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Effect of -Amylase and Glucoamylase Enzymes on Chemical Qualities of Vinegar from Banana Peel

Effect of α-Amylase and Glucoamylase Enzymes on Chemical Qualities of Vinegar from Banana Peel

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Abstract. The rate of banana consumption in Indonesia is reported to be increased. Abundant banana peels causes environmental problem. Peels of banana still have the potential to be utilized in the making of vinegar. Banana peels have high contents of fiber and carbohydrate, however breakage of polysaccharide bonds with enzyme is necessary. The α-amylase and glucoamylase are suitable enzymes to optimally break the polysaccharide bonds. The objective of this research was to determine the effect of α-amylase and glucoamylase on chemical qualities of banana-peel vinegar. The experimental design used was a Block Randomized Design with two factors and three replications. The 'A' factor was concentration of α-amylase at three levels (0.05%; 0.1%; 0.15%). The 'B' factor was concentration of glucoamylase at two levels (0.1%; 0.15%). Observation parameters included reducing-sugar, concentration of alcohol and acetic acid, and pH values. The high level of acetic acid was obtained from A_2B_1 (0.10% α-amylase; 0.10% glucoamylase) with an average value of 4.05% and pH of 3.52 on day-30 of fermentation period. An average value of reducing-sugar level on day-1 was 0.55% (g/ml) accompanied by alcohol content reduction of 0.386%. The best quality of banana-peel vinegar was resulted from the A_2B_1 treatment (0.10% α-amylase, 0.10% glucoamylase). Good quality vinegar was characterized by its aqueous liquid form, acetic acid odor with level at least 4%, residual alcohol at maximum level of 10%. The study has implications for the utilization of banana peel waste as natural preservatives.

1. Introduction

Vinegar is liquid containing acetic acid and used for food additives serving as both preservatives and marinade. Vinegar is condiment made from ingredients containing sugar or starch through alcohol or lactic acid fermentation [1]. Raw materials used for vinegar making may be classified into three groups, i.e. low-sugar materials (fruit juices), high-sugar materials (molasses, honey, syrups, corn), and high-starch materials (cereals, roots) [2]. Banana peels of Nangka variety are one of raw materials potentially applied in vinegar making for their high content of carbohydrate. Banana peel contains 59 % carbohydrate, 0.9 % protein, 19.2 mg/g calcium, 24.30 mg/g sodium, 0.61 Fe [3]. In addition, banana peels of Nangka variety is industrial waste of banana chips.

The process of making vinegar requires enzyme to hydrolyze carbohydrate into glucose before it is converted into alcohol by yeast. Carbohydrate bonds can be hydrolysed by α-amylase and glucoamylase. α-amylase hydrolyzes α-1,4 glycosidic linkages of amylose, amylopectin, and glycogen to form glucose and maltose while glucoamylase almost completely converts starch into D-glucose.

Conditions affecting activities of these enzymes, among others, are concentration of the enzymes and substrates, level of acidity, and temperature of reaction.

Two-step fermentation of vinegar making consists of alcoholization process in which there is conversion of sugar contained in the solution into ethanol using yeast and the following transformation of ethanol into acetic acid is performed by a static culture of acetic acid bacteria [4] called as acetification process. One of yeast able to be used in the alcoholization process is *Saccharomyces cerevisiae.* This yeast is widely applied in alcoholic fermentation for its high reproduction, resistance in high alcohol and glucose levels, and temperature activation range of at 4-32ºC. The best condition for *S. cereviseae* is anaerobic at 30ºC and pH 4.0-5.0. *Saccharomyces cerevisiae* reproduces under the presence of simple sugar such as glucose, sucrose, maltose, fructose, galactose, and maltoriose [5]**.** The inoculum percentage of two commercial strains of *S. cerevisiae* used as a starter for producing ethanol and acetic acid ranges from 15 to 30% [6]. While Saha and Banerjee [7] have used *S.cerevisiae* inoculum of 10% to produce vinegar from banana, the use of a small inoculum size of *S.cerevisiae* for making vinegar from coconut water at 1% [8].

Acetic acid constitutes a major component contained in vinegar produced from acetification process. In this fermentation, acetic acid producing-bacteria starters usedare *Acetobacter aceti*. This bacterium is mesophilic and grows at an optimum temperature of 25-30ºC and pH of 5.0-6.5. However some strains are able to grow at pH 2-4 [9]. *Acetobacter aceti* oxidizes ethanol into acetic acid. In a longer fermentation time, acetic acid is further oxidized into CO_2 and H_2O.

Vinegar may be made through one of two common fermentation methods, i.e. gradual and simultaneous fermentations. During gradual fermentation, alcoholization and acetification processes are performed separately whereas during simultaneous fermentation those processes are performed continuously in a reactor. This study was aimed to investigate the effects of α-amylase and glucoamylase enzymes on characteristics of vinegar made from banana peel of Nangka variety employing simultaneous fermentation.

2. Materials and methods

2.1 Material

Materials used in this study comprised of raw materials, supporting materials, and chemical materials. The raw materials were banana peels of 'Nangka' variety. Supporting materials included sucrose, α-amylase (NOVO), glucoamylase (NOVO), *S. cerevisie* and *A. aceti*; which were isolates of cacao pulp (collection of Microbiology Laboratory of Indonesian Center for Agricultural Postharvest Research and Development), ammonium phosphate and ammonium sulfate. Chemical materials included medium for microbial growth and materials for chemical analysis. Medium used were yeast extract, peptone, glucose, calcium carbonate, Mannitol Egg Yolk Polymixin Agar (MYP Agar), Potato Dextrose Agar (PDA), Plate Count Agar (PCA), and natrium chlorida (NaCl). Chemical materials for chemical analysis included NaOH 0.1N, phenolphthalein indicator 0.1%, DNS (Di-Nitro-Salicylic) solution, buffer phosphate solution, concentrated H2SO4, amylum indicator 0.1%, Na2S2O3 0.1 N, Luff Schrool solution, etc. Laboratory equipments used consisted of analytical balance, thermometer, autoclave, laminar air flow, micropipette, pH-meter, hot plate, incubator, colony counter, blender, UV-VIS and GC, glassware for chemical and microbial analyses as well as cooking ware (knifes, cutting boards, basins, containers, pots, gas stoves, spatula, sieves, funnels, and bottles).

2.2 Method

The process of vinegar processing from banana peel of Nangka variety was carried out using a modification of vinegar processing made from banana peels according Santoso (1995) [10]**.** The process was undertaken in several stages, i.e. (i) starter preparation, (ii) material preparation, (iii) vinegar-making process (cooking I, cooling I, grinding, sieving, cooking II, cooling II, fermentation I, fermentation II, sieving II, pasteurization).

During preparation of *S. cerevisiae* starter, the culture was first activated on PDA medium with pour plate method before being incubated at ambient temperature (25-30ºC) within 48 hours. Liquid medium as a propagation medium was used for a starter. One liter of liquid medium required 10 grams of yeast extract, 20 grams of peptones, and 20 grams of glucose diluted in aquadest and sterilized at 121ºC for 15 minutes. Once it was warm, yeast was poured and the medium was incubated at ambient temperature (25-30ºC) for 48 hours. Growth of *S. cerevisiae* was indicated by turbidity and strong, fresh yeast aroma of the medium. Preparation of *A. aceti* growth medium is as follows. One liter of liquid medium required 10 grams of yeast extract, 3 grams of glucose, and 10 grams of calcium carbonate diluted in aquadest and sterilized at 121ºC for 15 minutes. Propagation process conducted followed the *S.cerevisiae* propagation method. Growth of *A. aceti* was indicated by sedimentation, acidic and fresh aroma of medium.

In the process of material preparation, banana peels were stored in anaerobic condition for 24 hours before winded aired (24 hours) to stop pre-fermentation process. The Nangka banana peels were then cut into pieces and washed thoroughly. In the step of vinegar making, fermentation was performed simultaneously. The first fermentation using yeast occurred within 24 hours anaerobically. Subsequently, acetic acid bacteria were added without removing the solution which had contained alcohol until acetic acid was formed. Banana peels were boiled using a comparison of 1:1.5 (peels:water) within 1 hour at 100ºC with addition of α-amylase. Once they were cold, they were ground using a blender, filtered using filter cloths, followed by plastic-sieved. Filtrate then was heated for 10 minutes at 60ºC along with addition of sucrose, ammonium phosphate, and ammonium sulfate. It was alcoholic-fermented using 10% of *S.cerevisiae* at 30ºC for 24 hours anaerobically. Results from fermentation I then was further fermented using 10% of *A. aceti* aerobically at 30ºC for 30 days. This step was aimed to stop the fermentation process, inactivate enzymes, and destroy fungus/contaminants that may keep growing in vinegar.

Parameters of observation on the Nangka banana-peel vinegar included alcohol content, reducing-sugar content, acetic acid content, and pH value. The alcohol content was measured using GC on the basis of detectable ethanol gained from comparing retention time of sample and retention time of ethanol standard, calculated using equation bellow.

$$\text{Ethanol content (\%)} = \frac{\text{Width of sample area}}{\text{Width of standard area x Standard (\%)}} \quad (1)$$

The ethanol content of reducing sugar was calculated using DNS (3,5-dinitrosalicylic acid). In an alkali condition, reducing sugar reduces DNS forming compounds of which absorbance are able to be measured at wavelength of 550 nm. The content of acetic acid was determined using alkalimetric method. A 10 ml of sample solution was reacted with 0.1 N NaOH solution and phenolphthalein indicator. The mixture was titrated until a pink color was detected. Titration result was expressed as volume of NaOH reacting with the sample. Acetic acid content was calculated using formula bellow.

$$\text{Acetic acid content (\%)} = \frac{\text{(Vol.NaOH (ml) x N of NaOH x MW of NaOH x 100)}}{\text{(Vol. Sample (ml) x 1000)}} \quad (2)$$

2.3 Experimental design and statistical analysis

The research was designed using a Block Randomized Design with two factors and three replications. The A factor was α-amylase concentration (0.05%; 0.1%; 0.15%), while B factor was glucoamylase concentration (0.1%; 0.15%). Experimental results obtained were analyzed using Analysis of Variance involving two factors and three replications. Significant difference obtained allowed treatments to be further tested using Duncan Multiple Range Test (DMRT) method.

3. Result and Discussion

3.1 Reducing sugar concentration

Based on statistically analysis the treatments were affecting ($P<0.05$) the concentration of reducing-sugar. Observation results on reducing-sugar of all treatments on day-0 showed range values of 1.76-3.92% (Figure 1). These data suggested that the reducing-sugar content was relatively high in A_3B_1 (3.92%) which was a combination between 0.15% of α-amylase and 0.10% of glucoamylase concentrations. On day-0, the reducing-sugar content used to increased with an increased α-amylase level and glucoamylase level.

Amylase and glucoamylase may be used together to convert starch to simple sugars or reducing-sugars [11] such as glucose, also reducing disaccharide like maltose. The simple sugar such as glucose can be used to produce high fructose syrups or may become feedstock for bioethanol production [12].

Based on Figure 1, the alcoholic fermentation process underwent anaerobically in 24 hours on day-1. The 10% of *S. cerevisiae* was added into each treated sample in order to convert reducing- sugar (primary glucose) into alcohol. On day-1, reduction of reducing-sugar level occurred with range values of all treatments were 0.72-1.54%. The higher level of amylase and glucoamylase comparable with the reducing sugar level. In certain condition, may generate the contrast value. Hydrolysis time is one of the important factors for banana peel hydrolysis that effected to reducing sugar yield [13]. The optimal conditions for s α-amylase worked best at pH 5.5 and 75 ^{0}C [14] and glucoamylase worked best at pH 5.0 and 55 ^{0}C [15].

On day-30 of fermentation period, reducing-sugar levels were observed to find out the remaining glucose level used by *S. cerevisiae* as its nutrient and its substrate for alcohol conversion. Levels of reducing-sugar continuously decreased as both enzymes completely broke down all polysaccharide bonds and *S. cerevisiae* completely used up simple sugar for alcohol production. The range values of reducing-sugar levels were 0.03-0.34% (Figure 1). Data suggested that the A_2B_1 treatment (0.10% α-amylase, 0.10% glucoamylase) resulted in the lowest level of reducing-sugar (0.03%). Hence, the ratio of enzyme concentrations yielding in the lowest level of reducing-sugar after 30 days of fermentation period was 1:1.

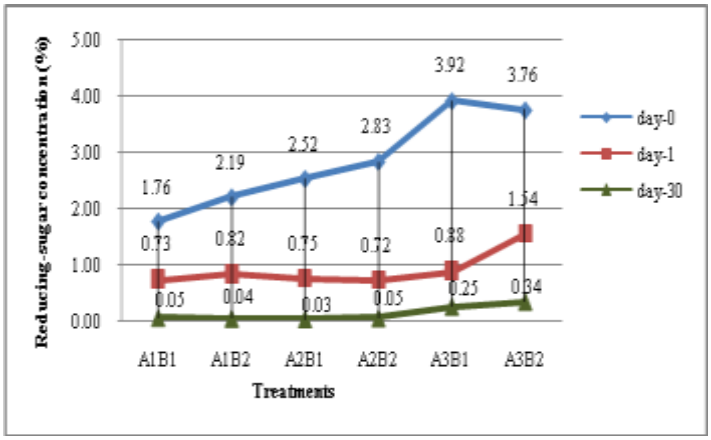

Figure 1. Reducing-sugar concentration of banana peel vinegar from different treatments

3.2 Alcohol concentration

The statistic analysis showed that the treatments were affecting ($P<0.05$) the alcohol contens of vinegar. Observation results on alcohol contents of treatments on day-0 showed the range values of 0.032-0.090% (Figure 2). Low concentration of alcohol found on day-0 possibly was derived from natural alcohol contained in banana peels. Based on [3]**,** one kind of substrate which contains alcohol is fruits. On these substrates, the alcohol and sugars are incompletely oxidized.

On day-1, the alcohol content increased after anaerobic fermentation using *S. cerevisiae*. The treatments showed range values of 0.313-0.779% with the highest alcohol content was obtained from A_3B_2 (treatment of 0.15% α-amylase; 0.15% glucoamylase). Addition of 10% sucrose during vinegar making was in order to optimize the nutrient availability for *S. cerevisiae* to grow so that it was capable to form a large amount of alcohol. Fermentation process through glycolysis pathway breaks glucose into pyruvic acid. Tthe pyruvic acid then is decarboxylated into acetaldehyde, and this acetaldehyde is reduced into ethanol (alcohol) and carbon dioxide [16].

Data on day-30 showed that all of treatments underwent a decrease in alcohol contents to 0.122-0.401%. The low alcohol content was obtained from A_1B_1 (0.122%) and A_2B_1 (0.125%). Average values of alcohol contents decreased during the fermentation process due to alcohol breakage into acetic acid by *A. aceti* in an acetic acid fermentation. The chemical reaction is showed that one molecule of alcohol or etanol (C_2H_5OH) will produce two molecules of CO_2 and three molecules of H_2O.

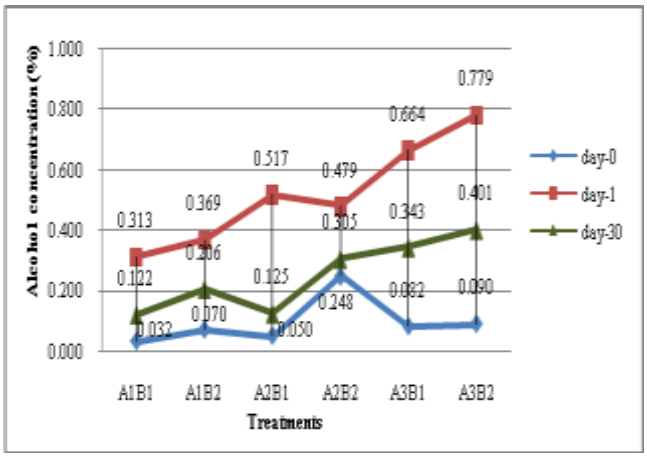

Figure 2. Alcohol concentration of banana peel vinegar from different treatments

3.3 Acetic acid concentration

Acetic acid content is one of parameters determining qualities of vinegar. Food and Drug Administration (FDA) specifies that vinegar contains at least 4 grams/100 mL (4%) of acetic acid. Indonesian National Standard also requires at least 4% acetic acid level in table vinegar and 12.5% in cooking vinegar [17]**.** In acetic acid fermentation, alcohol molecule are oxidized into acetic acid molecule by *Acetobacter aceti*. The acetic acid concentration of Nangka banana peel vinegar was affected by the treatments ($P<0.05$). Findings study showed that acetic acid content in all treatments from day-3 to day-9 did not undergo a significant increase, which was around 0.08-1.28% since *A.aceti* was in a adaption phase to the substrate.

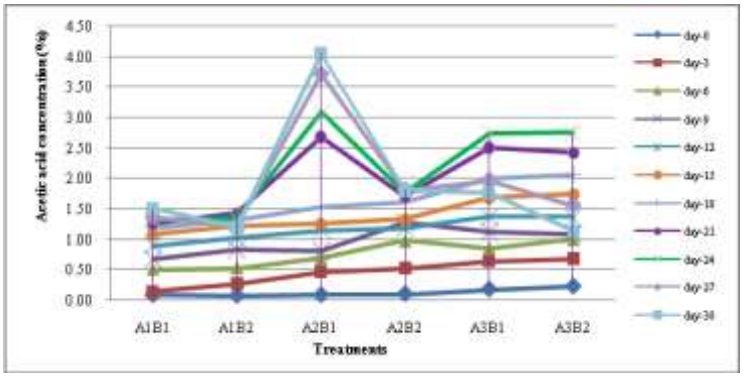

Figure 3. Acetic acid concentration of banana peel vinegar from different treatments

Based on Figure 3, the acetic acid content increased to 1.02-3.08% in all treatments on day-12 to day-24. Adams (1985) reported that acetification process occurs in a period of 12 days and is able to produce acetic acid up to a level of 3.5%. On day-7, the treatments of A_2B_2, A_3B_1 and A_3B_2 decreased in acetic acid content to 1.80%, 1.97%, and 1.56 %, respectively. This was possibly due to the fact that the treatments experinced an initial growth phase and a logarithmic phase in a faster period of time.

Furthermore, a significant increase occurred from the A_2B_1 treatment with acetic acid content of 3.72%. The acetic acid level of the A_2B_1 treatment rose again on day-30 to 4.05%. This result showed that concentration of amylase and glucoamylase 1:1 is optimal for production vinegar from banana peel of Nangka variety.

The acetification process was found to affect acetic acid formation. The longer the acetification process the larger the amount of alcohol oxidized. However a considerably long period of acetification raised an excessive oxygen supply (excessive oxidation) may cause the oxidation of acetic acid producing CO_2 and H_2O and as a consequence, there was a decrease in the acetic acid content [18].

3.4 pH value

The statistic analysis showed that the treatments were impacting ($P<0.05$) the pH value of Nangka banana-peel vinegar. Based on the research, the pH values were inversely correlated with acetic acid contents. The higher the acetic acid content the lower the pH value. In this research, concentrations of amylase and glucoamylase also contributed to pH values of banana-peel vinegar. The higher enzyme concentration the higher reducing-sugar level produced and the higher acetic acid content produced resulting in the lower the pH of Nangka banana-peel vinegar.

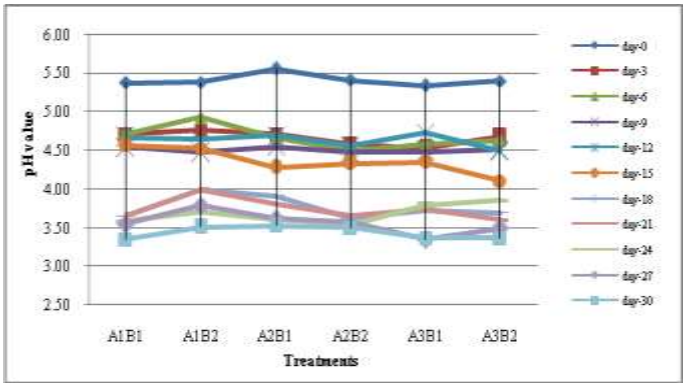

Figure 4. The pH values of banana peel vinegar from different treatments

The point of pH on day-0 from all treatments had range values of 5.34-5.55 (Figure 4). On day-0, pH of each treatment still adapted to environmental condition of α-amylase and glucoamylase which was pH of 5.5-9.5. Drop of pH values from each of treatments occurred significantly on day-18 resulting in range values of 3.62-3.99. This was possibly due to the fact that *A.aceti* had replicated its cell number and had converted alcohol contained in the filtrate of Nangkabanana banana-peel vinegar into acetic acid.

3.5 The ability of vinegar to inhibit pathogen microbes

Beside acetic acid, the vinegar also contain phenolic compound which has characterictic as antimicrobial agent. The phenolic compound in vinegar include gallic acid, 4-hydroxybenzaldehyde, catechin, vanillic acid, caffeic acid, syringic acid, vanillin, syringaldehyde, p-coumaric acid, m-coumaric acid, anisaldehyde, epicatechin, sinapic acid, salicylaldehyde, scopoletin, veratraldehyde and o-coumaric acid. The stages of antimicrobial action of vinegar phenolic compound are giving toxin to cell proplasm, destroying and penetrating the cell wall continued by precipitate the protein of microbe cells.

Based on the research result, vinegar concentration of banana peels and coconut water of one percent has inhibited the growth of the pathogen bacteria. Soaking the fresh meat (also chicken carcass) for one minute in vinegar solution could extent the storaging the fresh meat for 12 hours at ambient temperature and for 19 hours at cold temperature. Based on organoleptic test, the consumers still accept this fresh meat soaked in vinegar solution. This is because no differentiation in color, aroma, texture and acidity of fresh meat.

Through this research results, the banana peels and coconut water were not at all as waste of agricultural waste, while its processed to be safety biopreservative product. The processing of banana peels and coconut water potential to be developed as vinegar source because its manufacture were easy so that it can be applied especially in meat seller at traditional markets.

4. Conclusions

The findings can be concluded that the treatment generating the best acetic acid was 0.10% α-amylase and 0.10% glucoamylase (A_2B_1) with an average value of 4.05% and an pH value of 3.52 on day-30 of fermentation. Banana peel vinegar from A2B1 has accordance with the requirement of Indonesian National Standard (SNI) No. 01-4371-1996 (Fermentation of Vinegar). ‘

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