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Production of Single Cell Protein from Orange Peels Using *Aspergillus niger* and *Saccharomyces cerevisiae*

[1]Sadiq Azam, [1]Zeeshan Khan, [1]Bashir Ahmad, [1]Ibrar Khan and [2]Javid Ali

[1]Center of Biotechnology and Microbiology,
University of Peshawar, Khyber Pakhtunkhwa, Pakistan
[2]Pakistan Council of Scientific and Industrial Research Laboratories Complex,
Peshawar, Khyber Pakhtunkhwa, Pakistan

Abstract: The bioconversion of fruit wastes into certain valuable products like single cell protein (SCP) has the ability to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. However using wastes as substrate for the production of high nutritious product may also alleviate environmental pollutant up to some extent. In the light of this, an attempt was made in this study by selecting *Aspergillus niger* and *Saccharomyces cerevisiae* for the production of SCP. The orange peels were used as sole carbon source for preparation of fermentation media on which the two selected fungal strains were grown. *A. niger* biomass was produced by solid state fermentation while the *S. cerevisiae* biomass was obtained by submerged fermentation. The present research work can helps in SCP production from inexpensive and cheap agro waste material.

Key words: *Aspergillus niger* · *Saccharomyces cerevisiae* · *Citrus aurantium* · *Citrus sinensis* · *Citrus paradisi* · Single cell protein

INTRODUCTION

Proteins are mainly called as building blocks of all living organisms. Proteins are necessary for the growth and development of living organisms and carry out a number of different biochemical reactions in the form of enzyme. As compared to other macromolecules, our body requires abundant quantity of proteins and its destruction is sometime lethal [1].

Though post-modern world is replaced from the modern but still poverty is rampant in the world. The data collected by the Food and agriculture organization, UN (FAO), 25% of the world population has protein deficiency, which is a glaring example of protein gap. To rectify this deficiency, microbial proteins can be utilized to provide an alternative of protein to the economically marginalized segments of the societies [2]. For this purpose SCP was developed to produce microbial proteins [3]. These are the dead, dry cells of microorganisms like yeast, fungi, algae and bacteria, developed on substrates containing carbon. This technique has been in vogue in the contemporary world at the commercial level [4].

Pakistan is one of the major producers of different fruits and vegetables. The country is now an important global citrus producer and contributing fulfilling its demand by exporting it as well. Apart from that these fruits are also utilized by different industries for producing different industrial products and discard orange peels [5].These discarded peels can cause pollution which can affect the environment and also results various infectious diseases due to growth of microorganisms. These fruit wastes contain carbohydrates that are natural substrates for microorganisms. So by utilizing these cheap available substrates for the production of SCP will help in reducing pollutant up to certain extent [6, 7].

For the production of SCP a wide variety of microorganisms are used. Using algae for the SCP production it was revealed that it has high protein content as compared to other microorganisms and can be easily

Corresponding Author: Sadiq Azam, Center of Biotechnology and Microbiology,
University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

harvested, but apart from these merits such proteins are indigestible due to cellulosic wall and high concentration of metals [8, 9].

A number of different types of agro-industrial wastes, fruits and wooden pulps are used for growing yeast. Such cells are larger in size but similar to algae these cells are also poorly digestible. Temperature required for growing yeast cells ranges 30-34°C and pH 3.5-4.5. Similar to yeast cells, bacteria can also be grown on wide range of different substrates [10, 11].

Throughout the world a large number of different researches are going on in which low value products such as agro-industrial wastes are utilized and are successfully converted into products of high nutritious value [12-15].

MATERIALS AND METHODS

Collection of Orange Peels and Microbial Culture: Orange peels (*Citrus aurantium, Citrus sinensis* and *Citrus paradisi*) were collected from local market of District Peshawar in sterile bottles. By gently washing these peels with distilled water, removed dust particles. The peels were shade dried and crushed into fine powder in an electric grinder [16].

A. *niger* and *S. cerevisiae* were obtained from Microbial Type Culture Collections (MTCC).

The cultures were maintained on slant of Potato Dextrose Agar medium (Potato infusion 200g, Dextrose 20g, Agar 20g, Distilled water 1000ml) and stored at 4°C.

Sample preparation for *S. cerevisiae*: *S. cerevisiae* lacks the enzyme system which hydrolyzes the polysaccharides into monosaccharide. The peels were treated with 30% HCl and were kept in water bath at 75°C for one hour to obtain monosaccharides [16].

Proximate Analysis of the Substrate: The ash, crude fiber, crude fat and protein contents of the substrate was determined by AOAC methods [17].

Production and harvesting of SCP: Orange peels were inoculated with *A. niger* and *S. cerevisiae* and were incubated at various temperature and duration; 32°C for 8 days (Batch I), 30°C for 6 days (Batch II) and 28°C for 4 days other required conditions for the growth of these microorganisms were also provided. Each batch was observed on regular basis to avoid contamination [18].After production the *A. niger* and *S. cerevisiae* biomass was autoclaved at 121°C for 20 minutes [18]. Autoclavation of fungal biomass were done to reduce chances of toxin production by *A. niger*. As a result some of proteins were also digested into small peptides and make it fit for digestion. Mixture of each fungal biomass was then filtered through filter paper (Whattsman filter paper). For drying these cells adhered with filter paper, cells were transferred to aluminum foil and were kept at 50°C to remove all moisture contents. These cells were under regular check to avoid destruction of protein [19].

Analysis of Fungal Biomass: The fungal biomass of each batch was analyzed for its ash, crude fiber, crude fat and protein contents using the procedure mentioned in AOAC methods [17].

RESULTS AND DISCUSSION

Ash Contents: Ash content of *C. aurantium*, *C. sinensis* and *C. paradisi* peels were 4.4, 4.8 and 4.0%, respectively, as presented in figure 1. Ash content reflects the presence of organic matter in orange peels. As organic compounds are natural substrate for microorganisms so it will lower its content up to certain level.

The ash contents of the fungal biomass obtained in three different batches were also determined. The results revealed that the ash contents were decreased as shown in Figure 1.

In batch 1 the ash contents of *A. niger* was 3.62, 4.00 and 4.20% for *C. paradisi, C. aurantium* and *C. sinensis*, respectively. Similarly the ash content of *S. cerevisiae* was 3.60, 3.80 and 3.80% for *C. sinensis, C. paradisi* and *C. aurantium,* respectively. In batch 2, ash content of *A. niger* biomass was 3.38, 3.6 and 3.6% for *C. sinensis, C. paradisi* and *C. aurantium,* respectively, while ash content of *S. cerevisiae* biomass was 2.80, 3.0 and 3.2% for *C. sinensis, C. paradisi* and *C. aurantium* respectively (Figure 1).

Ash content of batch 3 was also determined the results showed that, for *A. niger* biomass it was 3.80, 4.00 and 4.20% for *C. paradisi*, *C. sinensis* and *C. aurantium* respectively, while for *S. cerevisiae* biomass it was 3.00, 3.20 and 3.40% for *C. paradisi*, *C. aurantium* and *C. sinensis*, respectively (Figure 1).

Crude Fat Contents: Crude fat content of citrus peels was determined and shown in Figure 2. The results revealed that all the samples showed a very low level of fat contents; 1.0% (*C. paradisi*), 1.5% (*C. aurantium*) and 1.5% (*C. sinensis*). As SCP must have low content of fats therefore by comparing crude fat contents of each batch it was found that *A. niger* biomass obtained in batch 2

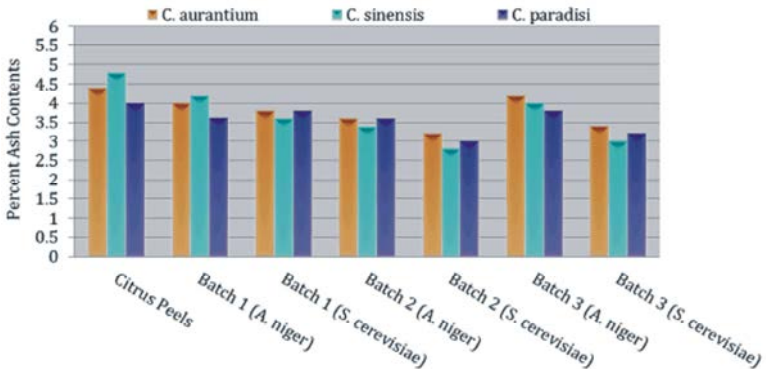

Fig. 1: Percent ash contents of citrus peels, *A. niger* and *S. cerevisiae* biomass of batch 1-3.

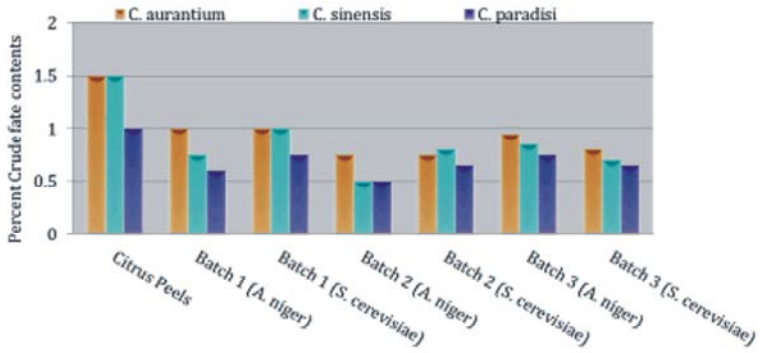

Fig. 2: Percent crude fate contents of citrus peels, *A. niger* and *S. cerevisiae* biomass of batch 1-3.

have lowest fat contents; 0.50, 0.50 and 0.75% for *C. sinensis*, *C. aurantium* and *C. paradisi*, respectively while in case of *S. cerevisiae* biomass, the fat contents were recorded as; 0.65, 0.75 and 0.80% for *C. paradisi, C. aurantium and C. sinensis* respectively. For batch 1, the fat content of *A. niger* biomass was 0.60, 0.75 and 1.00% for *C. paradisi*, *C. aurantium and C. sinensis,* respectively. Similarly low fat contents were shown by the *S. cerevisiae* biomass; 0.75% (*C. paradisi*) 1.00% (*C. aurantium*) and 1.00% (*C. sinensis*). In batch 3 the *A. niger* biomass the percent fate contents were; 0.75% (*C. paradisi*), 0.85% (*C. sinensis*) and 0.95% *(C. aurantium*) while in *S. cerevisiae* biomass the percent fat contents recorded were; 0.65, 0.70 and 0.80% for *C. paradisi*, *C. sinensis* and *C. aurantium*, respectively.

Crude Fiber Contents: As the fiber contents of fruits are high, the results obtained (figure 3) showed that high crude fiber content was recorded for *C. paradisi* (2.95%). The remaining citrus peels also showed high level of fiber contents; 2.85% (*C. aurantium*)and 2.65% (*C. sinensis*). The fungal biomass showed a low level of crude fiber contents as compared to the citrus peels. *A. niger* biomass produced in batch 2 showed lowest crude fiber contents; 1.85% (*C. paradisi*), 1.85% (*C. aurantium*) and 2.0% (*C. sinensis*) while *S. cerevisiae* biomass also showed low fat contents; 2.0, 2.1 and 2.2% for *C. sinensis*, *C. paradisi* and *C. aurantium*, respectively. The other two batches showed relatively high crude fat contents as shown in Figure 3.

Protein Contents: Single Cell Proteins are recommended as protein source, but like other organisms it also contains other biological macromolecules, like carbohydrate, lipids, nucleic acids and vitamins. However these macromolecules are given little importance by the nutritionists. Results obtained for protein contents of each citrus peel and fungal biomass are presented in figures 4. The *C. aurantium* showed highest protein content (13.37%) while in rest of the citrus peels the protein contents were recorded as 11.9 and 11.53% for *C. paradisi* and *C. sinensis*, respectively. The protein contents of each fungal batch were also determined. In case of *A. niger* it is revealed that highest protein contents were obtained in batch 2 that is 29.75% for *C. aurantium*, 27.15% for *C. sinensis* and 29.00% for *C. paradisi*. Similarly the *S. cerevisiae* biomass of

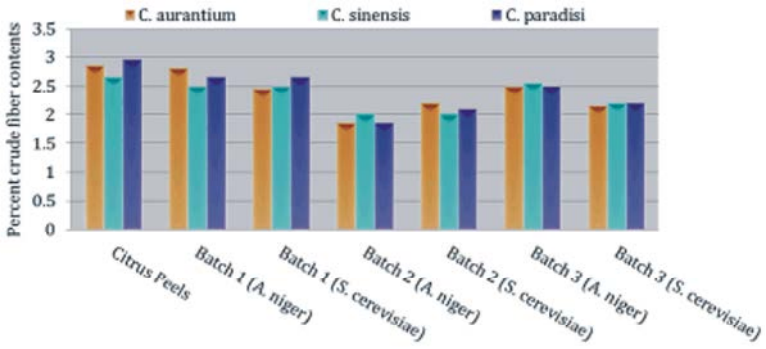

Fig. 3: Percent crude fiber contents of citrus peels, *A. niger* and *S. cerevisiae* biomass of batch 1-3.

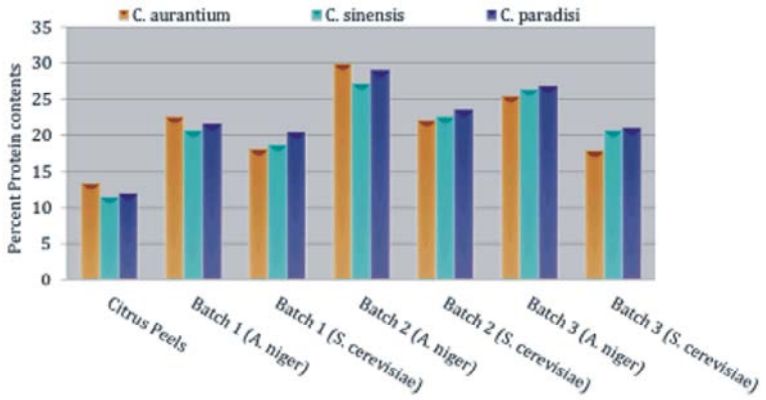

Fig. 4: Percent protein contents of citrus peels, *A. niger* and *S. cerevisiae* biomass of batch 1-3.

the same batch, also showed highest protein contents; 23.50% (*C. paradisi*).22.50% (*C. sinensis*) and 22.06% (*C. aurantium*).The *A. niger* biomass of batch 1 also showed high protein contents as compared to citrus peels; 22.50 (*C. aurantium*), 21.56% (*C. paradisi*) and 20.63% (*C. sinensis*). The protein contents of the *S. cerevisiae* biomass were recorded as 20.37% (*C. paradisi*), 18.68% (*C. sinensis*) and 18.0% (*C. aurantium*). In batch 3 for *A. niger* biomass, the percent protein contents were; 26.81% (*C. paradisi*), 26.37% (*C. sinensis*) and 25.37% (*C. aurantium*) while in *S. cerevisiae* biomass the percent protein contents recorded were; 21.06, 20.62 and 17.75% for *C. paradisi*, *C. sinensis* and *C. aurantium*, respectively.

CONCLUSION

Regarding SCP nutritionist are more concerned with its protein contents as compared to other characteristics. By comparing different batches of each inoculated sample of both microorganisms, it was found that in case of *A. niger* biomass it had shown a tremendous increase in its protein contents that is 29.75, 27.15 and 29.0% for *C. aurantium, C. sinensis* and *C. paradisi* which was 13.37, 11.53 and 11.99% for its original peels.

For *S. cerevisiae* protein contents were increased to 22.06, 22.50 and 23.50% from 13.37, 11.53 and 11.99% for *C. aurantium*, *C. sinensis* and *C. paradisi*, respectively. Such increase in protein contents is due to the availability of high monosaccharide for *A. niger* as compared to *S. cerevisiae.* Apart from it SCP had shown a remarkable decrease in crude fat, crude fiber and ash content.

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