

PRODUCTION OF BANANA ALCOHOL AND UTILIZATION OF BANANA RESIDUE

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Abstract

Aim of the study was production of alcohol from banana juice which use as complete replacement of malt in alcohol production by utilizing pure culture of *Sacharomyces cerevisiae* as fermenting organism. Banana juice was made from banana pulp by using pectinase enzyme. Optimization of amount of pectinase enzyme for juice production and optimization of pH of the final product were also aim of this study. Pectinase enzyme used for liquefying the pulp production was 0.0003% (w/v). The sugar percentage found in the banana juice was 18%. A sequential study has been done by consecutive pH levels of 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 in the final product. The best product was obtained at pH 6.0 with respect to taste; pH was regulated only after the complete fermentation of the banana juice but just before the filtration process. Alcohol percentage of the product was 8% (v/v) at 28°C. Total number of colonies detected was 21 in freshly prepared alcohol and total number of colonies detected was 20 in the beer after 5 months from production.

Another aim of the work was utilization of the banana residue for the production of fiber enriched cookies. High fiber enriched cookies were prepared using 5%-20% level of fiber obtained from banana residue. 7%-10 % fiber content was obtained as best parameter for cookie production and final moisture content of cookie was 3%.

Keywords: banana pulp, depectinization, pectinase enzyme, *Sacharomyces cerevisiae*, alcohol, banana fiber, cookies.

1. INTRODUCTION

Alcoholic drinks are fall into two broad categories: wines and beers. Wines are made from the fruits juice and beers from cereal grains [1]. Fermentation may be allowed to proceed spontaneously, or can be "started" by inoculation with a must that has been previously successfully fermented by *S. cerevisiae* or *ellipsoideus*. Many modern wineries eliminate the original microbial population of the must by pasteurization or by treatment with sulphur di oxide. The must is then inoculated with a starter culture derived from a pure culture of a suitable strain of wine yeast. This procedure eliminates many of the uncertainties and difficulties of older methods. At the start of the fermentation, the must is aerated slightly to build up a large and vigorous yeast population; once fermentation sets in; the rapid production of carbon dioxide maintains anaerobic conditions, which prevent the growth of undesirable aerobic organisms, such as bacteria and moulds. The temperature of fermentation is usually from 25 to 30°C, and the duration of the fermentation process may extend from a few days to two weeks. As soon as the desired degree of sugar disappearance and alcohol production has been attained, the microbiological phase of wine making is over. Thereafter, the quality and stability of the wine depend very largely on preventing further microbial activity, both during the "aging" in wooden casks and after bottling [2].

Bananas with its high carbohydrate content both in simple and complex sugar form should itself be a good substrate for the high alcohol yielding yeast *Saccharomyces cerevisiae*, *S. ellipsoideus* and *S. carlbergensis* [3].

In traditional beer making processes, the banana juice is also used to impart a certain aroma to the beer and flavor development in the final product [4].

In this alcoholic drink making process bananas are used which is a fruit, easily available and are at lower prices. Since the raw material is fruit and variations are done in the traditional beer making process. Hence, this kind of beer is known as to be the fruit beer in which the raw material are bananas [5].

The advantages of using banana extract for beer making because it contains higher amount of carbohydrates and sugars and minerals and it also has a lower pH value. Banana contains eleven vitamins; among them are vitamins A, B₁, B₂, B₆, B₉, and C [6]. Although fat and protein contents are very low, bananas are rich in some minerals, mainly phosphorus, and calcium. Banana used as a raw material is it contains high pertinacious (pectin) materials and fiber materials and is rich in minerals [7]. This pectin material is highly water absorbent and absorbs water to form a bulky material. So extraction of banana juice from the bulky material is carried out by the treatment of the pulp mass (bulk) with the pectinase enzyme

[8]. After processing the bananas for the extraction of banana juice, the sugar percentage found to be 15 to 20 percent which is actual percentage of sugar to carryout proper fermentation process [8]. The starch in the bananas converts into sugar only upon ripening. Usually once the banana starts ripening, it may have a sugar content of about 20 percent [8] while an unripe banana may have a sugar content of only 2 %.The fully ripened bananas which are soft and are not accepted by the consumers in the whole sale market .so it can be available at lower prices for the production of banana based alcoholic beverages.

A banana used as a raw material is it contains high pectinaceous (pectin) materials and fiber materials and is rich in minerals. This pectin material is highly water absorbent and absorbs water to form a bulky material. So extraction of banana juice from the bulky material is carried out by the treatment of the pulpy mass (bulk) with the pectinase enzyme. The pectinase enzyme breaks down the pectic substances present in the bananas and due to the breakdown of the pertinacious material the bulk or the banana pulp decreases its viscosity and on filtration by mechanical pressing or by press filter the clear banana juice is extracted out and the residue is dried (bone dried) and finely grinded, residue left on filtration has high fiber content which are used for the production of fiber enriched cookies [9].

The main objective of the study is to extraction of the banana juice with suitable dilution with water, obtaining juice of desired consistency [3] and followed by fermentation of the juice by addition of inoculums in aseptic condition. Filtration and clarification to obtain the alcohol of desirable strength (6-8%).Determination of quality of the product and utilization banana waste after juice extraction for fiber enriched cookie production.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Collection and Preparation of Raw Material

Bananas (Singapuri varieties) were collected from local market of Kolkata. The bananas were then washed with the good quality running tap water and followed by de-mineralized water. After that, bananas were peeled and banana mash/pulp was prepared (semi solid in nature) by a mixer grinder. Water is added with a ratio of 1:3 dilutions into the prepared pulp (1 part banana pulp + 3 parts water) and the Brix ($^{\circ}$) and pH was checked. Pectinase enzyme was added to the banana paste/pulp 0.0003% (w/v) for depectinization . Filtration was done with press filter or by muslin cloth, to extract out maximum non fibrous portion from the liquefied banana pulp and the Brix ($^{\circ}$) and pH was checked.

2.2 Methods

2.2.1 De-Pectinazation of Banana Pulp Using

Pectinase Enzyme

Pectinase enzyme was added to the banana paste/pulp at a concentration of 0.0003% (w/v) and left for 5-6 hours in incubation at 38°C, with occasional stirring.

2.2.2 Production of Alcohol

2.2.2.1 Preparation of Yeast Culture Medium

Activation of Dry Yeast

Two test tubes with 10ml Distilled water in each and with a pinch of active dry yeast (*Saccharomyces cerevisiae*) were incubated in between 24°C-27°C.

Preparation of Yeast Growth Medium

200 ml volume of growth medium was prepared with banana juice and distilled water (Brix=1). After wards, yeast extract-0.5% and peptone-0.5% was added. Final pH was maintained in between 4.0-5.0 and the medium was sterilized at 15 psig for 15 minutes and then cooled to 35-37°C and placed the media in a rotary shaker for 24hrs for growth of yeast.

Preparation of Fermentation Medium

In banana juice Potassium di hydrogen phosphate (KH_2PO_4) = 0.1 %, Ammonium sulphate (NH_4) $_2$ SO $_4$ = 0.05%, Yeast extract = 0.01 % was added. The pH was maintained in between 4.5-5. Then the medium was sterilized at 15psig for 15 minutes.

2.2.2.2 Flow Process of Alcohol Production

- Reducing sugar percentage of the filtered portion was checked.
- 10% of the active yeast medium was added to the growth medium and kept for aeration at 28°C for 24 hours.
- Add 10% - 12% of the growth medium to the fermentation medium and then subjected to aeration for 2 hours.
- After 2 hours stop the aeration, and allowed for anaerobic fermentation subjected to 30 hrs at 30°C
- The alcohol percentage and methanol content in the broth after fermentation was checked.
- Vacuum filtration was done after wards and bottled the sample for pasteurization. Pasteurization process was carried out at 70° to 80°C for 15 min and no haze development takes place. The banana alcohol was then stored at a low temperature around 3 - 4°C for better shelf life of the product.

2.2.3 Preparation of Banana Residue Powder

The residue obtained after the filtration of the pectinase treated banana pulp gets separated. The residue was then tray dried for almost 48 hours at 70^o to 80^oC. After drying, the residue was grinded till it becomes fine powder. This powder is highly hygroscopic in nature and readily absorbs moisture [10]. The grinded powder is again tray dried for about 24 hours at 70^o to 80^o C and kept at air tight containers and used in the fiber enriched cookie making process.

2.2.4 Production of Fiber Enriched Cookies

Cookies were prepared from prepared banana residue powder, for cookie production 100 grams flour, 65 grams sugar, 60 grams whole egg (1pc), corn flakes 15 grams, 2 grams baking powder and required amount of essence were taken. The banana fiber of the total flour was used 0 %, 5 %, 7 %, 10 %, and 20 % for cookie preparation. Cream shortening & sugar till light and fluffy added to the flour (flour+ banana fiber) sugar was grinded till it has a course texture. Eggs were beaten with vanilla and to the creamed shortening. The flour was sieved with the baking powder and fold into the creamed mixture. The hands were wetted with water the portions were divided into walnut size; the rounded portions were rolled in crushed corn flakes placed on the baking sheet an inch apart baked at 150^oC for 30 minutes [11].

2.2.5 Determination of Alcohol content in the

Product Sample

100 ml of sample product obtained from the filtration of the fermentation broth was taken in a 500 ml distillation flask along with few glass beads. Complete the distillation for 35 minutes at a temperature of 78^o-80^oC. The distillate was collected in a round bottom flask connected at the end of the condenser which was kept at an ice bath, to minimize the alcohol loss. The collected distillate was taken in a 100 ml of the measuring flask and make up to volume up to 100ml mark with distilled water and mix thoroughly. Place the alcohol meter in the measuring flask and take the reading which is in terms of specific gravity and ethanol content was 8%.

2.2.6 Determination of Methanol Content in the

Product

Chemicals Required

- Sodium Bisulphate
- Potassium permanganate
- Chromo tropic acid
- Concentrated Sulphuric acid

Procedure

- 1ml product (distilled sample) sample, 1ml distilled water, 2ml potassium permanganate solution was taken in a 50ml Nessler's tube (Color of the solution: Dark pink)
- Keep it in ice cold water (5^oC or less) for 30 minutes (Color of the solution: Dark pink.)
- Add a pinch of sodium bisulphate to decolorize the pink color of Potassium permanganate and shake well.(Color of solution: Colorless)
- Add 1ml of chromo tropic acid solution accurately
- Kept the tube in water bath at 80^oC and slowly 15ml concentrated Sulphuric acid was added with continuous shaking.
- Keep it for 20 minutes at 80^oC. Color of the solution will change from light dull yellow to violet & finally to deep brown.
- Cool and check the color absorbance at 575nm by spectrophotometer.

Calculation

$$\text{Ethanol (g/100 liters of absolute ethanol)} = (A_2 \times C \times D \times 1000 \times 100 \times 100) / (A_1 \times S)$$

Where,

A₂=Absorbance for sample standard solution

C = Concentration of methanol standard solution g/ml

D = Dilution factor for sample solution

A₁=Absorbance for methanol standard solution

S = Ethanol content of liquor sample in percent (v/v)

2.2.7 Determination of Microbial content in the

Banana Alcohol

Robert Koch invented this technique. In this technique successive dilutions of the inoculums serially are added to sterile Petri plates to which is poured and melted and cooled at 42^o to 45^oC agar medium and thoroughly mixed by rotating the plates which is then allowed to solidify. After incubation the plates are examined for the presence of individual colonies growing throughout the medium. The pure colonies which are different size, shape, color may be isolated or transferred into test tube culture media for making pure cultures.

2.2.8 Optimization of pH of Product after

Fermentation

The pH of the final product plays an important role in the taste of the banana based alcoholic beverages. A sequential study had been done by consecutive pH levels by 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 in the final product, showed in table: 1 on the basis of hedonic scale. Organoleptic study had been done to study the alcoholic beverage which tastes the best in a particular pH level. The pH of the alcoholic beverage is

regulated by addition of a sodium bi carbonate which acts as a buffer to the fermentation broth and regulates the pH around 6. Sodium bi carbonate used is 1 % (w/v) to the fermentation broth after the completion of the fermentation before filtration of the broth. Before fermentation, the fermentation medium is kept at a pH of 4.5 to 5 [7], because the yeast activity is optimum at this pH and effective anaerobic breakdown of sugar or fermentation takes place [12].

2.2.9 Proximate analysis of the Banana Residue

The total sugar, fat, protein, acid insoluble ash, moisture content and fiber content were measured by according to A.O.A.C. methods.

3. RESULTS AND DISCUSSION

Initially we carried out the fermentation process by using natural micro flora as fermenting organism using diluted banana and clarified banana juice as substrate. The sugar concentration in the diluted banana pulp sample was kept at 10- 12 % level. The following tables showed the result of the analysis.

3.1 Determination of Alcohol Content

The alcohol content found in the final product is 8% (v/v)

3.2 Determination Methanol Content

Methanol content of the final product was nil.

3.3 Determination of Microbial Content in the

Banana Alcohol

Number of Colonies Present /MI in the Banana

Alcohol Sample

Total number of colonies detected =21 (freshly prepared sample)

Total number of colonies detected = 20 (after 5 months from making)

[Number of dilutions =8, from the 5th dilutions the plate count is observed.]

3.4 Optimization of pH of Product after

Fermentation

The sequential studies (table: 2) had shown that the alcoholic beverage at pH 6.0 tastes the best. So the pH is regulated only after the complete fermentation of the banana juice and the pH regulated just before the filtration process and followed by filtration.

Table: 1 Taste parameters of fermentation broth at different pH level

pH of the fermentation broth	Color	Flavors	Taste
4.5	6.0	6.0	5.0 ±0.3
5.0	6.1	5.9	5.1±0.3
5.5	6.1	6.0	6.9±0.3
6.0	6.2	6.9	7.2±0.3
6.5	6.2	6.5	7.1±0.3
7.0	6.1	6.4	7.0±0.3

The values were based on Hedonic scale and the marks obtained were on the basis of total marks 10.

3.5 Proximate Analysis of Banana Residue

Table: 2 proximate analysis of banana residue

Raw material	% of total sugar	% of fiber	% of acid insoluble ash	% prot ein	% fat	% moist ure
Banana residue	Nil	20%	3%	12 %	Nil	9%

3.6 Production of Fiber Enriched Cookies

For preparation of fiber enriched cookies part of the flour in the cookies were replaced by the powder obtained from banana waste. Cookies were prepared by utilizing those fibers at 5to 20 levels and it was found that the cookies whose fiber level in between 7-10 % found to be optimum as per sensory evaluation. At higher fiber level cookies were relatively hard, with odd banana flavor. Moisture of cookie was almost 3%.

CONCLUSIONS

In the making of banana alcohol banana juice was used as an adjunct (to increase the fermentation efficiency) and to obtain higher ethanol content in the final product. Percentage of alcohol yield was 8% (v/v). So, the final product can be introduced as banana beer. At, 6.0 pH the taste parameters were best from others. The study on shelf life of banana beer kept at a temperature around 3⁰ to 4⁰C and at a lower light indicated that the shelf life of the banana beer was more than 5 months.

The study revealed that cookies can be successfully formulated using the banana residue. So, it can be effectively utilized for the fiber enriched cookie formation which have health benefit including pre-biotic effect and enhanced mineral absorption for the presence of high fiber content. The formulated cookies were well accepted by the panelists. Texture of such cookies was extremely liked by panelists. At higher fiber level cookies were relatively hard, with odd banana flavor.

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