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Production of acetic acid (Vinegar) from pineapple waste

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Abstract
In the present study utilization of pineapple waste viz pulp, peels and crown leaves for production of acetic acid (vinegar) which is commonly called as vinegar was done. Firstly the production medium was inoculated with *Saccharomyces cerevasae* procured from active dry yeast. The medium was kept for about seven days for alcoholic fermentation at room temperature. It was found that alcohol percentage for pulp was 3.75%, peels were 2.5% and crown leaves were 6.25%. After confirming alcohol percentage the medium was inoculated with *Acetobacter aceti* for acetic acid fermentation and it was incubated for the next 14 days at 37°C. The 9.6%, 10.2% and 10.8% of acetic acid was found to be produced from pulp, peels and crown leaves respectively. As from results it was found that increase in acetic acid concentration decreases pH of the medium**.** The present study also helpful in conversion of waste into the useful value added product like acetic acid (vinegar).

Keywords: Acetic acid, vinegar, pineapple

Introduction

Vinegar may be defined as a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic acid fermentation. Vinegar can be produced by different methods and from various raw materials. Wine (white, red, and sherry wine), cidar, fruit musts, malted barley, or pure alcohol are used as substrates. Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation in acetators. Vinegar traditionally has been used as a food preservative. Whether naturally produced during fermentation or intentionally added, vinegar retards microbial growth and contributes sensory properties to a number of foods (Tan, 2005) [31].

Vinegar fermentation is essentially a two stage process, firstly the anaerobic conversion of fermentable sugars to ethanol by yeasts, usually *Saccharomyces species*, and secondly the aerobic oxidation of ethanol to acetic acid by bacteria, usually *Acetobacter species*. Acid yield improvements can be achieved using high rates aeration during continuous production. *Acetobacter* is a genus of acetic acid bacteria characterized by the ability to convert alcohol, C_2H_5OH, (ethanol) to acetic acid CH_3COOH, in the presence of air by oxidation (Adams, 1998).

Vinegar was used earlier as a preservative for other fruits and vegetables. Vinegar is an inexpensive commodity, therefore economic considerations requires that a relatively low- cost raw material like pineapple peel be used in its production. All commercial found to have the potent bioactive effects which May vinegars are used primary in the food processing industry. It also benefits human health. The therapeutic properties of vinegar include antibacterial activity, blood pressure reduction, antioxidant activity, reduction in the effects of diabetes, and prevention of cardiovascular disease. Vinegar is very important food preservative in food industry. The vinegar can be obtained from various raw materials such as various fruits and fruit peels. Various agricultural waste materials can also be used as raw materials. It was observed during various investigations that enzymatic treatments of pineapple wastes had a significant effect on the saccharification process (Bitange, 2008) [9].

Most nations, whether economically advanced or at different stages of development are faced with the issue of disposal and treatment of wastes (Itelima *et al*., 2013) [17]. Agro-industrial wastes are generated in large amounts every year and their reuse in processes is of particular interest due to their availability, low cost, and characteristics that allow at obtaining different value-added compounds (Borghi *et al.*, 2009) [11].

Pineapple peels as agricultural wastes represent around 35% of the whole fruit mass. If these wastes are discharged to the environment untreated, they could cause a serious problem. So, it is necessary to have recycled waste raw material into useful product of higher value added products or even as a raw material for other industries or for use as food or feed after biological treatment (Aung, 2013) [3].

The generated waste is growing at increasing rate and the municipal council is not able to provide service for solid waste collection due to the rapid increase in the population and urbanization. A large amount of the solid waste generated by the market areas during working hours produced different types of waste which is not being properly managed, and might have detrimental effects on the environment, health and ecosystem (Abdulrasoul and Said, 2016) [1]. Thus in the present study an attempt was made to utilize the pineapple wastes to produce acetic acid (vinegar) which one would be the beneficial value added products.

Material and Methods

Collection of Sample

Sample of pineapple fruits were collected from vendors of local market of Akola city. The *Saccharomyces cerevisiae* was purchased from local market in the form of active dry yeast. The pineapple waste material collected were washed and then were manually cut into small pieces using knife and then chopped in an electric blender to obtain homogeneous mixture.

Production of Vinegar

Alcoholic Fermentation

Fermentation was carried out in aseptic condition by taking three types of waste samples which were pineapple fruit

peels, pulp and crown leaves. The peels solution was made up by mixing 50 gm of powdered peels and 450 ml distilled water in 1 L Erlenmeyer flask. The crown solution was made up by mixing 50gm of crown powder and 450 ml of distilled water in 1L Erlenmeyer flask. The 50ml of pulp and 450 ml of distilled water was added in 1L Erlenmeyer flask. All the flasks containing pineapple fruit waste solution was sterilized by heating in water bath for 50 min and later cool down at room temperature. The inoculum of *Saccharomyces cerevisiae* in the form of Active dry yeast was added in all the sterilized flasks. The flasks were then incubated for the fermentation process by keeping at temperature of 30ºC for 3 to 4 days in aseptic condition. The pH of the medium in flasks were checked regularly.

Determination of Alcohol Percentage

After the fermentation process the alcohol percentage was determined by potassium dichromate method using spectrophotometer. The reaction mixture contains 0.1 ml of culture supernatant and 2.5 ml of $K_2Cr_2O_7$ reagent, heated for 10 min at 60°C. Then, the solution is diluted with distilled water to make the volume 25ml and measure the absorbance at 600 nm to calculate the ethanol percentage from a standard curve which was made by using 5%, 10%, 15%, 20%, 25% ethanol.

Acetic acid fermentation and Extraction of acetic acid (vinegar) from fermentation medium:-

The culture inoculum was procured from Department of Microbiology, Shri Shivaji College, Akola. The inoculum was inoculated and allowed to grow in nutrient broth at a temperature of 37°C for 24 to 48 hours, and then prepared inoculum was added in all the flasks. The flasks were incubated for 14 days at 37°C for the acetic fermentation. After 14 days, the fermented medium was filtered and then some amount of filtrate was taken out in tubes and then it was centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed out and the extract was used for further tests.

Determination of acetic acid by titration method:-

The amount of acetic acid was measured using titration method (Bellankimath, 2017) [6]. The 10 ml of the extract was taken in a conical flask, in which 2-3 drops of phenolphthalein indicator was added and then it was titrated against 0.1N NaOH till the colour changes to pink which remains constant for 10-15 seconds then the reading was noted. The amount of acetic acid produced was then calculated by using the formula.

Number of grams of acetic acid = Molarity of NaOH x 1 mole of CH_3COOH x 60 g CH_3COOH x Vol. NaOH litre

Qualitative test for acetic acid production

Fermented pineapple fruit waste extracts from peels, pulp and crown was taken in a test tube and sodium bicarbonate also known as baking soda was added in the test tube. Positive result indicates formation of effervescence due to formation of carbon dioxide gas which is the product of reaction of vinegar and sodium bicarbonate. Negative result indicates absence of effervescence (Tumane *et al*., 2018) [35].

Determination of pH

The pH of prepared vinegar was determined by using digital pH meter.The glass electrode was first standardized by using buffer solution of pH 7 and the electrode was adjusted to that value. Then, the pH value of vinegar was measured and, the results obtained were noted.

Determination of Total Solids Content

All the prepared vinegar (10 ml) was placed in a previously weighed, clean, dry stainless steel dish and heated to dryness. Then, it was placed in an oven at 105ºC for 3 hours. After that, it was cooled in a desiccators for 15 minutes and weighed again. This procedure was repeated until a constant weight was obtained. The percentage of total solids content of vinegar was calculated as follows (Aye and KO, 2016) [5].

Weight of residue

$$\text{Total Solids Content (w/w/)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100\%$$

Results & Discussion

In the present study utilization of pineapple waste viz pulp, peels and crown leaves for production of acetic acid which is commonly called as vinegar was done. Firstly the production medium was inoculated with *Saccharomyces cerevasae* procured from active dry yeast. The medium was kept for about seven days for alcoholic fermentation at room temperature. After incubation the 100ml sample was removed from fermentation medium and utilized for determination of alcohol percentage. The estimation of alcohol produced was estimated by dichromate method using standard ethanol concentration for the determination. The optical density was noted at 620 nm and from standard graph (Fig-1) the percentage of ethanol was determined for each fermentation medium prepared using different substrates (Fig-2).

It was found that alcohol percentage for pulp was 3.75%, peels were 2.5% and crown leaves were 6.25%. After confirming alcohol percentage the medium was inoculated with *Acetobacter aceti* for acetic acid fermentation and it was incubated for the next 14 days at 37°C. After interval of two days the various parameters were checked from the fermentation media as acetic acid, pH, total solid contents and acid reaction with sodium bicarbonate.

The acetic acid produced after two days from pulp was 2.4%, from peels was 3% and from crown leaves was 3.6%. The acetic acid produced after 4 days from pulp was 3% from peels was 3.6% and crown was 4.2%. While after 6, 8, 10 days the acetic acid percentage was found to be 3.6%, 4.8% and 6% from pulp, 4.2%, 6%, and 7.2% from peels and 4.8%, 6.6% and 7.8% from crown leaves. The acetic acid was found to be increased after 12 days and 14 days and maximum yield found to be 7.2% & 9.6% from peels; 7.8% & 10.2%, from pulp and 9% & 10.8% from Crown leaves (Fig.-3).

In the study, percentage of acetic acid produced was higher than the other studies as Tumane *et al*., (2018) [35] produced Pineapple peel vinegar and percent of acetic acid was 4.60. Wang (2006) [40] estimated 4.77% of acetic from ripe Pineapple. Umaru *et al*., (2015) [36] estimated 3% of acetic acid during production of Pineapple peels.

The pH of the medium in which acetic acid were produced changes periodically (Fig.-4). The initial pH of the fermentation medium was 5.5 for pulp used as a substrate which decreased to 5, 4.5, 4.2, 3.8, 3.3, & 2.8 finally reaches

to 2.2 from 2 to 14 days interval. While the decrease in the pH of the medium prepared from peels as a substrate from acetic acid production was found from 6.0, 5.5, 5.0, 4.6, 4.1, 3.4 and 2.5 between 2 to 14 days of intervals. The medium prepared from Crown leaves showed initial pH 6 which then reduced to 5.5, 5.2, 4.9, 4.3, 4, 3.6, and 2.7 checked after 2 days of interval until 14 days.

As from results it was found that increase in acetic acid concentration decreases pH of the medium. This is in accordance with other studies, Ishiwu *et al.*, (2006) [16] reported decrease in pH to 4.0 during Pineapple peels vinegar formation. Patil (2013) [24] reported decrease in pH up to 1 during vinegar formation from Pineapple peels. According Walter (2005) [31] acetic acid and other organic acids determine the acidity of vinegar.

The qualitative test was also performed for the determination of acetic acid production. After addition of sodium bicarbonate to the extract, effervesces was observed which indicates formation of acetic acid because acetic acid and sodium bicarbonate when reacts results in formation of CO_2 which detected by formation of effervances. Similarly Tumane *et al.*, (2018) [35] also found the same observation. The total solid content test was also performed. All the prepared vinegar was placed in previously weighed, clean and dry dish and heated to dryness. Then it was placed in oven. After that, it was cooled and weighed again. This procedure was repeated until a constant weight was obtained and the percentage of total solid content of vinegar was determined. The TSS for Pulp, Peels and Crown leaves vinegar showed 2.8, 1.0 and 2.0 respectively. Chalchisa and Dereje (2021) [13] reported total solids ranged from 2.18 □ 2.31 brix. Aye and Ko (2016) [5] found 1% of total solid content for Pineapple peel vinegar.

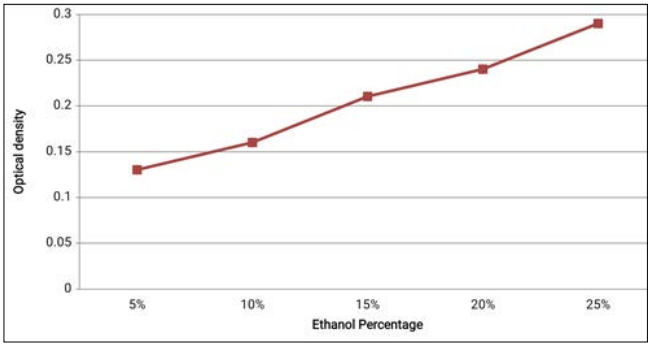

Fig 1: Standard graph for estimation of alcohol by potassium dichromate method

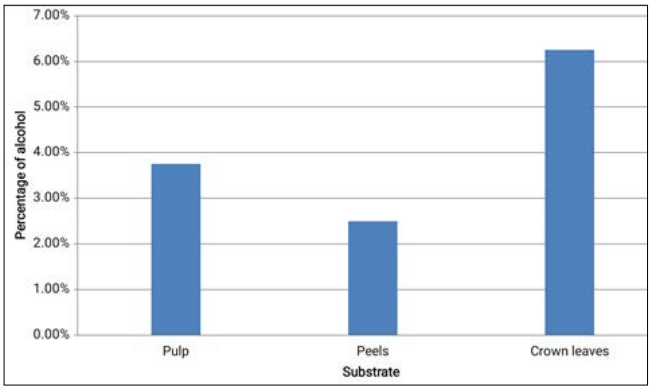

Fig 2: Estimation of alcohol percentage from Pineapple Waste fermentation

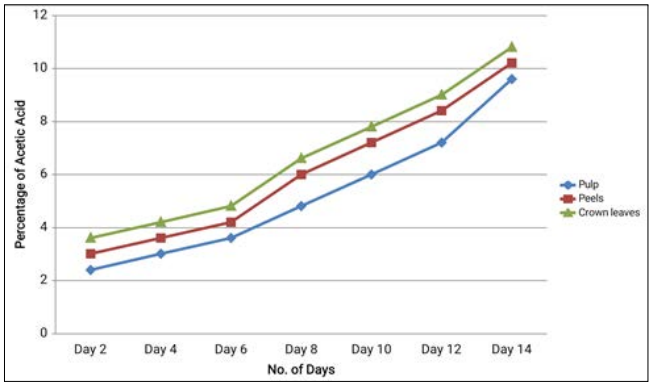

Fig 3: Production of acetic acid from pineapple waste

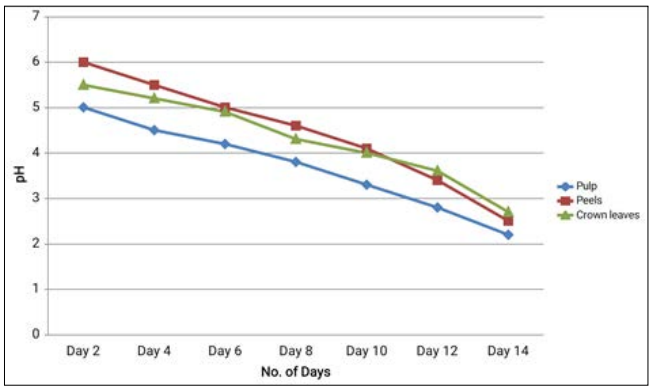

Fig 4: Production of pH of medium for acetic acid production at different time intervals

Conclusion

From the study it can be concluded that acetic acid (vinegar) can be produced from Peels, Pulp and Crown leaves of Pineapple. The 9.6%, 10.2% and 10.8% of acetic acid was found to be produced from Pulp, Peels and Crown leaves respectively. The present study also helpful in conversion of waste into the useful value added product like acetic acid (vinegar).

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