Production of coco-vinegar in a bubble biofermentor

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Abstract. The economic value of coconut water can be increased through its conversion into a functional drink of coco vinegar powder having antioxidant and anti-diabetic properties. The fermentation of coconut water into coco-vinegar is more beneficial and cheaper than the chemical routes. Nevertheless, to guarantee product stability and increase the fermentation rate, the fermentation process must be controlled. Bubble bio-fermentation is an applicable technique in upgrading the production scale and in preserving the coco vinegar quality due to its unique characteristics i.e.: simple design, fixed part, controlled flow and agitation, uniform space-time, larger contact area, lower energy input, larger capacity, and higher mass transfer rate. This research objective was to investigate the influence of the parameter process i.e. sugar and alcohol concentration towards the formation of acetic acid in the coconut water fermentation. The research showed that bubble fermentation of coconut water was proven as a potential method for the coco vinegar production process. The bubble bio-fermentation of coconut water conducted by the addition of 8% of sugar, 10% of ethanol and 15% of Acetobacteraceti inoculum and fermentation process for 21 hours was able to give a vinegar having acetic acid concentration up to 1.04%.

1. Introduction
Acetic acid fermentation is probably one of the oldest biochemical processes since the earliest known use of it can be traced back over 10,000 years ago [1]. Othman et al. [2] mention that a liquid contains a specified amount of acetic acid and fit for human consumption is the appropriate description for vinegar. Vinegar can be produced from any alcoholic material from alcohol-water mixtures to various fruit wines [1] [2]. Vinegar can also be produced from the water of coconut palm, botanically known as Cocos nucifera.

Samad et al. [3] stated that the sugar content of coconut water made it possible to be used as the material for vinegar production. Vinegar is usually used as a flavoring agent and food preservatives. Vinegar is commonly used for fruit and vegetable pickling in the preparation of food condiments. Nevertheless, the potential health benefits due to its bioactive components produce in the fermentation process has made it possible to be developed as functional food and drink. Soltan and Manal [4] mentioned that functional compounds found in vinegar such as organic acid, antioxidants, amino acids, and peptides are showing numerous health effects. Functional pharmacological properties of vinegar described include blood pressure reduction, antioxidant activity, antibacterial activity,
increased vigor after exercise, and prevention of cardiovascular disease. The antioxidant of various vinegar such as catechin and epicatechin can suppress oxidative stress significantly [1]. Several researchers are also reported that vinegar could act as an antidiabetic agent. Increasing population growth, urbanization, aging, and increasing prevalence of obesity and physical inactivity are considered as the causes of the appearance of diabetes in most countries. Diabetes is a metabolic disease that affects glucose, lipid and protein metabolism. Diabetes can lead to increased neuropathy, nephropathy, retinopathy, and cardiovascular mortality. In vivo tests of various vinegar showed the ability to reduce blood glucose significantly in the blood serum of diabetic mice [4].

Vinegar production methods can range from traditional methods to complex methods. The traditional methods are performed by employing wood casks and surface culture, meanwhile, the latest method is performed in a submerged fermentor [5]. Many technical devices and types of biofermentor have been developed to improve the industrial production of vinegar. Generally, these improvements increase the speed of the transformation of ethanol into acetic acid in the presence of acetic acid bacteria [1]. The fermentation of coconut water into coco vinegar is more beneficial and cheaper than the chemical routes. Nevertheless, to guarantee product stability and increase the fermentation rate, the fermentation process must be controlled. Bubble bio-fermentation is an applicative technique in upgrading the production scale and in preserve the coco vinegar quality due to its unique characteristics i.e.: simple design, fixed part, controlled flow and agitation, uniform space-time, larger contact area, lower energy input, larger capacity, and higher mass transfer rate.

Considering the advantages and uniques characteristics of the bubble fermentation process, hence this research was conducted to investigate the influence of parameter process i.e sugar and alcohol concentration towards the formation of acetic acid in the coconut water fermentation

2. Methods

2.1. Materials and apparatus
Coconut water was purchased from the local market in Semarang, Central Java. Meanwhile, the apparatus used in this research was a bubble biofermentor. The schematic of the bubble biofermentor is shown on figure 1.
2.2. Fermentation procedure

2.2.1. Acetobacter aceti inoculum preparation. 13 g of Nutrient Broth/NB was dissolved in 1 L of hot aquades and sterilized at 121 °C for 15 minutes. Two ose of 48 hours of Acetobacter aceti culture was inoculated in 10 ml of liquid medium and aseptically incubated at 37 °C for 48 hours. 10 mL of the culture was placed in an Erlenmeyer having 100 mL of liquid media and incubated at 37 °C for 48 hours. Then the culture was inoculated in 1000 ml of liquid media and incubated at 37 °C for 36 hours. The inoculum was then used for the fermentation process.

2.2.2. Coco vinegar fermentation. 10 liters of sterilized coconut water was loaded into the feed tank. A certain mass of sugar solution, ethanol, and inoculum was added to the feed tank. The sugar concentration was varied between 4-10%, the ethanol concentration was varied between 6-12% and the inoculum added was varied between 10-17%. The reaction mixture was then injected into the fermentor column. The samples were taken every 3 hours and analyzed for its acetic acid concentration.

3. Result and discussion

Vinegar is the result of the oxidative fermentation of ethanol by a genus of bacteria, Acetobacter. Therefore, vinegar can be produced from any alcoholic material from alcohol-water mixtures to various fruit wines. The raw material can contain either sugars or starch or both, starch and sugars by the process of double fermentation, alcoholic and acetous [1] [2]. In this research, vinegar was produced from the fermentation of water of Cocoa Nucifera which belongs to the family of Arecaceae (Palmae), an important member of monocotyledons. Within the cavity of the coconut, it can be found the juicy coconut water of the endosperm. Around 2 months after the natural opening of the inflorescence, coconut water begins to form [6]. Vigliar et al. [6] also mention that coconut water accounts for 25% of the weight of the fruit. The basic composition of the coconut water is 95.5% water. The other compound comprises 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorous, 0.5% iron, in addition to amino acids, vitamin C, B complex vitamins and mineral salts. In this research, the coconut water was added and mix with the sugar solution (4-10%), ethanol (6-12%) and inoculum of Acetobacter aceti (10-17%). The combination of the process parameter of the coconut water fermentation was tabulated in table 1.

| Formula | Sugar concentration (%w/v) | Ethanol concentration (%v/v) | Acetobacter aceti inoculum (% v/v) |
|---------|----------------------------|------------------------------|----------------------------------|
| A       | 4                          | 6                            | 10                               |
| B       | 6                          | 8                            | 12                               |
| C       | 8                          | 10                           | 15                               |
| D       | 10                         | 12                           | 17                               |

During the acetic acid fermentation, the ethanol will be converted into acetic acid by four consecutive reactions as follows: (i) formation of acetaldehyde, (ii) hydration of acetaldehyde, (iii) formation of acetic acid and (iv) electron transfer. The acetic acid concentration of the bubble biofermention of coconut water was shown in figure 2. Figure 2 showed that the highest acetic acid concentration was achieved from the fermentation conducted by the addition of 8% of sugar, 10% of ethanol and 15% of Acetobacter aceti inoculum. On 21 hours of the fermentation, the acetic acid concentration was found to be up to 1.04%. Meanwhile, the lowest acetic acid concentration was obtained from the bubble bio-fermentation conducted by the addition of 10% of sugar, 12% of ethanol and 17% of Acetobacter aceti inoculum.
The coco vinegar product obtained from the fermentation conducted by the addition of 8% of sugar, 10% of ethanol and 15% of Acetobacter acetii inoculum was also analyzed by using GC-MS. The spectra were shown in Figure 3. It was confirmed that the coco vinegar is having an acetic acid concentration of 0.95%. It was slightly different from the acetic acid value obtained by the volumetric analysis as shown in Figure 2.

Compared to the acetic acid of vinegar produced by traditional methods, the acetic acid of coconut water vinegar obtained from this bubble fermentation process is relatively high. Othaman et al. [2]
mentioned that 4 weeks of coconut water fermentation was able to produce vinegar of coconut water having at least 4% of acetic acid concentration. Meanwhile, Surana et al. [7] also mentioned that an acetic acid fermentation of coconut water for 8 weeks was able to give an acetic acid concentration of up to 3.319%.

4. Conclusion
Bubble fermentation of coconut water was proven as a potential method for the coco vinegar production process. The bubble biofermentation of coconut water conducted by the addition of 8% of sugar, 10% of ethanol and 15% of Acetobacter acetii inoculum and fermentation process for 21 hours was able to give a vinegar having acetic acid concentration up to 1.04%.

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