Identification of Single Cell Protein Producing Properties from Fruit Waste

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***Abstract* - The bio conversion of fruit waste by microbial fermentation into single cell protein has potential use for solving the worldwide protein deficiency in food .This single cell protein can be used as an economical product for food and feed. However utilizing the fruit waste as substrates for the production of high nutritious food products also reduce the environmental pollution. Single Cell Protein represents microbial cells grown in mass culture and harvested for use as protein, carbohydrate sources in food or feeds. In the present study, peels of Watermelon, Musambi, Muskmelon, Papaya and Pineapple (crown) were used as the carbon source for the media preparation and three different strains namely *Rhodopseudomonas, Saccaromyces cerevisiae* (baker's yeast), *Trichoderma harzianum* were used for microbial fermentation. Increasing the economic value of the waste obtained after 4 day fermentation process. Thus the present findings help in Single Cell Protein production from inexpensive, cheap, readily available agro waste material.**

Keywords: Single cell protein, fruit waste, agro waste, bio conversion

1. INTRODUCTION

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 (Adoki.A et al., 2008). Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. (Yabaya. A *et al*., 2008).Thus fruit processing wastes are useful substrates for production of microbial proteins. The utilization of fruit wastes in the production of Single Cell Protein will help in controlling pollution and also in solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food (Barton A.F.M, 1999).

The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technology (Omar and Sabry, 1991). Single cell proteins have application in animal nutrition as: fattening calves, poultry, pigs and fish breading in the foodstuffs area as: aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to-serve meals, in diet recipes and in the technical field as: paper processing, leather processing and as foam stabilizers.

Single Cell Protein (SCP) is one of the most important steps for this goal and is an alternative and an innovative way to successfully solve the global food problem. The term Single Cell Protein refers to dead, dry microbial cells or total proteins extracted from pure microbial cell culture and is produced using a number of different microorganisms including bacterium, fungus and algae. It can also be called biomass, bioprotein or microbial protein. The word Single cell protein is considered to be appropriate since most of the microorganisms grow as single or filamentous individuals. Besides high protein content (about 60-82% of dry cell weight), Single Cell Protein also contains fats, carbohydrates, nucleic acids, vitamins and minerals. Another advantage with Single Cell Protein is that it is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods. This protein can be used as additive added to the main diet instead of sources known very expensive such as soya bean and fish (Gour Suman *et al*., 2015).

Different types of microbes such as bacteria, fungi, mold, algae and yeasts can be used as the sources of Single Cell Protein. Algal single Cell Protein has limitations such as the need for warm temperatures and plenty of sunlight in addition to carbon dioxide, and also that the algal cell wall is indigestible. Bacteria are capable of growth on a wide variety of substrates, have a short generation time and have high protein content. Yeasts are probably the most widely accepted and used microorganism for Single Cell Protein (Singh Nee Nigam *et al*., 2009). Fungal species are also now widely used in single cell protein production (Mahmood Khan Yousuf, 2012). So this study focuses on the comparative study between fungal, bacterial and yeast for single cell protein production.

2. MATERIALS AND METHODS

2.1 Collection and preparation of fruit substrates

Watermelon, Musambi, Musk melon, Papaya and Pineapple waste were collected from the local markets of Hosur, Tamilnadu, India and washed several times with sterile water. The peels were separated, oven dried (at 40-50˚C), ground and sieved through mesh screen and stored

Figure 1: Represents the fruits used for single cell protein production

2.2 Microorganisms

The microorganisms used to ferment fruit wastes was *Rhodopseudomonas*, *Saccaromyces cerevisiae*, *Trichoderma harzianum* all the three microorganisms were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

2.3 Preparation of fruit hydrolysates

The fruit powders of the above fruit mixture were used as substrate for production of Single Cell Protein. The fruit powders were degraded to convert cellulose content into more available sugars by chemical treatments. 50 ml of 10% (w/v) Hydro chloric acid (HCl) was added to the 5 g fruit powder. The solution was placed in water bath at 100˚C for one hour and allowed to cool. The filtrates were collected using Whatman filter paper 42 pore size and autoclaved. The sterile solution was used as carbon and nitrogen source for biomass production. These fruit hydrolysates were used as the substrates for the three microorganisms

Figure 2: Represents the fruit hydrolysates after treatment of fruit powder with HCl

2.4 Biochemical analysis of the fruit wastes

The protein content was determined by the method of Lowry *et al.*, using BSA as standard. The total carbohydrates were estimated by the Anthrone method using glucose as standard (AOAC method of analysis.). The total phenolics content were determined by Folin-Ciocalteu method (Gao *et al.*, 2000). The crude fat, crude fibre, ash and moisture were determined by the method of Raghuramula *et al.*, The total flavonoids content were determined by employing aluminium chloride colorimetric assay method (Jia *et al.*, 1999).

2.6 Fermentation

Submerged fermentations were carried out with the Glucose Supplemented Fruit Hydrolysate (GFH) medium (NH4)2SO4 (1 gm), KH2PO4 (0.5gm), MgSO4.7H2O (0.25 gm), NaCl (0.05 gm), CaCl2 (0.05 gm) (pH-5.5) made up to 500ml with Fruit Hydrolysates (FH). The medium designated was Glucose Supplemented Fruit Hydrolysate (GFH).In the media, initial pH was adjusted to 5.5 using 1N H2SO4. 500 ml of the medium were divided equally and transferred to the three conical flasks and sterilized at 121˚C for 15 mins. The strains were inoculated in the media and incubated at 28˚c for 4 days.

2.7 Bioconversation of fruit waste and proximate analysis of the biomass

After fermentation for 4 days the biochemical constitutes of the three different biomass was analyzed for protein, carbohydrate, phenolics and flavonoids. The amount of protein was estimated by the method of Lowry *et al*., using BSA as standard. The total carbohydrates were estimated by the Anthrone method using glucose as standard (AOAC method of analysis.). The total phenolics content were determined by Folin-Ciocalteu method (Gao *et al*., 2000). The crude fat, crude fiber, ash and moisture were determined by the method of Raghuramula *et al*., The total flavonoids content were determined by employing aluminium chloride colorimetric assay method (Jia *et al.*, 1999).

3. RESULTS AND DISCUSSION

Table1. Proximate composition of the mixed fruit powder

1.	Carbohydrates (mg/ml)	20
2.	Protein (mg/ml)	3.45
3.	Phenolics (mg/ml)	0.6147
4.	Flavonoids (mg/ml)	2.564
5.	Ash content (%)	6.4
6.	Moisture content (%)	15.2
7.	Crude fat (%)	5.06
8.	Crude fiber (%)	12

The fruit peels powder is tested for different parameters such as carbohydrate, protein, ash content, moisture content, crude fat, crude fiber, phenolics and flavonoids. The carbohydrate content was found out to be 20 mg/ml, protein content was 3.45 mg/ml, phenolics and flavonoids content was estimated to be 0.6147 mg/ml and 2.564 mg/ml. The ash and moisture content was found out to be 6.4% and 15.2%.The crude fat and crude fiber was found out to be 5.06% and 12%.By this estimation it is clear that this fruit waste serve as the good carbon source for the cultivation of single cell protein with the carbohydrate content of about 25 mg/ml . Similar observation had been reported by Amit Kumar Mondal et al., by using *saccaromyces cerevisiae* to ferment the individual fruit waste of cucumber and orange peel hydrolysates but this study focuses on combining different fruit peels and using the combination of the different fruits in producing Single Cell Protein, giving a different approach in cultivating Single Cell Protein.

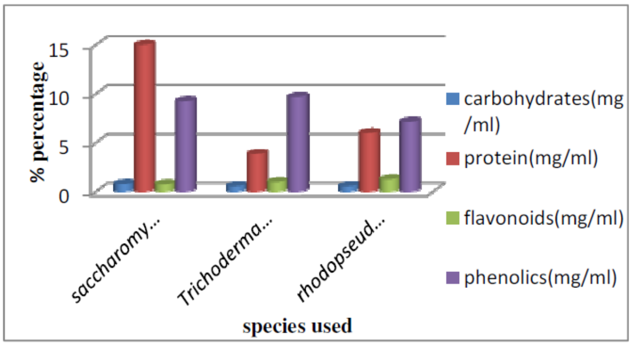

Figure3. The proximate analysis of wet biomass

The proximate analysis of the wet biomass after four days of fermentation process is represented in the figure.3. Estimation of highest protein percentage was found in the biomass of *Saccharomyces cerevisiae* (15 mg/ml) and *Rhodopseudomonas* (6mg/ml) but very less in *Trichoderma harzianum* (3.4 mg/ml) hence by percentage data comparing the protein of the three different species, the single cell protein production will be maximum if the fermentation of fruit waste was done with *Saccharomyces cerevisiae*. The fruit powder had the carbohydrate source and the nutrients which supported the microorganism growth but the sources of carbon and nutrients was well utilized by *saccharomyces cerevisiae* in comparing with the other two species because the protein estimation was said to be higher in the *Saccharomyces cerevisiae* in comparison with that of the other two species.

Based on fermentation observations the highest protein content was recorded on that biomass were *Saccharomyces cerevisiae* was used as inocula. Among the three micro organisms *S.cerevisiae* is effective in utilization of the carbon source from the fruit waste when compared to *Rhodopseudomonas, Trichoderma harzianum.* These findings were in agreement with the findings of Amit Kumar *et al*., (2008).

Instead of using single fruit peel as the substrate the combined mixture of the fruit peels observed good protein yielding capacity. Only 5 g of the fruit powder were hydolysed and used as the substrate it yielded about protein percentage of 15 mg/ml in *Saccharomyces cerevisiae*, 6mg/ml *Rhodopseudomonas* and 3.4 mg/ml in *Trichoderma harzianum* which is slightly greater than the findings of Amit Kumar *et al*., 2008 where about 40 g of fruit powder was taken for hydrolysis with HCl. Hence by comparison instead taking a single fruit waste extract for fermentation mixture of fruit peels can be taken for increased protein production

Glucose Supplemented Fruit Hydrolysate (GFH) medium were used for fermentation gave optimum .These findings were in agreement with the finding of Yakoub Khan M. and Umar Dahot M (2010).

Yeast is a probiotic component i.e. microorganisms which are good to human and animal body .This probiotic component can be utilized for single cell protein production thus, consumption of protein produced by yeast is good for health and is a great supplement for protein source .This proteinaceous product can be refined and further processed to obtain a pure single cell protein which can be used as food and feed. Similarly the agricultural waste, fruit waste and other vegetable waste from markets and industries can be used for single cell protein production thus greatly useful in controlling pollution and enables recycling of waste which are being throwed. Yeast is currently the most commonly used organism in the production of biomass, probably because it is already accepted both in human food and animal feed industries. Yeast based processes are the most advanced towards commercial production, followed by bacterial processes. Yeast may have many convenient characteristics, such as the ability to use a wide variety of substrates like hexose, pentose, and hydrocarbon, susceptibility to genetic variation, ability to flocculate and high nutritional value. In this study yeast showed promising result for single cell protein production.

4. CONCLUSION

Conclusion of this studies, the higher yield of single cell protein production can be obtained from the *Saccharomyces cerevsiae* in comparing to the other species the carbohydrate utilization is less and protein production is more in the *Saccharomyces cerevisiae*, the *Rhodopseudomonas* species also show promising results but in comparing to *Saccharomyces cerevisiae* the yield is less, the addition of glucose provided available carbon source for the organisms. Therefore, increasing the rate of Single Cell Protein production. This study reveals that Watermelon, Musambi, Musk melon, Papaya and Pine apple waste with good nutrient content can be converted into proteinaceous food and feed therefore properly utilizing them will bring an end to the protein deficiency around the world and good nutritious food can be supplied with least expenditure of cost and thereby converting fruit waste into protein rich food.

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