Industrial Microbiology

Explore–Journal of Research for UG and PG Students

ISSN 2278 – 0297 **(Print)**

ISSN 2278 – 6414 **(Online)**

http://www.patnawomenscollege.in/journal

Utilization of fruit wastes for the production of Single Cell Protein from Yeast

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Received : November 2013
Accepted : March 2014
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Abstract : *In the present study, yeast was grown on various fruit wastes such as banana peel, pineapple peel, orange peel, and sugarcane bagasse for the production of single cell protein. The utilization of fruit wastes for SCP production from Yeast is economical, easy to obtain in crude form, nutritive and can be made available as a food or feed additive to increase the nutritional value. A comparative study of SCP from fruit wastes revealed that orange peel generates highest amount of protein, followed by that of pineapple peel, sugarcane bagasse and banana peel respectively with 23.9%, 22.5%, 21.2% and 16.2% crude protein. Since disposal of wastes is a serious problem and their deposition poses health hazard for all living beings, utilization of these wastes will help in waste management. Besides this SCP may to some extent solve the problem of shortage of protein rich food.*

Key words: *Saccharomyces cerevisiae, Single Cell Protein, Fruit wastes, Food and feed additive.*

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Introduction :

The increasing demand for food and feed protein spurred the search for non-conventional protein sources to supplement the available protein source thereby a great deal of interest has been focused on the potential agricultural wastes to microbial protein or SCP. Single Cell Protein is a dehydrated cell consisting of mixture of proteins (50%), lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of other non protein nitrogenous compounds such as vitamins. SCP refers to the total protein content from dried microbial cultures. The term was coined in 1966 by Carol L Wilson.

India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. According to India Agricultural Research Data Book 2004, the total waste generated from fruits and vegetables comes to 50 million tons per annum. Fruit wastes rich in carbohydrate content and other basic nutrients support microbial growth (Mondal *et al*., 2012)The utilization of fruit wastes in the production of SCP will help in controlling pollution and also in solving waste disposable problem to some extent in addition to catering to the problem of shortage of protein rich food in the world. Yeast is suitable for the SCP production by fermentation on the substrates like fruit waste. A comparatively lower doubling period, lower nucleic acid content (which is a good chemical characteristic as high amounts in human diet cause potent health problems), big size which makes the downstream processing easy, widely accepted and consumed since time immemorial are some of the advantages of yeast over other microorganisms. Even though some bacteria have lower doubling time than yeast cells they are not employed in the present study keeping mind their higher nucleic acid content. The present project was undertaken keeping in mind the protein deficiency and the ever increasing protein demand across the globe. The need for exploiting different biotic fruit wastes as substrates like banana, orange, sugarcane and pineapple peels was felt, as they can be converted into useful products by employing economically feasible method.

Materials and Methods :

Before embarking on the present study, glass wares such as beakers, Petri plates, test tubes and conical flasks were thoroughly washed and sterilized and kept in a hot air oven for further use.

Collection of fruit Wastes : Fresh fruit residues of sugarcane (bagasse), orange (peel), banana (peel) and pineapple (outer skin) was collected from local juice outlets and fruit sellers located at Boring road and Patliputra areas of Patna.

Isolation of yeast for SCP production : Yeast was isolated from banana pulp on Yeast Extract Potato Dextrose (YEPD) media according to the protocol of Ojokoh *et al* (2005) with some modifications. The cultural and morphological characteristics of the isolate were determined by fungal staining with cotton blue and lactophenol and further, confirmation of the isolates was done by fermentation test, etc. (Adoki, 2008). The isolated culture was maintained on PDA slants for furthur use.

SCP Biomass Production

Preparation of fruit broth : After collection, the materials were thoroughly washed under running water and dried in hot air oven. It was then thoroughly ground and finely sieved through 1mm mesh screen into fine powder and stored at room temperature. 20 grams powder of each fruit was taken in separate flasks. 200ml of 10% HCl was added in each flask in order to make it a solution. Then, the solution was kept in waterbath at 100°C for 1 hour. After cooling, it was filtered through filter paper. The pH of the solution was adjusted to 4.5 with 2.5 M NaOH. Finally, the broth was ready for further inoculation & incubation (Khan *et al*., 2010 and Bacha *et al*, 2011).

Inoculation and Fermentation : The fruit broth was inoculated by adding a loopful of isolated yeast culture into the broth. Another set of fruit broth was inoculated with the suspension of yeast (procured as baker's yeast from the market and activated by taking 1 gm of sugar in 50 ml of lukewarm water to which 1gm of dried yeast was added and kept in dark, bubbling confirms the activation of yeast). Then, the inoculated fruit broths were incubated for 7 days at 26°C for fermentation.

Procurement of SCP biomass : At the end of the incubation, the solution was filtered through Whatman No. 1 filter paper. The yeast (filtrate) obtained was weighed for moisture content present in it and then kept in hot air oven for drying. The dried content was further analyzed for crude protein content present in it by Folin-Ciocalteau Method.

Results and Discussion :

The isolates on YEPD media were white, slimy, smooth , flat as recorded in Table 1 and Figure 1.

The isolates were Glucose and Fructose fermenting and produced both acid and gas in 48 hours as shown in Table 2.

Table 1. Characteristics of isolated Yeast culture on YEPD media plate.

Characteristics	Colony
Colour	White
Texture	Slimy
Margin	Smooth
Elevation	Flat
Reverse Pigment	Off-white

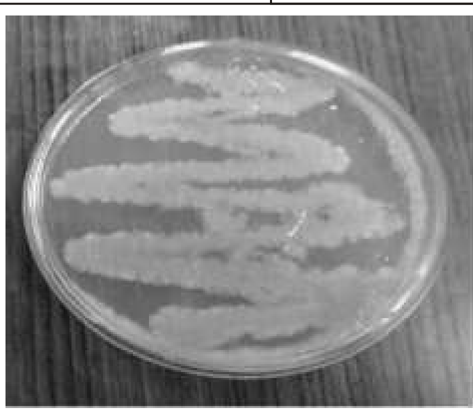

Fig 1: Yeast Colonies on YEPD media

Table 2. Observation of glucose and lactose Fermentation in 24 and 48 hours.

Sugar	24 hours		48 hours	
	Acid Formation	Gas Formation	Acid Formation	Gas Formation
Glucose	+	–	+	+
Fructose	+	–	+	+

On the basis of cultural, morphological and fermentation test , the isolate from banana pulp was confirmed as Yeast.

Estimation of SCP biomass : Maximum yield SCP biomass was obtained when orange waste was used as substrate for both the Baker's yeast and yeast isolated from banana pulp. Whereas, minimum SCP biomass was obtained when sugercane was used as substrate for both the Baker's Yeast and yeast isolated from banana pulp (Table 3).

Table - 3: total yield of SCP biomass in grams from different fruits wastes by the fermentation of Baker's yeast and yeast from banana pulp.

Yeast used / Fruit Wastes used	Weight of Baker's Yeast in g	Weight of Yeast Isolated from banana pulp in g.
Orange	3.217	4.924
Banana	1.640	0.799
Pineapple	1.531	0.673
Sugarcane	1.362	0.467

Crude Protein content of SCP biomass : The Protein content of the SCP biomass as determined by Folin's – Ciocalteau Method is shown in the Table 4.

Table 4. Total crude Protein concentration in the SCP produced from both the yeast culture

Yeast used / Fruit used	Baker's Yeast		Yeast isolated from banana pulp	
Orange	478 mg/ml	23.9%	466 mg/ml	23.3%
Banana	384 mg/ml	16.2%	264 mg/ml	13.2%
Pineapple	450 mg/ml	22.5%	394 mg/ml	16.7%
Sugarcane	424 mg/ml	21.2%	352 mg/ml	17.6%

A comparative study of fruit wastes revealed that orange peel generated highest amount of protein per 20 g of substrate used, followed by that of bagasse of sugarcane, peel of pineapple and peel of banana for the isolated yeast. In the present study, the amount of crude protein estimated was ranged from 13% to 24% (Table 4). Thus under uniform conditions of experimentation to achieve higher yield of mycelia biomass and as a consequence higher amount of fruit protein from Yeast , Orange peel is therefore comparatively the best substrate out of the four fruit wastes used. The estimation of protein content of SCP biomass showed that orange can be considered as the best substrate for SCP production.

The result of protein content of SCP biomass produced in the present study was in accordance with the protein content in the work carried out by Mondal *et al* [2012].

Comparative study of Protein Content of Fruit wastes and the SCP produced : The Protein Content of Fruit wastes originally were very less as compared to the crude protein content of the SCP biomass produced as shown in the table 5. Best results were obtained from orange as the fruit waste taken had negligible protein content but the SCP biomass produces from the substrate had 23% crude protein. Thus substrates with very less or no protein can produce SCP with an appreciable amount of crude protein.

Table 5. The crude protein content in the Fruit waste and SCP produced from it.

	% of crude protein in	
Fruit	**Fruit waste**	**SCP biomass**
Pineapple	0.6	22.5
Banana	5.0	16.2
Sugarcane	2.5	21.2
Orange	0	23.2

No supplements such as inorganic nitrogen sources, carbon and glucose sources were used to grow yeast on fruit wastes and therefore this process of SCP production becomes cheaper. However, many researchers in their studies have used inorganic supplements for the mycelial growth on waste materials (Ojokoh and Uzeh, 2005). Thus it can be concluded that orange waste was found to be most suitable substrate for SCP production. Therefore fruit and vegetable wastes should be exploited properly as a substrate for the production of cellular biomass of edible fungi instead of dumping them on roads, in the drains and water bodies so that they can be used as animal feed supplement and if suitable, for human consumption as well. This can help in waste management besides solving the worldwide problem of protein shortage.

These days people are becoming health conscious and consume large quantities of fruits and fruit juices leading to the accumulation of fruit wastes. The disposal of fruit waste in environment can pose health hazard to living beings (Yousufi, 2012).

A variety of fruit wastes have been used as substrates for the production of SCP by various researchers. Sweet orange residues have been used for SCP production by Nwabueze and Oguntimein. Sweet potato residue has been used for SCP production by Yang. Rahmat *et al.,* used apple pomace for the production of single cell protein from *Kloechera apiculata* and *Candida utilis* so as to improve stock feed. Pineapple cannery effluent has been utilized for SCP production by Nigam. Essien *et al.,*used banana peel as a substrate for mould growth and biomass production. Production of fungal biomass on fruit and other agricultural wastes shall not only minimize loads of pollutants but at the same time the atrophied people can have protein supplement at a reasonable cost. The study revealed that orange fruit waste generates highest amount of protein, followed by pineapple, sugarcane and banana (Table 5).

Thus, fruit and vegetable wastes should be utilized properly as a substrate for the production of cellular biomass of edible or food fungi instead of throwing them away on roads, in the drains and water bodies so that they can be used as animal feed supplement and if suitable, for human consumption as well with least expenditure.

Acknowledgement :

The authors are thankful to Dr. Sister Doris D'Souza, Principal, Patna Women's College, and Patna University for providing an opportunity to undertake this research work and for the facilities provided in carrying out this study.

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