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Application of *Saccharomyces cerevisiae* for wine production from star gooseberry and carambola

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The experiment was to produce wine from star gooseberry (*Phyllanthus acidus* (L) Skeels and carambola (*Averrhoa carambola* L.) by fermented with *Saccharomyces cerevisiae* for two weeks. Results showed that star gooseberry wine gave significantly higher total acid (%TA) than carambola wine at all formulations but the star gooseberry wine had lower acidity than carambola wine. Star gooseberry wine gave significantly higher in ethyl alcohol production (averaged 15.90%) than carambola wine (averaged 8.28%). Meanwhile, star gooseberry wine formulation 4 gave the highest ethyl alcohol (23.12%), and followed by carambola wine formulation 4 (14.37%), star gooseberry wine formulation 3 (17.25%), star gooseberry wine formulation 2 (13.75%), star gooseberry wine formulation 1 (9.5%), carambola wine formulation 3 (8.75%), carambola wine formulation 2 (6.5%) and the lowest ethyl alcohol production in carambola wine formulation 1 (3.5%). The amount of ethyl alcohol was analyzed in each formulation both in star gooseberry wine and carambola wine. It is demonstrated that all formulations of star gooseberry wine showed significantly higher amount of ethyl alcohol than all formulations of carambola wine.

Key words: *Saccharomyces cerevisiae,* star gooseberry (*Phyllanthus acidus* (L) Skeels, carambola (*Averrhoa carambola* L.)

Introduction

Wine is an alcoholic beverage typically made of fermented grape juice or variety of fruits. However, the natural balance of grapes is such that they can ferment without addition of sugars, acids, enzymes or other nutrients.Wine is produced by fermenting crushed grapes using various types of yeast. Pelczar *et al.* (1977) stated that the species involved in fermentation process is mostly *S. cerevisiae*. It is one of the most important fungus in the history of wine

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production in the world. This yeast is responsible for the production of ethanol in alcoholic drink. The process produces ethyl alcohol (ethanol) is the way of yeast to convert glucose into energy. *S. cerevisiae* has adapted in several important ways and be able to break down their foods through both aerobic respiration and anaerobic fermentation. It can survive in an oxygen deficient environment for a period of time. Kourkoutas *et al*. (2001) stated that a biocatalyst was reported to prepare by immobilization of *S. cerevisiae* strain AXZ-1 on apple pieces. The immobilized yeast showed the important stability without decreased in activity at low temperature from 1-12 ^{0}C. Especially, at 6 ^{0}C C the biocatalyst favored wine production within 8 days, that was less time than is required for the natural fermentation of grape which normally at 1 ^{0}C for wine production in a month. The presence of amyl alcohol proved to be temperature dependent and decreased with temperature decrease. Nidp *et al.* (2001) reported that sixteen yeast strains isolated from grapefruit (*Citrus paradis*), orange (*Citurs sinensis*) and pineapple (*Ananas comosus*) were characterized using standard microbiological procedures. The species were identified as *Saccharomyces uvarum, S. cerevisiae, S. carlbergensis* and *S. ellipsoideus.* Their abilities for wine production were evaluated by using sugar and ethyl alcohol tolerance tests. This report stated that the best biochemically active strain, *S. ellipsoideus* was along with commercially available baker's yeast *S. cerevisiae* was used to produce wine from grapefruit, orange and pineapple juices after fermentation for 14 days with *S. cerevisiae* and 21 days with *S. ellipsoideus*.

Moreover, Rosalinda *et al.* (2007) stated that the yeast biodiversity and dynamics during the production of sweet wine obtained from dried grapes were investigated and revealed that the capability of *S. cerevisiae* starter cultures was assessed by RAPD-PCR. The non-Saccharomyces yeasts e.g. *Hanseniaspora, Metschnikowia, Pichia, Candida, Torulaspora* and *Debaryomyces* and *S. cerevisiae* were isolated. After inoculation of the starter cultures, it revealed that only *S. cerevisiae* was observed. Ingledew *et al.* (1987) stated that the fermentation experiments have indicated that ethyl carbamate was not formed during fermentation, even in the presence of urea, ammonium phosphate or amino acid containing yeast foods at 12 times. The heating of end fermentation broths led to ethyl carbamate formation but only from fermentation supernatants where urea was used. Gonnzales *et al.* (2002) stated a temperature sensitive autolytic phenotype has been used to genetically improve a second fermentation, *S. cerevisiae* yeast strain by UV mutagenesis. The mutation was carried by the resulting strains affected cell morphology, growth, sporulation and release of nitrogenous compounds in an accelerated autolysis. This allows this species to live in many different environments. Thus, it is the reason to study the ability of the *S. cerevisiae* for fermentation of wine production from star gooseberry and

carambola which are commonly fruits in Laos. It was interested to study for promoting and value added of these fruits.

The objectives of this study was to determine the better conditions for wine production from star gooseberry (*Phyllanthus acidus* (L) Skeels) and carambola (*Averrhoa carambola* L.) to compare the quantity of alcohol from the wine fermentation process and to study the local fruit sources for wine production.

Materials and methods

This research work was used star gooseberry and carambola to produce wine through fermentation process for two weeks by inoculated *S. cerevisiae* (yeast) as starter. Either star gooseberry or carambola wines were performed with four formulations as follows: - formulation 1 consisted of fruit juice 150 g and sugar 170 g in 1 L of water, formulation 2 consisted of fruit juice 200 g and sugar 220 g in 1 L of water, formulation 3 consisted of fruit juice 250 g and sugar 270 g in 1 L of water and formulation 4 consisted of fruit juice 300 g and sugar 320 g in 1 L of water. Then, there were four formulations of star gooseberry wine and four formulations of carambola wine. The experiment was used Completely Randomized Design (CRD) and repeated at least three times. Data were subjected to analysis of variance and computed to compare the treatment by Duncan Multiple Range Test (DMRT) at P = 0.01.

Preparation of starter

Preparation of starter was performed by using water 400 ml and 80 g of sugar as the medium in Erlenmeyer flask, then autoclaved at 121^0C, 14 lbs/inch^2 for 20 min, after the medium cooled, pure culture of *S. cerevisiae* was transferred into the medium, incubated for 3 days at room temperature before use.

Preparation of fruit juices

The fruits of star gooseberry and carambola were selected only a good quality and cleaned by running water. The fruits were grouped and weighted at 150, 200, 250 and 300 g in order to follow the tested formulas. The fruit in each formula was macerated and filtered to get juice, then added water to reach 1000 ml and justified the pH level.

Wine fermentation

Before fermentation, the fruit juices were sterilized by boiling at 100 °C for 30 minutes and waited until cool, then transferred the starter to each treatment and incubated at room temperature approximately 27-30 °C for two weeks. After

fermentation, the growth of yeast was inhibited by boiling at 50-60 °C for 60 minutes to quit fermentation activity and kept at the room temperature until cool, then filtered through filter paper at the size of 0.4 microns and finally, storage in bottle and ready for drink. Data were collected as pH level, total acidity percentage, volume of wine and analysis of ethyl alcohol in each treatment.

Data analysis

The pH levels were measured by using the pH meter, percentage of total acidity and methanol which was used the formula as follows:

$$\%TA = \frac{n \times v \times m \times 100}{w \times 1000}$$

Where; n = concentration of base, m = mass of acid, w = weight of sample (per g or ml), v = Volume of mass (per ml). The analysis of methanol was analyzed by formula as follows:-:

$$W = \frac{V_2 \times 100}{V_1}$$

Where; W = which concentration of ethyl alcohol (%), V_2 = volume of fermentation and V_1 = volume of ethyl alcohol from filtration.

Results and discussion

Results showed that star gooseberry wine had lower pH or more acid and total acid than carambola wine. The pH of star gooseberry wine formulation 1, 2, 3 and 4 were 3.17, 3.15, 3.14 and 3.12 respectively. But, the pH of carambola wine formulation 1, 2, 3 and 4 were 3.42, 3.34, 3.28 and 3.42, respectively. This study was incubated yeast starter and fermentation process at normal temperature ca 25-27 ^{0}C as a natural fermentation. It is reported by Kourkoutas *et al*. (2001) that the yeast showed the important stability without decreased in activity at low temperature from 1-12 ^{0}C and at 6 ^{0}C the biocatalyst favored wine production within 8 days and the natural fermentation of grape which normally at 1 ^{0}C for wine production in a month. The presence study did not concern on various temperature regimes that would be done for further study. But it may prove that the temperature dependent would be one of a major factor affecting wine production.

The total acid of star gooseberry formulation 4 gave significantly highest total acid (1.49%), and followed by star gooseberry formulation 3 (1.27%), carambola wine formulation 4 (0.84%), carambola wine formulation 3 (0.65%), carambola wine formulation 2 (1.49%) and star gooseberry wine formulation 2 (0.52%). While, the lowest total acid was shown in star gooseberry wine

formulation 1 (0.40%) and carambola wine formulation 2 (0.49%) as seen in Table 1, Fig. 1). There are some reports stated that the total acid in fruit is affected to fermented process during incubation period and acid could help to inhibit the other contaminated microorganism. It is stated that in wine production during fermentation process, the include tartaric acid, malic acid, citric acid, tannic acid, lactic acid and acetic acid and cinamic acid (Champbel *et al.,* 1999).

Star gooseberry wine gave significantly higher in ethyl alcohol production (averaged 15.90%) than carambola wine (averaged 8.28%). Meanwhile, star gooseberry wine formulation 4 gave the highest ethyl alcohol (23.12%), and followed by carambola wine formulation 4 (14.37%), star gooseberry wine formulation 3 (17.25%), star gooseberry wine formulation 2 (13.75%), star gooseberry wine formulation 1 (9.5%), carambola wine formulation 3 (8.75%), carambola wine formulation 2 (6.5%) and the lowest ethyl alcohol production in carambola wine formulation 1 (3.5%). As a result, yeast (*S. cerevisiae*) play the important role to consume nutrients as starch and sugar, then released ethyl alcohol as fresh juice wine. With this, Nidp *et al.* (2001) reported that the starter as yeast must easy to propagate and increase the number of cells in a proper temperature including carbon dioxide concentration during fermentation process.

This preliminary study that produced wine from star gooseberry (*Phyllanthus acidus* L Skeels) and Carambola (*Averrhoa carambola* L.) by fermented with *S. cerevisiae* for two weeks as the natural fermentation which Kourkoutas *et al.* (2001) reported the natural fermentation usually for 30 days. In this study, the amount of ethyl alcohol production in star gooseberry wine and carambola wine showed that all formulations of star gooseberry wine showed significantly higher amount of ethyl alcohol than all formulations of carambola wine.

Table 1. Comparison of wine produced from star gooseberry and carambola.

Fruits sources	Formulations[1] (fruit juice:sugar, g)	pH	%TA[2]	% ethyl alcohol
Star gooseberry	1 (150:170)	3.17	0.40 e	9.5
	2 (200:220)	3.15	0.52 de	13.75
	3 (250:270)	3.14	1.27 b	17.25
	4 (300:320)	3.12	1.49 a	23.12
Carambola	1 (150:170)	3.42	0.37 e	3.5
	2 (200:220)	3.34	0.49 de	6.5
	3 (250:270)	3.28	0.65 d	8.75
	4 (300:320)	3.42	0.84 c	14.37
CV (%)	-	-	11.28	-

[1]Average of three replications. Means followed by a common letter are not significantly different at P=0.01.
[2]Formulation 1 consisted of fruit juice 150 g and sugar 170 g in 1 L of water, formulation 2 consisted of fruit juice 200 g and sugar 220 g in 1 L of water, formulation 3 consisted of fruit juice 250 g and sugar 270 g in 1 L of water and formulation 4 consisted of fruit juice 300 g and sugar 320 g in 1 L of water.

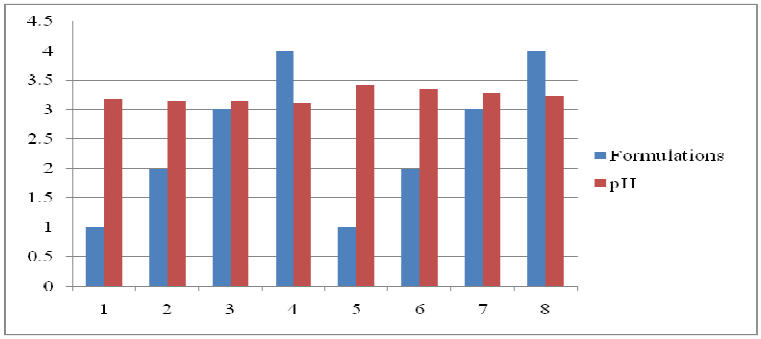

Fig. 1. The pH level of star gooseberry and carambola wines in different formulations.
Note: 1 to 4 is represented star goosebery wine and 5-8 is represented carambola wine where formulation 1 consisted of fruit juice 150 g and sugar 170 g in 1 L of water, formulation 2 consisted of fruit juice 200 g and sugar 220 g in 1 L of water, formulation 3 consisted of fruit juice 250 g and sugar 270 g in 1 L of water and formulation 4 consisted of fruit juice 300 g and sugar 320 g in 1 L of water.

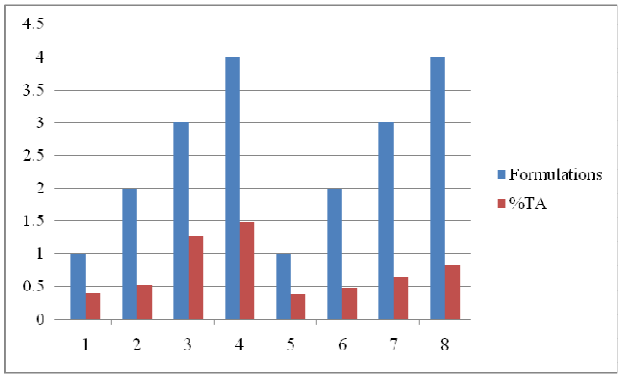

Fig. 2. The total acid of Star gooseberry and carambola wines in different formulations.
Note: 1 to 4 is represented star goosebery wine and 5-8 is represented carambola wine where formulation 1 consisted of fruit juice 150 g and sugar 170 g in 1 L of water, formulation 2 consisted of fruit juice 200 g and sugar 220 g in 1 L of water, formulation 3 consisted of fruit juice sap 250 g and sugar 270 g in 1 L of water and formulation 4 consisted of fruit juice 300 g and sugar 320 g in 1 L of water.

Table 2. Percentage of ethyl alcohol from star gooseberry and carambola wines.

Fruit sources	**Formulations of wine**				
	1	**2**	**3**	**4**	**Average**
Star gooseberry	9.51	13.75	17.25	23.12	15.90 a[1]
Carambola	3.5	6.5	8.75	14.37	8.28 b
Average	6.5 d	10.12 c	13 b	18.75 a	-

[1]Average of two repeated experiments. Means followed by a common letter are not significantly different at P=0.01.

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