek acetic acid})}{\text{volume of sample (mL)}}$$

### Total Phenolic Content Analysis

Analysis of total phenolic content using a gallic acid standard curve and Folin Ciocalteu reagent. The standard solution of gallic acid (concentrations 0, 10, 20, 30, 40, 50, and 60 ppm) was measured in 200 µl of each concentration and placed into a test tube, then 1.5 ml of Folin Ciocalteu reagent (10x dilution) was added and mixed with a vortex until homogeneous. A mixture of gallic acid and Folin Ciocalteu reagent followed by 1.5 ml Na<sub>2</sub>CO<sub>3</sub> (prepared from 75 g/L) was added and stirred until homogeneous. The mixed solution was incubated for 180 minutes at room temperature under dark conditions. The absorbance value was measured with a spectrophotometer at λ = 765 nm. A linear regression equation curve was generated using the absorbance value of the standard gallic acid solution. Testing of the total phenol content of samples was conducted in the same manner as the standard curve, namely by replacing 200 µl of gallic acid solution with the sample extract (Zhou et al., 2014).

### Antioxidant Analysis

Antioxidant activity testing was conducted with DPPH, and the antioxidant activity value was expressed as IC<sub>50</sub> according to the method used in prior work (Rubiati, 2021). Ascorbic acid was used as a control at concentrations of 6 ppm, 8 ppm, 10 ppm, and 12 ppm. Red rice vinegar samples were prepared at concentrations of 5, 10, 15, and 20 ppm. The sample was treated with 0.2 ml of a 0.4 mM DPPH solution and methanol. The solution was incubated in the dark for 60 minutes before analysis with a spectrophotometer at 517 nm. DPPH standards were prepared at concentrations of 5, 10, 15, 20, and 25 ppm. Vinegar samples were prepared at 5, 10, 15, and 20 ppm concentrations, followed by the addition of 0.2 ml of 0.4 mM DPPH and methanol.

### Sensory Analysis

Analysis of the sensory properties of vinegar used a hedonic quality test to determine the panelists' impressions of the sensory characteristics of red rice vinegar, including aroma, taste, and color. The sensory test involved 25 panelists aged between 19 and 21 years, all of whom were non-smokers and free from any impairments affecting their five senses. Panelists received an initial briefing on perceptual alignment before testing and underwent primary taste recognition. Panelists were given a closed questionnaire containing answer scores ranging from 1 to 5 for each sensory attribute (Eshak, 2016). The research passed ethical clearance with certificate number 0030/EC/KEPK/UNUSA/2023.

### Statistic Analysis

Data were analyzed using one-way analysis of Variance (ANOVA) for chemical tests (p < 0.05) between fermentation times. Sensory

test data were analyzed using the Kruskal-Wallis test in SPSS 27.0. Data for all measurements were obtained in triplicate and expressed as mean  $\pm$  SD.

### 3. Result and Discussion

Figure 1 shows the properties of the red rice vinegar created in this investigation. Different fermentation times result in red rice vinegars with similar colour characteristics, particularly yellow.



**Figure 1.** Red rice vinegar with different spontaneous fermentation times

#### pH and Alcohol

After 30 days of fermentation, the pH is  $3.7 \pm 0.11$ , whereas after 60 days of fermentation, it is  $3.2 \pm 0.22$ . There was a drop in pH, with the 30-day fermentation treatment having a higher pH than the 60-day fermentation treatment. The pH of red rice vinegar with spontaneous fermentation was significantly influenced by fermentation time ( $p < 0.05$ ) (Table 1). As the fermentation time increases, the acid content of the product rises due to bacteria breaking down alcohol, making the fermentation conditions acidic and causing the pH to decline. The pH decrease is caused by  $H^+$  ions released from acetic acid produced during the acetate fermentation process. Therefore, the more acetic acid formed, the lower the pH (Zubaidah & Veronica, 2014). The degree of acidity, or pH, is a vital component in fermentation because it affects microbe proliferation and product synthesis (Fadilah et al., 2018). Vinegar's pH level defines its quality based on the amount or strength of acid in the vinegar (Suwardiyono et al., 2014). Previous research by Rahayu et al. (2017) on cocoa vinegar showed that changing pH has the opposite effect on acetic acid levels. Cocoa vinegar with the greatest pH value of 3.8 produces acetic acid levels of 1.55%, while the lowest pH value of 3.3 yields the highest acetic acid levels of 2.64%.

As shown in Table 1, the alcohol content of red rice vinegar had not yet developed by the 30th day of fermentation. The result was 0. Meanwhile, on day 60 of fermentation, the alcohol concentration was 1.48%. The alcohol content of red rice vinegar differed significantly between 30 and 60 days of treatment ( $p < 0.05$ ). The fermentation period influences the chemical parameters (alcohol content) of red rice vinegar made through spontaneous fermentation (Table 1). The alcohol percentage of

red rice vinegar after a 30-day fermenting period is low. This is due to the low sugar content of the components, which is necessary for microbial growth as food. However, the alcohol level of red rice vinegar in the 60-day treatment was higher because bacterial activity to convert sugar into alcohol had already developed and was more effective. This is because acetic acid fermentation is affected by alcoholic fermentation; therefore, if the alcohol fermentation process has not yet produced alcohol, the amount of acetic acid formed will be reduced. Low alcohol content is closely related to the sugar content that is transformed through alcoholic fermentation (Pohan et al., 2019). The rise in alcohol content corresponds to increased fermentation time induced by yeast growth and metabolism to generate alcohol. In the fermentation process, yeast aids the conversion of pyruvic acid from the glycolysis process (the conversion of lactose into glucose) into alcohol (Lestari et al., 2018).

#### Acetic Acid

Table 1 showed that after 30 days of fermentation, red rice vinegar had an acetic acid level of  $1.08 \pm 0.04\%$ . After 60 days, the acetic acid content increased to  $1.22 \pm 0.05\%$ , and acetic acid levels vary considerably with fermentation time ( $p < 0.05$ ). Acetic acid levels were determined using the titration method. The length of fermentation influences the acetic acid concentration in red rice vinegar made through spontaneous fermentation. This can occur because a shorter fermentation time results in less acetic acid since the substrate is not fully decomposed. Vinegar fermented for 60 days contains more acetic acid, probably due to the higher alcohol content produced compared to vinegar fermented for 30 days. Acetic acid is formed during the fermentation process of red rice vinegar, which converts alcohol to acetic acid. The longer the fermentation, the greater the amount of acetic acid produced. *Acetobacter* bacteria performed more efficiently in converting alcohol into acid, resulting in an increase in total acid (Arnata, 2015).

#### Total Phenolic Content

Red rice vinegar, after 30 days of fermentation, had a phenolic acid concentration of  $73.02 \pm 4.91$  mg GAE/G. In the 60-day fermentation treatment, the phenolic compound content was  $83.02 \pm 14.14$  mg GAE/G. The results demonstrate that the quantities of phenolic compounds in red rice vinegar increased from 30 to 60 days of fermentation. The total phenol content increased with increasing fermentation time. Table 1 shows that fermentation time has a significant effect ( $p < 0.05$ ) on the total phenolic content of red rice vinegar. There are numerous types of microorganisms from the bacteria and yeast groups that can metabolize to create flavonoid compounds via enzymatic processes, influencing the overall amount of phenol. The length of fermentation might affect the amount of phenolic compounds produced since microbial activity can continue over time, producing more phenolic compounds. Phenolic compounds are chemical components that influence the antioxidant activity of a substance. The majority of antioxidants in components produced

from plants are phenolic chemicals (Hardoko et al., 2019). Fermentation time significantly affects the chemical properties and bioactive compounds in fruit vinegars (Hammouda et al., 2023). Research indicates that fermentation time and storage conditions significantly influence the antioxidant activity and total phenolic content of apple vinegars, peaking at week 3 (Budak, 2021).

The fermentation process increases the amount of phenolic compounds because there is a decarboxylation process of cinnamic acid components, such as trans-4-hydroxy-methoxycinnamic acid (ferulic acid (FA)) and trans-4-hydroxycinnamic acid (p-coumaric acid (PCA)), to form phenolic compounds, namely 4-vinylguaiacol (4-VG) and 4-vinylphenol (4-VP), by microorganisms (Coelho et al., 2017). Yeast decarboxylates cinnamic acid into vinyl phenol by the activity of the enzyme phenol reductase. Phenolic chemicals are closely associated with antioxidant activity. The capacity of phenolic compounds to release protons, form chelates, and produce radical dismutase contributes to their antioxidant properties. Phenolic substances donate hydrogen atoms from the hydroxyl groups to radical molecules, resulting in stable phenoxy radical compounds. Therefore, the assessment of total phenolic compounds is highly significant in determining the antioxidant activity of plant extracts (Aksoy et al., 2013).

**Table 1.** Chemical analysis red rice vinegar with different spontaneous fermentation time

Analysis	Fermentation Time (day)		p-value
	30	60	
pH	3,7±0,11	3,2±0,22	0,006*
Acetic acid (%)	1,08±0,04	1,22±0,05	0,001*
Alcohol (%)	0,00±0,00	1,48±0,35	0,013*
Total Phenolic content (mg GAE/g)	73,02±4,91	83,02±14,14	0,009*
Antioxidant activity (IC50)	87,78±1,81	47,63±1,55	0,021*

Data are displayed as average and standard deviation (n=3), and analyzed using One Way Anova (p<0.05)

**Antioxidant Activity**

Red rice vinegar fermented for 30 days has an antioxidant activity value (IC50) of 87.78±1.81, while vinegar fermented for 60 days has an IC50 value of 47.63±1.55. Red rice vinegar fermented for 60 days has greater antioxidant activity, with an IC50 value of less than 50. Table 1 shows that fermentation time has a significant effect (p < 0.05) on antioxidant activity. The time of fermentation influences the chemical properties, namely the antioxidant activity of red rice vinegar with spontaneous fermentation. The increased antioxidant activity results from vinegar culture bacteria metabolism during the fermentation process. As fermentation time increases, microorganisms will

remain active and perform biotransformation activities, converting raw material components into molecules with improved antioxidant potential. This process can increase the antioxidant content of fermented products. The total phenolic content and antioxidant activity may increase with fermentation time (Hapsari et al., 2021). Fermentation can enhance antioxidant activity in plant-based products (Yan et al., 2019). The increase in antioxidant activity was mediated by free phenols produced during the fermentation process. Higher levels of total phenolics and flavonoids correspond to stronger antioxidant and antibacterial effects (Sartini et al., 2019). Vinegar contains various amounts of antioxidants, one of which depends on the raw materials used to produce it (Laily et al., 2019). Vinegar's phenolic content also affects its antioxidant activity (Taweekasemsombut et al., 2021). Researchers have identified a connection between total phenolic content and antioxidant activity. This phenolic component is found in rice grains that are fermented into rice vinegar (Wijaya & Romulo, 2021).

**Sensory Analysis**

**Aroma**

The aroma of red rice vinegar after 30 and 60 days of fermentation was more likely to be yeasty compared to the panellists' judgement (Figure 3A), and there was no difference since the majority of the panellists agreed that red rice vinegar had a yeast aroma. The statistical test results revealed no significant difference (Table 2) in the scent of red rice vinegar (p > 0.05). Additionally, spontaneous fermentation involves a mixture of microorganisms that could reach a balanced state early on, preventing further noticeable changes in scent. Yeast typically contains bacteria that assist the fermentation process and serve as a culture medium for these microorganisms. Acetobacter is one of the microorganisms found in yeast, along with other types of bacteria and mould. Acetobacter is a bacterium that produces acetic acid and can convert ethanol into vinegar. This study supports previous research on Wuluh starfruit vinegar, indicating that the yeast scent is associated with the presence of ethanol and CO<sub>2</sub> in the product. As is known, in fermentation, yeast works to convert sugar and produce metabolites in the form of alcohol and carbon dioxide (Laily et al., 2019).

**Table 2.** Statistic analysis of sensory red rice vinegar with different spontaneous fermentation time

Fermentation Time (days)	Aroma	Taste	Color
30	3,04±1,36	3,28±1,02	2,52±1,32
60	2,76±1,50	3,64±1,38	2,56±1,22
p-value	0,480	0,215	0,928

Data are displayed as average and standard deviation (n=3), and analyzed using Kruskal Wallis (p<0,05)

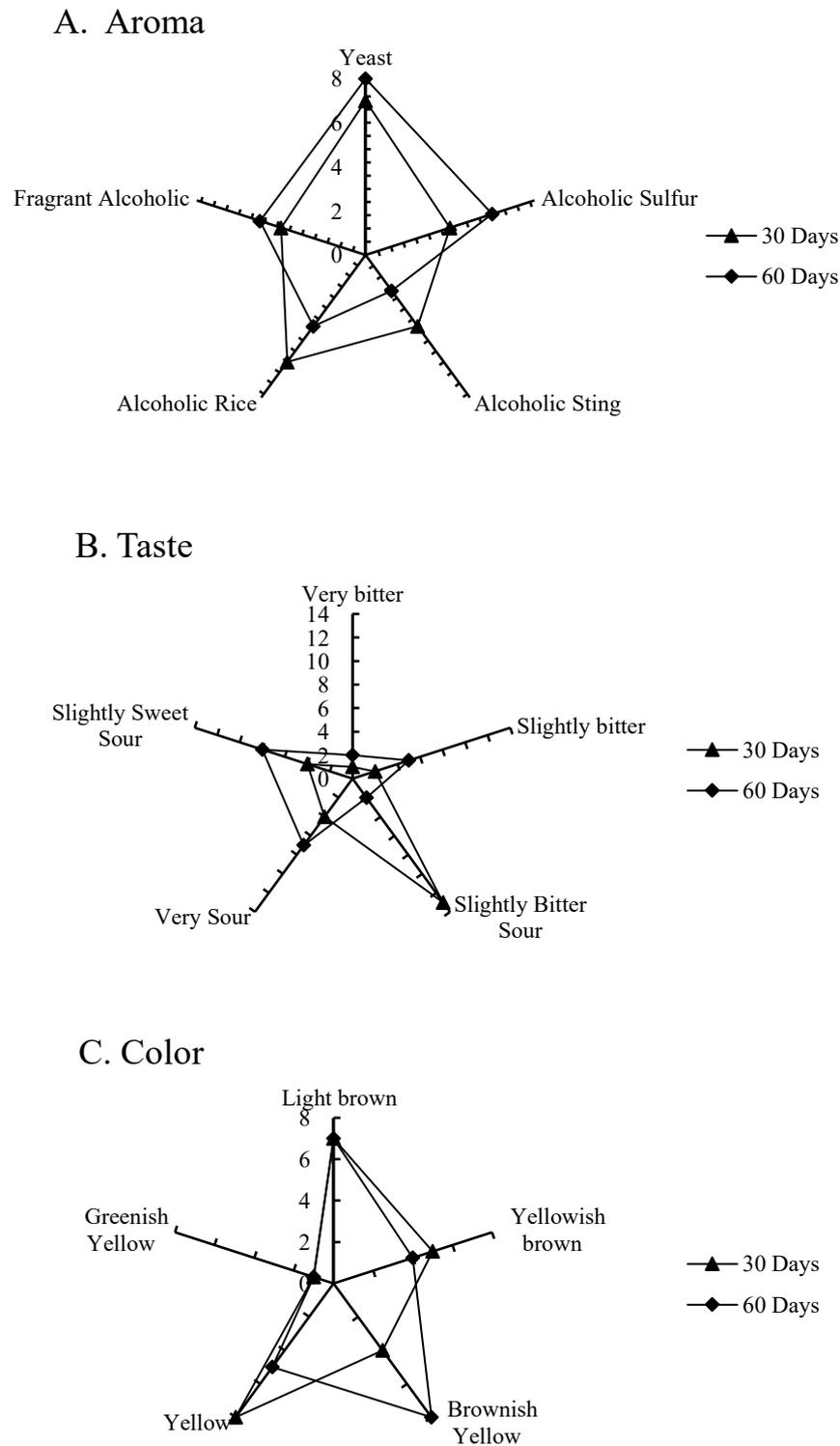


Figure 3. Sensory evaluation of aroma (A), taste (B), and color (C) in red rice vinegar with different spontaneous fermentation time.

**Taste**

Red rice vinegar treated for 30 days has a slightly bitter, sour flavor (Figure 3B). According to Table 1, there is no significant difference in the flavor of red rice vinegar after 30 or 60 days of fermentation ( $p>0.05$ ). The duration of fermentation has no effect on the sensory qualities (taste) of spontaneously produced

red rice vinegar. The bitter taste is likely due to the pH being higher than during the 60-day fermentation. Bitter taste in vinegar is typically caused by acetic acid, which forms during alcoholic fermentation by *Acetobacter* bacteria. *Acetobacter* bacteria then convert alcohol to acetic acid, producing vinegar. The 60-day treatment had a slightly sweet sour flavor; this sweet

taste was associated with the phenolic content, and it was known that the phenolic content of red rice vinegar after 60 days was higher than after 30 days of fermentation. The sour taste of red rice vinegar after 30 or 60 days of treatment is most likely due to its high acetic acid content. Additionally, the alcohol content may influence the vinegar's sour taste. This is because alcohol concentration affects the progression of acetic acid fermentation. Meanwhile, the taste of red rice vinegar is unaffected by its antioxidant activity or phenolic compounds. Phenolic compounds in vinegar, such as phenolic and polyphenolic substances, can contribute flavor and aroma, but their relationship to bitterness is usually indirect. Phenolics often impart a sour, sweet, or astringent taste but do not produce substantial bitterness (Praja, 2015). The fermentation time can affect the sugar content in vinegar because microorganisms convert sugar in the raw material into acetic acid during alcoholic fermentation.

#### Color

According to the results of the colour sensory test in Figure 2C, the majority of panellists agreed that the red rice vinegar produced after 30 days of vinegar treatment was yellow, whereas the red rice vinegar produced after 60 days of vinegar treatment tended to become brownish yellow. The colour shift is induced by changes in fermentation time, which result from the microbial consortium's ability to break down colour. Table 1 demonstrates that there is no significant difference in the colour of red rice vinegar between 30 and 60 days of fermentation ( $p > 0.05$ ). The colour of red rice vinegar produced through spontaneous fermentation is unaffected by variations in fermentation time. The findings of this study are consistent with previous research that found honey pineapple vinegar to be brownish yellow. The brownish colour results from the browning process. The brownish colour results from the Maillard process, which occurs when amino acids in sugar condensate and rearrange themselves. A browning process in vinegar can occur during fermentation, especially if fermentation is conducted over a long period of time (Mizrotun, 2020). Colour is the primary characteristic of a food or beverage product that can influence the panellists' subsequent evaluation. Colour is also a highly essential part of visual assessment and can affect the analysis of other sensory components (Lemmetti et al., 2014).

#### 4. Conclusion

With spontaneous fermentation, the chemical parameters of red rice vinegar (acetic acid content, pH, alcohol content, antioxidant activity, and phenolic compounds) are influenced by fermentation time. However, fermentation time has no effect on the sensory qualities (aroma, taste, and colour) of red rice vinegar with spontaneous fermentation. Red rice vinegar fermented for 60 days contained higher amounts of acetic acid, phenolic compounds, and antioxidant activity than vinegar fermented for 30 days. A prolonged fermentation treatment of 60 days produced red rice vinegar with a yeast scent, slightly sweet bitter flavour, and brownish yellow colour, which was the best treatment based on sensory qualities. Future study should focus

on the utilisation of microorganisms in fermentation to enhance the functional qualities of red rice vinegar.

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