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COCONUT WATER VINEGAR: NEW ALTERNATIVE WITH IMPROVED PROCESSING TECHNIQUE

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Abstract

Vinegar is a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation. Vinegar can be produced via different methods and from various types of raw material. A new alternative substrate for vinegar production namely mature coconut water has been tested and was compared with 2 common substrates which were coconut sap and pineapple juice. Substrates such as sap and juices have been found to have high amount of total soluble solids which corresponding to high sugar content in the substrates which is more than 14^{o}Brix. Therefore, both substrates could be directly used for vinegar production without requirement of other carbon sources. However, coconut water which showed low Brix value need to be adjusted to 14^{o}Brix by adding sucrose prior to the fermentation process. Substrates fermented with *Saccharomyces cerevisiae* have yielded 7-8% of alcohol within 7-10 days aerobic incubation at room temperature. The alcoholic medium were then used as a seed broth for acetic fermentation with *Acetobactor aceti* as inoculums and fermented for approximately 2 months to obtain at least 4% of acetic acid. Investigation on the effect of inoculum sizes and implementation of back-slopping technique were performed to improve the processing method for coconut water vinegar production. The results show that 10% of inoculum size was the best for acetic acid fermentation and the back-slopping technique has helped to reduce the process time of coconut water vinegar production.

Keywords: Coconut water vinegar, Ethanol fermentation, Acetic acid fermentation, Inoculum size, Back-slopping technique, Spray drying.

1. Introduction

The vinegar is described as "a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing either starch or sugars, or both, starch and sugars by the process of double fermentation, alcoholic and acetous, and contains a specified amount of acetic acid" [1]. Natural vinegar is a better food additive than synthetic vinegar as it carries essential amino acids from its fruit source and is reported to act as medicine for many illnesses. The acetic acid in vinegar elicits beneficial effects by altering metabolic processes in the gastrointestinal tract and in the liver [2]. For the substrates, fruit juices, plant sap, fruit musts, malted barley, rice and wine have been practiced [3]. Traditionally, vinegar has been applied as a food preservative as it inhibit the microbial growth and contributes to sensory properties to a number of foods such as sauces, mayonnaise, etc.

However, the production of natural vinegar is unfavorable among the manufacturers. Many of the producers refuse to produce natural vinegar due to several reasons such as the availability of the substrates and long fermentation time (6-8 weeks). Moreover, the price of synthetic vinegar is still much lower than natural vinegar in local market. Natural vinegar production has only been practised as a cottage industry in many states in Malaysia using various types of agro-based products and by-products as substrates such as coconut sap, nipah sap and matured fruit juice. There was no standard practice being followed by the local farmers and entrepreneurs thus, have resulted in interference of contaminants, non-hygienic processes, and un-standardized percentage of acetic acid in the marketed vinegar.

In Malaysia, coconut own large industry and has a fast-growing market. Malaysia is the 10^{th} largest coconut producer in the world with the production of 555,120 tons per year [4]. Although producing large quantity of coconut, it is still not sufficient to supply the local market especially during festive season. Thus, importing from foreign countries such as Thailand and Indonesia is one of the applying options. With a huge market size, the coconut industry leaves huge waste products to the environment including the mature coconut water. For that reason, mature coconut water has been tested for its suitability as vinegar substrate in comparison with 2 commonly used substrates namely the coconut sap and pineapple juice. The process of vinegar production has been improved to shorten the processing time as well as yielding a high concentration of acetic acid and thus resulting in a low cost and high quality production of vinegar from agricultural by-products. Study on the feasibility of spray drying process has also been performed in order to expand the vinegar products in the market and meet the industrial need. This study will lead to the diversification of coconut products and consequently will increase the income of local farmers and small entrepreneurs. Therefore, the objectives of this study were to investigate the suitability of mature coconut water as a substrate for vinegar production, to improve the production of coconut water vinegar and to study the feasibility of producing vinegar powder by spray drying.

2. Materials and Methods

2.1. Sample collection

Fresh coconut water (from mature coconut) was collected from selected local market. Sample was kept in sterile container and directly brought to laboratory. Pineapple was also bought from the same market and the juice was extracted and kept in sterile container. Coconut sap (nira) was collected from the cottage industry in Malaysia. To standardize the initial carbon source, all substrates were adjusted to a minimum of 14° Brix by adding sucrose and pasteurized at 90°C for 20 min prior to the fermentation.

2.2. Yeast and bacterial strain

Inoculum for the fermentation of vinegar has been obtained from *Andreas Fischerauer A-8212 Pischelsdorf, AUSTRIA*. The cultures for inoculums which consist of *Saccharomyces cerevisiae* and *Acetobacter acetii var*. Europeans were supplied as dry granules (*Saccharomyces cerevisiae*) and also in liquid culture (*A. aceti*) with the pH of 3.16 and 3.6% acidity of acetic acid. The cell concentration of *A.aceti* in the stock culture was approximately 7.93×10^7 CFU/ml (Colony forming unit per milliliter). The bacteria are active between the temperatures of 20°C-33°C.

2.3. Ethanol and acetic fermentation

Alcoholic seed broth was prepared through the fermentation of *S. cerevisiae* in coconut water, coconut sap and pineapple juice with the inoculum size of 1% (v/v). The inoculated substrate was incubated for 7-10 days at room temperature under static condition prior using it as a seed medium for acetic acid fermentation. After that, 10% of *A. aceti* stock culture was inoculated into the seed broth and incubated at the room temperature under static condition for 2 months. Samples were withdrawn at 1 month interval to quantify acetic acid concentration through titration assay. Proximate analysis was performed on all produced vinegars to obtain its profile.

2.4. Inoculum size on acetic fermentation

Study on the inoculum size for acetic fermentation was performed to elucidate its effect on the acetic acid production in coconut water medium. The inoculum was prepared by introducing 10% of *A. aceti* stock culture in fresh alcoholic coconut water medium and fermented for 3 days. The concentrations of inoculum were varied in the range of 5%, 10%, 15% and 25% (v/v) and were introduced in new alcoholic coconut water medium. All the samples were incubated at 30°C and shaken at 150 rpm for 4 weeks.

2.5. Back-slopping technique

A fresh pasteurized coconut water medium with brix value of 14° Brix was used as substrate. Approximately 10% of the previous fermented broth was used as a starter culture for the next round of alcoholic fermentation. The cycle was repeated until 3 rounds. The working volume of this experiment was 4 L per round. After inoculation, the substrate was left to ferment for 7–10 days. Samples were withdrawn and determined for pH changes and alcohol concentration. The alcohol concentration was measured by ethanol detection kit (Sigma Aldrich). For the acetic acid fermentation, the working volume used for this experiment was 2 L per round. Alcoholic media was taken from the second round of the back-slopped alcoholic fermentation (10 days) and used as a seed broth. The fermentation was performed at room temperature, in static condition and sampling was done to determine acetic acid concentration. The acetic acid concentration was measured by titration assay (0.5M NaOH, 10 ml sample volume).

2.6. Spray drying

A 100 ml of feed solutions (vinegar + encapsulant agents) were prepared accordingly as mentioned in Table 1. Spray drying was performed using a laboratory scale spray dryer (B-290, Buchi - Switzerland. The mixture was fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump rotation rate. The pressure of the compressed air was maintained at 3 bars throughout the experiment. Inlet and outlet temperatures were maintained at 150 and 80°C, respectively. Process yield was calculated from the relationship between the total solid content in the produced powder and the total solid content in the mixture.

Table 1. Combination of Encapsulation Agents for Freeze Dried Vinegar.

Run	Maltodextrin (%)	Arabic Gum (%)
1	10	1
2	10	2
3	10	3
4	20	1
5	20	2
6	20	3
7	30	1
8	30	2
9	30	3

2.7. Statistical Analysis

Each fermentation was carried out in duplicate and mean values ± standard deviations are reported. The MINITAB version 14 (Minitab Inc., PA, United States) was performed to evaluate statistical significance (level of $p < 0.05$) of differences between the substrates and to compare the means among the samples.

3. Results and Discussion

3.1. Proximate analysis

Vinegar fermentation is essentially a two-step process comprising the anaerobic conversion of sugars to ethanol and the aerobic oxidation of ethanol to acetic acid [5]. Alcoholic seed broths which were prepared from the fermentation of *S. cerevisiae* in coconut water, coconut sap and pineapple juice were used for acetic acid fermentation. A total 10% of *A. aceti* stock culture from *Fischerauer* was inoculated into all seed broths. After inoculation, all samples were incubated at room temperature under static condition. Samples were withdrawn after 4 weeks and 8 weeks to quantify the acetic acid concentration through titration assay (Fig.

1). Proximate analysis for all samples was performed before and after production of vinegar. Profiles of the proximate analysis are shown in Table 2.

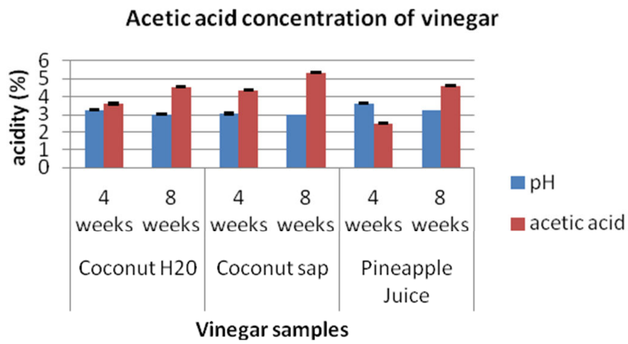

Fig. 1. Percentage of Acidity of Produced Vinegar from Local Substrates.

Table 2. Proximate Analysis of Vinegars and its Substrates.

	Coconut Water	Coconut Water vinegar	Coconut Sap	Coconut Sap vinegar	Pineapple juice	Pineapple vinegar
Moisture/water (%)	95.97	97.72	85.31	98.4	85.99	97.59
Fat (%)	0.06	0.142	0.084	0.162	0.117	0.102
Ash (%)	0.47	0.45	0.24	0.28	0.24	0.46
Protein (%)	0.51	0.16	0.2	0.18	0.54	0.38
Nitrogen (%)	0.097	0.026	0.032	0.029	0.087	0.061
Crude Fibre (%)	0.08	0.035	0.028	0.045	0.037	0.044
Carbohydrate (%)	2.91	1.493	14.134	0.933	13.076	1.424

From the literature, the chemical composition of the mature coconut water is 94.45% water, 0.15% fat, 0.47% ash, 0.52% protein, and 4.41% carbohydrate [6]. The proximate analysis that has been performed showed almost similar result as shown in Table 2. Due to the low carbohydrate content, coconut water was added with sucrose until 14^{o}Brix to support fermentation. Pineapple juice and coconut sap were also adjusted to 14^{o}Brix prior fermentation to standardize the initial carbon source. After the completion of vinegar production, the carbohydrate content for all substrates was in the range of 0.93 to 1.49%. No major changes were found in total percentage of fat, crude fiber and ash composition. However, there was a sign of reduction on protein after all substrates turned to vinegar. Result in Table 2 also shows no much difference in the chemical composition of coconut water vinegar in comparison to coconut sap vinegar and pineapple vinegar. This indicates that mature coconut water is a suitable substrate in vinegar making thus, have the good prospect on the environmental protection and can be included in the term of “waste to wealth” creation.

3.2. Acetic acid fermentation

The initial concentrations of ethanol prior acetic acid fermentation in all samples were between 5 – 7% with the pH ranging between 5.00-5.15 (data not shown). During acetic acid fermentation, ethanol will be oxidized to acetic acid by two-step reaction, the oxidation of ethanol to acetaldehyde, followed by the oxidation of acetaldehyde to acetate [7]. After acetic acid fermentation, the acidity of all samples reached between 2.45 – 4.36% (4 weeks) and 4.55 – 5.34% (8 weeks) and the pH lied between 2.98 – 3.64 (4 weeks) and 3.01 – 3.23 (8 weeks). As shown in Fig. 1, after 4 weeks of fermentation, coconut water vinegar showed higher acidity than pineapple juice vinegar but was lower than coconut sap vinegar. However, coconut water vinegar showed the lowest acidity among all samples tested after 8 weeks of acetic acid fermentation. This indicated that the natural sugar found in the pineapple juice and coconut sap is a better carbon source for the production of acetic acid than the sucrose that was added into coconut water. Although the lowest acidity was produced in coconut water, it still managed to pass the commercial standard of vinegar acidity which is 4% as mandated by the U.S. Food & Drug Administration (USFDA) [8].

3.3. Inoculum size

After discovering that the mature coconut water is a suitable candidate for vinegar substrate, the next strategy was to focus on how to improve the process of vinegar making especially in order to shorten the time of acetic acid fermentation and also maturation time. Two strategies that have been applied in this study are the application of the best inoculum size and implementation of the back slopping technique.

The influence of the concentration of inoculated cell line on acetic acid production and pH in shake flask by *Acetobacter acetii* was investigated by varying the inoculum size at 5, 10, 20 and 25% in the production medium. After 48 h of the alcoholic fermentation, *A. aceti* was then inoculated into the medium. The results shown in Fig. 2 indicated that at 5% of inoculum size, *A. aceti* was unable to produce high concentration of acetic acid as compared to other inoculum sizes. While at 10% of inoculum size, the highest concentration of acetic acid was produced. The same trend of pH reduction was observed in which 10% of inoculum size has shown the highest pH reduction (3.23 ± 0.06) as compared to initial pH at 5.13. The concentration of acetic acid at 20% inoculum size was lower than 10% of inoculum size and at 25% of inoculum size, the concentration of acetic acid was lower than 20% of inoculum size. This reduction was associated to the capability of *Acetobacter* species which could have oxidized acetic acid to water and carbon dioxide during the depletion of ethanol [9]. Therefore, 10% inoculum size is considered as the best inoculum size for acetic acid fermentation of coconut water vinegar.

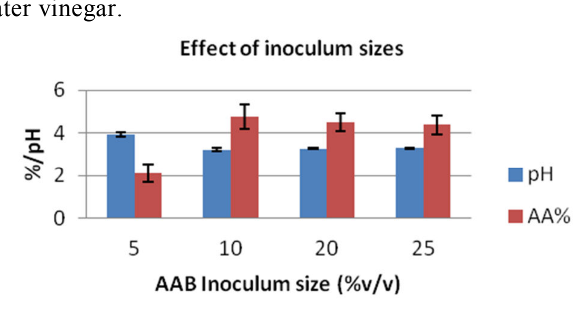

Fig. 2. Effect of Inoculum Size on pH and Acetic Acid Concentration.

3.4. Back-slopping technique

In order to ensure the continuity production of alcohol, the alcoholic fermentation was run repeatedly. Instead of using freshly prepared inoculums for each fermentation round, the back-slopping technique was applied. Approximately 10% of the previous fermented broth was used as starter culture and each round was fermented for up to 10 days. Figure 3 shows the result of alcohol concentration from 3 rounds of back-slopping of alcohol fermentation. Approximately, 5-6% of alcohol concentration was obtained in every back-slopping cycle. In all round, alcohol was still being produced after 7 days of fermentation. Alcohol concentration was continued to increase after every round of fermentation. In third round, the concentration of alcohol reached 6% after 7 days of fermentation instead of 10 days in the first round of alcoholic back-slopping fermentation. Thus, the back-slopping technique served as a better approach in producing fast and high alcoholic seed broth for acetic acid fermentation in vinegar production rather than using freshly prepared inoculums.

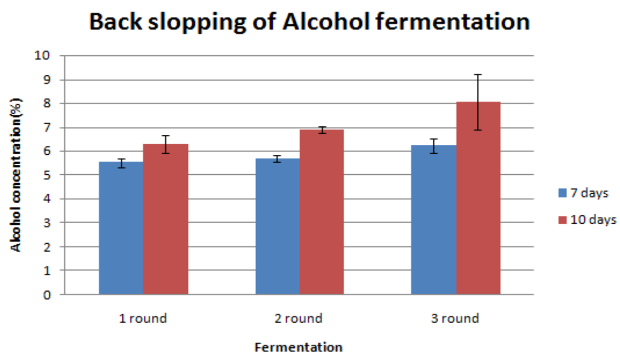

Fig. 3. Back-Slopping of Coconut Water into Alcohol Seed Broth.

A back-slopping technique for acetic acid production has also been adopted. Approximately 10% of the previous fermentation broth or vinegar was used as the starter culture. Figure 4 shows the percent of acidity through acetic acid fermentation resulting from the first round and the second round of back-slopping technique. It was observed that in the second back-slopping fermentation, higher concentration of acetic acid was produced when compared to the first round of acetic acid back-slopping fermentation. The concentration of acetic acid in the vinegar reached 4% after 6 weeks of fermentation in the first round of acetic acid back-slopping fermentation, while more than 6% of acetic acid was obtained within 4 weeks of fermentation during the second round. This suggests that the acetic acid from the first round of acetic acid back-slopping fermentation might gave the booster effect and enhanced the production of acetic acid in the second round of acetic acid back-slopping fermentation. According to Holzapfel (2000), the inoculums from the previous batch of fermented medium contains large numbers of desirable microorganism in an active state, thus accelerate the fermentation process [10]. The back-slopping technique has been practised in numerous tradition processes in which material from a previous successful batch was added to facilitate the initiation of a new process. Thus, shortened the time for fermentation process and reduced the risk of fermentation failure [9].

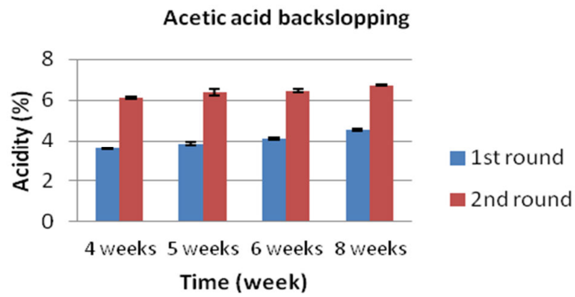

Fig. 4. Back-Slopping for Vinegar Production with Alcohol Seed Broth from Coconut Water.

The end product of coconut water vinegar was tested for the feasibility of spray drying process. Spray drying with encapsulant agents may promote protection to the volatile compound against adverse condition such as heat and reduce the hygroscopicity of the powder. Figure 5 shows the combination of 2 encapsulant agents that was tested in this experiment and the process recovery.

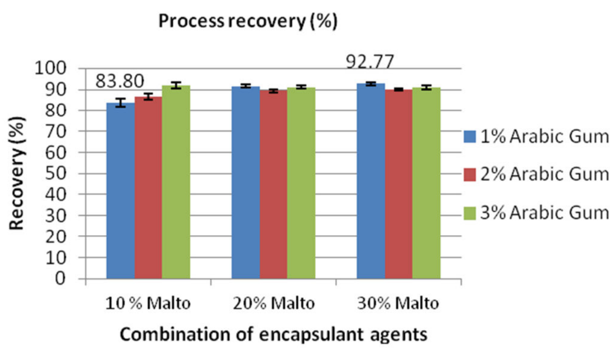

Fig. 5. The Process Recovery (%, Per Mixture Weight) for Each Formulation of Spray Dried Vinegar.

The process recovery for each of the formulation was ranging from 83.80 to 92.77%. The process recovery was not significantly affected by the addition of different concentration of gum Arabic. On the other hand, the use of maltodextrin has influenced the process recovery. The highest recovery (92.77%) was obtained with the addition 30% of maltodextrin and 1% of gum Arabic into the vinegar suspension before the liquid feed was atomized into hot-medium drying chamber. Theoretically, maltodextrin is added to help the pulverization of the vinegars as gum Arabic is added to prevent moisture-absorption of the vinegar powder and thus, enhance the stability of the powder [11]. However, through the observation made during sample collection, the higher the maltodextrin content, the more powder stuck to the drying chamber thus, resulted in the reduction of powder recovery. As reported by Nurhadi et al. [12], the addition of filler (maltodextrin) to encapsulate honey by spray drying had decreased its Tg, thus increased the stickiness of the feed sample. This experiment showed that the spray drying of coconut water vinegar is feasible with the process recovery was more than 80%. Nevertheless, to ensure the survival of spray dried vinegar product in the market, more analyses such as analysis of moisture, acetic acid content and vinegar powder stability need to be done.

4. Conclusions

The production of vinegar from mature coconut water supplemented with sucrose was studied. The following conclusions were drawn from the analysis.

- Mature coconut water is a suitable alternative substrate for the vinegar production.
- The process has been improved by using 10% inoculum size in acetic acid fermentation with the application of back-slopping technique in alcoholic and acetic acid fermentation.
- The fermentation time was shortened where the concentration of alcohol reached 6% in 7 days and acetic acid concentration reached 4% in 4 weeks.
- The spray drying of the coconut water vinegar is feasible with the highest recovery was attained at the optimum formulation of 30% maltodextrin and 1 % gum Arabic for the encapsulation purpose.

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