Preparation of Vinegar from Coconut Water Using Baker's Yeast and *Acetobacter aceti* TISTR 102 Starter Powder

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Abstract

In order to produce vinegar, coconut water was fermented through two stages: alcoholic fermentation with baker's yeast and followed by acetous fermentation with *A. aceti* TISTR 102 starter powder. The baker's yeast and the sugar concentration significantly effected on the alcoholic fermentation ($p \leq 0.05$). Baker's yeast at 0.4% (w/v) was added to 1,500 mL of coconut water at 12% (w/v) sugar content that adequately produced approximately 6% (v/v) ethanol concentration within 1 day. The ethanol was used as the substrate for acetification with *A. aceti* TISTR 102 starter powder. The addition of *A. aceti* TISTR 102 starter powder at 0.5% (w/v) completely produced 6.27±0.02% acetic acid within 18 days, thus attaining 89% fermentation efficiency. In the sensory evaluation test, the coconut water vinegar was rated with acceptable scores for all of the sensorial attributes (appearance, odor, sourness and overall acceptance). Vinegar from coconut water is one considered and application in household scale.

Keywords : *vinegar, coconut water, baker's yeast, A. aceti, starter powder*

1. Introduction

In tropical and subtropical regions, concretely in South-East Asia countries, coconut (*Cocos nucifera* L.) is available in several varieties with a relatively large planting area. It has provided a lot of substantial benefits for economy and human life. Among the edible parts of coconut, coconut water is notably used in processing and increasingly consumed because of its delicious taste and essential nutrients.

However, a large amount of coconut water in overly-mature coconut normally is disposed, up to 200,000 tons, after dehusking for production of coconut oil or coconut milk (Unagul *et al*., 2007). No matter how good in control and how much in utilization, the overly-mature coconut water are wasted with the number has been still increased year by year. Thereby, fermenting coconut water into vinegar is considered to be an attractive

means of utilizing waste from coconut industry. In the past, several researches into vinegar production from coconut water were carried out with complicated designed apparatus and longtime procedure (Truong and Marquez, 1987, Longtong *et al*., 1989). Besides, the nutrients in coconut water have been considered as ability to promote the growth of many microorganisms such as *Schizochytrium mangrovei* Sk-02 (Unagul *et al*., 2007) and *Lactobacillus confuses* TISTR 1498 (Seesuriyachan *et al*., 2011).

Vinegar is an acidic liquid containing acetic acid as the main component and made from fermentable carbohydrate source within two steps: alcoholic fermentation and acetification (Brian, 1998). Generally, the alcoholic fermentation involves to activities of yeasts to convert fermentable sugar into ethanol; and acetic acid bacteria oxidize ethanol into acetic acid in the second stage. Vinegar is normally used in house cooking and it is widely applied in dressing industry with enormous products (e.g. salad dressing, mayonnaise, ketchup, hot sauce) and in food industry as acidulant or flavoring agent (Huskin, 2008). Vinegar so far has been still used for preservative purpose (i.e. pickling method) and several useful applications in human living such as cleaning, sanitizing, and deodorizing (Perlman, 1976). Besides, vinegar has been well-known and proved as the healthy beneficial drinking in various types and flavors (Kim *et al*., 2012).

Generally, in fermentation, starter culture has been preferentially used for improvement in fermentation efficiency and for controlling the final products' qualities. The concept of starter culture in powder form (starter powder) has been introduced and recently available in market for conveniences in use, storage and preservation (e.g. baker's yeast). As the results, *Acetobacter aceti* TISTR 102 strain was also prepared in powder form for vinegar fermentation (Wongsudaluk, 2012). The use of starter powders, including baker's yeast for alcoholic stage and *A. aceti* TISTR 102 starter powder for acetous stage would be an convenient process for vinegar production in terms of fermentation time and application procedure. This research is also considered as guidance for applying starter powder in vinegar production outside laboratory, especially for household scale. Consequently, this result also could contribute to the diversity of the local vinegar in the South of Thailand, beside the traditional palm sap vinegar.

2. Materials and Methods

2.1 Coconut water

Overly-mature coconut water was collected from the market in Pattani province, Thailand, and was filtered through a thin cotton cloth. Commercial sugar (Milk Phor) was added to increase the sugar content in coconut water that then was boiled for 10 minutes.

2.2 Starter cultures
2.2.1 Baker's yeast

Commercial baker's yeast (Angel Yeast Gold Baker's) was bought from the local market was used in this research. The yeast concentration was 2.1×10^9 CFU/g.

2.2.2 Preparation of A. aceti TISTR 102 starter powder

Acetobacter aceti TISTR 102 strain was bought from the Thailand Institute of Scientific and Technological Research was inoculated in GYC agar (D-glucose, 100g/L; yeast extract, 10g/L; agar, 20g/L and calcium carbonate 10g/L).

The culture was incubated at 30°C for 24 hrs, subsequently stored at 4°C and subcultured every month to preserve the pure culture.

Acetobacter aceti TISTR 102 was cultured in coconut water with 50% (v/v) added banana juice for 18-24 hrs, 30°C and 120 rpm. The pellet (cells) was collected by centrifugation (Hettich Zencrifuhen ROTINA 420R) with 9600g at 4°C for 10 minutes and washed with phosphate buffer 50 mM 6.5 pH twice times. The washed cells were suspended into 20% (w/v) sucrose solution within 1 hr. The cell suspensions consequently were mixed with sterilized rice bran with ratio 4mL: 10g and dried at 35°C for 12 hours. After drying, the starter powder contained 1.26×10^7 CFU/g with 7.17 ± 0.17 (%) moisture content and 0.41 ± 0.18 water activity.

2.3 Experiments

2.3.1 Alcoholic fermentation

The pasteurized coconut water was transferred in 2,000 mL round bottom flask with approximately 1,500 mL working volume. Effects of baker's yeast concentration and initial sugar concentration on the fermentation process were consequently investigated. The commercial baker's yeast was varied into 0.2, 0.4 and 0.6% (w/v) and put directly into 14% (w/v) coconut water. The coconut water contained approximately 2.61 % sugar content that is not sufficient carbon source for fermentation. So, in this experiment, coconut water was adjusted into 12, 16 and 20% (w/v) sugar contents and fermented with 0.4% (w/v) baker's yeast. Fermentation process performed in round bottom flask fitted with cotton at room temperature (30±2°C) in 7 days. Samples were collected every day for ethanol and sugar analysis in each treatment. Consumed sugar was measured by phenol sulfuric acid method (Dubois *et al.*, 1956), while ethanol concentration results were obtained from Ebulliometer equipment (Wongsudaluk, 2012). The pH and total soluble solid were monitored using a digital pH meter (Schott Instrument, Lab 850) and refractometer (Atago N1), respectively.

2.3.2 Acetification

Coconut wine attained approximately 6% ethanol was subsequently applied for acidification process at different *A. aceti* TISTR 102 starter powder concentrations. The starter powder concentrations at 0.5, 1.0, 1.5 and 2.0% (w/v) were put directly into 1,500 mL coconut wine in 2,000 mL round bottom flask fitted with cotton. Fermentation process performed at room temperature (30±2°C) within 30 days. The samples were collected every 3 days to check the pH (Schott Instrument, Lab 850). Acidity was measured by titration with NaOH 0.1N (AOAC, 2000) and expressed in terms of acetic acid, while ethanol concentration results were obtained from Ebulliometer equipment. The fermentation efficiency (FE) for the optimal treatment was calculated as follow

$$FE = \frac{\% \, Acidity \left(\frac{w}{v}\right)}{\% \, Ethanol \left(\frac{v}{v}\right) x \, 1.043} \, x100 \quad (1)$$

2.3.3 Sensory evaluation

Coconut water was fermented into vinegar with baker's yeast and *A. aceti* TISTR 102 starter powder. The palm sap vinegar traditionally fermented within 3 months was bought in the market was the control sample. The quality of two vinegars including acidity, residual ethanol, total soluble solid and pH were determined.

The sensory evaluation for appearance (i.e. colour and turbidity), odor, flavor and overall acceptance was carried out with 9-point hedonic scale and 30 panelists between 20–35 year ages. Samples were served in clean transparent glasses (tumblers) which had been labeled with 3-digit random numbers. Questionnaires and water for mouth rinsing between each tasting were provided. Prior to evaluation, the panelists were asked to read through the questionnaires, and the meaning of each attribute (appearance, odor and sourness) was explained to the panelists to avoid any misinterpretation. The panelists were requested to use small spoons to take samples and taste the sourness. The scoring used was 1-dislike extremely; 2-dislike very much; 3-dislike; moderately; 4-dislike slightly; 5-neither like nor dislike; 6-like slightly; 7-like moderately; 8-like very much; 9-like extremely. The scores from all the panelists were expressed as mean ± standard deviation.

2.4 Statistical analysis

All experiments were carried out in triplicate, using a completely randomized design (CRD). The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3. Results and Discussion

3.1 Effect of baker's yeast concentration

The results of ethanol production and sugar consumption as fermenting the 14% (w/v) coconut water by different baker's yeast concentrations 0.2, 0.4 and 0.6% (w/v) were represented in Figure 1. Generally, at 14% (w/v) sugar content, the maximal ethanol content attained approximately 9 (%v/v) for all of the treatments. Baker's yeast at 0.2% (w/v) produced ethanol content that linearly increased within 3 days before stagnating during later time. Correspondingly, the ethanol contents were recorded with 3.64±0.08, 7.76±0.20 and 9.92±0.18 (% v/v). Likewise, the ethanol content increased sharply to 6.18±0.22 and 9.81±0.18 (%) within 2 days and statistically remained during later when coconut water was fermented at 0.4% (w/v) baker's yeast. Similar trend was found in with 0.6% (w/v) baker's yeast concentration, but the significantly higher ethanol contents were 7.57±0.24 and 9.86±0.10 (%), correspondingly.

The increase in ethanol content resulted in decrease in the sugar content. At 0.6% (w/v) baker's yeast, the sugar content sharply declined to 2.70±0.15 and 0.07±0.02 (g/100mL) within 2 days that then statistically stagnated. Also, a slightly decreasing trend was observed at 0.4% (w/v) baker's yeast with 6.19±0.58 and 0.13±0.01 (g/100mL) sugar contents were recorded after 2 fermentation days before being statistically constant in the further time. Otherwise, baker's yeast at 0.2% (w/v) took almost 3 days to completely consume sugar at 0.34±0.04 g/100mL. Similar trend was found in results of total soluble solid; while there was no significant difference in pH values of these treatments, exception in later on when fermentation had been completed (Data not shown).

With the maximal ethanol content attained approximately 9% (v/v) for all of the treatments, it was obvious to see that the baker's yeast concentration did not

influence on the ethanol content. However, it was a significant factor on fermentation process performance. The higher baker's yeast concentration was inoculated; the ethanol contents were produced in the shorter time, resulted in the higher fermentation rate. The fermentation rate was calculated by the quotient between the produced ethanol and the fermentation time required to reach that concentration. Therefore, the ethanol production rates were 3.31, 4.91 and 4.93 (% per day) when the baker's yeast inoculated at 0.2, 0.4 and 0.6% (w/v), respectively.
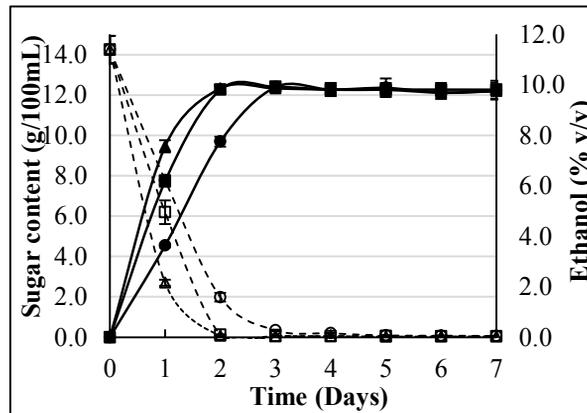
Figure 1. The profile of ethanol production (line with full symbol) and consumption sugar (dotted line with opened symbol) as the function of baker's yeast concentrations and time. Baker's yeast at 0.2% (circle), at 0.4% (square) and at 0.6% (triangle)

The similar trend was found in several previous studies with the yeast concentrations were less than 1.0% (w/v) (Akin-Osanaiye, 2008; Kumoro *et al*., 2012). The yeast concentrations more than 30.0 g/L or 3.0% (w/v) could cause the minor inhibition in the ethanol yield (Matloob, 2014). The explanation possibly due to the high propagation of yeast cells in fermentable substrate and their competition in nutrient consumption (Ndip *et al*., 2001). Additionally, the high ethanol content in the beginning of fermentation, which usually performed with the rapid fermentation rate, possibly caused negative effect on further growth of the yeast cells (Kumoro *et al*., 2012).

3.2 Effect of sugar content

The results of ethanol production and sugar consumption as fermenting coconut water at 12, 16 and 20% (w/v) with 0.4% (w/v) baker's yeast were represented in Figure 2. Obviously, there was a proportional relationship between the substrate concentration and the ethanol production. At 12% (w/v) sugar content, the ethanol content linearly increased to 6.27±0.14% within 1 day to complete fermentation as the ethanol content remained statistically constant during the later time. The coconut water at 16% (w/v) sugar content produced ethanol content up to 9.61±0.17% (w/v) within 2 days before stagnating in the further time. Otherwise,

the increase in ethanol production prolonged within 3 days to attain 12.37±0.06% ethanol content with coconut water at 20% (w/v) sugar content.

Obviously, the higher sugar content took longer time to be consumed completely by baker's yeast, and the increase in the initial sugar content also increased the residual sugar content. Coconut water contained 12.55±0.71 g/100mL sugar content that was completely consumed within 1 day and remained 0.44±0.00 g/100mL without any more consumption thereafter. Otherwise, at 16% (w/v) sugar content, baker's yeast took longer time to consume almost the sugar and metabolized into ethanol. Indeed, the initial sugar content was 16.11±0.46 g/100 mL that linearly decreased to 5.11±0.11 and sharply dropped to 0.25±0.01g/100mL within 2 days. At 20% (w/v) sugar content, a linear decline from 21.12±0.19 g/100mL sugar content to 2.41±0.49 g/100mL was also observed in the first two days. There was a slight decrease in the next day to 1.12±0.34 g/100 mL before almost completing fermentation at 0.41±0.02 g/100 mL. Similar trend was found in results of total soluble solid; and pH values also decreased in fermentation time, but the lower values were obtained with the increase in substrate concentration (Data not shown)
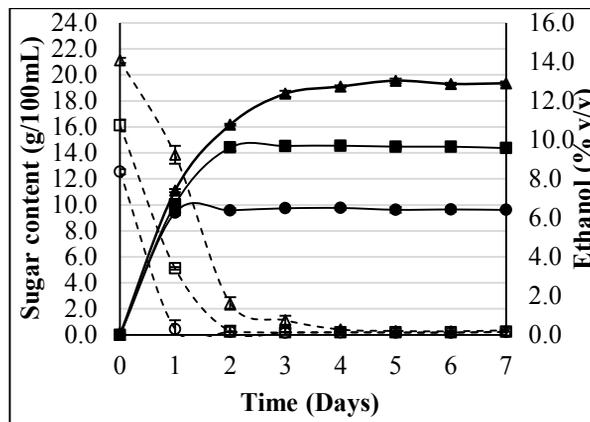
Figure 2. The profile of ethanol production (line with full symbol) and consumption sugar (dotted line with opened symbol) as the function of sugar contents and time. Coconut water at 12% (w/v) sugar content (circle), at 16% (w/v) sugar content (square) and at 20% (w/v) sugar content (triangle)

The higher substrate concentrations may achieve the higher ethanol production, but a longer incubation time was required for fermentation completion. As the results, the fermentaion rates 6.27, 4.80 and 4.12 (% per day) were calculated for fermentation of coconut water at 12, 16 and 20% (w/v) sugar contents, respectively.

The experimental results or the increasing trends in ethanol production strongly agreed to several previous researches (Asli, 2010, Kumoro et al., 2012). However, at the higher sugar contents up to 25% (w/v) were reported to cause decrease in ethanol production that could be explainedy by the osmotic effects (Charoenchai et al., 1998, Grahovac et al., 2012, Lin et al., 2012). The high substrate concentration might cause the osmotic shock that affected directly on the rate of

glycolysis-an important biochemical metabolism to support energy for the survival of yeast cells (Nagodawithana *et al*., 1974). In addition, possibly when the high ethanol concentrations formed and by-products accumulated, resulted in the pH change to cause inhibition (Lin *et al*., 2012).

3.3 Effect of *A. aceti* TISTR 102 starter powder concentration

The 6% (w/v) ethanol was proved to be the proper substrate for *Acetobacter aceti* TISTR 102 to grow well (Wongsudaluk, 2012). Therefore, the coconut water at 12%

(w/v) sugar content with 0.4% (w/v) baker's yeast performed in 24 hours to obtain approximately 6% (w/v) ethanol concentration that was the substrate for vinegar fermentation. The starter powder (*A.aceti* TISTR 102 strain) was consequently inoculated into alcoholic substrate at different concentrations of 0.5, 1.0, 1.5 and 2.0% (w/v) for acetificcation at 30±2ºC within 30 days. The results of ethanol consumption and acidity production were shown in Figure 3.
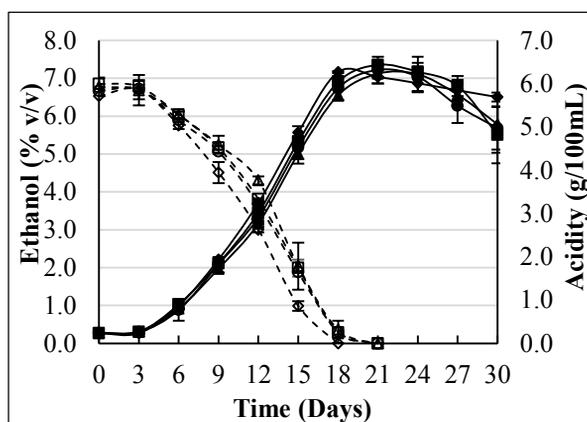
Figure 3. The profile of ethanol consumption (dotted line with opened symbol) and acetic acid production (line with full symbol) as the function of starter powder concentration and time. Starter powder at 0.5% (w/v) (diamond), at 1.0% (w/v) (square), at 1.5% (w/v) (circle) and at 2.0% (w/v) (triangle)

Generally, after inoculating starter powder, the ethanol content remained almost 3 days before sharply decreasing during the later time. In all of the treatments, the ethanol content intended to decline linearly that started from the 6th day onwards until the ethanol was run out. The treatment of 0.5% (w/v) starter powder showed the slightly higher consumption rate, which could be inferred from the significantly lower residual ethanol content at any sampling time. At any sampling time, there was no statistical difference in

residual ethanol content was obsevred among the treatments of 1.0, 1.5 and 2.0% (w/v) starter powder. Obviously, the ethanol was alomost run out after 18 days at 0.5% (w/v) starter powder. Otherwise, at the same time, the ethanol contents were 0.29±0.05, 0.31±0.18 and 0.37±0.23 (% v/v) at 1.0, 1.5 and 2.0% (w/v) starter powder, respectively.

The decrease in ethanol content increased the acetic acid content during the fermentation time. The acetic acid started to be produced after 3 days from inoculating

and also increased linearly before going to be stationary. Obviously, the treatment of 0.5% (w/v) starter powder produced the statistically higher acetic acid content as compared to those obtained from the other treatments at the same time. The acetic acid increased within only 18 days and the higher acid content was obtained at the lower starter powder concentration. Indeed, acetic acid 6.27±0.02% was recored at 0.5% (w/v) starter powder that was significantly different from 6.04±0.14, 5.89±0.27 and 5.73±0.12% obtained at 1.0, 1.5 and 2.0% (w/v) starter powder, respectively. Acetic acid content stagnanted for the further time, even declined at the end of fermentation process.

In all of the treatments, the pH decreased significantly within the first 18 days correspondingly when the acetic acid produced. The treatment that produced the higher acetic acid obviously had the lower pH values (Data were not shown). The final pH values were 3.23±0.00, 3.26±0.01, 3.31±0.01 and 3.36±0.01 at 0.5, 1.0, 1.5 and 2.0% (w/v) starter powder, respectively.

From the results, no changes in both consumed ethanol and produced acidity within the first three days possibly reflected the adaptation of the starter (lag phase) after inoculating into the fermenting substrate. This phenomenon also represented for disruption of yeast activity by *Acetobacter* biological oxidation (Claro *et al.* 2007) or intracellular pH changes (Valli *et al.*, 2005). The exponential phase could be observed when the acetic acid increased significantly via linear trend; and obviously every 1% (v/v) ethanol concentration could produce approximate 1% (w/v) acetic acid content. The optimal results of ethanol consumption and acetic acid produced at treatment of 0.5% (w/v) starter powder was possibly due

to the higher oxygen uptake during fermentation process. In fact, for starter powder production, rice bran played role as the carrier in which the bacteria suspension immobilized for drying and fermentation process. Therefore, in static condition, the starter powder inoculated into alcoholic substrate with more concentration had thicker sediment layer could prevent bacteria from oxygen uptake for their activity. As the results, the acetification rate attained 3.48 g/L/d and the fermentation efficiency was 89% with 0.5% (w/v) starter powder inoculation. The results in this experiment were considered to be comparable to previous studies with the short fermentation time, and relatively high efficiency (Kocher *et al.*, 2006; Sossou *et al.*, 2009).

3.4 Primarily sensory evaluation

Coconut water was fermented into vinegar with baker's yeast and *A. aceti* TISTR 102 starter powder for 20 days. It was a cloudy and slightly-yellow liquid with a distinct flavor; contained 5.71±0.26% acetic acid content, 0.3±0.10 (% v/v) residual ethanol, 5.4±0.2 °Brix and pH 3.11±0.03. The control sample was traditional palm sap vinegar that contained 4.5±0.50% acidity, 0.50±0.20 (% v/v) residual ethanol, 5.7±0.2°Brix and pH 3.09±0.02. The two samples were shown in Figure 4; while the sensory scores were represented in Figure 5.

In this study, coconut vinegar showed high acetic and to agree with the Thai Community Product Standard of fermented vinegar.

Appearance attribute of palm sap vinegar was rated at 7.10±1.56 that significantly preferred to the appearance of coconut vinegar with 5.77±1.54 scores. The scores of odor attribute were 6.03±1.90 and 5.19±1.80 for coconut vinegar and palm sap

vinegar, respectively. The coconut vinegar had 6.55±1.69 scores for sourness, while the sourness of palm sap vinegar attained 6.30±2.13 scores. For overall acceptance, the panelists accepted coconut vinegar and palm sap vinegar with the scores were rated at 6.48±1.23 and 6.41±1.72, respectively.

Figure 4. Coconut vinegar (left) and palm sap vinegar (right)
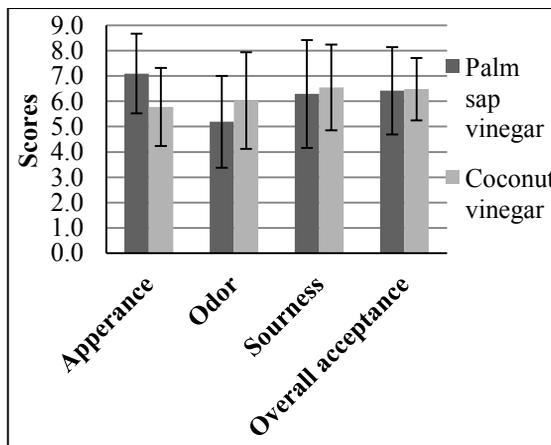
Figure 5. The sensorial scores of coconut vinegar and palm sap vinegar

The high standard deviation is unavoidable in sensory evaluation when the results were done by intuition/attitude of panelists about the samples. However, the lower standard deviation possibly reflects the more reliability on the results. In spite of significant differences in scores, the panelist rated slightly higher scores for coconut vinegar (exception appearance attribute) with the lower standard deviations as compared to the counterpart. The lower score for appearance was probably due to the turbidity and lower lightness as fermenting with starter powder and its carrier (rice bran). So, coconut vinegar produced by baker's yeast and starter powder possibly was comparable to traditional palm sap vinegar in sensory qualities. In the future, the clarification treatments should be studied to improve the appearance of the final product and the other researches on application of coconut

vinegar in foods also could be carried out for adding values and market expanding.

4. Conclusions

From the results, it was possible to produce vinegar from coconut water with baker's yeast and *A. aceti* TISTR 102 starter powder. The coconut water at 12% (w/v) and 0.4% (w/v) baker's yeast were adequate to produce approximately 6% (w/v) within 1 day for the later acetous fermentation. For acetification, the starter at 0.5% (w/v) sufficiently produced 6.27±0.02% acidity after approximately 18 days, attaining 89% fermentation efficiency. The coconut vinegar was evaluated with acceptable scores for most of attributes such as appearance, odor, sourness and overall acceptance. The obtained results possibly develop and add value to agricultural waste product by fermentation. Using coconut water to make vinegar also reduces the cost and contributes to diversify the local vinegar market.

5. Acknowledgement

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