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RESEARCH ARTICLE

BANANA PEEL EXTRACTS FOR THE PRODUCTION OF SINGLE CELL PROTEIN BY USING *SACCHAROMYCES CEREVISIAE.*

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Abstract

Protein deficiency is a state of malnutrition which results from eating a diet in which proteins are not enough or present in an inadequate amount**.** It is a major factor responsible for a variety of ailments including mental retardation, kwashiorkor and even death, with millions of affected people as it increases an individual's susceptibility to and severity of infections. The escalating prices of traditional protein sources have intensified this problem. The present study aims to produce low cost protein by using waste material like banana peels which can alleviate protein deficiency of masses while considerably reducing environmental pollution. Bioconversion of waste banana peels into valuable product like single cell protein (SCP) has the potential to solve the problem of protein deficiency worldwide. Here, banana peels were used as substrate; peels were treated with hydrochloric acid 10% (w/v), followed by inoculation with pure culture of the yeast i.e. *Saccharomyces cerevisiae* and left for eight days for fermentation. Temperature was maintained at 30º C for the growth of the yeast. Chemical analysis to quantify the protein content in the fermented media revealed an increase in the protein content as compared to the unfermented media.SCP is rich in certain essential amino acids which are limiting in most plant and animal sources. So, the protein thus produced is considered to be beneficial for the mankind for reducing illness and death. It is a method that can aid in reducing the burden of waste materials on the mother Earth.

Introduction:-

Protein is a nutrient needed for normal growth and maintenance of human body [1]. Proteins (made up of amino acids) are important dietary constituents because they are required for the structure, function, and regulation of the body's major tissues and organs. Protein deficiency in food may pose a problem because essential amino acids, which can't be synthesized by the body itself, are not replenished [2]. Single cell protein can be a potential alternate to solve the global protein deficiency problem [3]. SCP or microbial proteins are edible unicellular microorganisms, used especially as a substitute for protein-rich foods. It is rich in certain essential amino acids like lysine and methionine which are limited in conventional sources of protein like animal proteins [4]. India grows the second largest quantity of fruits and vegetables [5]. Each fruit generates about 40% of its total mass as waste that includes peels, pulp and seeds. Banana (*Musa sapientum*) is a popular fruit consumed worldwide. Once the peel is removed,

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the fruit can be eaten raw or cooked and the peel is generally discarded. It is an abundant and low cost agricultural waste residue and is easily available in large quantities [6]. Banana peel constitutes about 18-20% proportion of fruit which is wasted [7]. These peels are sometimes used as feedstock for cattle, goats, pigs, poultry etc. as they are rich in carbohydrates, and various vitamins and mineral elements [8].Various microorganisms that are used for the production of SCP are bacteria (*Cellulomonas*, *Alcaligens*, etc.), algae (Spirulina, Chlorella, etc.), molds (*Trichoderma*, *Fusarium*, *Rhizopus*, etc.) and yeast (*Candida, Saccharomyces*, etc.). Yeast is a suitable candidate for single cell protein production because of its superior nutritional quality and efficiency to use low cost raw materials [9]. Moreover, yeast cells are easy to harvest because of their large size (than bacteria), high malic acid and lysine content and their ability to grow at acidic pH.

Thus, in the present study, submerged fermentation has been carried out to produce single cell protein of *Saccharomyces cerevisiae* by using banana peels as a source of energy to convert into a high quality of protein. The banana peels were treated with acid to obtain monosaccharide as *Saccharomyces cerevisiae* lack the enzymes that hydrolyze the polysaccharide into simpler glucose units. In this study, the effect of addition of dextrose as carbon source when added to banana peel extracts is compared with banana peel extract without dextrose supplementation.

Material and Methods:-

Requirements: Lyophilized culture, inoculating loop, incubator shaker, laminar air flow, Erlenmeyer flask (500ml), measuring cylinder, distilled water, autoclave, ethanol (70%), cotton, refrigerator, centrifuge, colorimeter, etc.

Culture revival of *Saccharomyces cerevisiae*:-

Medium components: Pure culture of yeast (*Saccharomyces cerevisiae*) used was MTCC170. It was revived on Yeast extract potato dextrose (Yeast Extract 3.0g; Peptone 10.0g; Dextrose 20.0g; Agar 15.0g; distilled water 1.0 L) at 30^0 C for 48 hrs and was stored at 4^0C[10].

Procedure:-

- Pure culture was obtained in lyophilized form from MTCC (microbial type culture collection), IMTECH.
- Culture was revived in Yeast extract potato dextrose broth medium.
- Culture was spread on the Yeast extract potato dextrose agar (YEPDA) medium plates using spreader, under sterile conditions and incubated at 30 ^{0}C for 24hrs. Optical density of the culture was checked in colorimeter at 680nm.
- Sub culturing was done to maintain culture for further use.

Collection of Banana peels:-

The banana peels were collected from the local market in Chandigarh, India and washed several times with sterile water.

Sample preparation and Pre-treatment:-

Medium components- Banana peels (60gm), hydrochloric acid 10%(w/v), Erlenmeyer flask (500ml), measuring cylinder, distilled water, water bath at 100^0C, Whatman filter paper no. 1, autoclave, dextrose (4gm/100ml).

Procedure:-

- The Banana peels were degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure of Lenihan 2010 [11].
- 100 ml of 10% (w/v) Hydrochloric acid was added to 60gm of banana peels in conical flasks.
- The mixture was placed in water bath at 100^0C for one hour.
- After being allowed to cool, Banana peel extract was filtered through Whatman filter paper no: 1.The filtrates (98ml) were used as substrates and dextrose was added (4gm/100ml) in one group i.e. banana peel dextrose (abbreviated as BPD) and one was used without dextrose supplementation i.e. Banana peel extract (abbreviated as BPE).
- The two media were autoclaved at 121^0C for 15 min.

The sterile broths thus prepared were used as carbon and nitrogen sources for biomass production.

Fermentation and harvesting of single cell protein:-

- Submerged fermentation was carried out in different Erlenmeyer flasks containing 98ml of the media.
- Media were aseptically inoculated with 2ml of inoculum of *Saccharomyces cerevisiae* (2×10^7 cfu/ml).
- Static fermentation was carried out at 28^0C in a laboratory incubator for 8 days.

Drying of the final sample after fermentation:-

Procedure:-

- After 8 days of fermentation, media (BPE and BPD) were poured into different Eppendorf centrifuge tubes.
- The Eppendorf tubes containing media were centrifuged at 10,000rpm for 15min.
- Supernatant was removed and pellet along with Eppendorf tube was weighed (wet biomass).
- Chemical analysis of the wet pellet was done to find the protein content.
- Hot air oven was set at 60^0C. The Eppendorf tubes containing pellet were kept in pre-heated oven (60^0C) for 24hrs.
- After drying, the dry weight of the sample was weighed.

Results:-

Biomass content of Saccharomyces cerevisiae:-

Table 1:- Final percentage increase in the Biomass content after fermentation of 8 days:

Growth media	Biomass content increase (%age)
Banana Peel Extract	72.4%
Banana Peel Ext+Dextrose	84.6%

In submerged process [12], the banana peel substrate used for fermentation contains the nutrients to support the growth of microorganisms. The results obtained in the study showed that there was difference in dried biomass and the protein content when comparing the BPE and BPD media after fermentation.

The results showed that there was an increase in the biomass content in the banana peel extract with and without dextrose supplementation after fermentation. The utilization of sugar from the extract corresponded to the biomass production**.** There was a constant increase in total biomass content in banana peel extract supplemented with dextrose and banana peels without dextrose supplementation, during fermentation. The biomass content of BPD was found to be more than BPE. Although, it was also observed that the difference between the percentage biomass content of the two media was not too big. This demonstrated that BPE and BPD both have the nutrients that could be needed by an SCP microorganism for its growth.

Protein estimation:-

Protein was estimated in the sample by the method of Lowry et al (1951).

Table 2:- Protein content in banana peel extract at concentration of 60% after fermentation.

Growth media	1^{st} day (mg/g)	8^{th} day (mg/g)
Banana Peel Extract	2.5	11.7
Banana Peel Extract + dextrose	3.1	12.9

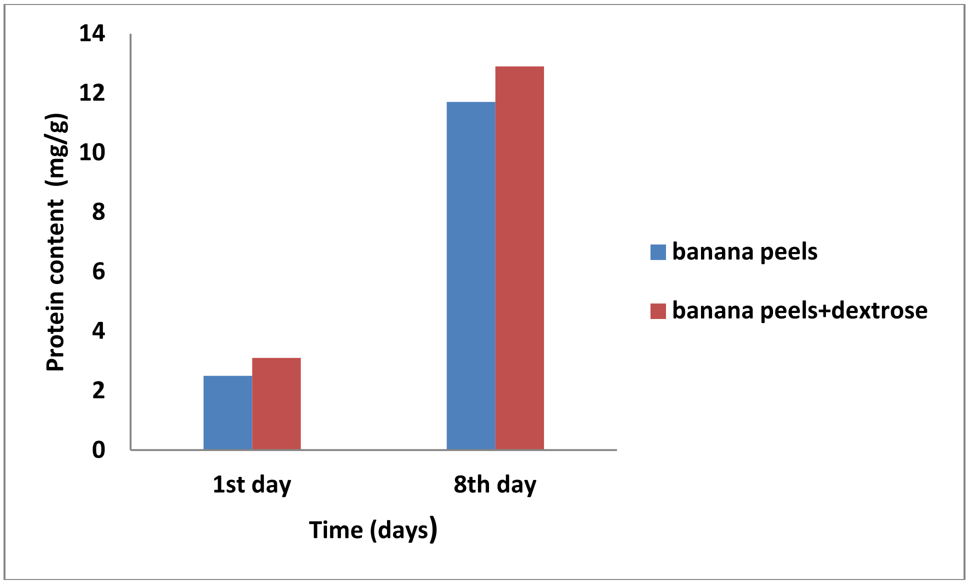

Figure 1:- Concentration of protein in banana peel extracts after fermentation.

SCP from yeast provides superior and better nutritional value in diets than other sources. This may be due to its acceptability, palatability and digestibility compared to the other SCPs [13]. Crude protein content of yeast cells is one of the major factors to study in different growth media.

During the 8 days of fermentation, the protein content has significantly increased in the media. When examined the two different media on first day of fermentation, a low content was found in both the media. However, the protein content in BPD (3.1mg/g) was little higher than that of BPE (2.5mg/g) on the first day of fermentation as shown in Figure-1. While on the final day of fermentation the resultant protein content of BPE (11.7mg/g) and BPD (12.9mg/g), when tested, uncovered that supplementation with dextrose resulted in the enrichment of protein content in BPD as compared to BPE. Nevertheless, the difference between the media was not very large. This showed that supplementation of dextrose supported the growth of *Saccharomyces cerevisiae* in the banana peel substrate and made source of carbon and energy readily available for the yeast during the fermentation process.

In the present study, the experimental results clearly showed that high residual sugar content in the banana peels could be a suitable substrate for multiplication of yeast in the form of single cell protein leading to considerable increase in protein content of the bio processed product [10].

Discussion:-

The results obtained from this study revealed that fermentation can bring about desirable changes in the nutrient composition of banana peel extracts. Yeast showed potential to increase the protein content of the banana peel extracts. The peels fermented with *Saccharomyces cerevisiae* had an increase in the protein content to 12.9mg/g from 3.1mg/g (for BPD) and 11.9mg/g from 2.5mg/g (for BPE). This implied that yeast had significant ($P< 0.05$) effect on protein content. Both the media i.e. BPE and BPD had a considerable surge in the biomass and protein contents. Since the difference between biomass and protein contents of the two media was not colossal and both the media produced relatively good results, banana peel extract can be used for the SCP production, with or without dextrose supplementation. This observation, further confirmed that banana peels had enough nutrients for the growth of *Saccharomyces cerevisiae*.

Conclusion:-

The data obtained in this study indicated that banana peel provided necessary nutrients for the *Sacchromyces cerevisiae* to grow and synthesize proteins. In conclusion, a promising yield of SCP production by *Saccharomyces cerevisiae* was achievable by fermentation using banana fruit peels. The present findings suggested that banana peels can be used as a potential substrate for products with relatively high protein content as produced by submerged state bio processing of banana waste by *Saccharomyces cerevisiae* that successfully enriched its protein content by utilizing various ingredients available in banana peels and there is a scope of converting waste fruit peels to proteinaceous feed and food. Thus, banana fruit peels should be exploited properly as a substrate for the production of cellular biomass of edible or food grade yeast instead of dumping them, so that they can be used as animal feed supplement and for human consumption with minimal expense of money.

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