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Preparation of Wine from Jackfruit (*Artocarpus heterophyllus lam*) Juice Using *Baker yeast*: Effect of Yeast and Initial Sugar Concentrations

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Abstract: The overproduction of jackfruit (*Artocarpus heterophyllus Lam*) during harvest season and its short shelf-life have caused serious losses for farmers. Fortunately, high sugar content of the fruit pulp makes the juice a potential substrate for wine production. This work was purposed to investigate the effect of yeast and initial sugar concentrations on jackfruit juice wine fermentation. Clarified jackfruit juice of 14 % w/w sugar concentration was fermented using 0.5 to 2.0 % w/v Baker's yeast *(Saccharomyces cerevisiae)* under anaerobic condition at 30°C for 14 days. Samples were collected daily for ethanol and sugar contents analysis. The profile of sugar and ethanol concentration as function of fermentation time, showed that higher yeast inoculums rate and initial sugar concentrations inhibited growth of yeasts. The fermentation of original jackfruit juice of 14 % w/w sugar concentration using 0.5% w/v yeast for 9 days was the best to produce a good quality wine with 12.13% v/v of ethanol and specific jackfruit aroma.

Key words: Wine % Jackfruit % Yeast concentration % Sugar concentration % Ethanol % Time

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L) is indigenous to India and is widely grown in Bangladesh, Burma, Sri Lanka, Malaysia, Indonesia, Philippines, Brazil and other tropical countries [1]. In 2009, the Ministry of Agriculture of the Republic of Indonesia recorded about 57,000 hectares of jackfruit plantations that produced 653,000 tonnes of fruit [2]. When ripe, jackfruit (Figure 1) contains about 7-15% weight sucrose with a very specific aroma and taste. Hitherto, this aromatic fruit is usually consumed fresh. Unfortunately, the shelf life of the ripe jackfruit pulps is only about two days under room condition and the pulps will start to wither and rot thereafter. Being aware to this fact, local people have transformed the fruit pulp into various food products to improve its economic value [1].

Wines are healthy beverages that have been seen as a natural remedy for man's illness from early days and are said to aid recovery during convalescent period [3].

Fig. 1: Ripe jackfruit pulp

Wine is defined as an alcoholic beverage having alcohol content within 8-15% v/v, usually produced from the fermentation of grape juice or juice of other fruits [4]. The natural balance of grapes composition is such that it can be fermented without addition of sugars, acids, enzymes or other nutrients making it a favourite substrate for wine making. In order to meet the diversity of consumer needs, extensive researches have also been carried out to find other possible sources of wine making

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process such as banana, pineapple [5], kiwi [6], apple [7], mango [8], orange [9], cashew apple [10], longan [11], carambola [4, 12], gooseberry [12], pomegranate [13] and other fruit juices. Basically, the fruit juice should contain at least 14% w/w of sugar to be converted into alcohol. If the sugar content is less than 14 % w/w, some amount sugar must be added to compensate the lack of sugar content. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production. The initial sugar concentrations, fermentation temperatures, SO_2 concentrations and specific yeast strains are key factors in determining successful fermentative processes of fruit wine [14].

At the moment, most of the wine production processes are relying on *Saccharomyces cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations [15]. *Saccharomyces cerevisiae* can be obtained from commercially available Baker's yeast or ragi tapai. Yeast starter cultures that are specifically selected for the winemaking process on the basis of scientifically verified characteristics typically complement and optimize the raw material quality and individual characteristics of the wine, producing a more desirable product [16]. Yeasts are the prominent organisms involved in wine production and determine important characteristics of the wine, including the flavour, by a range of mechanisms and activities [17]. Wines produced by selected yeasts have a better quality than those produced by spontaneous fermentation [18]. Ragi tapai is a consortium of microbes containing *Saccharomyces cerevisiae* and other microbe strains, which may result to inconsistent quality of the wine produced. Therefore, Baker's yeast is more preferred than ragi tapai for the production of wine from fruit juices.[4] This important microorganism converts sugars contained in the fruit juice to alcohol and organic acid, that later react to form aldehydes, esters and other chemical components [19]. *Saccharomyces cerevisiae* have adapted themselves in several important ways that enables them to break down their foods through aerobic respiration, but they do better that activity under oxygen deficient environment for a period of time through anaerobic fermentation [20].

Previous researchers have reported some studies on the use of Baker's yeast in wine making process. Reddy and Reddy [21] utilized Baker's yeast of 1% w/v in the production of mango wine from ripened mango juice by addition of sugar at 16-18% w/v to produce wine with 7-8.5% v/v alcohol content. Ban-Koffi and Han [22] fermented the juice of pineapple waste containing 11.7 % w/w of fermentable sugar using *Saccharomyces cerevisiae* 0.2% w/v with addition of sugar to a sugar concentration up to 14.4 % w/w to yield pineapple wine with 8% v/v of alcohol in 24 hours. Recently, Napahde *et al*. [12] reported star fruit wine making process using *Saccharomyces cerevisiae* without addition of sugar to produce carambola wine containing 8% v/v of alcohol. The latest report was from Sibounnavong *et al*.[4] with which they successfully made gooseberry wine from gooseberry juice using *Saccharomyces cerevisiae* with addition 17% w/v sugar to obtain 15% v/v of alcohol. Therefore, an investigation on the production of wine from high sugar content fruit juice such as clarified jackfruit juice without addition of sugar using Baker's yeast is necessary. The study becomes very interesting for promoting and adding value to this fruit. This article reports the effect of yeast inoculums rate and initial sugar concentrations on the production of jackfruit wine from clarified jackfruit juice using *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Materials: Freshly ripened jackfruit pulp was purchased from traditional market in Bandungan-Semarang, while Baker's yeast was obtained as a free donation from Inter-University Food and Nutrition Centre, Gadjah Mada University-Yogyakarta. Analytical grade chemicals (min purity 99.5% mole) were purchased from Sigma-Aldrich Singapore Pte Ltd. and used in this research without pre-treatment.

Method: Freshly ripened jackfruit pulps were washed using tap water. The fruit pulps were then cut into smaller pieces and juiced using laboratory scale juicer/juice extractor (Green Power Co. Model GP-E1503 Gold, Anaheim, CA, USA) to obtain seedless jackfruit juice. The extracted juice was clarified and filtered twice to remove the remaining pulp and other impurities. Di-ammonium phosphate was added into the clarified juice at 0.05% w/v prior to fermentation study.

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°C, 14 lbs/inch^2 for 20 minutes. After the medium cooled, *Saccharomyces cerevisiae* was transferred into the medium, incubated for 3 days at room temperature before use.

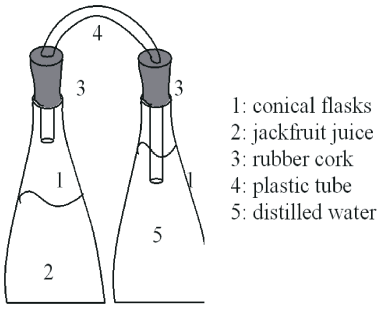

Fig. 2: Experimental set-up

Every sample of 100 mL of clarified jackfruit juice was pasteurized at 60°C for 30 minutes as suggested by Alobo and Offonry [23] and cooled. A starter of *Baker* yeast containing *Saccharomyces cerevisiae* strain was then transferred into each jackfruit juice sample and incubated in a conical flask fitted with a rubber cork under anaerobic condition at room temperature for two weeks. To prevent explosion by carbon dioxide produced from the fermentation, the conical flask incubator was connected to another conical flask containing distilled water using a bent plastic tube (Figure 2). After fermentation, the growth of the yeast was inhibited by boiling at 50-60°C for 60 minutes to terminate the fermentation activity and kept at room temperature until cool. The fermentation broth was then filtered through filter paper at the size of 0.4 microns to obtain clear yellow wine. Samples were collected for ethanol and sugar content analysis in each treatment. Effect of yeast and initial sugar concentrations and time on the fermentation process was investigated. Sugar concentration in the samples was determined by the Shaffer and Somogyi micro method [24]. Total sugars were analyzed by the method of Dubois *et al*. [25], while ethanol content was quantified chromatographically following the method developed by Antony [26].

|RESULTS AND DISCUSSION

Effect of Yeast Concentration: The profile of ethanol and sugar concentrations as a function of time at various yeast concentrations are presented in Figure 3 and 4. As expected, the increase in yeast concentration will increase the ethanol concentration and reduce the sugar concentration at all fermentation time. The reduction in sugar concentration indicated that some sugar contained in the jackfruit juice has been consumed by yeast as a substrate to grow and converted it to ethanol. Increasing yeast concentration from 0.5 to 1.0 % w/v resulted in significant increase in ethanol concentration and decrease in sugar concentration at all the time investigated. However, further addition of yeast concentration from 1.0 to 2.0 % w/v gave no significant changes in both fermentation parameters. This fact suggests that the number of yeast cells to be grown in the culture medium cannot be too many. Nidp *et al*. [27] 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. When the yeast cells concentration in the culture medium is too high, the competition amongst yeast cells to consume nutrients becomes very high. Surprisingly, Nagodawithana *et al*. [28] have also found that inoculating high level concentration of yeast cells during sugar fermentation at 30°C causing high cell death rate even though nutrients were not apparently limiting. In addition, high ethanol content as a result of rapid fermentation rate in the beginning of wine making process may inhibit further growth of the yeast cells. The use of 0.5% w/v of *Baker* yeast in jackfruit wine making seemed to be the best as ethanol concentration increased almost linear within legal standard specification of wine within the studied period of time [29].

In general, Figure 3 and 4 show that as the time go by, sugar concentration decreased and ethanol concentration increased almost linearly when 0.5% w/v of *Baker* yeast was used in the jackfruit wine making process. As can be seen in Figure 3, almost a half of the sugar has been consumed by yeast in 7 days. This phenomenon agrees well with that reported by Wang [30] where primary wine fermentation lasts for approximately one week; during that time most of the sugar originally presents in the juice is converted to ethanol and yeast cells, with the evolution of carbon dioxide. However, when higher *Baker* yeast concentrations were used, the rate of ethanol production and sugar consumption decreased after day 8 as indicated in Figure 3 and 4 by an inflection of the sugar and ethanol concentrations curves where ethanol concentrations were within 14.5-15.5% v/v. A slight curve inflection was also found in the fermentation using 0.5% w/v of *Baker* yeast at day 11, which coincided with ethanol concentration of 15.4 % v/v. This fact was likely to be caused by ethanol inhibition of yeasts [31]. Although ethanol is the major product of the oenological fermentation of fruit juice, it is inhibitory to the cells that produce it and constitutes a major stress factor during fermentation [32]. Wine yeasts in particular seem to be extremely tolerant of high ethanol levels, up to 12% (v/v) and more [33]. Ethanol inhibition stems mainly from its protein-denaturing properties. By diffusing freely

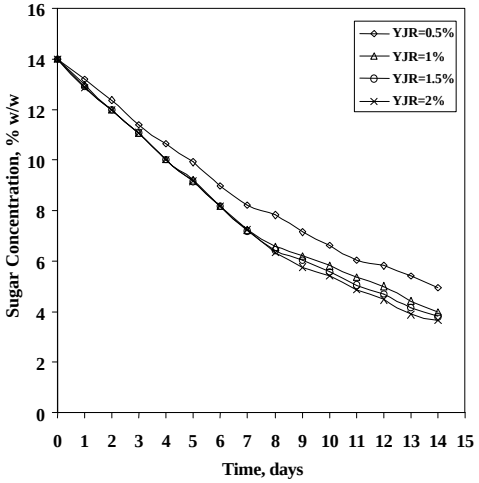

Fig. 3: Profile of sugar concentration as function of yeast concentration (YJR) and time

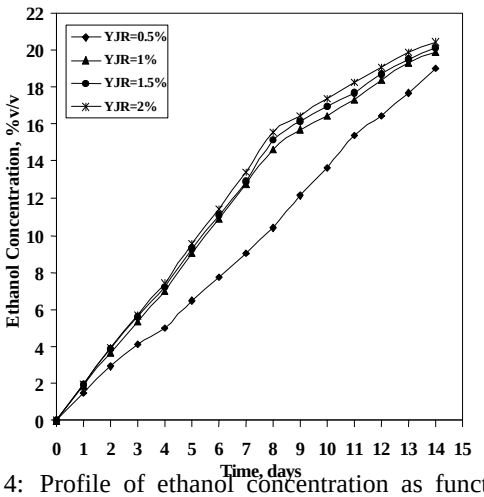

Fig. 4: Profile of ethanol concentration as function of yeast concentration (YJR) and time

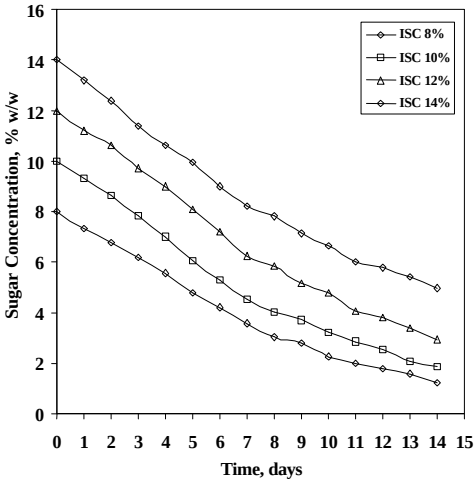

Fig. 5: Profile of sugar concentration as function of initial sugar concentrations (ISC) and time

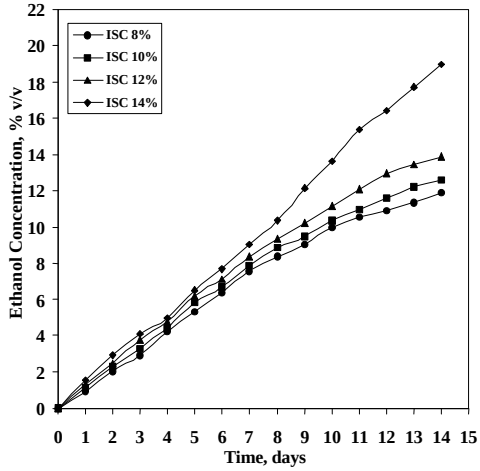

Fig. 6: Profile of ethanol concentration as function of initial sugar concentrations (ISC) and time

through the yeast plasma membrane, ethanol causes damage not only to membrane proteins and to the phospholipid bilayer, but also to intracellular enzymes and structures [34].

This preliminary study revealed that wine production from jackfruit juice by fermentation using *Saccharomyces cerevisiae* of *Baker* yeast can be done in less than two weeks and is faster than the natural fermentation which Kourkoutas *et al.* [35] reported the natural fermentation usually for 30 days. The use of 0.5% w/v *Baker* yeast was adequate to produce jackfruit wine where ethanol concentration of 12.13% v/v was obtained after 9 days of fermentation. While fermentation of jackfruit juice using *Baker* yeast concentration of 1.0 to 2.0 % w/v yielded jackfruit wines containing ethanol concentrations of about 12-13.5 % v/v in just 7 days. All the jackfruit wines obtained were clear yellow in colour and brought strong jackfruit aroma.

Effect of Initial Sugar Concentration: To investigate the possibility of producing jackfruit wine from low sugar content jackfruit juice, the effect of initial sugar concentrations on the production of jackfruit wine using 0.5% w/v *Baker* yeast was studied. The initial sugar concentration of jackfruit juices was varied by diluting the original jackfruit juice (14 % w/w sugar) to as low as 8% w/w sugar concentration. Figure 5 shows the profiles of sugar concentration as a function of initial sugar concentrations of the processed juice and time, while Figure 6 presents the profiles of ethanol concentration at various initial sugar concentrations of the processed juice and time.

It is clearly shown in Figure 5 that as the initial sugar concentration increased the residual sugar concentration also increased. This is because the fermentation rate was slower when high initial sugar concentration was used. This finding is in good agreement with Charoenchai *et al* [36] who reported that *Saccharomyces cerevisiae* gave decreased biomass growth rate at higher sugar concentration and Attri[10] who found a reduction of fermentation rate as a result of an increase in initial sugar concentration during the production of cashew apple wine. Strehaiano and Guma [37] also observed that high initial substrate concentration strongly inhibits the growth rate of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* during fermentation of sugar at 30°C. According to Nagodawithana *et al.* [28] this substrate inhibition may affect the rate of glycolysis, from which in turn may restrict the energy supply for the survival of yeast cells. This deficiency with respect to energy may ultimately cause severe death rate to the cell.

The indication of slow fermentation rate was further verified by calculating the value of conversion of sugar as the ratio of sugar concentration difference at a given time and the initial sugar concentration. As the initial sugar concentration in the jackfruit juice increased from 8 to 14% (w/w), the sugar conversion decreased from 0.56 to 0.42 after 7 days fermentation. In addition, the overall sugar conversion after 14 days fermentation decreased from 0.85 to 0. 65 as the initial sugar concentration increased from 8 to 14% (w/w).

Figure 6 shows that ethanol concentration increased with an increase in initial sugar concentration. A similar phenomenon was also reported for sugar fermentation using *Saccharomyces cerevisiae* isolated from fermented siahe sardasht grape pomace [38]. At lower concentration of sugar, the production of ethanol was associated with yeast growth only for a short period of time and hence will require less time to complete the fermentation. Pramanik observed that ethanol became inhibitory when its concentration reached about 14 % (v/v) [39], but in this research this phenomenon was not observed as low yeast concentration was used. In general, all the raw materials used could produce wine with alcohol content within legal standard specification of wine [29] after 14 days of fermentation. Unfortunately, the jackfruit wines obtained from fermentation of jackfruit juice with initial sugar concentrations of less than 14 % (w/w) had weaker jackfruit aroma.

CONCLUSIONS

From the experiments, it can be concluded that both yeast and initial sugar concentrations affected the production of jackfruit wine. Higher yeast and initial sugar concentrations were found to inhibit the growth of the yeast cell. The use of 0.5% w/v *Baker* yeast and original jackfruit juice with 14 % (w/w) sugar concentration was adequate to produce jackfruit wine where ethanol concentration of 12.13% v/v was obtained after 9 days of fermentation. No significant difference in ethanol concentration was observed when the yeast concentration was varied from 1.0-2.0 % w/v and wines with ethanol concentrations of about 12-13.5 % v/v were obtained in just 7 days fermentation. All the jackfruit wines obtained from fermentation of original jackfruit juice were clear yellow in color and brought strong jackfruit aroma.

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