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From waste to food: utilization of pineapple peels for vinegar production

Abstract

The present study is aimed at producing vinegar from fermented pineapple byproducts (peels). The vinegar was produced after fermenting the peels of pineapple usig three selected strains of acetic acid bacteria's. such as propionic bacterium acidipropionici, panteo agglomerans, and pantea dispersa. This article introduces a new type of acetic acid bacteria's strains for the production of vinegar from pineapple peels. Three fermentation times (24hr, 48hr, and 72hr) and three acetic acid bacterias (propionic bacterium acidipropionici, panteo agglomerans, and pantea dispersa) were considered and arranged in a factorial experimental design. The fermentation was performed in 500 mL Erlenmeyer flasks containing 200 mL of medium at 28°C. Aeration rate, temperature, and carbon source were set as constant factors. pH, total soluble solids, total residual reducing sugar and titratable acidity were evaluated. The results showed that the vinager samples were in the range of 3.5 to 4.31, 1.3 to 2.31 °brix, 0.50 to2.47% and 3.13 to 6.15 mg/100g of pH, total soluble solids, total residual reducing sugar and titratable acidity respectively. There were significant differences ($P<0.05$) in the values obtained. Therefore bacterial strain and fermentation time were the most important factors affecting vinegar production. From the experimental results and interaction effects, the optimum yield of acetic acid production was found to be 6.15 g/L at fermentation time of 72h by propionic bacterium acidipropionici acetic acid bacterial strain.

Keywords: acetic acid bacteria's, fermentation time, pineapple peel, vinegar

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Teklu Chalchisa, Belay Dereje
Department of Food Process Engineering, Wolkite University, Ethiopia

Correspondence: Teklu Chalchisa, Department of Food Process Engineering, Wolkite University, Ethiopia, Tel +251921564983

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Introduction

Our world is faced with the issues of disposal waste materials which are generated from different food industries. Pineapple is one of the tropical fruits that is produced in different countries and it's placed on the second. Hence, after the production of different food items from it results in huge waste generation and pineapple peels are among the wastes which is estimated around 40%. These wastes contains a valuable components such as sugars, particularly sucrose, glucose and fructose and other constituents including minerals and vitamins which makes them a useful and cheap raw materials for the production of different food items. Indeed, some efforts have been made in order to utilize pineapple wastes, which have already been used as the substrate for the production of bromelain and organic acids,[1] fiber and phenolic antioxidants,[2] ethanol and biogas[3] vinegar.[4] In Ethiopia, most consumers of fruits in general and pineapple in particular usually discard the peels extensively as waste parts after consumption, in this manner constituting stoppable pollution. Hence, it is important assessing the potential of pineapple peel in vinegar production instead of discarding the peels as waste materials.

Vinegar is a traditional fermented product that can be made from a variety of raw materials.[4] It can be made from alcohol-water mixtures to different fruit wines from all alcoholic content.[5] It is the result of a bacterial genus, Acetobacter, that is, converts ethyl alcohol into acetic acid. In this case pineapple peels is found to be a potential raw material in various forms, which can be used as a nutrient substance in culture broth for microbes that can be concomitantly converted into value-added products.[4] Recently, Tanamool et al.[4], produced vinegar from pineapple peels using the newly isolated thermo tolerant acetic acid bacteria and their resut showed that that using whole pineapple peel with the addition of diammonium phosphate (DAP) and $MgSO_4$ at an initial pH of 5.5 gave a slightly higher acetic acid content than that produced from the squeezed juice. The authors also observed an increase in sucrose concentration led to the high production of ethanol, which resulted in the suppression of acetic acid production.

Yet no studies have been available for acetic acid bacterial strains for vinegar fermentation of pineapple peels and this study is the foremost documented methodology to study acetic acid strains for vinegar production. In this study, three acetic acid bacterial strains (*propionic bacterium acidipropionici*, *panteo agglomerans*, and *pantea dispersa*) used for fermentation of vinegar using the pineapple peels as a raw materials. The effect of fermentation time and bacterial strains on the amount of acetic acid production was studied. This study also evaluates some properties of the vinegar produced from pineapple peels were also discussed.

Materials and methods

Pineapple peels

Pineapples (*Smooth Cayenne*) variety fruits were collected from Jimma agricultural research centre and afterwards, it was kept at 22 °C afore undergoing the saccharification process. The pineapples were washed and then the by-product peels were separated from the edible pulp and the crown. The peels were manually cut into small pieces using a knife and then chopped in an electric blender to obtain a homogeneous mixture and thenafter the samples were stored in a freezer -18 °C before use.

Acetobacter bacteria

The acetic acid bacterial strains used for fermentation of vinegar (*propionic bacterium acidipropionici, panteo agglomerans,* and *pantea dispersa)* were obtained from the institute of biodiversity found in Addis Ababa.

Juice extraction: The pineapples peels which was separated from the pulp were washed and shredded in a small-scale commercial system with a centrifugal grating mill (Voran Machinery). The juice was roughly strained and stored at 8°C in a plastic bucket for some hours and then frozen at -18°C until preparation for fermentation.

Alcoholic fermentation: 800 mL of pineapple peels juice was poured into 1L of flask and then the juice was heated to 50 °C in a conventional oven for 20 minutes and later cooled at room temperature, before inoculation of yeast. The yeast strain *saccharomyces cerevisiae* which was obtained from BGI Ethiopia brewery S.c was inoculated at inoculation concentration of 80 g/hL. Then the fermentation process was started at a temperature of 20°c by closing the flask with rubber cape and the fermentation lock was mounted. After 21 days of fermentation, the fermentation process was stopped and chemical analysis was conducted.

Acetous fermentation: After alcohol fermentation 200 mL of fermented wine was poured into three cleaned 500 mL of Erlenmeyer flasks and the three selected acetic acid bacterial strains were inoculated. The flasks which contain the inoculated wine were placed in dark at a temperature of 28°C and mounted with an aeration system. The fermentation was run over 72 hrs, and then the products were roughly filtered using muslim clothes to remove the produced slime.

Chemical analysis

Total soluble solids

Total soluble solids (TSS) was measured as ^{0}Brix with a portable digital refractometer. The measurements were made in triplicate for each sample.

Total reducing sugar

Benedict"s solution was designed to detect the presence of reducing sugars. In hot alkaline solutions, reducing sugars reduce the blue copper (II) ions to brick red copper (I) oxide precipitate. As the reaction proceeds, the colour of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the colouration developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained. The concentration of total reduced sugar (TRS) content of hydrolysates which obtained from hydrolysis is determined using digital spectrophotometer by using measuring absorbance v_s sugar concentration at 540nm wavelength. To do that standard glucose dilution series solution is prepared at different concentration of 0,1, 1.23, 1.25, 1.38 and 1.5 g. Then, 0.5 mL was pipetted from each of the dilution series into the labelled test tube, each containing 5 mL of Benedict's solution mixes the solution by shaking. The labelled test tubes were heated at 90 °C in the water bath for 5 minutes. Each of the test tubes was removed from the water bath and filtered using filter paper to remove red precipitate formed when the reduced sugar in the sample reacted with Benedict reagent. After filtered precipitate, % absorbance is measured using a spectrophotometer at 540nm. A calibration curve was plotted to show the % of absorbance blue light by the standard glucose solution.

CSRSUS = (absorbance of unknown sample) – (y-intercept)/slope

Where *CSRSUS* = concentration of total reducing sugar of unknown sample

pH

The pH was performed using edge® Multiparameter pH Meter (HI202001) attached digital electrodes (Hanna Instruments, Inc). Before taking measurements, a three-point calibration with three buffers (pH 7.0, 4.0, and 10.0) was performed.

Titratable acidity

The content of acid in the vinegar was analyzed by titration methods. 10 mL sample was taken in a conical flask and 10 mL distilled water and 3 to 4 drops of phenolphthalein indicator were added. The solution was titrated against 0.1 N NaOH solution till the colour of the solution was changed to pink.

Results and discussion

Chemical analysis for pineapple peels

The chemical composition of the fresh pineapple peels (*smooth cyaned variety*) were 9.81, 0.96%, 7.5% , and 4.8 of total soluble solids, titratable acidity, reducing sugar, and pH, respectively. The results thus obtained are compatible with the reported values for pineapple peels by USDA.

Characterization of pineapple peels wine

During the fermentation of pineapple peel at different pH levels the complex pattern of pH changes was observed. During the initial days of fermentation, the pH of the must be 4.8 which showed continuous fall and on last day a slight rise in its value. Pineapple peel wine with pH 4.8 showed the highest fall in pH on last day of fermentation 4.35. The wine show alternate slight rise and fall in pH during fermentation. Various factors such as pH, temperature, the concentration of sugars can affect the physicochemical parameters of wine during its fermentation. For the fermentation of the juice in a glass of high quality wine, pH is an important factor. Low pH prevents unwanted microflora from growing and can therefore increase the quality of the final product. Soluble solids (Brix) represents the percent sugar and other dissolved solids in the solution. The decrease in soluble solids was observed during the fermentation of pineapple peel must from 9.81 to 2.6 °brix. This decrease was due to the utilization of sucrose by microorganism. The titratable acidity of pineapple wine was increased from 0.96 to 1.42 after fermentation, while the pH was decreased. Metabolic activity of yeast and other microorganisms present in must probably be the responsible factor for the increase in titratable acidity and decrease in pH.[6–10] Similar observations are reported by other authors for banana wine.[6,11]

The concentration of RRS decreased from 7.5% to 2.88% at the end of the fermentation process. This decrease was due to utilization of sucrose by the yeast. This was attributed to a reduction in soluble solid and rise in alcohol percent during fermentation by yeast. The alcohol content of pineapple peel wine after 21 days of fermentation was 5.96% (v/v). The starting alcohol should be lower than 7.5 % (v/v) for the production of vinegar. Therefore the obtained result from this research indicated as it was suitable for the production of vinegar.

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Characterization of vinegar

pH

The initial pH value of pineapple peel wine was 4.35. A medium at an initial pH. of 5.0 was generally favourable for acid production. The pH of the vinegar ranged from 3.5–4.31 as shown in the Figure 1 with the control sample having the highest pH value at 24 hour fermentation time and the sample fermented with PAP acetic acid bacterial strain at 72 hour fermentation time having the lowest pH values. This value is close to the value 3.55 to 3.68 reported by Tessaro et al.[12] for orange vinegar. There was significantly difference (P<0.05) in the value of pH as the hours of the fermentation increased the values of pH slightly decreased. The same trend was observed for all the samples including the control (sample fermented without inoculating with the acetic acid bacterial strains). These variations were attributed to the acetic acid bacterial strain and fermentation time differences. The results thus obtained are compatible with Oguntoyinbo et al.[13]. PH is an important factor for the fermentation of fruit juice into a bottle of good quality wine. Low pH inhibits the growth of unwanted microflora and thus can improve the quality of the final product. The different maximum average yield variation in pH with individual organism was influenced by the spasmodic behaviour of organisms in acid production than by the change in pH.

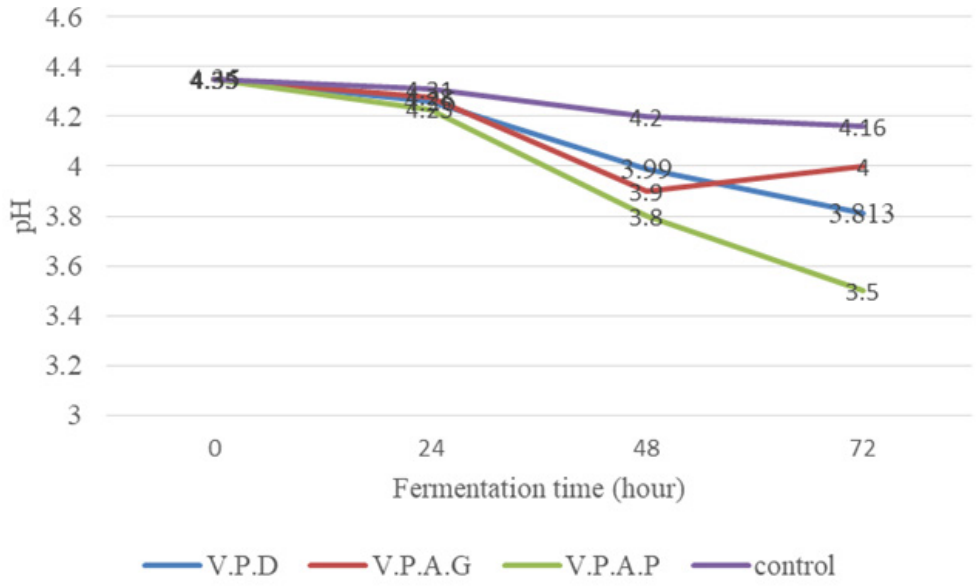

Figure 1 Effect of fermentation time and bacterial strain on pH of vinegar.

Total soluble solid

Total solids ranged from 2.18 to 2.31 ^{0}brix as shown in Figure 2. There were significant differences (P < 0.05) in the values of the total solids as the days of fermentation increased from 24 to 72 hours and among the different strains. The values decreased as the fermentation times increased. TSS represents the percent sugar and other dissolved solids in the solution.

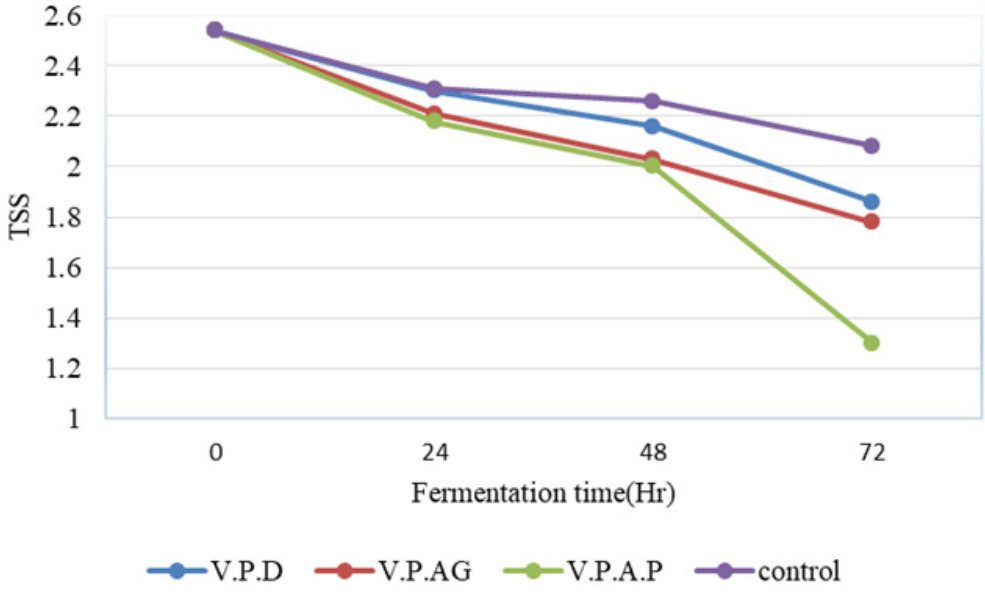

Figure 2 Effect of fermentation time and bacterial strain on TSS.

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Total amount of residual reduced sugar

This study showed that the initial concentration of residual reducing sugar before inoculation of selected acetic acid bacteria for vinegar production was 2.8793% and then after inoculation of acetic acid bacteria for vinegar fermentation the value was found in the range of 0.50 to 2.47% and showing significant (P<0.05) differences among the bacterial strains and fermentation time. Total sugar contents were within the range specified by Ishiwu et al.[14]. The low value of the sugar content in all the vinegar samples is an indication of the effectiveness of acetous fermentation stage by Acetobacter aceti. Polysaccharides, monosaccharide's and disaccharides also influence vinegar, enhancing sourness and viscosity.[15] The maximum TRRS value was recorded in control sample which was vinegar fermented without acetic acid bacteria at 24hr and the minimum value was on vinegar produced with PAP acetic acid bacterial strain at 72hr. The graphical illustration of RRS variation with time and selected strain of acetic acid bacteria strain is shown in Figure 3. The concentration of RRS decreased as the fermentation time increased. This decrement was due to the utilization of sucrose by an acetic acid microorganism. From this study, the fermentation time and bacterial strain had a significantly different (p<0.05) in the value of residual reduced sugar.

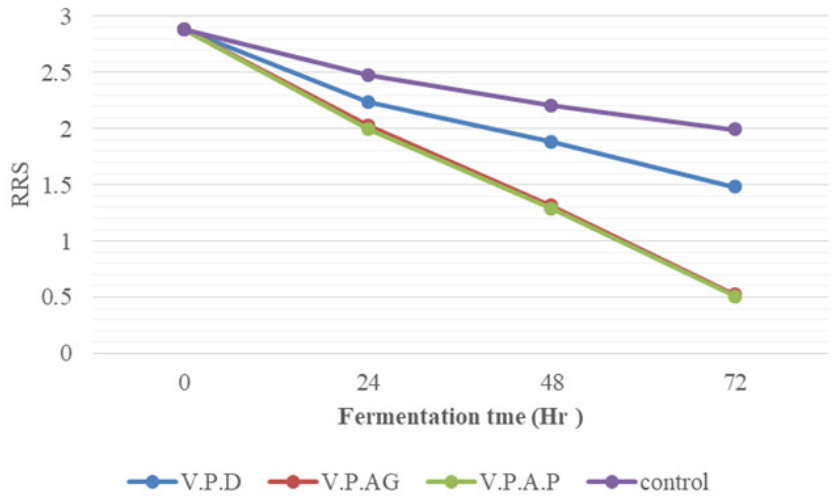

Figure 3 Effect of fermentation time and bacterial strain on residual reduced sugar.

Total acidity

Total acidity content ranged from 3.13 to 3.54, 3.85 to 4.92, 4.81 to 6.15 and 3.04 to 3.18 expressed as acetic acid (w/v) for P.D, P.A.G, P.A.P and control sample respectively as shown in Figure 4. The present result showed that fermentation time had a significant effect on the yield of acetic acid. The acetic acid concentration obtained from an acetic acid strain of P.A.P in the fermented product ranged from 4.81 to 6.15% (w/v). This value indicates a high rate of conversion of ethanol to acid, as the substrate (pineapple wine) had an alcohol concentration of 6.28% (w/v) which was used for microbial strain mentioned in this study. The product meets the minimum requirements established by Brazilian law, which defines vinegar as having a minimal volatile acidity of 4 g/100 mL, expressed as acetic acid.[16] This value indicates that the product can almost be classified as vinegar. But as showed in the following figure 4 the other strain which was used for this study had lesser acetic acid value as compared to strain P.A.P strain of acetic acid bacteria whereas P.A.G strain of acetic acid bacteria meets the standard stated by Brazilian law for vinegar. However, P.D strain does not meet the stated standard which means it couldn't be used for commercial purposes.

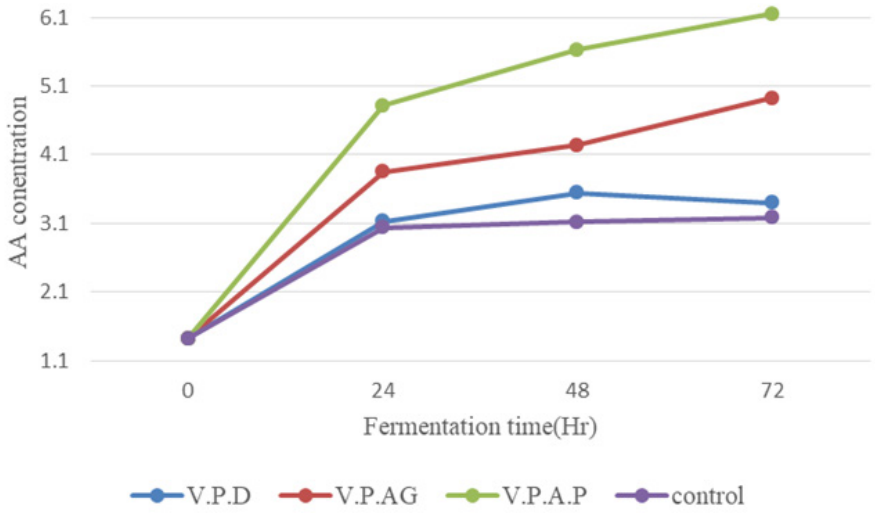

Figure 4 Effect of fermentation time and bacterial strain on acetic acid concentration.

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Evaluation of bacterial strain

Bacterial starter cultures were evaluated to find a culture that could be suitable for pineapple peel vinegar production in the fermentation system that was designed in this study. P.A.P and P.A.G bacterial cultures produced an acidity of 6.15%, and 4.92% respectively. It can therefore strictly be marketed as spoonfuls of vinegar. But P.D bacterial strain did not show the capacity to produce the required amount acetic acid concentration which is stated by international law to be considered as vinegar. Each strain of acetic acid bacteria has its fermentation characteristic, affecting the quality of the produced vinegar.[17] Thus depending on their capacity to produce acetic acid it was possible to select the best bacterial culture for further experiments. Commercial starter cultures consist of many different strains, and thus the differences between the starters can be large.[18–21]

Conclusion

The results of this research work have revealed that it is possible to produce vinegar from pineapple peels. This was done via the use of yeast (Saccharomyces cerevisae) as an aerobic degradation of sugar to ethanol and Acetobacter aceti oxidizers ethanol to acetic acid (vinegar) by using different strain of acetic acid bacterias. The various parameters evaluated compared favorably with the standard values. Considering all the quality values, pineapple peels at 72 hours of fermentation gave the best vinegar. The study also revealed that while cleaning the environment, an added value can be achieved through recycle or conversion of supposed wastes into useful products.

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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