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Effect of fermentation on quality of red beetroot *(beta vulgaris l.)* based spirit

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Abstract:
Introduction: Method: Results:
Background*: Red beetroot (Beta vulgaris L.) contains more sugar than other vegetables and is a rich source of micronutrients such as folate (vitamin B9), iron, and potassium as well as antioxidants such as phenolics, flavonoids, and vitamin C. It is also low in fat and high in protein. But beetroot has earthy flavor, which influence in consumer's consumption behavior. Wine fermentation is a good way to preserve nutritional and sensory quality of red beetroot as well as to extend its shelf-life. This work studied the effect of fermentation temperature and yeast inoculation on the physical, antioxidant, and sensory properties of red beetroot spirit.*
Materials and Methods*:Red beetroot must was either naturally or yeast fermented at 3 different temperature conditions (20, 25 and 30^{0}C). Physical, antioxidant, and sensory properties were determined to study changes in quality of red beetroot after fermentation.*
Results*: Alcohol content of yeast fermented beetroot spirit was significantly higher than that of natural fermented samples while fermentation temperature did not affect the alcohol strength. Higher pH value was also observed in yeast inoculated samples than in natural fermented samples. pH value was not influenced by fermentation temperature. The data for titratable acidity was reversed by the pH value. The TSS of yeast inoculated wine increased when increasing fermentation temperature (20^{0}C, 25^{0}C, 30^{0}C). The natural fermentation did not follow this trend. In order to characterize changes in bioactive compounds of red beetroot spirit after fermentation; vitamin C, antioxidant activity, total betalain pigment and total phenolic content were analyzed. After fermentation, the bioactive compound was decreased significantly. Comparing 2 fermentation methods, the yeast fermentation was the best method to minimize bioactive compound loss as well as to provide better sensory quality of red beetroot spirit. In 3 different fermentation temperature (20, 25 and 30^{0}C), 25^{0}C was the best fermentation temperature to preserve antioxidant compounds.*
Conclusion: *Inoculated yeast play an important role in preserving bioactive compound and sensory quality as well as extending shelf-life.*
Key Word*: Red beetroot spirit, fermentation temperature, yeast fermentation, natural fermentation, bioactive compound*

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I. Introduction

Red beetroot (*Beta vulgaris L.*) is known as a member of *Chenopodioideae* family. It contains high sugar and nitrate content, which is used as one of natural food to boost energy in athletes (1). It has a deep red color feature derived from betalain. Betalains are composed of red-violet betacyanins (e.g. betanin and isobetanin) and yellow betaxanthins (e.g. vulgaxanthin I and II), which are classified as water-soluble nitrogenous pigments (2). The colour of betalains is mostly stable between pH 3 and 7 (3, 4). At pH values lower than 3 the colour turns more violet and at pH higher than 7 it becomes more yellowish-brown. Due totheir structure, betalains are reported to be higher in antioxidant activity than ascorbic acid and other molecules with similar effect (5). It was reported that the high antioxidant activity of betanin was linked to an increasing of its electron donation ability (6).

In addition to betalains, other valuable, active compounds such as carotenoids, glycine betaine, saponins, betacyanines, folates, betanin, polyphenols and flavonoids are present in beetroots (Beta vulgaris) with high level (3, 7). Wootton-Beard and Ryan, 2011 (8)reported that beetroot juice contains many micronutrients like potassium, magnesium, folic acid, iron, zinc, calcium, phosphorus, sodium, niacin, biotin, B6 and soluble fibre, which are together with biologically accessible antioxidants to promote health effect.

Many studies indicated that consumption of red beetroot (*Beta vulgaris L.*) can contribute to many health positive effects such as cancer prevention and diminishing risks associated with cardiovascular diseases or ageing – chronic diseases (3, 7, 9). To get natural protection from cancer, eating foods rich in bioactive compounds like beetroot is a promising option (9). However, beetroot juice consumption may not be as popular as other fruit and vegetable juice because of its earthy flavor. This unpleasant flavor comes from geosmin and pyrazine derivatives (6). Wine fermentation is a good way to preserve nutritional and sensory quality of red beetroot as well as to extend its shelf-life.

Fruit-based wines which have been fermented are nutritious, tasty, and function as a pleasant stimulant. Alcohol content of fruit wines ranges from 5 to 13 percent (10). They are not distilled and include majority of the nutrients found in the original fruit juice. Wine fermentation is a process in which yeasts work on sugar to produce beneficial product like alcohol. Apart from ethanol, some by-products produced during fermentation such as carbonyl compounds, alcohols esters, acids, and acetals, which may influence wine quality (11). During the wine fermentation, under condition of low pH, high sugar content, anaerobic environment, and phenolic compounds, many yeast species grow and enrich over other microbes (12). Thanks to the metabolic activities of yeast, the wine's aroma and flavor can be impacted. The aim of this study was to investigate changes in chemical property, antioxidant capacity and sensory characteristics of beetroot wine after fermentation in different conditions.

II. Material And Methods

Beetroot juice preparation

The red beetroots were obtained from MM Mega Market An Phu, An Phu ward, Thu Duc city, HCMC, Vietnam. The roots was ground with deionized water with the ratio 1:4 (w/v) by a blender (Philips HR2118, Indonesia) after being washed with clean water and being peeled off. The red beetroot juice was extracted immediately by filtering the slurry with a filter cloth to get the clarified juice.

Yeast strain

Saccharomyces cerevisiae Lalvin EC-1118 (Canada) was in freeze - dried form. It was mixed with sucrose and deionized water together with the ratio 1:1:5 (w/w/v). The solution was incubated for 15 minutes to activate the yeast.

Wine fermentation

TTS of the filtered beetroot juice were 8^0Brix, which was adjusted to reach 22 ±1 ^{0}Brix by adding sucrose and deionized water while pH of the juice was not adjusted (4.5). Fermentation step was done in sterilized containers. A starter culture was prepared by inoculating yeast culture into the filtered beetroot juice to reach a yeast cell count of 1 x 10^7 CFU/ml at different temperature (20^0C, 25^0C and 30^0C) for 14 days. Another batches were done in the same fermentation condition for temperature and duration but without yeast supplement.After 14 days, the crude wine was clarified by using centrifuge (Hettich Universal 1406, Refrigerated, Germany) with 4500 RPM at 20^0C for 15 min to remove yeast body and the suspended solid. All data were used in triplicate for each coordinate. The clarified fermented wine samples were pasteurized at 70^0C for 10 minutes before storing at - 20^0C until use.

Physical measurement

Total soluble solids (TSS) and pH values of the red beetroot juice or wines were measured with a refractometer (Atago rx 5000 alpha) and a pH meter (HANNA instruments HI 2216), respectively.

The alcohol content was determined by using distillation, which was based on the method ofMartins*et al.*(13)with some modifications. In the experiment, 20 ml of samples (V1) was poured into distillation flask and heated to 78^0C. The volume of collected alcohol was measure (V2) and the alcohol content was determined by the following equation.

$$\text{Alcohol content} = \frac{V_2}{V_1}$$

Total phenolic content (TPC)

Determination of total phenolic content was done according to Apak *et al.*(14), using the Folin–Ciocalteu colorimetric method. Before measuring, the TPC was extracted by soaking 2 ml of samples with 8 ml of 1.2M HCl in 50% methanol at 60^0C for 2 hours in dark condition (15). The extracted solution was immediately centrifuged with 4500 RPM at 4^0C for 15 mins after incubation. The supernatant was collected and store in freezer until using for analyzing purpose. The mixture of 1 ml of supernatant, 1 ml of 10% Folin-Ciocalteu's reagent, 8.4 ml of water, and 5 ml of 10% disodium carbonate solution was mixed well and incubated at room temperature for 2 hours. The absorbance was measure at 760 nm wavelength. Distilled water will be used as the blank and gallic acid was used as a calibrated solution. The results was expressed as miligram of gallic acid per 100ml of alcoholic beverages (mg GAE/100 ml).

Total sugar content

To analyze total sugar content, the phenol-sulfuric acid method described by Masuko *et al.*(16) was applied with minor modifications. 1 mL of sample solution was mixed with 1 mL of 5% phenol (v/v) at room temperature. 5 mL of 99% concentrated sulfuric acid was then added directly to the solution. The mixture was allowed to stand for 10 min before being incubated in a 30oC water bath for 20 min. The developed yellow-orange color was then measured using the spectrophotometer at 490 nm. Distilled water was used as blank.

Vitamin C content

The content of vitamin C was quantified by titration with iodine solution, which was based on the method described by Babashahi *et al.* (17). Iodine solution was prepared by mixing KIO_3, KI, and 3M H_2SO_4 with the ratio 1:0.054:6 (w:w:v), respectively. After dissolving, the volume of the mixture was filled up to 100 ml for titration purposes. 25 ml of diluted sample (1:10) was poured together with 5 - 6 drops of 1% starch solution into the volumetric flask for titration. The mixture was titrated with iodine solution until dark blue color was obtained. The content of vitamin C in samples is expressed in mg/100 mL.

Titratable acidity (TA)

Titratable acidity was measured using the titration method following the procedure of De Freitas & Mitcham (18)with some modifications. 4 mL of sample was diluted with 20 ml of distilled water. The diluted sample was titrated with 0.1 N NaOH until a pH of 8.2 (endpoint) was reached. The used volume of NaOH was recorded. Malic acid equivalents were calculated based on the following equation:

$$TA = \frac{(\text{mL of NaOH titrated}) \times \left(\text{N of NaOH in } \frac{\text{mol}}{\text{liter}}\right) \times (\text{Eq.Wt.of acid})}{(\text{mL of sample}) \times 10}$$

Where: Eq.wt. is the equivalent weight of malic acid = 67.04

DPPH Radical Scavenging Activity

Extraction for the DPPH assay was based on the procedure of Nurliyana *et al.*(19) with adjustments. 1 ml of samples was extracted with 4 ml of 70% methanol solution (v/v) at 60^oC in darkness for 2 hours with occasional shaking. The extracts were then centrifuged at 3000 rpm for 15 minutes at 4^oC. The supernatants were kept at -20^0C until being used. The DPPH assay was measured by the modified method described byBakar *et al.*(20). To perform the experiment, 0.1 ml of supernatant was mixed with 4.9 ml of 0.05 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent in absolute methanol. The tubes was incubated at room temperature in dark for 30 min before being measured by spectrophotometer at 517 nm wavelength. The 70% ethanol was used as a blank and Butylated hydroxyanisole (BHA) acted as the standard compound. The test was done in triplicate.

Determination of Betalain content

To extract betalain in samples, the procedure described by Georgiev *et al.*(6)was applied. 20 ml of samples was poured into distillation flask and heated to 65^0C until dry. The residues were dissolved in 20 mL of 70% methanol, then the methanol-sample mixtures were diluted 50 times with methanol before measurement.

Betalain content was determined according Wruss *et al*.(21). It was calculated as the sum of the concentrations of betacyanins and betaxanthins. The measurement was done spectrophotometrically with betacyanins content at 536 nm and betaxanthins at 485 nm. The absorption was measured and concentrations were calculated using the following formula:

$$\text{Betacyanins (Betaxanthins)content (in mg/L)} = \frac{A \times DF \times MW \times 1000}{\epsilon \times i}$$

where A = A_{536nm} - A_{650nm} (betacyanins) or A_{485nm} -A_{650nm} (betaxanthins); DF = dilution factor; MW (molecular weight) = 550 g/mol (for betacyanins) or 339 g/mol (for betaxanthins); ε= 60,000 (molar extinction coeffificient in L x mol^{-1} x _x 0005_ cm^{-1} for betacyanins) or 48,000 (for betaxanthins); i = path length (cm).

Sensory evaluation

Before being sensory evaluated, wine samples was microbiological tested. The investigated samples should meet the satisfactory level of microbiological standards for alcohol drink in QCVN 6-3:2010/BYT, which is less than 10^3 CFU/mL of sample . The sensory evaluation was carried out to know the acceptability of 30 untrained panelists (selected based on their availability and objectivity) using a five-point Hedonic scale. 15ml of each qualified sample was served in and labeled with a 3-digit code with Latin square order. 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, aroma, taste, overall acceptability.

Statistical analysis

All experiment results were analyzed using Minitab software (Version 21, IBM Corp., USA). The data were analyzed statistically by ANOVA and followed by multiple range comparisons using Fisher pairwise comparisons ($P<0.05$). All numerical data were expressed as mean ± standard deviations of triplicate measurements.

III. Result

Effect of fermentation on physico - chemical properties of fermented red beetroot spirit

Effect of yeast and fermentation temperature on physico - chemical properties of fermented red beetroot spirit was indicated in Table 1. It is shown that pH value of fermented red beetroot spirit was lower than the must after fermentation. pH of the must was 5.23 (not shown in the data) but it reduced within the range of 2.97 and 3.45 after fermentation. Yeast addition caused higher pH values than the natural fermentation.

Table 1. Changes in physical and chemical characteristics of fermented red beetroot spirit

Parameters	W/ yeast			W/O yeast		
	Fermentation temperature					
	20^0C	**25^0C**	**30^0C**	**20^0C**	**25^0C**	**30^0C**
pH	3.45 ± 0.05^a	3.38 ± 0.09^a	3.35 ± 0.06^a	2.97 ± 0.07^c	3.08 ± 0.05^b	3.07 ± 0.05^b
Total soluble solids (°Brix)	5.42 ± 0.02^f	6.39 ± 0.04^b	6.91 ± 0.03^a	6.25 ± 0.01^c	5.54 ± 0.03^e	6.21 ± 0.01^d
Alcohol content (%)	13.73 ± 0.37^a	13.79 ± 0.59^a	14.19 ± 0.77^a	3.80 ± 0.17^c	3.87 ± 0.06^c	4.96 ± 0.07^b
Residual sugar (g/L)	5.12 ± 0.14^c	6.09 ± 0.04^b	6.61 ± 0.03^a	5.95 ± 0.01^b	5.24 ± 0.03^c	5.91 ± 0.01^b
Titratable acidity (g/L)	5.42 ± 0.31^c	5.82 ± 0.57^c	6.02 ± 0.37^c	8.41 ± 0.43^a	7.66 ± 0.31^b	7.71 ± 0.40^b

Titratable acidity expressed as citric acid; Residual sugar expressed as glucose. Data were reported as mean ± standard deviation of 3 replications. Means followed by different letters (a to d) within the same row are significantly different from each other (P <0.05) according to Fisher pairwise comparisons.

The fermentation temperature did not influence pH value significantly except for the natural fermentation at 20^0C. In this research there is a negative correlation between pH and acidity of the samples. The higher the acidity, the lower the pH of the wine. In contrast with pH, yeast inoculated fermentation had lower acidity than the natural one. The titratable acidity of fermented samples was from 5.42 to 8.41 after fermentation, which was higher than that of must (5.52) except for the yeast fermented samples at 20^0C.

Table 1 showed that the TSS and residual sugar of fermented samples ranged from 5.42 to 6.91 ^{0}Brix and from 5.12 to 6.61 g/l, respectively. The TSS of yeast inoculated wine increased when increasing fermentation temperature (20^0C, 25^0C, 30^0C). The natural fermentation did not follow this trend. Fermentation

at 25^{0}C had lowest total soluble solids (5.54^{0}Brix). Red beetroot wine fermented by yeast shows a significantly higher alcohol content than natural fermented samples. The alcohol strength of yeast fermented wine was between 13.73 and 14.19% but not significant when raising fermentation temperature at an experimental range of 20 to 30^{0}C. Fermentation temperature did affect alcohol content of natural fermented samples only at 30^{0}C, which was 4.96% compared to 3.8% at 20^{0}C.

Effect of fermentation on bioactive compounds of fermented red beetroot spirit

In order to characterize changes in bioactive compounds of red beetroot spirit after fermentation; vitamin C, antioxidant activity, total betalain pigment (Betacyanin and Betaxanthin) and total phenolic content were analyzed (Table 2 and Figure 1).

Table 2. Effect of fermentation temperature on vitamin C, antioxidant activity and total betalain pigment (Betacyanin and Betaxanthin)

Parameters	Must	W/ yeast			W/O yeast		
		Fermentation temperature					
		20^{0}C	25^{0}C	30^{0}C	20^{0}C	25^{0}C	30^{0}C
Vitamin C (mg/100ml)	15.16 ± 0.38	3.91^b ± 0.19	3.87^a ± 0.13	4.54^e ± 0.08	2.82^c ± 0.08	3.7^h ± 0.07	3.83^b ± 0.08
DPPH radical scavenging activity (%)	72.39 ± 0.44	56.84^b ± 0.61	63.12^a ± 0.23	56.08^c ± 0.3	43.37^e ± 0.32	45.49^d ± 0.54	46.86^d ± 0.32
Betacyanin (mg/L)	175.24 ± 10.89	134.9^b ± 0.53	145.45^a ± 1.15	91.67^c ± 2.55	69.67^e ± 1.65	83.42^d ± 1.65	41.25^f ± 1.65
Betaxanthin (mg/L)	92.64 ± 2.27	64.03^b ± 0.54	68.27^a ± 1.34	61.21^c ± 0.74	41.08^e ± 0.2	43.08^d ± 1.83	31.55^f ± 2.3
Total Betalain (mg/L)	267.87 ± 9.8	198.94^b ± 1.00	213.72^a ± 2.41	152.88^c ± 3.05	110.75^e ± 1.46	126.5^d ± 2.75	72.80^f ± 0.85

All values are means ± standard deviation of data from three independent experiments. Different lowercase letters (a-f) in the same row indicate significant difference (P < 0.05).

The vitamin C content of the fermented samples significantly reduced after fermentation (Table 2). While the must contained 15.16 mg/100ml of vitamin C, the fermented samples decreased significantly from 2.82 to 4.54 mg/100ml after fermentation. Natural fermentation caused more reduction in vitamin C than yeast fermentation. The natural fermented beetroot spirit had the lowest pH (2.97) and retained the lowest vitamin C (2.82 mg/100ml). Yeast fermentation caused less vitamin C reduction than the natural fermentation. When assessing the effect of yeast fermentation temperature on vitamin C content, 30^{0}C was the best condition to minimize vitamin C loss.

To evaluate the free radical scavenging ability of fermented red beetroot spirit, DPPH assays were done . The result for radical scavenging activity of fermented samples were found to be within the range of 43.37 - 63.12% (table 2). The free radical scavenging effect was changed to the range of 56.08 and 63.12% for yeast inoculated wine and to the range of 43.37 - 46.86% for natural fermented spirit from 72.39%.

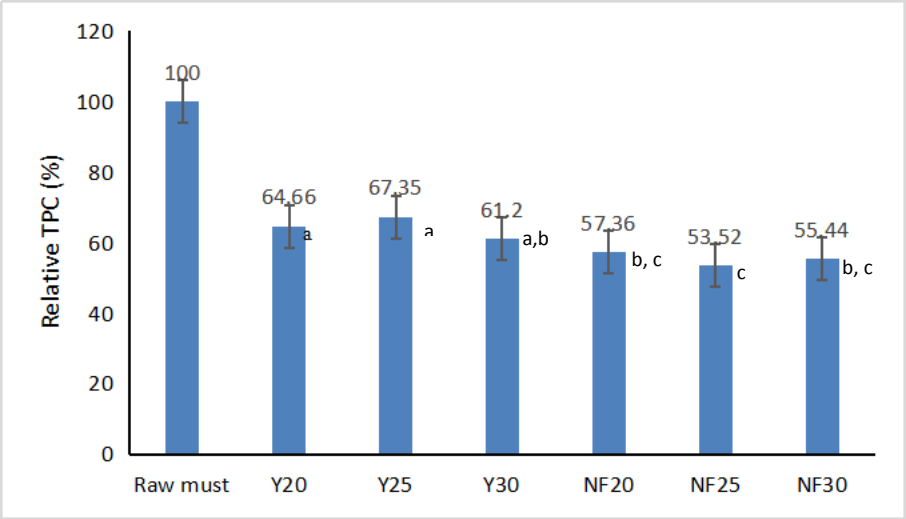

Figure 1. Relative total phenolic content of fermented beetroot spirit at different fermentation temperature. Bars with same letter are not signifificantly different (p < 0.05; Fisher comparison test). Means ± SE presented. Y20, Y25, Y30: yeast fermented samples at 20, 25 and 30^{0}C, respectively. NF 20, NF25 and NF30: natural fermented samples at 20, 25 and 30^{0}C, respectively.

Natural pigments containing antioxidant properties provide many health benefits. Betalain is one of them. Effect of fermentation temperature on total betalain pigment (Betaxanthin and Betacyanin) was analyzed and presented in table 2. 25^0C was the best temperature for total betalain retention (213.72 mg/l) as well as the highest scavenging effect (63.12%). Betacyanin and Betaxanthin amount in the fermented samples had the same trend. Fermentation at high temperature (30^0C) resulted in a serious color degradation.

After fermentation, the TPC of red beetroot spirit reduced significantly (Figure 1). Compared to yeast fermentation, natural fermentation cause the most TPC reduction. Specifically, the relative TPC of natural fermented beet root spirit samples was recorded from 54 to 57% while yeast fermented samples retained more than 60% of relative TPC. Fermentation with yeast at 25^0C results in the least TPC degradation but not significantly when assessing effect of fermentation temperature on TPC in yeast inoculated samples.

Effect of fermentation on sensory characteristics

The verbal descriptions of fermented beetroot spirit's sensory properties were presented in Figure 2.

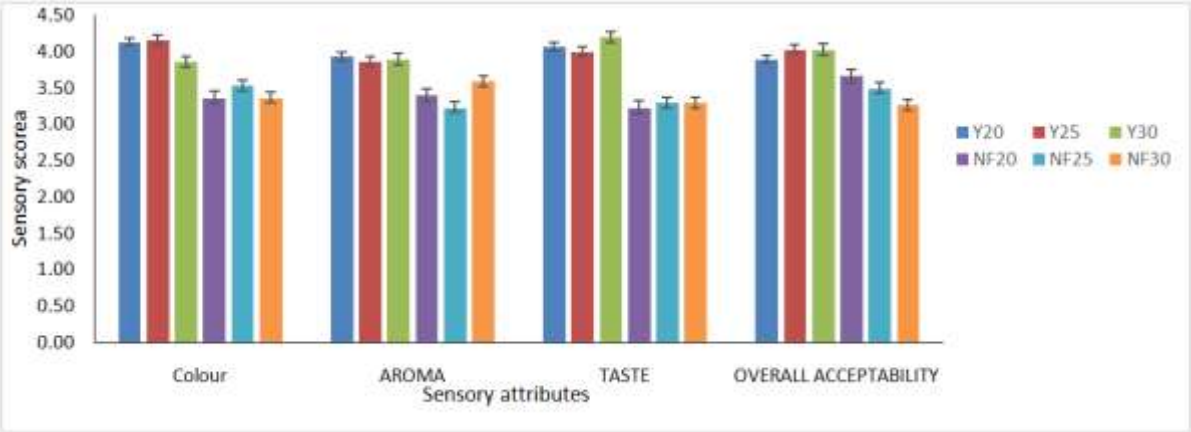

Figure 2*. Sensory properties of fermented beetroot spirit at different temperature. Means ± SE presented. Y20, Y25, Y30: yeast fermented samples at 20, 25 and 30^0C, respectively. NF 20, NF25 and NF30: natural fermented samples at 20, 25 and 30^0C, respectively.*

In general, samples fermented with yeast got higher score than the natural fermented ones. Especially, there was significant difference in taste and aroma attribute between yeast and natural fermented samples for all fermentation temperatures (taste score ranged from 4.0 to 4.2 for yeast beetroot wine and ranged from 3.23 to 3.3 for natural fermented samples; aroma score ranged from 3.87 to 3.93 for yeast beetroot wine and ranged from 3.23 to 3.60 for natural fermented samples).

IV. Discussion

Physical property was positively affected by yeast addition after fermentation (higher pH, higher alcohol content). Fermentation temperature did not significantly change pH value, alcohol content and acidity for both fermentation methods. The data of pH value is similar to the reference data (22, 23). Reason for pH reduction after fermentation was probably linked to the formation of acetic acid (13). The higher pH in fermented samples may be explained by strong activity of yeast to convert sugar into ethanol instead of acid. pH value of wine plays an important role in the taste of wine (22). Low pH wine will taste tart and crisp, while higher pH wines will taste weak and flabby. Apart from sensory property, pH affects the microbial and physicochemical stability of wines. In addition, the pH helps to stabilize the color. When the pH is greater than 5.5, the color of the wine does not stable (13). Total solid and residual sugar are related to sweetness, which influences the preference and choice of fruit juice products in sensory evaluation as well as alters the physico-chemical characteristics of wine (24, 25).The TTS of yeast fermented samples in 30^0C was the highest value, which explained why taste property in sensory test shows the highest score. The higher in TA of fermented samples was as a result of an increase in organic acids formation after fermentation. The result for TA also has an agreement with the data reported by (22). The stronger alcohol content in yeast fermented samples may

demonstrate a better yeast cell growth as well as a better yeast activity of yeast inoculated samples to convert sugar into ethanol. Compared with some other fermented fruits, beetroot produced wine with higher alcohol content than apple wine with 10.9% at 20^0C (26) or grape wine with 7.7% at 20^0C (27).

After fermentation, the bioactive compounds were decreased significantly but yeast fermentation preserved them better than the natural method. Burdurlu*et al.*(28)stated that degradation of vitamin C was the most in in acid solutions, which can explain why there was vitamin C loss after fermentation of red beetroot. Yeast fermented samples at 25^0C had highest antioxidant activity. Similar to vitamin C value, the antioxidant activity was better preserved in yeast inoculated samples than in natural fermented samples.Pavlov *et al.*(29)found that there is a positive correlation between the concentration of the betalains and the antiradical activity of the ethanol extracts, which is the same in the case of our yeast fermented samples. The result for Betacyanin and Betaxanthin concentration in this research is much higher than the value in study ofSkalicky et al(30), which can be explained by the different variety of beetroot. It was reported that total phenolic content in beetroot juice was 2 - 3 times higher than other juice (8). In beverage processing, pasteurization, fermentation and clarification are processing methods which can cause loss of phenolics (31). In this research, the loss of phenolics after fermentation should come as no surprise.Although after fermentation at 14 days, the TPC of beetroot spirit reduced significantly, it was still high compared to other fruits and vegetables, in the range between 947 and 1150 mg GAE/L for our fermented beetroot spirit and in the range of 474 and 695 mg GAE/L for other fruits and vegetables (8).

Regarding to sensory result, the higher score in taste and aroma properties in yeast fermented samples than in the natural fermented samples can be explained by alcohol strength, which hid the earthy flavor of raw beetroot juice. It is very interesting that after fermentation and pasteurization, panelists still appreciated color attribute for the case of yeast fermented beetroot wine. This data is in agreement with data for betalain concentration. It is indicated that fermentation temperature did not affect all sensory properties in both yeast and natural fermented samples.

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. 25^0C 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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