Influence of sucrose and *Acetobacter aceti* addition to acetic acid content on coconut water vinegar

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Abstract. Vinegar can be made from coconut water through a fermentation process in two stages, namely alcohol fermentation used yeast in an anaerobic state and acetic acid fermentation using Acetobacter aceti in aerobic. The present work was to investigate the influences of sucrose addition on alcohol fermentation and *Acetobacter aceti* on acetic acid fermentation to the acetic acid and phenol content of product vinegar. Method of the experiment was used in a completely randomized design by factorial with two replications. The first factor was sugar concentration (A) consisting of 2 levels i.e 15% and 20%, whereas the second factor was the concentration of inoculum of *A. aceti* consisting of 3 levels i.e 10%, 15% and 20%. Observations determined for ethanol content, pH, specific gravity, acetic acid and phenol content. The results showed that ethanol and acetic acid contents tended to increase, and the pH value decreased, while the specific gravity almost constant. Then, for acetic acid fermentation was showed increasing the acetic acid content for all treatment in days 49, especially for the addition of 15% sucrose and 10% *A. aceti*.

1. Introduction

Vinegar contains phytochemicals such as organic acid, amino acids, and phenolic compounds. Because of this, vinegar can be used for preservative, seasoning sauce, mayonnaise and soft drink. In the house cooking, vinegar is widely applied in the dressing industry with enormous products (e.g. salad dressing, mayonnaise, ketchup, hot sauce) and in the food industry as flavouring agent [1]. However, the coconut water vinegar can be used for preservative to extend shelf life of chicken carcass. Besides, vinegar has been well-known and proved as the healthy beneficial drinking in various types and flavours [2]. Vinegar is a product of a mixed fermentation of yeast followed by acetic acid bacteria. Generally, the alcoholic fermentation involves two activities of yeasts to convert fermentable sugar into ethanol; and acetic acid bacteria oxidize ethanol into acetic acid in the second stage [3]. The first stages of fermentation for about 4 or 5 days produced alcohol (ethanol) used *Saccharomyces cerevisae* gave the characteristic flavour and aroma of vinegar. Then, it continued inoculated with mother vinegar containing acetobacter bacteria, i.e acetic acid fermentation. Acetic acid fermentation affected by the concentration of bacterial inoculum of acetic acid [4]. Pre-treatment has been made to the effect of *A. aceti* inoculum addition to the acid content. The addition of sucrose each of 15% (w/v), inoculum *A. aceti* was added as much as 10% and 15% (v/ v) showed the differences of acetic acid contents were 3.54% and 3.56%, respectively on the
44th days. It has been reported that the addition of sugar (sucrose) in coconut water will affect fermentation process as well as quality of vinegar [5]. The good quality of vinegar contains not less than 4% acetic acid [6].

The present work was to investigate influences of sucrose addition on alcohol fermentation and *Acetobacter aceti* on acetic acid fermentation to the acetic acid content of coconut water vinegar and also phenol content..

2. Materials and Methods

2.1. Microorganism

*Acetobacter aceti* FNCC 016 strain was bought from Food and Nutrition Culture Collection, PAU-UGM and was inoculated in *Glucose Calcium Yeast Extract Agar* (GCYEA) for preservation and *Plate Count Agar* (PCA) for calculating of *A. aceti* inoculum.

2.2. Fermentation

Fermentation process carried out two stages, i.e alcohol and acetic acid fermentation. Firstly, coconut water filtered by filter cloth, then filtrate was pasteurized at 63°C for 30 minutes. The coconut water filtrate as a raw material was used for fermentation process.

2.3. Alcohol fermentation

Alcohol fermentation was used from ITDI which has modified. Coconut water was filtered by filter cloth, continued pasteurized at 63°C for 30 minutes; furthermore 15% sucrose was added. After that, the substrate was cooled at room temperature and pour to the container. Subsequently, it was added 1% yeast, 0.03% (NH₄)₂HPO₄ and 0.5% (NH₄)₂SO₄. The container was closed and left for fermentation aerobically for 4 days. On the day 0 and day 4, it was taken sampling for determining alcohol content, total dissolved solids, specific gravity, pH and acetic acid content.

2.4. Acetic acid fermentation

*Acetobacter aceti* inoculum with concentrations ranging from 0.57-11.5 x 10⁷ CFU/ml was added to the substrate. The fermentation was done in room temperature for 49 days. After that, every 7 days, it was taken for sampling of product.

The research was conducted using Completely Randomized Design with two replications. The first factor is the concentration of sugar consisting of 2 levels ie 1: 15% and 2: 20%, whereas for the second factor is the concentration of inoculums addition of *A.aceti* consists of 3 levels ie 1: 10%, 2: 15% and 3: 20%. Observation was determined of ethanol content, total soluble solids, specific gravity, pH, acetic acid concentration (day 0 and days 4 fermentation alcohol), total polyphenols. The remark of treatment as followed:

SA 1 : Sucrose 15 % and *A.aceti* 10%
SA 2 : Sucrose 15 % and *A.aceti* 15%
SA 3 : Sucrose 15 % and *A.aceti* 20%
SA 4 : Sucrose 20 % and *A.aceti* 10%
SA 5 : Sucrose 20 % and *A.aceti* 15%
SA 6 : Sucrose 20 % and *A.aceti* 20%

2.5. Determination of ethanol content

The ethanol content was measured by GC (Gas Chromatography) which is equipped with FID detector at temperature 250°C, column DEGS, length 4 m, temperature programmed from 50-180° C/5° C/minutes. Carrier gas was nitrogen and hydrogen with flow rate 20–50 ml/minutes. Injection volume 1 μl.
2.6. Determination of Acetic acid by titrimetric methods [7].

2.7. Polyphenol content
The polyphenol content was carried out using the Folin-Ciocalteu method [7] with modification.

3. Results and Discussion

3.1. Characteristic of coconut water
Addition Characteristic raw material seen in Table 1. The value of total soluble solids gave 2.9°Brix, this is not sufficient to be used for alcohol fermentation. The value of total soluble solids gave 2.9°Brix, this is not sufficient for alcohol fermentation. Coconut water, which is shown the low °Brix value needs to be adjusted to 14 -22 °Brix by adding sucrose before the fermentation process [8]. Therefore, if the sugar content is too high, it will inhibit the growth of yeast cells because it increases the osmotic pressure of the substrate on cells [9; 10]. Sugar is one of the sources of carbon for microorganisms as the building material of the cell [11]. The type of sugars contained in coconut water i.e glucose, fructose and sucrose [12]. Sucrose will be hydrolyzed to glucose and fructose by S. cerevisiae with the invertase enzyme. Then, glucose will be assimilated into pyruvic acid through the pathway of glycolysis (Embden-Meyerhof-Parnas), while fructose firstly converted into fructose-6-phosphate which is a compound between the glycolytic processes [13]. While pyruvic acid will be fermented into ethanol and carbon dioxide with pyruvate decarboxylase enzyme [14].

| Characteristic         | Value |
|------------------------|-------|
| Total soluble solid (°Brix) | 2.9   |
| Acetic acid content (%)  | 0.109 |
| pH                     | 4.38  |
| Ethanol content (%)     | 0.546 |
| Specific gravity (g/mL) | 1.0096|

Acetic acid and pH values are also factors that influence the metabolism of the yeast S. cerevisiae during alcohol fermentation process. The optimum pH value of the alcohol fermentation substrate is between 3.8 and 4.5 [9].

3.2. Alcohol fermentation
The result of alcoholic fermentation showed that there was an increase of ethanol content from day 0 to day4 fermentation times (Table 2). It has been reported that one of the factors affecting sugar changes to ethanol is the amount of sugar in the substrate [15]. Wrasati et al. [16] also reported that ethanol content from the addition of 20% sucrose was gave higher than the addition of 15% sucrose with4 days alcohol fermentation time. The increased of sucrose addition will increase the amount of ethanol produced during sugar levels in the range of 9 and 17% [9; 10].

It can be seen that there was a decrease in specific gravity during alcoholic fermentation (Table 3). This is because of the breakdown of sugar into ethanol. Ethanol has a lighter density than water that allows for the decrease of specific gravity. Specific gravity of ethanol is about 0.79 g/ml [17]. Raikar [18], also reported that there was a decrease in the weight of grape juice during alcoholic fermentation.
due to the conversion of sugar to ethanol. The decrease in pH results from an increase in acetic acid levels in fermented substrates (Table 2).

Table 2. The alcohol and acetic acid content of alcohol fermentation in various treatment.

| Treatment                  | Fermentation time |          |          |
|----------------------------|-------------------|----------|----------|
|                            |                   | Day 0    | Day 4    |
|                            | Ethanol (%)       | Acetic acid (%) | Ethanol (%) | Acetic acid (%) |
| Sucrose 15 % and A. aceti 10% | 1.62              | 0.38     | 10.86    | 0.56           |
| Sucrose 15 % and A. aceti 15% | 1.52              | 0.38     | 13.62    | 0.58           |
| Sucrose 15 % and A. aceti 20% | 1.46              | 0.40     | 11.67    | 0.60           |
| Sucrose 20 % and A. aceti 10% | 1.45              | 0.45     | 13.91    | 0.63           |
| Sucrose 20 % and A. aceti 15% | 1.39              | 0.48     | 12.54    | 0.66           |
| Sucrose 20 % and A. aceti 20% | 1.48              | 0.47     | 13.01    | 0.68           |

Table 3. The specific gravity and pH of alcohol fermentation in various treatment.

| Treatment                  | Fermentation time |          |          |
|----------------------------|-------------------|----------|----------|
|                            |                   | Day 0    | Day 4    |
|                            | Specific gravity  | pH       | Specific gravity | pH   |
| Sucrose 15 % and A. aceti 10% | 1.041            | 4.59     | 1.008    | 3.88  |
| Sucrose 15 % and A. aceti 15% | 1.041            | 4.59     | 1.009    | 3.73  |
| Sucrose 15 % and A. aceti 20% | 1.016            | 4.64     | 1.007    | 3.85  |
| Sucrose 20 % and A. aceti 10% | 1.047            | 4.48     | 1.004    | 3.83  |
| Sucrose 20 % and A. aceti 15% | 1.044            | 4.50     | 1.004    | 3.90  |
| Sucrose 20 % and A. aceti 20% | 1.047            | 4.54     | 1.006    | 3.92  |

The pH decreased occurs during the alcohol fermentation process is caused by the generation of alcohol as the primary metabolite by yeasts. However, the acetic acid content was showing increasing with the length of the fermentation process. Total soluble solids ranged from 16.59 ± 0.19 to 20.08 ± 0.38°Brix on day 0, however, it was tended to decreased around 50% on the days 4. This is because of the change process sugar to alcohol.

3.3. Acetic acid fermentation
Acetic acid fermentation was showed the decreasing of pH from 4.01 to the lower 2.83 (Figure 1). The acetic acid content of vinegar coconut water was showing increasing for the length of the fermentation process. The highest content was from the treatment of the minimal addition of sucrose and A. aceti (15 % sucrose and 10% A.aceti). Herefore, the lowest was from the maximal addition of sucrose and A. aceti (20 % sucrose and 15% A. aceti).
Figure 1. Effect of fermentation time to pH of vinegar
SA 1 : Sucrose 15 % and \textit{A. aceti} 10%;  SA 4 : Sucrose 20 % and \textit{A. aceti} 10%
SA 2 : Sucrose 15 % and \textit{A. aceti} 15%;  SA 5 : Sucrose 20 % and \textit{A. aceti} 15%
SA 3 : Sucrose 15 % and \textit{A. aceti} 20%;  SA 6 : Sucrose 20 % and \textit{A. aceti} 20%

Figure 2. Effect of fermentation time to acetic acid content
SA 1 : Sucrose 15 % and \textit{A. aceti} 10%;  SA 4 : Sucrose 20 % and \textit{A. aceti} 10%
SA 2 : Sucrose 15 % and \textit{A. aceti} 15%;  SA 5 : Sucrose 20 % and \textit{A. aceti} 15%
SA 3 : Sucrose 15 % and \textit{A. aceti} 20%;  SA 6 : Sucrose 20 % and \textit{A. aceti} 20%

The addition of 10% \textit{A. aceti} inoculum was expected to create favourable competitive conditions for obtaining nutrients so that the acetate metabolism can take place optimally. Othaman \textit{et al.} [8], the addition of 10% acetic inoculums on fermentation of coconut water could be given the highest levels of acetic acid and the decrease pH value. The addition of 15% and 20% \textit{A. aceti} inoculums of this treatment was predicted to cause a high level of competition to obtain nutrients so that the metabolism of \textit{A. aceti} not optimal.

The total content of polyphenols from fermented vinegar was obtained ranging from 467.38 to 572.31 mgGAE/100 ml. This value was higher than the total content of polyphenols from the raw material of
coconut water (60.16 mgGAE /100ml). The higher of total polyphenol value might be occurred because of the addition of sucrose, which is being broken into glucose and fructose [19].

![Figure 3](image)

**Figure 3.** Polyphenol content of vinegar for 49 days fermentation.
SA 1 : Sucrose 15 % and *A. aceti* 10%; SA 4 : Sucrose 20 % and *A. aceti* 10%
SA 2 : Sucrose 15 % and *A. aceti* 15%; SA 5 : Sucrose 20 % and *A. aceti* 15%
SA 3 : Sucrose 15 % and *A. aceti* 20%; SA 6 : Sucrose 20 % and *A. aceti* 20%

4. Conclusion
The addition of sugar and *A. aceti* concentration on fermentation of coconut water vinegar was gave effect to the acetic acid content in vinegar. The best yield of acetic acid (4.29%) was given from the treatment of 15% sucrose and 10% *A. aceti*. Standard for acetic acid content in coconut water vinegar is 4%.

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