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Production of fungal single cell protein using *Rhizopus oligosporus* grown on fruit wastes

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ABSTRACT : In the present investigation the *Rhizopus oligosporus* was grown on various fruit wastes such as papaya waste, cucumber peelings, pomegranate fruit rind, pineapple fruit skin and watermelon skin. The disposal of wastes is a serious problem and their deposition poses health hazard for all the living beings. The problem was undertaken because on one hand there is disposal problem and on the other majority of our population is living below poverty line and is suffering from malnutrition. The protein from microbes is cheap, easy to obtain in crude form, nutritive and can be made available as a food or feed additive to increase its nutritional value. A comparative study of fruit wastes revealed that papaya fruit waste generates highest amount of protein per 100 g of substrate used, followed by cucumber peelings, pomegranate rind, pineapple fruit skin and water melon skin respectively with 59.5 mg, 57.3 mg, 51.6 mg, 48.0 mg and 43.2 mg crude protein respectively. The paper deals with details of SCP production using *R. oligosporus*.

INTRODUCTION

The term protein is derived from the Greek word ‘Proteios’ which means first. The name is so given because they are the first among the natural polymers that are vital for the growth and maintenance of life. Proteins are present in all living tissues as building block components of the body. Proteins are important dietary constituent for the supply of nitrogen as well as sulfur. When necessary, proteins may be catabolized to produce energy. Proteins are also the major structural components that provide mechanical support to the body. Many hormones in the living body are proteins that help in regulating or controlling various cell functions from metabolism to reproduction. Proteins are the essence of life processes and are important for proper growth and development of all the living beings. Its deficiency may lead to a number of health disorders in an individual. A huge population in this world, especially those who are living below poverty line is suffering from malnutrition. This is because there is a big gap between the demand of protein-rich food and its supply to the ever-increasing world population. In order to bridge this gap, Single cell protein (SCP) is an innovative and an alternative way to this direction. SCP may be used directly as human food supplement or it may be used in animal feed to at least partially replace the currently used protein-rich soybean meal and fish proteins and even cereals, which can be diverted for human consumption (Singh, 1998).The impetus behind single cell protein production lies partly in the need for more protein and partly in the commercial increase in the economic advantages gained by substitution of microbial protein for the conventional protein supplements used in livestock feeding (Khan *et al.*, 1992).

The present study was carried out to utilize fruit and vegetable wastes in the production of single cell protein by growing microorganisms which can be used in food as such or as animal feed. The protein from microbes is cheap, easy to obtain in crude form and nutritive. Land use is negligible. Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper (Anupama and Ravindra, 2000). Microorganisms can utilize waste materials that cause pollution problem and also sanitary hazards. The use of wastes would help in controlling pollution and also in solving waste disposal problem to some extent. SCP production has the potential for feeding the ever increasing world population at cheaper rates. As a source of protein, it is very promising with the potential to satisfy the world shortage of food while population increases (Najafpour, 2007).

MATERIAL AND METHODS

Fruit residues of papaya, pineapple, pomegranate, watermelon and cucumber were collected and washed thoroughly under running tap water and then transferred into beakers and conical flasks after weighing a specific quantity of residue. The flasks were then subjected to autoclaving at 121ºC and 15 Psi pressure for 45 minutes. After cooling, the sterilized material was then transferred to pre-sterilized Petriplates and inoculated with the inoculum of *Rhizopus oligosporus*. The plates were then incubated at 28 ± 1ºC for 5-7 days. After the growth of fungal biomass, the mycelia were filtered on a filter paper (Whatmann filter paper No.1) and washed with distilled water to remove adhering particles if any. The filter papers containing the mycelia were dried at 90 ± 2ºC for 24 hours to get moisture free fungal contents. The protein content of the fungal biomass was estimated by Kjeldahl method (Mathur and Mehta, 1967).

RESULTS AND DISCUSSION

After inoculation with *R. oligosporus* and 5 days of incubation using wastes of papaya, cucumber, pomegranate, pineapple and watermelon at a temperature of 28 ± 1ºC , it was found that wastes of papaya supported higher mycelial growth and biomass, followed by that of cucumber peelings, pomegranate rind and skin of pineapple.

However, skin of watermelon could not support the higher growth of mycelial biomass.

Increased mycelial biomass on a substrate varies directly with the amount of protein for a particular fungal species. In the present investigation, the amount of protein (crude) estimated by Kjeldahl Method was found to be 59.5 mg, 57.3 mg, 51.6 mg, 48.0 mg and 43.2 mg respectively from the mycelial growth on papaya waste, cucumber peelings, pomegranate fruit rind, pineapple fruit skin and watermelon skin. Thus under uniform conditions of experimentation to achieve higher yield of mycelial biomass and as a consequence higher amount of fruit protein from *R. oligosporus*, papaya fruit waste is therefore comparatively best substrate out of the five fruit wastes used. The results are per 100g of the substrate used.

Table 1 : Describes the biomass production by *Rhizopus oligosporus* in various fruit wastes taken in the present investigation.

Substrate	Biomass Production (mg/100g of substrate)
Papaya waste	59.5 mg
Cucumber peelings	57.3 mg
Pineapple fruit skin	48.0 mg
Rind of pomegranate	51.6 mg
Skin of watermelon	43.2 mg

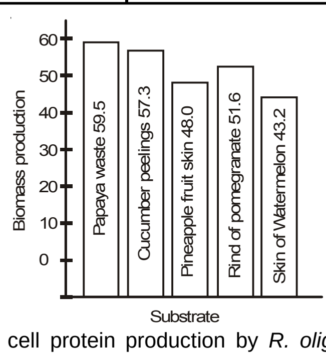

Fig. 1. Single cell protein production by *R. oligosporus* using various fruit wastes

It should be noted that *R. oligosporus* was able to grow on various fruit wastes without any supplementation with inorganic nitrogen sources, carbon and glucose sources, the addition of which makes the SCP production expensive.

However, many workers in their investigation have used inorganic supplements for the mycelial growth on waste materials. Ojokoh and Uzeh (2005) utilized glucose (2% w/v) and $(NH_4)_2HPO_4$ (0.25% w/v) as a nitrogen source supplement for the production of *Saccharomyces cerevisiae* biomass in papaya extract medium. Similarly, Adoki (2008) studied various factors influencing cell biomass production with *Candida* species using citrus fruit wastes (orange, plantain and banana wastes) and found that the test strain was capable of meeting its amino acid requirements in culture when supplied with inorganic nitrogen sources. Supplementation of media with 0-15% and 0-6% w/v combination of dextrose and ammonium nitrate respectively resulted in optimal growth at a pH of 4.6 after 60 h.

In urban areas the people are health conscious and consume large quantities of a variety of fruits at home as well as on eateries and juice parlours. This leads to the accumulation of fruit skins/rinds and the residue left over after extraction of juice. This poses a very serious problem for their disposal and the dumping of such wastes into the drains causes choking and overflow of foul smelling water on the roads causing problem to pedestrian and vehicular movements. This waste can be exploited as a substrate for the growth of food fungi such as *Rhizopus oligosporus*, *Aspergillus oryzae* and various species of *Agaricus* and *Morchella*, the sources of protein, which after proper testing may be utilized as a feed supplement for domestic animals and cattles and if found suitable for human consumption as a protein source. Several different fruit wastes have been utilized as a substrate by a number of researchers such as dates (Kamel, 1979), beet pulp (Ghanem, 1992), sugarcane bagasse (Azin and Moazami, 1989), banana skins (Enwefa, 1991) and guava peel (Moharib, 2003) for the production of single cell protein.

Natural rubber waste serum has also been used for the production of SCP as a substrate for *Rhizopus oligosporus* by Mahat and MacRae (1992). Yabaya and Ado (2008) studied the mycelial protein production by *Aspergillus niger* using banana peels.

It is a fact that majority of our people live below poverty line and are malnourished. Production of fungal biomass on fruit and vegetable wastes shall not only minimize loads of pollutants which also enter water bodies and by their rich nutrient contents make it eutrophic, but at the same time the malnourished people can have protein supplement at a very cheaper cost. The wastes from edible substrates have been used for the production of microbial biomass protein by Steinkraus (1986).

The degree of mycelial biomass growth depends on the type of substrate used. The present investigation (Table 1) clearly reveals that for *R. oligosporus*, papaya fruit waste is the best substrate followed by that of cucumber, pomegranate rind and pineapple. However, because of high water content watermelon skin could not support higher growth of mycelial biomass and the yield was only 43.2 mg/ 100 g of the substrate used.

Thus, instead of dumping fruit and vegetable wastes on the roads, in the drains and water bodies, it should be exploited by properly managing it as a substrate for the production of fungal mycelial biomass of edible or food fungi to be used as supplement in animal feed and if suitable for human consumption with least expenditure of money.

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