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To evaluate the biochemical parameters of fruit waste infested with fungi

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Abstract : *In the present investigation, the various fruit wastes used included papaya waste, orange waste, pomegranate rind, watermelon skin, pineapple skin and mango peel. A total of five different combinations of fruit waste were inoculated with two fungi i.e. Aspergillus niger and Rhizopus oliogosporus. These different wastes were taken for analysis of different biochemical parameters like analysis of protein, carbohydrate and fatty acid contents. The maximum protein content with Apergillus niger was found to be 999 µg/ 500 mg substrate when used in combination with watermelon skin and mango peel. However, the maximum protein content in Rhizopus oligosporus was found to be 990µg/ 500 mg substrate when used in combination with watermelon skin and pomegranate rind .The maximum carbohydrate content in Apergillus niger was found to be 848µg/500 mg of substrate when used in combination with Pineapple skin and pomegranate rind. However, the carbohydrate content in Rhizopus oligosporus was found to be 821 µg/ 500 mg substrate when used in a combination with papaya skin and orange waste. The maximum fatty acid content in Apergillus niger was found to be 0.0196µg/500 mg of substrate when used in combination with pomegranate rind and watermelon skin. However,The fatty acid content Rhizopus oligosporus was found to be 0.0252µg/ 500mg substrate when used in combination with pineapple skin and pomegranate rind. The study suggests that molds use fruit refuse to built up their protein, carbohydrate and fatty acid content.*

Keywords: *Biochemical Parameters, Aspergillus niger, Rhizopus oligosporus, Fruit Wastes.*

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Introduction :

Fruit wastes are produced in large quantities in the markets and constitute a source of environmental pollution. Increasing world population and food crisis has shifted the emphasis on use of wastes for alleviating food shortage.

Fruits store energy in the form of

carbohydrates, proteins and fat which may be converted into any other form of energy through thermo-chemical conversion or biochemical conversion process (Nirmala et al, 1996).

According to Indian Agricultural Research Data Book 2004, the total waste generated from fruits comes to 50 million tons per annum. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth (Yousufi et al., 2003 and Yousufi, 2013). Thus, fruit processing wastes are useful substrates for production of microbial proteins. Essential fatty acids are a special type of "good fat".The protein from microbes is easy to obtain in crude form and is nutritive. Anupama and Ravindra (2000) studied the bioconversion of agricultural and industrial wastes to protein rich food and fodder stock. 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 wastes disposal problem to some extent.

The present study deals with the estimation of biochemical parameters viz. proteins, carbohydrates and fatty acids of various combinations of fruit waste infested with fungi viz. *Aspergillus niger* and *Rhizopus oligosporus.*

Materials and Methods :

Fruit waste used : Different fruit wastes used in the present investigation included papaya waste, orange waste, pomegranate rind, watermelon skin, pineapple skin, mango peel. These fruit wastes were collected from local fruit juicecorner, Patna.

The collected fruit wastes were transferred into sterilized distilled water after proper cleaning. Thereafter, different combination of fruit wastes were prepared in the ratio of 1:1 and transferred to different conical flasks. The flasks containing different fruit wastes were steam sterilized. The sterilized fruit wastes were aseptically transferred to pre- sterilized petriplates. Two standard fungi i.e. *Aspergillus niger* and *Rhizopus oligosporus* respectively were transferred as inoculum to the plates. The plates were transferred in an incubator at (28ºC) for growth and development. After 7 days of growth of fungal mycelium were transferred on a filter paper (Whatmann filter paper No.1) and washed with distilled water to remove undesirable fruit waste. The filter paper containing the mycelia were dried at 90ºC for 24 hours to get moisture free fungal content (Yousufi, 2013).

Estimation of biochemical component : The method of Lowry et. al. (1951) was followed for protein estimation. The estimation of carbohydrate and fatty acid was done by using Anthrone reagent and Phenolphthalein respectively.

Preparation of extract and method for protein test : 500mg of fruit waste combinations was added in10mL acetate buffer and crushed and centrifuged at 2500 rpm for 10 minutes and filtered.After that, 1 mL of the filtrate was added in 5mL alkaline solution (0.1(N) NaOH + 2% Sodium Carbonate + 1% Copper Sulphate + 2% Sodium Potassium Tartarte) and left undisturbed for 10 minutes. 0.5 mL of Folin- Ciocalteau reagent was added. Absorbance was taken at 750 nm. (Lowry et al., 1951).

Preparation of extract and method for carbohydrate test : 500mg of fruit waste combinations was dissolved in 12.5mL of 6.25 N concentrated sulphuric acid and neutralized with it with solid sodium carbonate until the effervescence

ceased. Then it was centrifuged at 3000 rpm for 10 minutes. One mL of the filtrate was added to 10 mL of Anthrone reagent. It was heated for 8 minutes in a boiling water bath and left undisturbed. Absorbance was read at 630 nm.

Preparation of extract and method for fatty acid test : 500mg of fruit waste combinations was crushed with 10mL distilled water and centrifuged at 2500 rpm for 10 minutes and filtered. 0.5 mL of the fruit waste combinations was taken in a conical flask and 12.5mL neutral solvent (Diethyl ether: 95% ethanol) + four drops of Phenolphthalein indicator was added. The solution was titrated against 0.1 (N) KOH till the persistent pink color was obtained.(Phenolphthalein indicator reagent).

Results and Discussion :

In the present study, the fruit waste combination included papaya and orange, pineapple and pomegranate, papaya and pineapple, watermelon and pomegranate and watermelon and mango . The combinations are considered as a good source of protein, carbohydrate and fatty acid.

(a) Extent of protein : In *Aspergillus niger* strain the protein content was maximum in watermelon and mango waste combination i.e. 999µg/mg and least in papaya and pineapple waste i.e. 130µg/mg (Table 1). In *Rhizopus oligosporus* strain the protein content was maximum in watermelon and pomegranate waste combination being 990µg/mg and least in papaya and orange waste i.e. 172µg/mg. Such increased protein contents was due to the availability of high monosaccharide for *Aspergillus niger* as compared to *Rhizopus oligosporus*. But according to Yousufi et al. (2003), the maximum protein content determined with *Aspergillus oryzae* was 57.3 mg per 100g of substrate on the combination of pomegranate rind and guava peel, least protein content was found to be 24.2 mg per 100g of substrate on the combination of apple waste and watermelon skin and with *Rhizopus oligosporus*, it was found to be 61.2 mg per 100g of substrate on the combination of pineapple skin and pomegranate rind having least protein content being 31.3 mg per 100g of substrate in the combination of orange waste and guava peel.

Table 1. Estimation of protein content in fruit wastes

S. No.	Combinations of fruit wastes	Combination ratio	Protein Content (in µg/500mg of substrate)	
			Aspergillus niger	*Rhizopus oligosporus*
1.	Papaya waste and Orange waste	1:1	152	172
2.	Papaya waste and Pineapple skin	1:1	130	380
3.	Pineapple skin and Pomegranate rind	1:1	887	877
4.	Pomegranate rind and Watermelon skin	1:1	988	990
5.	Watermelon skin and Mango peel	1:1	999	969

The best fruit waste combination that produced maximum protein content was found to be in pomegranate rind and watermelon skin with *Rhizopus oligosporus.* However, with *Aspergillus niger,* the best fruit wastes combination was found to be in watermelon skin and mango peel (Yousufi et al., 2003).

(b) Extent of carbohydrate : In *Aspergillus niger* strain the carbohydrate content was maximum in pineapple and pomegranate waste combination being 848 µg/ml and this value being least in watermelon and mango waste which was 132 µg/mg (Table 2). In *Rhizopus oligosporus* strain the carbohydrate content was maximum in papaya and pineapple waste combination is 821 µg/mg and least in papaya and orange waste was 179µg/mg.(Kareem et al., 2010), Screening of stock-cultures of *Aspergillus niger* and pineapple waste, the carbohydratecontent obtained to be 42.2% (Yousufi et al.,2013). The maximum carbohtdrate content was obtained on orange fruit waste medium with *S. cerevisiae* i.e. 10.0%and with *C. tropicalis* i.e. 10.2% and with mango fruit waste medium.

Table 2. Estimation of carbohydrate content in fruit wastes

S. No.	Combinations of fruit wastes	Combination ratio	Carbohydrate Content (in µg/500mg of substrate)	
			Aspergillus niger	*Rhizopus oligosporus*
1.	Papaya waste and Orange waste	1:1	212	179
2.	Papaya waste and Pineapple skin	1:1	189	821
3.	Pineapple skin and Pomegranate rind	1:1	848	209
4.	Pomegranate rind and Watermelon skin	1:1	749	795
5.	Watermelon skin and Mango peel	1:1	132	195

The best fruit waste combination that produced maximum carbohydrate content was found to be in papaya waste and pineapple skin with *Rhizopus oligosporus.* However, with *Aspergillus niger* the best fruit wastes combination was found to be in pineapple skin and pomegranate rind (Yousufi et al., 2003).

(c) Extent of fatty acid : In *Aspergillus niger* strain the fatty acid content was maximum in watermelon and pomegranate waste combination and least in pomegranate and pineapple waste (Table 3). In *Rhizopus oligosporus* strain the fatty acid content was maximum in pineapple and pomegranate waste combination and least in watermelon and mango waste. (Azam et al., 2014), *Saccahaoromyces cereviisae,* the total fat produced from orange peels were 0.80% and *Aspergillusniger*the total fat produced from orange peels were 0.75%.

Table 3. Estimation of fatty acid content in fruit wastes

S. No.	Combinations of fruit wastes	Combination ratio	Fatty acid Content (in µg/500mg of substrate)	
			Aspergillus niger	*Rhizopus oligosporus*
1.	Papaya waste and Orange waste	1:1	0.0142	0.0196
2.	Papaya waste and Pineapple skin	1:1	0.0112	0.0142
3.	Pineapple skin and Pomegranate rind	1:1	0.0084	0.0252
4.	Pomegranate rind and watermelon skin	1:1	0.0196	0.0224
5.	Watermelon skin and Mango peel	1:1	0.0168	0.0084

The best fruit waste combination that produced maximum fatty acid content was found to be in pineapple skin and pomegranate rind skin with *Rhizopus oligosporus.* However, with *Aspergillus niger,* the best fruit wastes combination was found to be in watermelon skin and pomegranate rind.

Conclusion:

The study concluded that, fruit wastes can be used in different combinations to produce protein, carbohydrate and fatty acid. Fruit waste infested with fungi can be also used in future as food supplement. The benificial part of this utilization is

that, the use of fruit wastes would help in controlling environmental pollution and also in solving waste disposal problem to great extent.

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