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Effect Of Fermentation On Physicochemical, Microbiological Properties Of Brown Rice Flour And The Related Brown Rice Bread Properties

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Abstract:
Background*: Brown rice bread is one of the most popular non-gluten products suitable for celiac patients. Brown rice (BR) contains a variety of nutrients, but its high phytic acid content inhibits dietary mineral absorption. Fermentation is one of the most effective treatments for reducing the antinutrient phytic acid via the action of microorganisms and grain phytases. The objective of this study was to investigate effect of fermentation (fermentation method and temperature) on physicochemical (pH, pasting property, phytic acid, iron, and total phenolic content) and microbiological properties of BR flour and its related bread properties.*
Materials and Methods*: BR flour was fermented at RT (25ºC) for 15 hours, either naturally or with yeast, and the fermented flour was then used to make bread. The differences in properties between samples from the two fermentation methods and the control were used to determine which fermentation method is superior.*
Results*: Fermentation decreased the viscosity, increased the microbiological count as well as lowered the pH values of fermented samples, released more phenolic compounds, and reduced the amount of phytic acid to enhance mineral bioavailability in fermented BR flour and BR bread. Natural fermentation was the best fermentation method, with the highest amount of reduced phytic acid and iron combined with the reserved total phenolic compound and a high sensory evaluation score. Three levels of temperature 25ºC, 30ºC, and 35ºC were then used to determine the best fermentation temperature using natural fermentation for 15h. With increasing fermentation temperature, the microbiological counts increased, the pH value decreased, and the total content of phenolic compounds in BR flour and BR bread products.*
Conclusion: *Natural fermentation at 30°C was the most effective fermentation condition for reducing antinutrients, increasing mineral bioavailability, and preserving the total phenol content of BR, with the highest overall acceptability score.*
Key Word*: brown rice, phytic acid, natural fermentation, yeast fermentation, brown rice bread*

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I. Introduction

In Vietnam, rice (Oryza sativa) is the most important source of carbohydrates. It can be used as a wheat substitute in bakery products. Wheat gluten maintains the texture, volume, and satisfying crumb of bread; however, its consumption results in gluten-related disorders, such as coeliac disease, dermatitis herpetiformis, and non-coeliac gluten sensitivity (Šmídová & Rysová, 2022). Rice is a possible substitute for wheat because it is more widely available and less allergenic. Ilowefah et al. (2014) reported that rice is a unique crop due to its colorless, soft taste, low sodium levels, easily digestible carbohydrates, and hypoallergenic properties. Therefore, its flour is an attractive food material to be used for making gluten-free foods (Gujral et al., 2003). Rice, on the other hand, is commonly consumed after it has been polished or whitened and is considered a high glycemic grain due to its high starch content. The outer bran layer is removed during rice milling, resulting in a loss of nutrients, dietary fiber, and bioactive components (Saleh et al., 2019). Several rice bran components, including phenolic acids, falvonoids, γ - oryzanol, aminobutyric acid (GABA), α-tocopherol, and γ -tocotrienol, have been reported to have biological activities. Brown rice consists of 1–2% pericarp, 4–6% aleurone plus nucellus and seed coat, 1% embryo, 2% scutellum, and 90–91% endosperm (Juliano, 2016). Therefore, BR was found to contain a greater amount of those nutrients, and phytochemical compounds than refined white rice (Saleh et al., 2019). These nutrients help reduce blood pressure, prevent cancer, and heart disease, and have antioxidative effects (Fukushima et al., 2020). Brown rice bread, according to Ilowefah et al. (2017), is one of the most popular non-gluten products suitable for celiac patients, with rising demand.

However, due to the presence of the antinutrient phytic acid, brown rice is not a good source of metabolizable micronutrients (Wei et al., 2012). Antinutrients are substances that reduce the maximum

utilization of nutrients, particularly proteins, vitamins, and minerals, preventing optimal nutrient utilization in a diet and lowering nutritional value (Fekadu Gemede & Retta, 2014). Phytic acid, also known as inositol hexaphosphate (IP6) or phytate, the primary phosphorus storage form, is a common component of bran (Konietzny & Greiner, 2003). This antinutritional factor reduces mineral bioavailability and inhibits mineral absorption by strongly binding to metallic cations, particularly Zn^{2+}, Ca^{2+}, Fe^{2+}, and Mg^{2+} (Ram et al., 2020).

According to Liang et al. (2008), fermentation is one of the most effective treatments for reducing the antinutrient phytic acid (56% - 96% removal) via the action of microbiological and grain phytases. The process activates starch-hydrolyzing enzymes such as α-amylase and maltase to catalyze the breakdown of starch into maltodextrins and simple sugars, respectively (Nkhata et al., 2018; Osman, 2011). The starch modification will influence the gelatinization, pasting, and texture properties. These effects of fermentation are related to amylopectin chain changes due to amorphous-region hydrolysis and changes in the ratio of amylose to amylopectin (Lu et al.,2007). Furthermore, fermentation increases carbon dioxide and ethanol production by decreasing starch content in millet varieties, and a significantly lower pH activates the phytase enzyme (El-Hag et al., 2002). Phytases degrade the hexa form of phytic acid (IP6, myo-inositol 1,2,3,4,5,6-hexakisphosphate) into lower forms such as IP5, IP4, IP3, IP2, IP1, and myo-inositol (Ragon et al., 2008), which have the lower metal binding capacity (Agte et al., 1997).

II. Material And Methods

Ground brown rice preparation

Phu Minh Tam brown rice (rice with its bran intact) was purchased from the local supermarket BigC. Brown rice was first soaked in distilled water at a ratio of 1:2 w/v for 12h (Ilowefah et al., 2015) to weaken protein-starch interactions and loosen the structure of rice kernels, resulting in the production of small particle flours with little starch damage (Chiang & Yeh, 2002). After being drained, each batch of 100g brown rice grains was ground into flour for 10min with 20 cycles of 30s and power level 3 using a Philips grinder to pass through a 500µm sieve. The ground brown rice was then packaged into airtight zip bag and stored at -18°C in a freezer until use.

Fermented brown rice flour (FBRF) preparation

The ground brown rice was fermented in 15h with the 1:2 w/v addition of distill water. The fermentation was done with 1% of yeast at different temperature (RT, 30°C, 35°C). The Baker's yeast (Saf Instant red label) was used in the yeast inoculated fermentation. The supernatant was used to analyze pH and microbiological properties after fermentation, and the fermented brown rice slurry was dried in an air-dry oven at 50°C until the moisture content reaches 14%, ground to flour using a Philips grinder in 5min of 10 cycles of 30s for each batch of 100g, sieved in a 500µmsieve, and verified as fermented brown rice flour (FBRF) (Ilowefah et al., 2017). The flour was stored in an airtight zip bag in a freezer at around -18°C for further analysis. Each treatment and control was performed in triplicates.

Preparation of brown rice bread

The brown rice bread was produced using a basic recipe developed by Ilowefah et al. (2017) with some modifications, as shown in Table 1. All the dry ingredients was combined in a mixer for 3min on low speed. Water and oil was then added and thoroughly mixed. To make dough, the mixture was mixed at high speed for 5min. The bowl containing the dough was then wrapped in plastic wrap and allowed to ferment for 45min before being poured into the mold. After that, the dough was baked at 175°C for 35min (Cornejo & Rosell, 2015). After baking, the bread was removed from the mold and allowed to cool to 35°C before being packed in the air tights zip bags and stored in the freezer for further analysis.

Table 1. The fomula of brown rice bread making

Ingredients	Amount (%)
FBRF	100
Sugar	8
Salt	1.5
Instant dry yeast (red)	1.5
Vegetable oil	4
Water	90

Physical measurement

pH was measured by a pH meter.

Pasting properties of the flours were measured using a micro visco-amylo-graph. The pasting properties of the slurry were recorded as the visco-amylo-graph program described as gelatinization temperature, maximum viscosity, trough viscosity, final viscosity, breakdown, and setback.

Microbiological analysis

Yeast was determined according to ISO 21527-1:2008. The samples was diluted with peptone water from dilution 10^{-1} to 10^{-4}. 1ml of each diluted suspension was spread on the sterilized Dichloramphenicol Rose Bengal Agar dishes. These dishes was incubated at 25ºC for 5 days. The number of microorganisms per g of food was calculated using the equation below.

$$Count\left(\frac{CFU}{g}\right) = \frac{average\ number\ of\ colonies\ from\ duplicate\ plates}{dilution\ factor\ \times volume\ plated\ (ml)}$$

According to ISO 15214:1998, the total lactic acid bacteria were enumerated on a selective media De Man, Rogosa and Sharpe agar. The samples were diluted with saline peptone water from dilution 10^{-1} to 10^{-6} then the lactic acid bacteria was grown using pour plate method. Petri dishes were incubated at 35ºC for 72h. After 72h, lactic acid bacteria were enumerated by Standard Plate Count.

Phytic acid content

Phytic acid content was determined by a procedure described by Ilowefah et al. (2015). 0.06g of brown rice flour or fermented brown rice flour was soaked for 3h at room temperature with 10mL of 0.2N HCl. After that, the mixture was centrifuged at 2000g for 10 mins. The supernatant (0.5µL) was pipetted into a test tube, and 1 mL of ammonium iron sulphate was added, which was made by dissolving 0.2g of $NH_4Fe\ (SO_4)_2.12H_2O$ in 100 mL of 2 mol/L HCl and diluting to 1000mL with distilled water. The mixture was then incubated for 30min at 100°C. After cooling to room temperature, a 2mL (1% v/v) 2′,2-bipyridine solution was added to the mixture, and the absorbance was immediately measured at 519nm against distilled water using a UV–Vis spectrophotometer. The results were expressed as mg phytic acid per g sample using a standard curve prepared by diluting phytic acid sodium salt hydrate stock solution.

Total phenolic content (TPC)

The total phenolic content (TPC) was determined using a modified spectroscopic method (Ainsworth et al., 2007). First, the powdered samples were sonicated in 60% ethanol (ratio of 1:20 w/v) for 40 minutes at 50°C in an ultrasound bath (Tabaraki et al., 2011). Following the extraction, the sample was centrifuged at 4500 rpm for 10 minutes before being filtered using Whatman filter papers. The mixture was then prepared by combining 1ml of sample solution, 1 ml of 10% Folin-reagent, Ciocalteu's 13ml of distilled water, and 5ml of 7% Na_2CO_3 solution. The mixture was thoroughly mixed and stored in the dark at room temperature for 2h. The blank was also prepared by substituting 1ml of distilled water for 1 ml of sample solution. A spectrophotometer with a wavelength of 760nm was used to measure absorbance. Gallic acid was used to generate a calibration curve, and the results were expressed in mg of gallic acid per g of sample (mg GAE/g). This test was carried out in triplicates.

Sensory evaluation

The sensory analysis was carried out to evaluate the acceptability of 30 untrained panelists (selected based on their availability and objectivity) using a nine-point Hedonic scale. Each panelist did the sensory evaluation in an individual booth under controlled humidity and temperature (25^0C) to prevent error. The hedonic scale is from extremely dislike to extremely like in terms of appearance, color, flavor, taste and overall acceptability.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to determine significant difference at $p \leq 0.05$ among means. To assess which of the samples is statistically different, Tukey test was utilized. The experimental analysis and calculations were carried out in triplicate by using the SPSS program (Version 26, IBM Corp., USA).

III. Result

Effect of fermentation on microbiological count of fermented brown rice flour (FBRF)

The number of yeast and lactic acid bacteria (LAB) in FBRF at different temperature conditions is shown in table 1.

Table 1. Microbiological properties of FBRF

Fermentation condition	Yeast count (log CFU/g)	Lactic acid bacterial count (log CFU/g)
Control	3.48 ± 0.43^{cx}	6.11 ± 0.21^{cy}
25ºC	4.91 ± 0.37^{bx}	8.40 ± 0.06^{by}
30ºC	6.01 ± 0.11^{ax}	9.35 ± 0.46^{ay}
35ºC	6.72 ± 0.03^{ax}	9.22 ± 0.39^{aby}

The values are mean ± standard deviations of three replicates. Values within the column followed by different superscript letters (a, b, c) are significantly different (p≤0.05) using Tukey's test. Values with the same row followed by different superscript letters (x, y, z) are significantly different (p≤0.05) using Tukey's test.

The number of microorganisms increased significantly after fermentation and increased with increasing temperature. As the fermentation temperature increased from 25ºC to 30ºC and 35ºC, the number of yeast increased from 4.91 log CFU/g to 6.01 and 6.72 CFU/g, and the number of LAB increased from 8.40 log CFU/g at 9.35 and 9.22 CFU/g. The microbiological count of fermented BR flour at 30ºC and 35ºC was not significant different.

Effect of fermentation on physical properties of fermented brown rice flour (FBRF) and brown rice (BR) bread

Effect of fermentation temperature on physical properties of FBRF and BR bread was indicated in Table 2 and Table 3.

pH value of the fermented BR flour at 25ºC was the highest at 4.33, the second highest was the pH value of fermented BR flour at 35ºC at 4.04 and the pH of fermented BR flour at 35ºC was the lowest with 3.73.

Table 2. pH value of FBRF and BR bread

Fermentation condition	pH of fermented flour	pH of bread
control	6.43 ± 0.05^{ax}	6.11 ± 0.02^{ay}
25ºC	4.33 ± 0.05^{bx}	4.26 ± 0.01^{bx}
30ºC	3.73 ± 0.02^{dy}	4.11 ± 0.02^{cx}
35ºC	4.04 ± 0.01^{cy}	4.07 ± 0.01^{dx}

The values are mean ± standard deviations of three replicates. Values within the column followed by different superscript letters (a, b, c and d) are significantly different (p≤0.05) using Tukey's test. Values with the same row followed by different superscript letters (x, y) are significantly different (p≤0.05) using Tukey's test

The number of microorganisms in 30ºC and 35ºC fermentation were higher than that of 25ºC fermentation. During fermentation, LAB produces lactic acid as a main product which reduce the pH value of the samples.

Table 3. Effect of fermentation on pasting properties of fermented BR flour at different temperature condition

Fermentation temperature	Peak viscosity (BU)	Trough viscosity (BU)	Final viscosity (BU)	Breakdown (BU)	Set back (BU)	Pasting temperature (ºC)
Control	946.33 ± 0.58^{a}	391.33 ± 4.16^{a}	728.00 ± 4.58^{a}	555.00 ± 4.58^{b}	305.00 ± 1.00^{b}	69.23 ± 0.12^{c}
25ºC	912.67 ± 7.51^{c}	376.33 ± 8.08^{b}	710.33 ± 13.28^{b}	536.33 ± 0.58^{c}	297.00 ± 20.80^{c}	70.07 ± 0.12^{b}
30ºC	847.00 ± 3.27^{d}	342.17 ± 0.27^{d}	678.00 ± 11.59^{d}	505.33 ± 0.37^{d}	305.33 ± 20.61^{b}	71.17 ± 0.39^{a}
35ºC	941.33 ± 0.84^{b}	350.07 ± 0.42^{c}	698.07 ± 0.72^{c}	591.33 ± 7.38^{a}	316.33 ± 0.85^{a}	70.00 ± 0.21^{b}

The values are mean ± standard deviations of three replicates. Values within the column followed by different superscript letters (a, b, c, d) are significantly different (p≤0.05) using Tukey's test. Values with the same row followed by different superscript letters (x, y, z) are significantly different (p≤0.05) using Tukey's test.

The pasting profile of fermented BR flour at different fermentation temperatures determined by Rapid Visco Analyzer (table 3). Different fermentation temperature caused significant difference in all pasting properties of BR flour including peak viscosity, trough viscosity, final viscosity, breakdown, set back and pasting temperature.

All pasting properties decreased with increasing fermentation temperature from 25°C to 30°C but increased significantly at a fermentation temperature of 35°C. For example, the peak viscosity dropped from 912.67 BU (25°C fermentation) to 847.00 BU (30°C fermentation) but increased remarkably to 941.33 in the 35°C fermentation. This may be different degrees of fermentation between samples at different fermentation temperatures.

Effect of fermentation on chemical properties of fermented brown rice flour (FBRF) and brown rice (BR) bread

Phytic acid content

The phytic acid contents of BR flour and BR bread from different fermentation temperatures are described in Figure 1. The phytic acid content of fermented BR flour at different temperature was in the range of 8.60 – 8.94 mg/g and that of BR bread was in the range of 6.034 – 8.47 mg/g. The results showed that the difference in fermentation temperature did not cause a significant difference in the phytic acid of the fermented BR flour samples. Although not significantly different, there was still a slight decrease in phytic acid content in the bread product as the fermentation temperature increased from 25°C and 30°C to 35°C, specifically 8.22 and 8.47 mg/g to 6.03 mg/g.

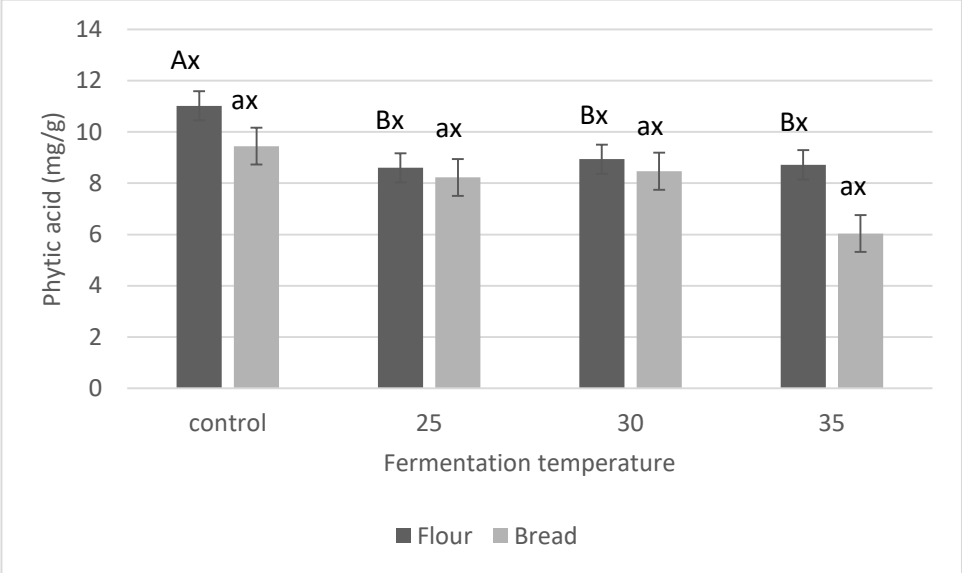

Figure 1. Effects of fermentation temperature on phytic acid content of BR flour and BR bread.

The values were presented as mean ± SD (Standard Deviation). Means with different capital letters (A, B,) indicated significant differences among the flour samples (p<0.05). Means with different small letters (a, b) indicated significant differences among the bread samples (p<0.05). Means with different small letters (x and y) in the same pairs of markers indicated significant difference between the flour samples and the bread samples with the same fermentation temperature (p<0.05) using Tukey test.

It can be explained that the low pH of the fermentation product and the fermentation temperature can facilitate phytase activity, although the hydrolysis of phytic acid by endogenous phytase enzymes and microflora causes most of the antinutrient loss during fermentation (Dhankher & Chauhan, 1987). It is found that phytic acid is more susceptible to breakdown by fermentation rather than temperature. The degree of reduction in phytic acid, as well as polyphenols, appears to be a function of fermentation time. Therefore, studies on the effect of fermentation duration on phytic acid need further investigation.

Total phenolic content (TPC)

The total phenol content (TPC) of fermented BR flour and BR from different fermentation temperature are described in Figure 2.

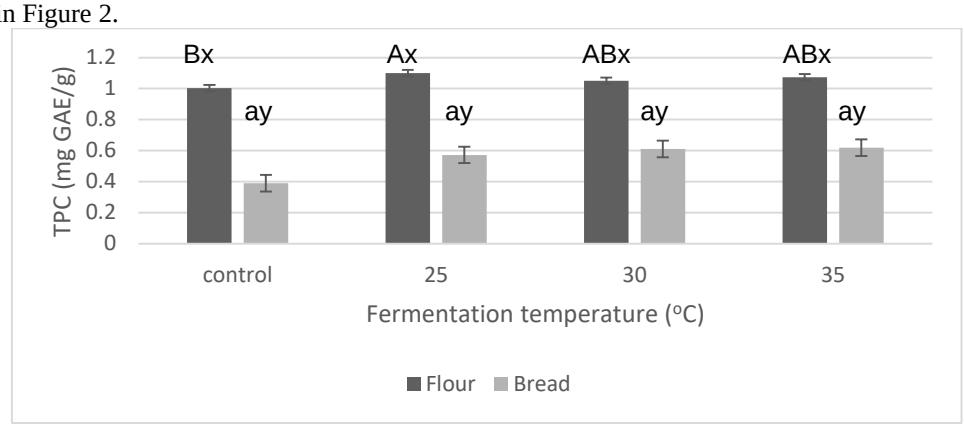

Figure 2. Effects of fermentation temperature on TPC of BR flour and BR bread.
The values were presented as mean ± SD (Standard Deviation). Means with different capital letters (A, B) indicated significant differences among the flour samples ($p<0.05$). Means with different small letters (a, b) indicated significant differences among the bread samples ($p<0.05$). Means with different small letters (x and y) in the same pairs of markers indicated significant difference between the flour samples and the bread samples with the same fermentation temperature ($p<0.05$)

The TPC of fermented BR flour at different temperature was in the range of 1.05 – 1.10 mg GAE/g and that of BR bread was in the range of 0.57 – 0.62 mg GAE/g. The results showed that the difference in fermentation temperature did not cause a significant difference in the TPC of the fermented BR flour samples as well as the BR bread samples.

Effect of fermentation on sensory properties of BR bread

The 9-hedonic scale was used for sensory evaluation of BR bread products based on appearance, color, aroma, texture, sourness, and overall acceptability, and the results are described in Figure 3. The evaluated score of all attributes ranged from 5.38 to 5.46. Bread sample using 35°C fermented BR flour got the lowest score in aroma and overall acceptability.

Figure 3. Sensory evaluation result of BR bread product made from natural fermented BR flour at different temperatures.

IV. Discussion

Fermentation temperature caused significant effects on microbiological properties, pH value, pasting profile, and iron content. With increasing fermentation temperature, the microbiological counts increased, pH

value decreased, and the viscosity first decreased in 30ºC fermented BR flour and then increased in 35ºC fermented BR flour. The increase in the fermentation temperature also increased the total content of phenolic compounds and the iron content of bread products. In combination with the low pH of the fermentation product, the fermentation temperature facilitated the activity of phytase, which hydrolyzes phytic acid and reduces the antinutrient amount in BR flour and BR bread.

Fermentation temperature did affect the microorganism amount. The increasing fermentation temperature was, the more microorganism was found until 30^0C. There was no more increasing microbial count when fermentation temperature was more than 30^0C. Taal et al. (2013) found that the optimal temperatures for growing most LABs are in the range of 30-40°C. Therefore, it is understandable that the LAB counts at 30°C and 35°C were higher than those at 25°C. It was reported that *S. cerevisiae* provides the right atmosphere containing sufficient CO_2 to favor LAB growth. In all samples, LAB amounts were higher than yeast amounts, which agrees with the research that reported LAB are the dominant microorganisms in fermented rice flour supernatant (Lu et al., 2008). With the coexistence and cooperation of yeast and lactic acid bacteria in fermented beverages and foods, the optimal growth of LAB in the range of 30-35°C led to a corresponding increase in the number of yeasts. The LAB was found to be the dominant microorganism in brown rice; therefore, it is explainable that the amount of LAB in all samples were higher than yeast amount. Although higher temperatures could improve enzyme reactions to increase fermentation rates (Roza et al. 2003), too high temperatures could result in a faster decline of yeast cell populations (Lu et al., 2017).

Physical property was positively affected by yeast addition after fermentation (lower pH, lower viscosity). The significant decrease in the pH values of fermented BR flour at 30ºC and 35ºC compared to that of the fermentation at 25ºC correspond to the higher microbiological count of the fermented BR flour. In addition, the pH of BR flour fermented at 30°C was lower than that of BR flour fermented at 35°C due to the higher amount of present LAB. The significant increase in the pH of the bread at the fermentation temperature of 30°C and 35°C (from 3.73 and 4.04 to 4.11 and 4.07) may be due to the addition of yeast during the baking process. According to Sieuwerts et al. (2017), *S. cerevisiae* has been shown to consume lactic acid in a dough environment, which slows acidification through the production of lactic acid by LAB, leading to an increase in pH. According to Kati et al. (2004), high acidity or low pH can have undesirable effects on the taste and texture of products; therefore, the pH of fermented BR flour should not be too low. The decrease in peak viscosity is related to the reduction in amount of starch which became more fragile due to acidification (Ilowefah et al., 2015). Damaged starch absorbs more water than the undamaged starch. Excess damaged starch results in excessive susceptibility of attack by alpha amylase resulting in sticky crumb and weak bread structure. This phenomenon was observed in the bread making step, where with the same amount of water, some rice bread doughs were more viscous than the other. Therefore, the viscosity of fermented BR flour should not be decrease to too low level.

The antinutrient, phytic acid, was reduced after fermentation but the fermentation temperature did not affect the phytic acid amount. The total phenolic content was affected by fermentation but not fermentation temperature. The similar trend is reported in the previous study by (Dhankher & Chauhan, 1987) that variations in temperature did not have a significant effect on the concentration of phytic acid or polyphenols in the fermented product.

Regarding to sensory result, with the highest score in overall acceptability, fermentation at 30°C was the most effective fermentation condition for reducing antinutrients and preserving the total phenol content of brown rice.

V. Conclusion

Results from this work demonstrate that inoculated yeast play an important role in preserving bioactive compound and sensory quality as well as extending shelf-life. 30^0C was the best fermentation temperature to produce beetroot wine with high quality and high nutrient. Further research on fermentation is recommended to reduce nutrient loss.

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