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Comparative study of single cell protein production by utilising different fruit and vegetable wastes

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Abstract
The fruit and vegetable waste i.e. peels, pomace and seeds can be utilized to produce various products such as ethanol, single cell protein, hydrogen. Single Cell Protein (SCP) is one of the product that can be isolated by use of fruit and vegetable waste. SCP refers to dried microorganisms that can be used as protein dietary supplement. The production of SCP majorly depends upon the type of substrate being provided to the microorganism. Studies have shown that peels have high nutritional value and hence can be used to produce a value added product. Comparison was made in the single cell protein production by using different fruit and vegetable wastes. Saccharomyces cerevisiae was allowed to grow on different substrates like peels of pomegranate, orange, carrot and banana to produce SCP. The comparative study showed that carrot waste produced maximum amount of Single Cell Protein.

Keywords: fruit and vegetable waste, single cell protein, saccharomyces cerevisiae, protein dietry supplement, waste management

Introduction
Food waste are the parts of fruits, vegetables or any other food stuff which are unconsumed and unused morphological characteristics of the commodity, lack of proper handling operations, or simply discarded for diverse reasons (Sagar *et. al*., 2018) [6]. Starting from production stage to consumer domain, about 1.3 billion tons per year food is wasted or lost. Out of all the food wastes commodities, fruits and vegetables account for 44%. When a raw material enters food processing line, both product and wastes are produced. About 25-30% of fruit waste is in form of peels, pomace and seeds (Salim *et. al.,* 2017) [7]. Dicing of papaya produces 8.5% of peels and 6.5% of seeds as waste product whereas mango processing produces 11% of peels, 13.5% of seeds and 18% of inoperable pulp (Sagar *et. al*., 2018) [6]. A lot of study has been going on utilization of these wastes to produce value added products. Peels can be used for production of methane, hydrogen and ethanol. They have high nutritive content and thus can be used as single cell protein by carrying out fermentation process (Kodagoda *et. al.,* 2017) [3]. Proteins are building blocks of all the living beings. Proteins are important for life processes, for proper growth and development in living beings. Its deficiency lead to a number of disorders because essential amino acids which cannot be synthesized by the body itself are not replenished. The most common deficiency is known as kwashiorkor (Hermann, 1990) [2]. Single Cell Protein (SCP) is one of the easiest, cheap and innovative ways to solve the protein deficient problem. In the late 1960s, the production of Single Cell Protein began. Single Cell Protein refers to dead, dry cells of micro-organisms such as yeast (*Candida, Saccharomyces, etc.)*, bacteria (Cellulomonas, Alcaligens, etc.), algae (Spirulina, Chlorella, etc.), molds (Trichoderma, Fusarium, Rhizopus, etc.). The term SCP is appropriate as most of the microorganisms grow as single or filamentous individuals. SCP is rich in protein content (about 60-82% of dry cell weight), fats, carbohydrates, nucleic acids, vitamins and minerals. SCP is rich in most of the essential amino acids such as lysine, methionine. SCP can be used instead of the expensive protein source like soybean and fish (Gour *et. al.,* 2015) [1]. Different micro-organisms such as bacteria, fungi, yeast and algae are used in the production of Single Cell Protein. Out of all yeast is consider to be the best microbes for the production of SCP as it is easy to harvest because of their large cell size, lower nucleic acid content, high lysine content and ability to grow on the acidic conditions. Disadvantages of using bacteria cell are it has small cell size and high nucleic acid content. To decrease the level of nucleic acid certain processing step has to be added which increase the production rate. Disadvantages of using fungi cell are it has low growth rate and lower protein content. Algae have cellulosic cell walls which cannot be digested by the human digestive system (Nasseri *et. al.,* 2011). In the production of fruits and vegetables, India is considered to be second largest producer. Approximately 40% of total mass as waste that includes its peels, pulp and seeds are generated from fruits. According to researchers, the fruit wastes have high nutritional value e.g. banana peels have more protein content than banana pulp itself (Pereira *et. al*., 2013) [5]. Thus fruit wastes are suitable as it is cheap substrate for the production of SCP. This will also help in solving the waste disposal problem to some extent and control pollution and will also reduce the protein shortage. The objectives of this study were to utilize the fruit waste to produce value added product, production of low cost protein and to meet the protein demand of growing population.

Materials and Methods
Procurement of sample
The wastes of pomegranate, banana, orange and carrot were obtained from fruit and vegetable vendors of local market of sector 36-A, Chandigarh.

Revival of Saccharomyces cerevisiae
The yeast (Royal dried yeast) was bought from local market. It was suspended in potato dextrose broth and was allowed to grow on slants of yeast extract potato dextrose agar (YEPDA) (Fig 1, Fig 2).

Fig 1: Culture of Saccharomyces cerevisiae preserved on slants

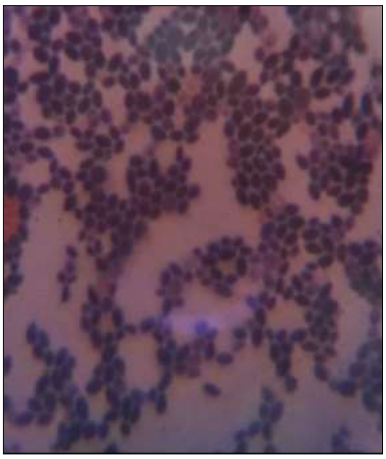

Fig 2: Microscopic view of *Saccharomyces cerevisiae*

Sample preparation and pretreatment
Sample (60 gm) was taken in a conical flask and 10% HCl (100 ml) was added to it. The flask was kept in water bath at 100^0C for 1 hour. The contents were cooled and filtered through Whatman filter paper no. 1. A carbon source i.e. dextrose (4 gm) was added to it. The media was then autoclaved.

Counting cells using hemocytometer
Dilution (1:1) of the cell suspension in methylene blue dye was prepared. Cover slip was placed and 0.1ml of methylene blue cell suspension was transferred to one of the chamber of the hemocytometer using auto pipette. The cells were viewed under microscope at 100X magnification and the total number of cells were counted as:

Fermentation and harvesting of single cell proteins
Autoclaved media (98ml) was inoculated with 2×10^7 CFU/ml *Saccharomyces cerevisiae* culture which was estimated using a hemocytometer. The media was then incubated at 27^0C for 9 days.

Isolation of single cell proteins
After incubation, the media was poured in preweighed centrifuge tubes (10 ml) and was centrifuged at 10,000 rpm for 15 minutes at room temperature. Supernatant was discarded and wet pellet was collected for further analysis.

Analysis of pellet
Amount of biomass produced
It was done to check the amount of yeast cells produced in the media. The weight of centrifuge tubes was noted prior to centrifugation. The sample (after incubation) was poured into the tubes and was centrifuged at 10,000 rpm for 15 minutes at room temperature. The supernatant was removed and again weight of the tubes was taken.

Calculations
Amount of biomass produced = Final weight-Initial weight

Protein content
Protein content of all the samples was estimated by using Folin Lowry method (Lowry *et. al*, 1951).

Results and Discussions
This study was conducted in order to compare the amount of single cell proteins produced by different fruit and vegetable wastes by analyzing the protein content and amount of biomass produced. The fruit and vegetable wastes were used as substrate for growth of Saccharomyces cerevisiae and above said parameters were studied. The differences are explained below.

Amount of biomass produced
It was calculated by centrifugation method and is reported in Table 1.

Table 1: Amount of biomass produced using different substrates

Sr.no.	Substrates	Amount of biomass produced (g/100ml)
1	Orange	1.4
2	Banana	2.6
3	Pomegranate	3.5
4	Carrot	1.5

Table 2: Protein concentration of different substrates

Sr.no.	Substrates	Protein concentration (mg/ml)
1	Orange	0.114
2	Banana	0.271
3	Pomegranate	0.282
4	Carrot	0.435

The results indicated that amount of biomass produced using orange, carrot, banana and pomegranate as substrate was 1.4g, 1.5g, 2.6g and 3.5g per 100ml respectively (Fig 3). According to study conducted by Sharma *et. al*., (2017), inoculating *Saccharomyces cerevisiae* on banana peel the increase in amount of biomass was found to be 84.6%. The possible reason for the same could be high amount of residual sugar in the banana peel. A similar study was conducted by Khan *et. al.*, (2015) that showed that the increase in amount of biomass produced using orange peel as a substrate was 30.5%. This indicates that orange peel has low residual sugar content.

Protein concentration
A standard curve was plotted between absorbance at 660 nm and concentration (mg/ml) using BSA as standard. The protein content of the samples was analyzed by comparing

the absorbance with concentration (Fig 4). The results indicated that protein content of *S.cerevisae* grown on orange, banana, pomegranate and carrot waste was 0.114 mg, 0.271 mg, 0.282 mg and 0.435 mg per ml respectively. According to the study conducted by Khan *et. al*., (2015), *Saccharomyces cerevisiae* was inoculated on banana, pomegranate and orange peels. The banana peels (58.62%) were found to contain more protein content as compared to pomegranate (54.28%) and orange peel (26.26%). The possible reason could be that banana peels have high residual sugar content followed by pomegranate and orange.

To conclude the study it can be said that the study was conducted to compare the production of single cell proteins using different fruit and vegetable wastes such as orange, banana, pomegranate and carrot. These wastes were used as a substrate for growth of *Saccharomyces cerevisiae.* The study showed that when carrot was used as a substrate, the protein content of yeast cells was maximum i.e. 0.435mg/ml as compared to orange, banana and pomegranate. But, the amount of biomass produced was found to be maximum in case of pomegranate i.e. 3.5g/100ml. This indicates that despite of the fact that amount of biomass produced is maximum in case of pomegranate, carrot waste is a good source of single cell proteins as it produces maximum amount of proteins. Thus, using these wastes as a source of microbial proteins solves two important purposes i.e. utilisation of food wastes and production of a cheaper source of protein for growing population.

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References

1. Gour Suman, Mathur Nupur, Singh Anuradha and Bhatnagar Pradeeep. Single cell protein production. Int. J Curr. Microbiol. app. Sci. 2015; 4 (9):251-262.
2. Hermann, Janice R. "Protein and the Body". Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources, Oklahoma State University, 1990; T3163(1):T3163-4.
3. Kodagoda Gethmini, Marapana Upul. Utilization of fruit processing by-products for industrial applications: A review. International journal of food science and Nutrition. 2017; 24:2455-4898.
4. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193:265-275.
5. Pereira Paulo, Henrique Fernandes, Kelly Cristina C. Carvalho Benini, Cintia Yumi Watashi, Herman Jacobus Cornelis Voorwald, *et al*. "Characterization of high density polyethylene (HDPE) reinforced with banana peel fibers." BioResources. 2013; 2351-2365
6. Sagar NA, Pareek S, Sharma S, Yahia EM Lobo. M.G. Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Compr. Rev. Food Sci. Food Saf. 2018; 17:512-531.
7. Salim NSMd, Singh A, Raghavan V. Potential utilization of fruit and vegetable wastes for food through drying or extraction techniques. Novel Techniques in Nutrition and Food Science, 2017, (2).