Research Paper

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Bioconversion of Papaya Peel Waste in to Vinegar Using Acetobacter Aceti

MICROBIOLOGY

KEYWORDS : Papaya peels, Acetic acid, Acetobacter aceti , Saccharomyces cerevisiae.

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ABSTRACT

Vinegar is one among the organic acid based product with many potential industrial applications in the fields of food, chemical, fabrication and therapeutics. Vinegar production essentially involves the anaerobic conversion of sugars to ethanol and the subsequent aerobic oxidation of ethanol to acetic acid. In spite of its potential application, the cost of production remains as a major hindrance for large scale production especially in developing and under developed countries. The main focus of this research work is to carry out the fermentative production of vinegar using the discarded papaya peel waste. The precise nutritive composition in the peels was analyzed prior to fermentation. The anaerobic fermentation yielded 8.11% of alcohol content. The titrable acidity after acetic acid fermentation was found to be 5.23%. Thus the present study highlights a methodology of recycling peel waste rather than its careless discharge which brings a lot of environmental issues.

INTRODUCTION

Vinegar is defined as the condiment made from sugary or starchy materials by alcoholic or acetic acid fermentation (Cruess, 1958). Vinegar majorly consists of acetic acid along with other organic acid in trace amounts. Vinegar production, irrespective of the nature of substrates used involves four main steps. In the first step the complex starch molecules in the substrate are converted in to simple fermentable sugars by amylases produced by yeast (S.cerevisiae) followed by anaerobic conversion of sugars to ethanol (Dobereiner, 1822). Further ethanol is dehydrogenated to form acetaldehyde followed by hydration of acetaldehyde to acetic acid.

Production of organic acid from cheap raw materials like agro waste has gained attention due to its impact in the reduction of cost of production. Papaya (Carica papaya) is an oblong tropical fruit, often seen in orange red, yellow green and yellow orange hues with a rich orange pulp. Papaya has high content of vitamin A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties.

This research work attempts to exploit the nutritive potential of papaya peels and involves bioconversion of nutrient rich papaya peels in to commercially important acetic acid. Thus the present study highlights a methodology of effective utilization and recycling of papaya peel waste rather than its careless discharge which brings a lot of environmental issues.

MATERIALS AND METHODS

Collection of raw materials

Papaya peels was collected from fruit shops in Coimbatore, Tamil Nadu, washed several times with distilled water and used as substrate for vinegar production.

Microbial cultures

Dry yeast (Saccharomyces cerevisiae) grown in YEPD broth was used as inoculum for alcoholic fermentation. For acetic acid fermentation, lyophilized culture of Acetobacter aceti (MTCC 2633) obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India was used. The culture was maintained in YPM agar medium during the course of study.

Analysis of nutrient profile in papaya peels

A) Analysis of moisture content

The moisture content of papaya peel was determined by A.P.H.A, 2005 method.

The % of moisture content was calculated using the following formula:

$$\% \text{ Moisture} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100$$

B) Analysis of ash content

The ash content of papaya peel was determined by A.P.H.A, 2005 method.

The % of ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

C) Analysis of crude protein content

The crude protein content of papaya peel was determined by Lowry's et al., method using BSA as reference standards.

D) Analysis of Lipid content

The Total Fat content of papaya peel was determined using A.O.A.C, 2005 method. About 1g of the sample was weighed and a piece of filter paper was folded in such a way that it could hold the sample. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus. The weight of the empty extraction flask was weighed initially. Approximately 150 ml of petroleum ether was added into the Soxhlet extractor and the sample was extracted for 6 hours without interruption at 60-80°C. It was then allowed to cool and the solvent was evaporated on a steam or water bath until no odour of ether remains. The extract was allowed to cool at room temperature. Any dirt or moisture outside the flask was removed carefully and the weight of the extraction flask along with the dried extract was weighed finally. The weight of the extraction flask along with the dried extract minus the weight of the empty extraction flask gives the weight of the total fat being extracted.

The % of total fat was calculated using the following formula;

$$\text{Total Fat \%} = \frac{\text{Weight of the fat (g)}}{\text{Weight of the sample (g)}} \times 100$$

E) Analysis of crude fiber content

The crude fiber content of fruit peels were determined by A.O.A.C, 2005 method. About 1g of the sample was taken and 100 ml of 1.25% sulfuric acid was added into it and was allowed to boil for 30 minutes. After boiling, the sample was filtered through muslin cloth and washed several times with hot water till it is free from acid. Then 100ml of 1.25% Sodium hydroxide solution was added into the acid free sample and was allowed to boil for 30 minutes. After boiling, the sample was filtered through muslin cloth and washed several times with hot water till it is free from alkali. The sample was then dried in the hot air oven at 105°C till it loses the moisture. After drying, the dry weight of the sample was noted. Finally, the dried sample was transferred into a crucible and kept in muffle furnace and ignited at 550-600°C for 2 hours. It was then cooled in a desiccator and the weight of the ashed sample was noted.

The % of Crude Fiber in was calculated using the following formula

$$\%\ \text{Crude Fiber} = \frac{\text{Weight aft drying} - \text{Weight after ashing}}{\text{Weight of the sample}} \times 100$$

F) Estimation of total sugars in the fruit peels

Total sugar content in the fruit peels were determined by anthrone method (Hedge et al., 1962).

G) Estimation of reducing sugars in the fruit peels

Reducing content estimation was done post acid hydrolysis using DNSA method as described by Miller (1959).

Preparation of hydrolysates for fermentation

The modified method of Puimput et.al., was used for substrate hydrolysate preparation. About 8gram of papaya peelwas steam exploded in an autoclave at 121°C for 20min .Sterile water was added to the wet pretreated material to make the volume of 200ml and boiled at 80°C for 30 min. Later the hydrolysate was recovered by filtration with cheese cloth. Acid hydrolysis of hydrolysate was carried out to cleave the oligosaccharides in to monomeric sugars by autoclaving at 121°C with concentration of 1% HCl v/v for 30min (Pumiput, 2008). The pH of the hydrolysate after hydrolysis was adjusted with CaO to 6-6.8 and the CaSO4 precipitate formed was removed by filtration with Whatmann filter paper No.1 (Pumiput, 2008). This was used as substrate for frmentation.

Alcoholic fermentation (Anaerobic Fermentation)

To 100ml of papaya hydrolsate in a conical flask, 1% of glucose and 1gm dry yeast culture was added and incubated initially in shaker incubator at 28°C 300rpm for 2 hrs. Later the flask was covered with aluminum foil and left for anaerobic fermentation in dark for 48 hrs at room temperature.

Estimation of alcohol content in fermentation broth

After anaerobic fermentation period the residual alcohol content of the fermentation broth was estimated by specific gravity method (De Clerck, 1958).

Acetic Acid fermentation (Aerobic fermentation)

The fermentation broth after anaerobic fermentation period was centrifuged at 8000rpm for 15min. The supernatant was transferred in to sterile conical flask under aseptic conditions and inoculated with Acetobacter aceti (5%) culture precultivated in YPD both. The inoculated medium was incubated in a shaking incubator at 37°C for 72hrs. After the fermentation period the pH and total acidity of the fermented broth was analysed.

Estimation of pH and Total Acidity

pH of the fermented broth was determined using pH meter (ELICO). Total acidity was determined by titrating the fermentation medium against 0.1N NaOH using phenolphthalein as indicator and the result was expressed as percentage acetic acid content (AOAC, 1990). The stochiometry of the titration is given by the equation:

CH3COOH + NaOH = CH3COONa + NaOHThe Titrable Acidity was calculated using the formula:

$$\text{Total Acidity (\%)} = \frac{\text{Vol of NaOH consumed x Normality of NaOH x Gram Eq wt of Acetic acid}}{\text{Sample weight x Volume of fermentation broth used for titration}}$$

RESULT AND DISCUSSION

The moisture content reflects the loss in weight of water when heated under specified condition. Moisture content in the papaya peels was found to be 75.13%. Total Ash, an index of the mineral content in peel was found to be 3.23%. Crude protein content in the peels was found to be 1.24%. Total lipid content analysis was done using the fat extraction using petroleum ether and was observed to be 6.93%. Crude fiber consists largely of cellulose lignin along with some mineral matter. It represents 60-80% of cellulose and 4-6% of lignin. During the acid and subsequent alkaline treatment of sample, oxidative hydrolytic degradation of native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gave the crude fiber content and it was found to be 1.72%. The total sugar content (9.13%) was revealed by anthrone method and reducing sugars (7.49) by DNSA method.

Table 1: Nutritive profile of papaya peels, Alcoholic content and Total Acidity

S.No	Nutritive Parameter	content
1	Moisture	75.13 %
2	Ash	3.23 %
3	Crude protein	1.24 %
4	Lipid	6.93 %
5	Crude fiber	1.72 %
6	Total sugars	9.13 %
7	Reducing sugars	7.49 %
8	pH	4.23
9	Alcohol content	8.11 %
10	Total Acidity	5.62 %

Figure 1: Nutritive and Fermentative profile of papaya peel

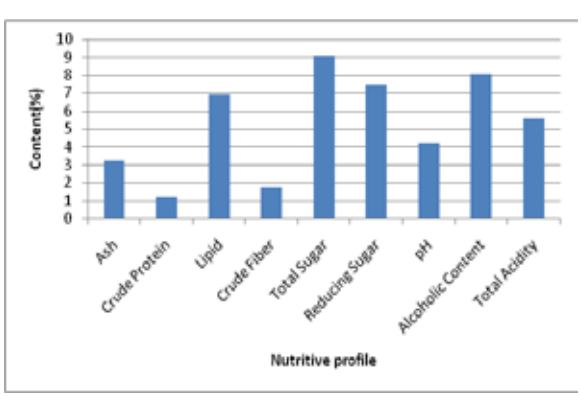

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Figure 2: Papaya Vinegar

The current study revealed that dilute acid hydrolysis resulted in the conversion of complex sugars in the papaya hydrolysate in to simpler fermentable sugars. During the anaerobic fermentation, amylases from yeast initially break the starchy residues further in to monomeric residues. These residues are utilized for ethanol production. The total ethanol content was found to be 8.11%. Finally the acetic acid fermentation was carried out via the conversion of ethanol to hydrated acetaldehyde and dehydrogenation of acetaldehyde to acetic acid by aldehyde dehydrogenase produced by Acetobacter aceti.

CONCLUSION

Production of vinegar from papaya peels suggested an adoptable methodology for tuning an potential waste in to a commercially important organic acid which significantly reduces production cost.

REFERENCE

• AOAC, 2005.18th edition. Official Methods of Analysis of A.O.A.C, edited by K.Helirich, Association of Official Analytical Chemists, Inc., Arlington,VA. | • APHA, AWWA and WEF, 2005.21st edition. Standard methods for the examination of water and waste water, Washington,D.C. | • Lowry, O.H., Rosebrough.,N.J., Farr, A.L., and Randall., R.J. (1951). Protein measurement with the Folin's phenol reagent. Journal of Biological Chemistry 193,265-275. | • Hedge, J.E., and Hofreiter, B.T, 1962. In: Carbohydrate Chemistry 17 (Eds Whistler R.L. and Be Miller,J.N.), Academic Press New York. | • Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 31: 426-427. | • Pushparani Mudaliyar, Lathasharma, and Chandrasekar Kulkarni, (2012).Food waste management - Lactic acid production by Lactobacillus species. International Journal of applied Biological Research.2 (1):34 -38. | • Mridul umesh and Preethi.,K. (2014). Fermentative utilization of fruit peel waste for Lactic Acid production by Lactobacillus plantarum. Indian Journal of Applied Research. 4(9):62-64. | • De Clerck and Jean. (1958). A text book of brewing, Chapman and Hall Ltd. | • Pumiput , P., Chuntranuluck,S., Kitpreechavanich , V., Punsuron , V., and P.Vaithanomsat (2008). Production process of hydrolysate from steam explosion of oil palm trunk for xylitol fermentation. Kasetsart journal (National Science), 42:73-78. |