Wine Making of Dragon Fruit and Orange Fruit by Fermentation with Their Isolated Yeasts

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Abstract

In this study, the two fruit samples: dragon fruit (*Selenicereus grandiflorus* (L.) Britton and Rose) and orange fruit (*Citrus sinensis* L.) were used for isolation of natural yeasts. Four natural yeasts were isolated from dragon fruit and six natural yeasts were isolated from orange juice by directly spread method on three selective media. Isolated strains were identified as *Candida* spp., *Saccharomyces* spp., and *Saccharomyces cerevisiae*. Among ten isolates, two strains were chosen to investigate biochemical changes of two fermented fruit juices. Acid content percent of fermented dragon fruit juice gradually increased from 0.25 to 0.71 during 360 hrs. Its alcohol percent was ranged between 0.0 and 7.5 during 360 hrs. Its pH was ranged between 5.5 and 3.0 during 360 hrs. Sugar content percent of fermented dragon fruit juice decreased from 100 to 30 during fifteen days. At 312 hrs, sugar content percent of fermented dragon fruit juice decreased to 35. Acid content percent of fermented orange juice gradually increased from 0.18 to 0.84 during 360 hrs. Its alcohol percent was ranged between 0.0 and 8.0 during 360 hrs. Its pH was ranged between 5.0 and 3.0 during 312 hrs. Sugar content percent of fermented orange juice decreased from 100 to 30 during fifteen days. At 288 hrs, sugar content percent of fermented orange juice decreased to 40. Therefore, day 13 fermentation with alcohol (6.5%) and sugar content (35) at pH 3.5 was the best for the dragon fruit juice whereas day 12 fermentation with alcohol (6.0%) and sugar content (40) at pH 3.5 was the best for the orange juice to make wine.

Keywords: *Citrus sinensis* L., *Selenicereus grandiflorus* (L.) Britton and Rose, Fermented wine

Introduction

Dragon fruit (*Selenicereus grandiflorus* (L.) Britton and Rose, synonym Cactus grandiflorus or Night blooming cereus, is a plant species of the family Cactaceae. Dragon fruit is an important fruit cultivated in tropical and subtropical areas (Wu *et al*., 2019). Dragon fruit is harvested several times a year; in the northern hemisphere from June to November (Polturak and Aharoni, 2019). Dragon fruit is a valuable raw material for fermented beverage production because of its high nutrient content (Huan and Min, 2009). Ethanol and methanol levels with the treatment of sugar and fermentation time observed in dragon fruit wine. Natural yeasts were isolated from dragon fruits to produce a high-value wine product (Teddy and Dimero, 2018).

Orange fruit (*Citrus sinensis* L.) belongs to Rutaceae family. Orange originated in a region encompassing Southern China, Northeast India and Myanmar. Yeast strains from orange fruits for wine production have been isolated (Aye Aye Nwe and Nwe Ni Haling, 2017). Yeast isolates from orange fruit and juice in a spontaneous fermentation were identified and classified by two molecular techniques (Mingorance-Cazorla et al., 2002). Yeast consumes the sugar present in the fruit juice producing alcohol and carbon dioxide as by products.

The objectives of this research are to collect the two selected samples (dragon fruit and orange fruit), to isolate natural yeasts from dragon fruit juice and orange fruit juice, and to investigate biochemical changes of selected juice samples for wine making.

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Materials and Methods

Collection of fruit samples

The two samples of dragon fruit (*Selenicereus grandiflorus* (L.) Britton and Rose) and orange fruit (*Citrus sinensis* L.) were used to isolate natural yeasts in Figure 1. All the samples were collected from the local market in South Dagon Township, Yangon Region.

Dragon fruits

Orange fruits

Figure 1. The selected fruit samples

Isolation of natural yeasts

All selected samples were screened for the isolation of natural yeast strains. All the experiments were carried out at Microbiological Laboratory, Department of Botany, Dagon University. Natural yeasts from dragon fruit and orange fruit were isolated by directly spread method by Hartman (2011).

Direct spread method was carried out as follow:

1) Prepare the juice samples of dragon fruit and orange fruit.
2) Take 0.5 ml of sample juice with a clean and sterile pipette and applied the center of the surface of an agar plate.
3) Spread the samples on each medium by shaking the plate gently

Isolation media for yeasts culture

1. Yeast Peptone Dextrose Agar medium (YPD)
2. Yeast Malt Extract Agar medium (YM)
3. Yeast Extract Phosphate Agar medium (YEP) were used according to the producers of described by Harrigan and Mac Cane (1966).

Production of fermented pure dragon fruit juice

Freshly extracted, filtered (through muslin cloth), the dragon fruit juice in the glass bottles was pasteurized at 70ºC temperature for 20 sec. Then, the bottles were cooled at room temperature.

Isolated yeast inoculum (2%) was then added to the cool pure dragon fruit juice. The bottles were aseptically incubated at room temperature for 15 days and periodically studied for biochemical changes (acidity, alcohol content, pH and sugar content daily) (Marsh, 2014).

Production of fermented pure orange juice

Freshly extracted, filtered (through muslin cloth), the orange juice in the glass bottles was pasteurized at 70ºC temperature for 20 sec. Then, the bottles were cooled at room temperature.

Isolated yeast inoculum (2%) was then added to the cool pure orange juice. The bottles were aseptically incubated at room temperature for 15 days and periodically studied for biochemical changes (acidity, alcohol content, pH and sugar content) daily (Marsh, 2014).

Determination of biochemical changes in fermented juices

(a) Determination of the titratable acidity (T.A)

The titratable acidity (T.A) utilizes the endpoint of titration to determine the result. The titration was determined by taking 10 ml of the juice sample into the beaker. Two drops of phenolphthalein are added into the sample in the beaker, 0.1M sodium hydroxide was used to titrate the juice sample, with shaking of the beaker at interval so that the indicator was mixed until a change in color occurs. The titratable acidity is calculated by using following formula.

$$\text{T.A (\%)} = \frac{T \text{ x } Normality\ of\ alkali \text{ x } 9}{Volume\ of\ sample\ used\ (i.e. 10\ ml)}$$

Where: T is the mean titre (in ml) of 0.1M sodium hydroxide solution required to neutralize the acidity in 10 ml of the juice sample while 9 is the factor of acetic acid in accordance with Bitange et al., 2009.

(b) **Determination of alcohol content**

Alcohol levels of the two samples at different age of fermentation are determined by using alcohol meter. The alcohol contents of fermented juices are periodically recorded for 15 days.

(c) Determination of pH

The pH of the different age of fermentation of the two samples was determined by pH paper strip. The pH meter could not be used for measuring the pH because the amount of fermented juices was not enough for sampling. The pH of each fermented juice was daily recorded.

(d) Determination of sugar content

Sugar concentration of the two samples at different age of fermentation was determined by using hand refractometer. The sugar content of each fermented juice was daily recorded.

Results

Isolation of natural yeasts

Ten natural yeasts were isolated from the two selected samples, and gave the temporary names S1 to S10. Strains S1 to S4 were isolated from the dragon fruit juice and S5 to S10 from orange juice.

Wine making of two selected samples

Among ten strains, two strains: strain S3 (*Saccharomyces* sp.) from dragon fruit juice and strain S9 (*Saccharomyces cerevisiae*) from orange fruit juice were selected for wine making according to their more outgassing in biochemical tests.

Biochemical changes of fermented dragon fruit juice

Acid content percent of fermented dragon fruit juice gradually increased from 0.25 to 0.71 during 24 hrs to 360 hrs. pH of fermented dragon fruit juice was ranged between 5.5 and 3.0, and gradually decreased to 3.0 at 360 hrs. Alcohol percent of fermented dragon fruit juice was ranged between 0.0 and 7.5 during 24 hrs to 360 hrs. Sugar content percent of fermented dragon fruit juice decreased from 100 to 30 during fifteen days. At 312 hrs, sugar content percent of fermented dragon fruit juice decreased

to 35 (Table 1 and Figures 2 to 5). Thirteen day-old fermentation (312 hrs) with alcohol (6.5%) and sugar content (35) at pH 3.5 was the best for dragon fruit wine making.

Table 1. Biochemical changes of dragon fruit juice

Fermentation/ Storage (in hours)	Parameter of Dragon Fruit Juice			
	Acid content percent	Alcohol percent	pH	Sugar content percent
24	0.25	0.0	5.5	100
48	0.31	1.0	5.0	90
72	0.36	1.5	4.5	90
96	0.40	2.0	4.5	85
120	0.43	2.5	4.5	80
144	0.47	3.0	4.0	70
168	0.50	3.5	4.0	65
192	0.52	4.0	4.0	60
216	0.56	4.5	4.0	55
240	0.59	5.0	3.5	50
264	0.62	5.5	3.5	40
288	0.65	6.0	3.5	35
312	0.67	6.5	3.5	35
336	0.69	7.0	3.5	30
360	0.71	7.5	3.2	30

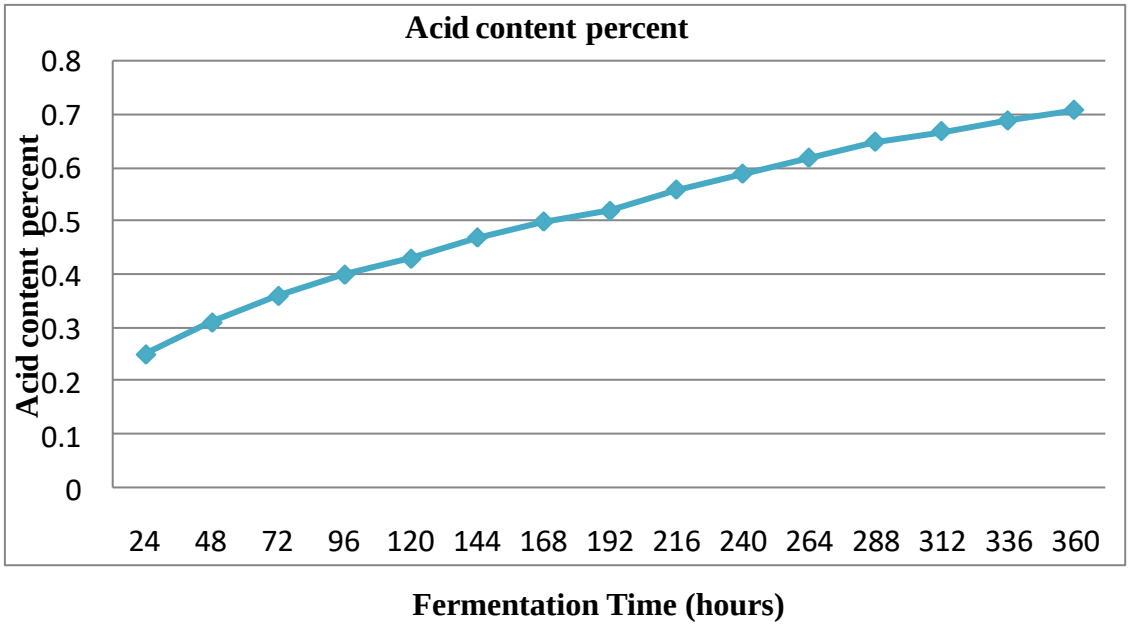

Figure 2. Biochemical changes (acid content percent) of dragon fruit juice

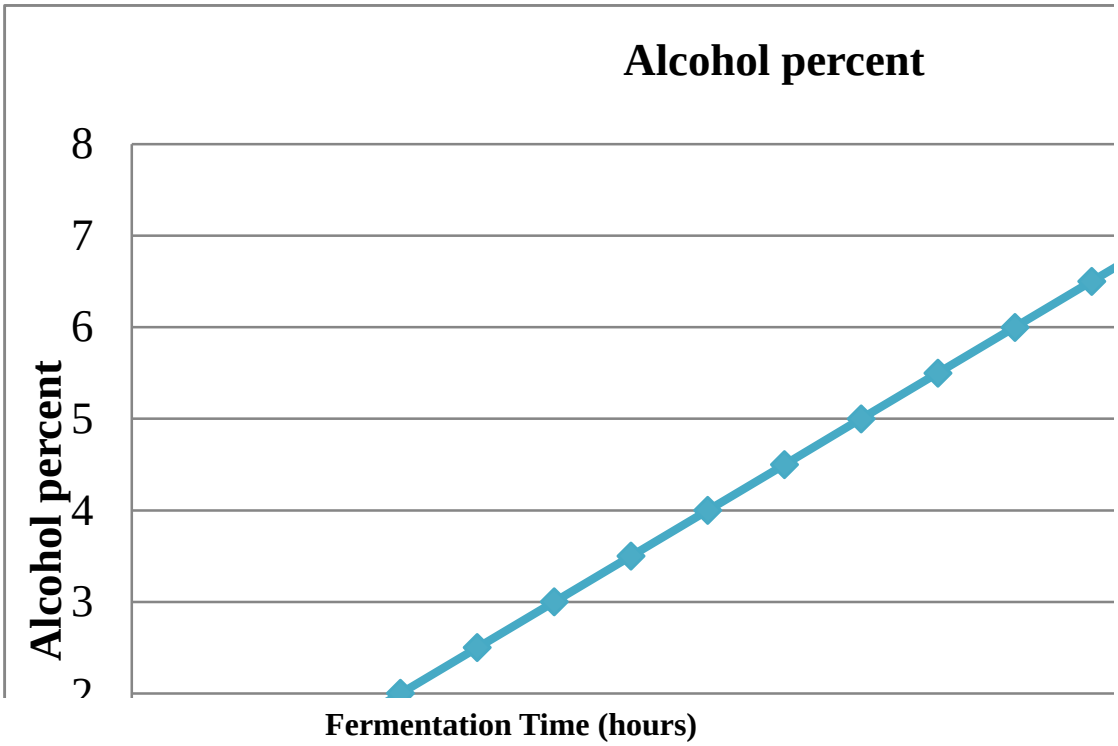

Figure 3. Biochemical changes (alcohol percent) of dragon fruit juice

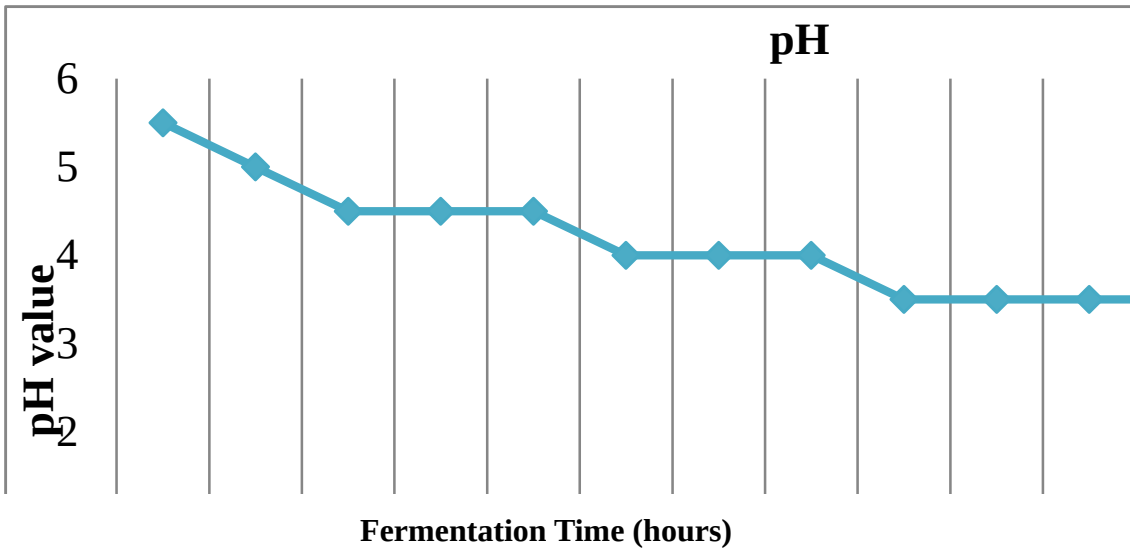

Figure 4. Biochemical changes (pH) of dragon fruit juice

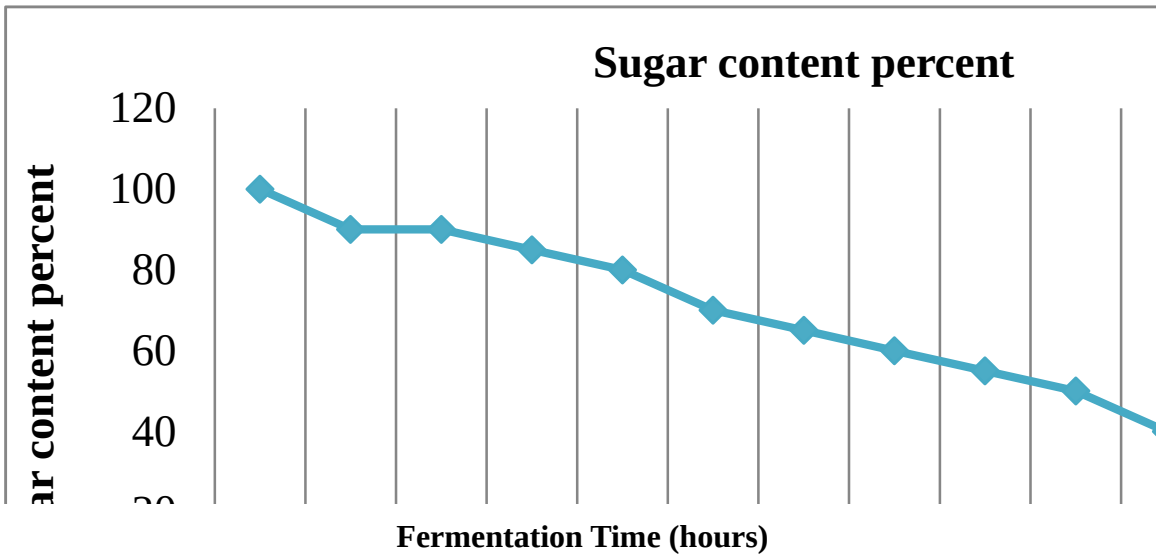

Figure 5. Biochemical changes (sugar content percent) of dragon fruit juice

Biochemical changes of fermented orange juice

Acid content percent of fermented orange juice gradually increased from 0.18 to 0.84 during 24 hrs to 360 hrs. The pH of fermented orange juice was ranged between 5.0 and 3.0, and gradually decreased to 3.0 at 360 hrs. Alcohol percent of fermented orange juice was ranged between 0.0 and 8.0 during 24 hrs to 360 hrs. Sugar content percent of fermented orange juice decreased from 100 to 50 during fifteen days. At 312 hrs, sugar content percent of fermented orange juice decreased to 50 (Table 2 and Figures 6 to 9). Twelve days-old fermentation (288 hrs) with alcohol (6.0%) and sugar content (40) at pH 3.5 was the best for orange wine making.

Table 2. Biochemical changes of orange juice

Fermentation/ Storage (in hours)	Parameter of Orange Juice			
	Acid content percent	Alcohol percent	pH	Sugar content percent
24	0.18	0.0	5.0	100
48	0.23	0.0	5.0	100
72	0.29	1.0	4.5	90
96	0.34	1.5	4.5	85
120	0.37	2.0	4.5	85
144	0.41	2.5	4.5	80
168	0.46	3.0	3.5	75
192	0.50	4.0	3.5	75
216	0.52	4.5	3.5	60
240	0.58	5.0	3.5	55
264	0.63	5.5	3.5	50
288	0.69	6.0	3.5	40
312	0.74	7.0	3.0	40
336	0.80	7.5	3.0	35
360	0.84	8.0	3.0	30

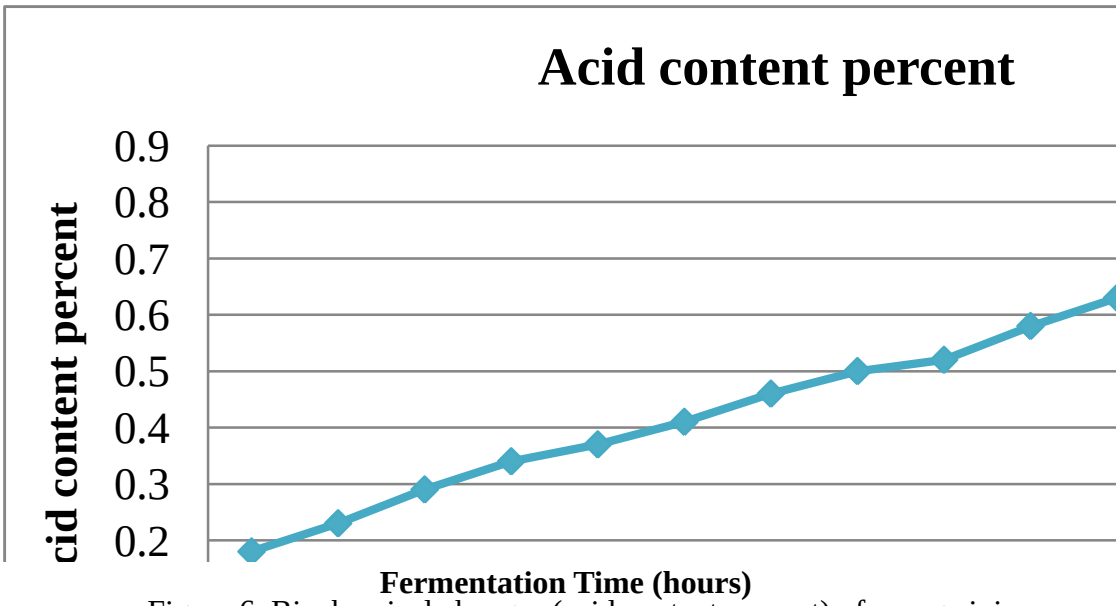

Figure 6. Biochemical changes (acid content percent) of orange juice

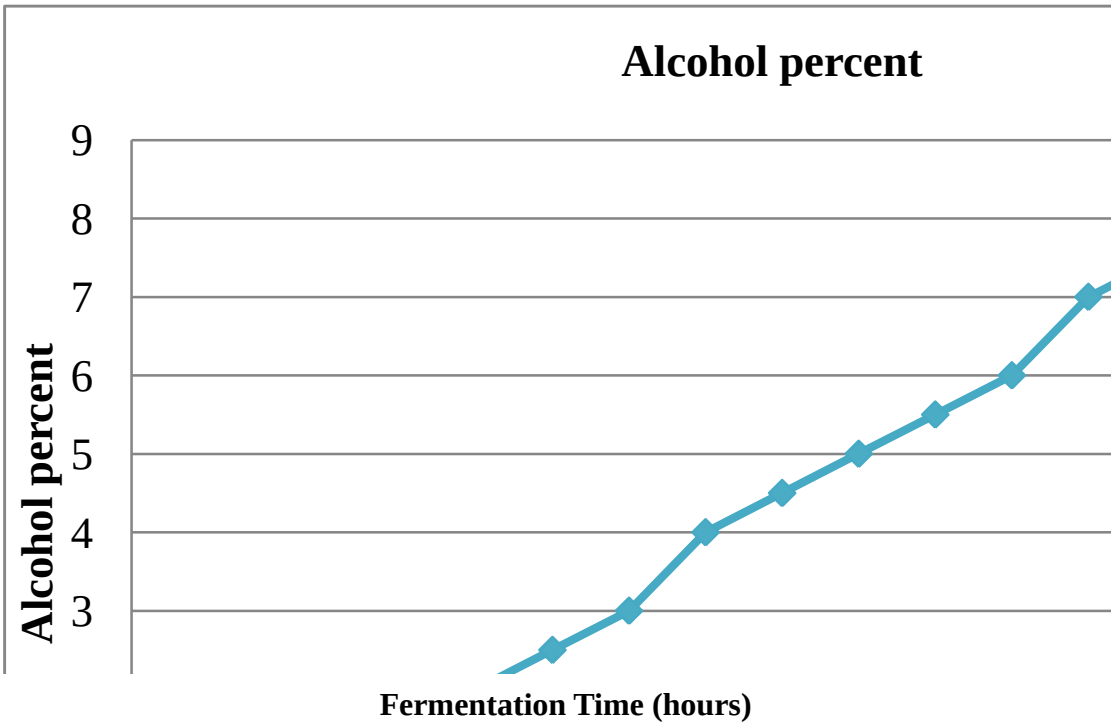

Figure 7. Biochemical changes (alcohol percent) of orange juice

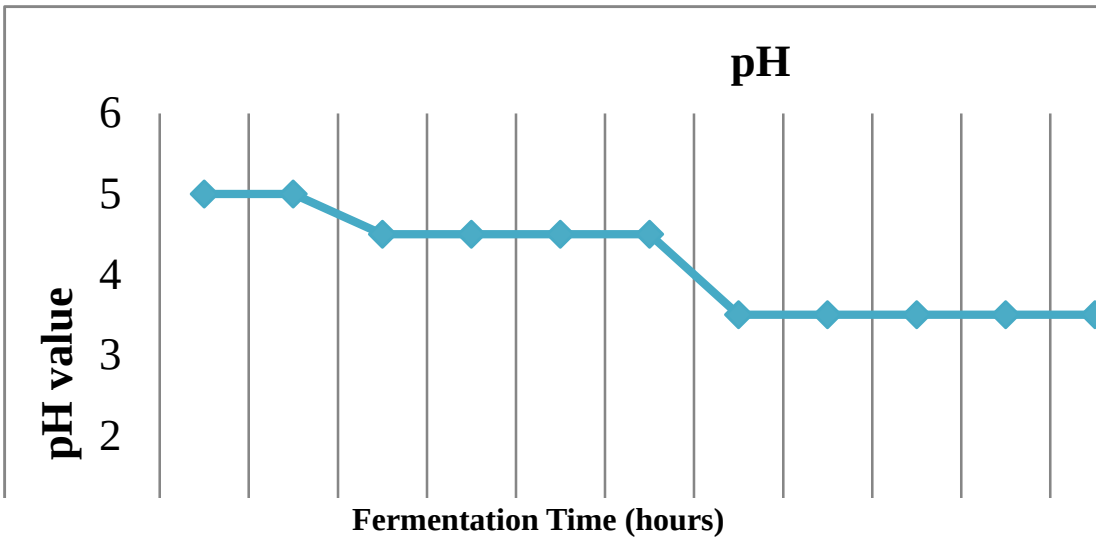

Figure 8. Range of pH of fermented orange juice

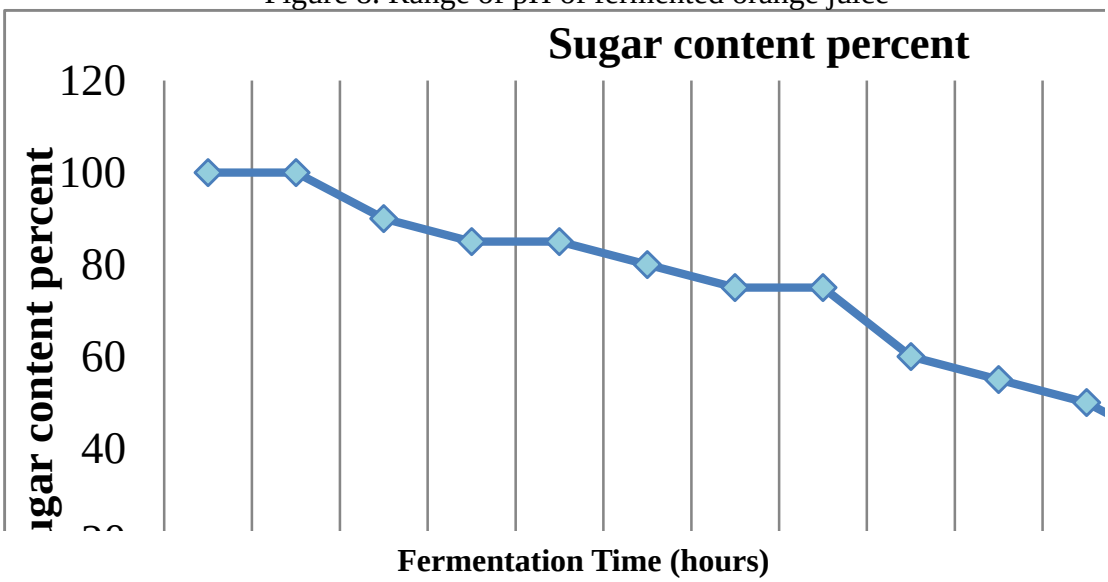

Figure 9. Biochemical changes (sugar content percent) of orange juice

Discussion and Conclusion

In this study, four natural yeasts were isolated from dragon fruit (*Selenicereus grandiflorus* (L.) Britton and Rose) and six natural yeasts were isolated from orange juice (*Citrus sinensis* L.).Teddy and Dimero (2018) have isolated natural yeasts from dragon fruits to produce a high-value wine product. Mingorance-Cazorla L. *et al*. (2002) have isolated yeast strains from orange fruit and juice in a spontaneous fermentation. Aye Aye Nwe and Nwe Ni Haling, 2017 isolated natural yeasts from orange fruits for wine making.

In this study, among isolated yeast strains, two strains (*Saccharomyces* sp. and *Saccharomyces cerevisiae*) were used to study their biochemical changes for wine making. The pH (3.5), sugar content percent (35) and alcohol content (6.5) were the best condition for dragon fruit fermentation for 13 days. Sudiarta *et al.* (2020) investigated the effect of sugar levels and the best fermentation time on characteristics of red dragon fruit wine, they showed that the pH (4.5 to 3.65) and alcohol content (12-18%) by the end of the aging period (14 days).

In this study, the pH (3.5), alcohol percent (6.0) and sugar content (40) were the best condition for orange fermentation at 12 days old fermentation. Aye Aye Nwe and Nwe Ni Haling, 2017 examined preparation, characterization of orange wine and

comparison of some physical parameters of different wines, and they exhibited that pH (3.4 to 3.9) and alcohol content percent (16.98) during 15 days.

In conclusion, fruits are very essential for our health and they are used for the production of various alcoholic beverages like wine. Red wine can lower bad cholesterol levels and promote heart health. It is rich in antioxidants and reduces the risk of cancer. White wine may not have health benefits as red wine so that red wine is healthier than white wine. Therefore, among two selected strains, strain S9 (*Saccharomyces cerevisiae*) isolated from orange fruit should be chosen for red wine making because it produced the red pigment.

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