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UTILIZATION OF FRUIT WASTES IN PRODUCING SINGLE CELL PROTEIN

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Abstract: The bioconversion of fruit wastes into single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. Using food processing leftovers in the production of single cell protein as substrate would alleviate pollution. In this work cucumber and orange peels were evaluated for the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation. Results showed that tested fruit wastes were highly susceptible to hydrolysis. A comparative study of fruit wastes revealed that cucumber peel generates higher amount of protein followed by that of orange with 53.4% and 30.5% crude protein respectively per 100 gm of substrate used. Percentage of protein in single cell protein was much lower (17.47%) when *Saccharomyces cerevisiae* was grown on supplemented fruit hydrolysate medium that contained inorganic nitrogen sources but devoid of glucose. Addition of glucose to the supplemented fruit hydrolysate medium enhanced the protein content (60.31%) within the yeast cell. Thus the single cell protein production by yeast depends on the growth substrates or media composition.
Key words: Single cell protein, *Saccharomyces cerevisiae*, cucumber and orange peel extracts.

Introduction

The continual population growth in developing countries has required an increase in animal and human food supply. The increasing world demand for protein rich food led to the search for the formulation of alternative protein sources to supplement the conventional protein sources. 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 (1).

The term SCP 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 (2). It can also be called biomass, bioprotein or microbial protein. The word SCP 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), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals (3, 4). Another advantage with SCP 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 soybean and fish (1, 5).

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 could support microbial growth (6, 7). Thus fruit processing wastes are useful substrates for production of microbial proteins. The utilization of fruit wastes in the production of SCP will help in controlling pollution and also in solving waste disposable problem (8) to some extent in addition to satisfy the world shortage of protein rich food.

Different types of microbes such as bacteria, fungi, mold, algae and yeasts can be used as the sources of SCP. 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. Their use is somewhat limited by poor public acceptance of bacteria as food, small size and difficulty of harvesting and high content of nucleic acid on dried weight basis. Yeasts are probably the most widely accepted and used microorganism for single cell protein. So it will be beneficial to focus on yeast single cell protein rather than bacterial and algal single cell protein.

Over the last few years, a lot of research has been done for reprocessing and reuse of different fruit wastes for the conversion of valuable and nutritive products (9, 10, 11). Therefore the present investigation was carried out to assess the potential of various fruit wastes for cost effective yeast biomass production. In this work cucumber and orange peels were introduced as a potential substrate for fermentation to produce bioprotein which can be used in food as such or as animal feed.

2. Materials and Methods

2.1 Collection and preparation of substrates

The cucumber and orange fruit wastes were collected from the local markets of Shibpur, West Bengal, India and washed several times with sterile water. The peels were separated, oven dried (at 40-50^{0}C), ground and sieved through mesh screen. The samples thus prepared were packed in transparent Zip-lock polythene bags and stored at room temperature until further study.

2.2 Microorganism

The microorganisms used to ferment fruit wastes was *Saccharomyces cerevisiae* obtained from Department of Food technology and Biochemical Engineering, Jadavpur University, Kolkata. The yeast was grown on Yeast Extract Potato Dextrose Agar (YPDA) and subcultured every 3 weeks and incubated at 28-32^{o}C.

2.3 Preparation of fruit Extracts

The fruit peels were used as substrate for production of SCP. Peels were degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure of Lenihan *et al* (12). 50 ml of 10% (w/v) HCl was added to the each waste (40 gm) in conical flask respectively. The mixture/solution was placed in water bath at 100^{0}C for one hour. After being allowed to cool, it was filtered through Whatman filter paper. The filtrates were diluted with sterile distilled water at varying concentrations and autoclaved at 121^{0}C for 15 mints. The sterile solution/broth thus prepared was used as carbon and nitrogen source for biomass production.

2.4 Chemical analysis

The method for the determination of crude fat, crude fibre and total soluble solids were determined as described by AOAC (2006) (13). Total carbohydrate content was determined using Anthrone reagent (14). Ash content was obtained by igniting the fruit wastes in a muffle furnace at 55^{0}C as previously described by Pearson (15). Moisture content was determined by the method based on the principle of drying to constant weight. The biomass was expressed in terms of total protein content. The protein estimation was determined according to the method described by Lowry *et al* (16).

2.5 Inoculums Preparation

Saccharomyces cerevisiae culture prepared from 4 days growth on YPDA slants incubated at 28^0C was used as inoculums. It was prepared by washing the growing culture with 25 ml sterile distilled water. The spore suspensions were rubbed and adjust to final concentration of 10^7spores/ml. The suspension inoculums were kept in chiller at 4^0C for further use.

2.6 Fermentation and harvesting of single cell protein

Submerged fermentations were carried out in Erlenmyer flasks with three trial media. One of these designated Supplemented Fruit Hydrolysate (SFH) had the following composition a $(NH_4)_2SO_4$ (2 gm), KH_2PO_4 (1gm), $MgSO_4.7H2O$ (0.5 gm), NaCl (0.1 gm), $CaCl_2$ (0.1 gm) (pH-5.5) made up to 1 litre with Fruit Hydrolusates (FH). The second medium designated Glucose Supplemented Fruit Hydrolysate (GFH) had all the compositions of SFH and glucose (2 gm/l). The third had the Fruit Hydrolysate medium (FHM) only.

In all the media, initial pH was adjusted to 5.5 using 1N H_2SO_4 and/or 1N NaOH. Each medium (98 ml) was transferred into 250 ml Erlenmeyer flask and sterilized at 121^0C for 15 mints. Inoculums of 2 ml from suspension of *Saccaharomyces cerevisiae* was aseptically transferred into each medium. Fermentation was carried out at 28^0C under static condition followed by determination of biomass and other parameters after 6-day intervals.

2.7 Bioconversion of fruit waste and proximate analysis of SCP

After fermentation biomass was separated from culture broth by vacuum filtration and washed with sterile water. Before taking the weight of the biomass, it was transferred into an aluminum disk and was oven dried at 105^0C for one hr followed by cooling in desiccators to balance the temperature and weight. The biochemical constitutes of separated biomass (both wet and dry) such as crude protein, total carbohydrate content, reducing sugar and mineral content were studied.

3. Results and Discussion

The results of the chemical analysis of the fruit peel extracts were presented in Figure 1. The composition of all the waste samples was significantly different for all the tested parameters i.e. crude protein, crude fat, total carbohydrate, ash and moisture contents.

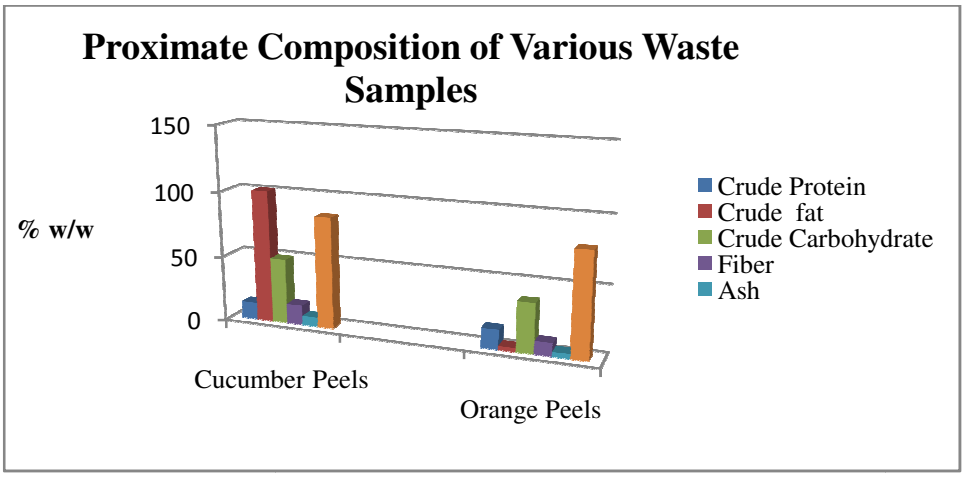

Figure 1. Proximate composition of various waste samples

Figure 2 represents the proximate composition of yeast biomass produced after fermentation which revealed that the amount of crude protein produced from cucumber peels by *Saccahaoromyces cereviisae* had the highest yield of 53.4% and that orange peels with *Saccaharomyces cerevisiae* had 30.5%. The total carbohydrate produced from orange and cucumber peels were 39.66% and 50.5% respectively.

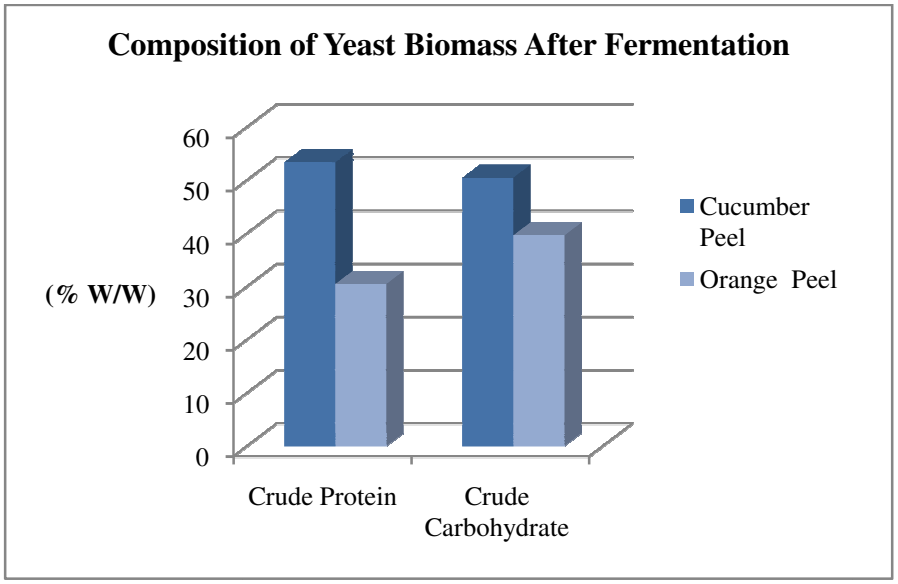

Figure 2. Composition of yeast biomass after fermentation

Data in Figure 1 illustrated that the fruit peel extracts contained variable ingredients such as carbohydrates, proteins, fats, minerals which are supposed to be useful for the growth of the yeast in the production of SCP. The findings of the present study for proximate chemical

composition of fruit wastes are corroborated with the results of various other studies (17, 18). Cucumber peels contained higher amount of available carbohydrates and minerals which might have favorably affected yeast biomass production. This type of composition has been reported to enhance biomass production by Lehnihan *et al* (19). Though orange peel also contained high concentration of carbohydrates but supported less biomass production. This may be due to the less mineral content in orange peels (3.55%) than that of in cucumber peels (6.96%) and hence resulted in lower growth of yeast biomass. Similar observations have also been reported earlier by Bacha *et al* (17). Furthermore orange peels had higher crude fat content especially limonene (antimicrobial) which makes hindrance in digestion process of microbes thus depriving yeast cells from essential nutrients (20) resulting in supported less biomass production.

The effect of addition of nutrient supplements for the yeast growth in the production of SCP was shown in Figure 3. The results clearly indicated that higher percentage of protein (60.31%) was found in yeast biomass when *Saccaharomyces cerevisiae* was grown on Glucose Supplemented Fruit Hydrolysates (GSF) indicating that biomass yield can be increased when a carbon source like glucose is added to the medium. Similar observation had been reported by Yakoub and Umar with *Penicilliun expansum* (21). The low yield of protein (53.4%) obtained from FHM could be as a result of limited concentration of nutrients particularly carbon source required for microbial growth. This highlights the importance of supplementation to increase biomass yield. In contrast, protein content in fermented biomass was much lower (17.47% only) in Supplemented Fruit Hydrolysates (SFH) compared to that of in Fruit Hydrolysates Medium (FHM). Here nitrogen supplementation decreased SCP production. Hence supplementation with inorganic nitrogen may have a suppressive effect on *Saccaharomyces cerevisiae* as the biomass yield was very low in the presence of these compounds.

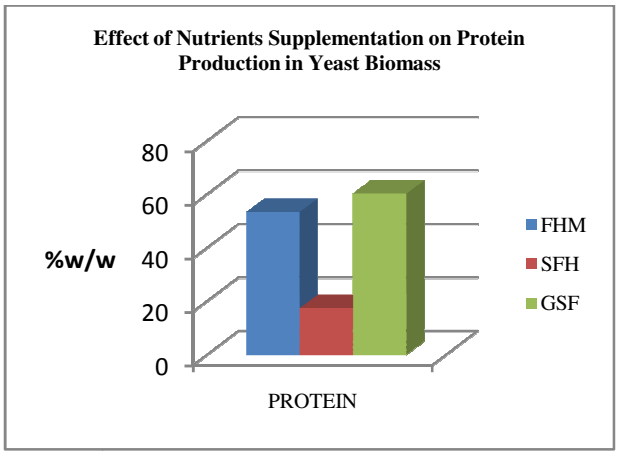

Figure 3. Effect of nutrients supplementation on protein production in yeast biomass

FHM: Fruit Hydrolysates Medium
SFH: Supplemented Fruit Hydrolysates
GSF: Glucose Supplemented Fruit Hydrolysates

From the above results it may be said that *Saccaharomyces cerevisiae* was able to grow on fruit wastes without supplementation of inorganic carbon and nitrogen sources, the addition of which makes SCP production expensive. Under uniform conditions of experimentation to achieve higher yield of yeast biomass and as a consequence higher amount of protein from *Saccaharomyces cerivisae* cucumber peels are comparatively better substrate out of the two fruit wastes used.

4. Conclusion

In conclusion higher yield of single cell protein production from *Saccaharomyces cerevisiae* was possible by submerged fermentation of both substrates. The degree of SCP production depends on the type of substrate used and also on media composition. For *Saccaharomyces cerevisiae* cucumber waste is a better substrate followed by orange peels. The addition of glucose provided available carbon source for the organisms thereby enhancing SCP production. The present finding reveals that cucumber and orange peels were used as potential source for product with higher protein content by utilizing various ingredients present in them and there is a possibility of converting these fruit wastes to proteinaceous feed and food. Thus fruit wastes should be exploited properly as a substrate for the production of cellular biomass of edible yeast instead of dumping them. So they can be used as feed supplement with least expenditure of money.

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