J. Sc. & Tech. Univ. Peshawar, 2016, 40 (2), 01-07

OPTIMIZATION OF LABORATORY REQUIREMENTS THROUGH EXPERIMENTAL DESIGN FOR THE PRODUCTION OF INDIGENOUS *SACCHAROMYCES CEREVISIAE* FROM ORANGE PEELS

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Abstract: Orange peels are one of the main by-products of the citrus processing industry. Due to their microbial support capability on one hand, if discarded these may be a threat to a different form of life while on the other hand, they contain essential nutrients to support the growth of beneficial microbes for production of single cell microbial protein. For maximum microbial growth, the process of response surface methodology was utilized to optimize different parameters such as temperature, pH, shaking (revolutions per minute) and glucose concentration. It was observed that 2.36% glucose,10% substrate, 0.5% ammonium nitrate,1% yeast extract, pH 5,32ºC temperature, and shaking at 150 rpm were best growth parameters for indigenous *Saccharomyces cerevisiae* and to obtain their proteins. Obtained single cell microbial protein was then evaluated for the presence of different amino acids. Results showed that concentration of aspartic acid was high followed by leucine i.e. 17.82±3.97% and 15.90±2.33% respectively on the other hand produced single cell protein was deficient in methionine. These results were analyzed through High-Performance Liquid Chromatography (HPLC) using Ortho Phthalaldehyde (OPA) as afluorescent agent. It is concluded that using response surface methodology different parameters can be effectively optimized for the growth of indigenous *S. cerevisiae*. As single cell microbial protein is the good source of amino acids these can be used as a protein source for human, animal and poultry consumption.

Keywords: Orange peels, *Saccharomyces cerevisiae*, HPLC, Single cell protein, Amino acids.

Introduction

Orange peels are one of the mainby-products of the citrus processing industry. Dry orange peels have high contents of cellulose, pectin, and hemi cellulose that are used for fermentation after hydrolysis by chemical or enzymatic methods. Chemically orange peels are treated with 30% HCL while enzymatically these are hydrolysed with pectinase, cellulase and beta-galactosidase that breaks the bonds present in polymers and converts them into short monomers(Kandari & Gupta, 2012). As orange peels are a good source of cellulose and lignocellulose, so instead of discarding it that can cause some environmental pollution like increasing biochemical oxygen demand and some serious health problems, these can be used to produce single cell microbial protein.

Microbial proteins which are also called as Single Cell Protein (SCP) have been produced by growing different microbes like algae, bacteria, fungi and yeast using different agricultural wastes and cheap raw materials (Zepka, Jacob-Lopes, Goldbeck, Souza-Soares, & Queiroz, 2010). These microorganisms are grown in large-scale culture systems for use as protein sources in human food or animal feed.(Wang, Kim, Kim, & Kim, 2013). Single cell microbial protein is

the most important due to the presence of large protein contents. It is the most valuable unconventional optional protein as compare to animal protein, because these are economic and environmental friendly (Ravindra, 2000).
To predict responses, Central Composite Design (CCD) of response surface methodology is used which are based on some sets of experiments, containing dependent and independent variables within a given range. In past response surface methodology was used for the production of metabolites, optimization of microbial growth and microbial media (Li, Bai, Cai, & Ouyang, 2002; Ramírez, Gutierrez, & Gschaedler, 2001; Vazquez & Martin, 1998).

Keeping in view the value of microbial proteins, the present research work was designed to evaluate the possible use of orange peels for the growth of indigenous *S. cerevisiae*. Different parameters like glucose concentration, shaking, incubation temperature and pH of the medium were optimized through experimental design of response surface methodology and produced biomass was then evaluated for its amino acids composition.

Materials and Methods

Isolation and identification of indigenous Saccharomyces cerevisiae

Indigenous *S. cerevisiae* was isolated and identified by their respective procedures (Barnett, Payne, & Yarrow, 1983; Kurtzman, Fell, & Boekhout, 2011; Martorell, Querol, & Fernández-Espinar, 2005). Pure cultures of indigenous *S. cerevisiae* were then maintained by subculturing.

Collection and Preparation of Substrates

Orange peels were collected from local market of University of Peshawar in a sterile plastic bag and were transferred to the research facility for further investigation. After collection, orange peels were washed with sterile distilled water and were cut into small pieces. Orange peels were then blended in a clean blender to make the slurry type material.

The slurry material was then treated with chemicals by their respective procedure to obtain more available sugars (Bacha, Nasir, Khalique, Anjum, & Jabbar, 2011).Acids treated slurry material was then filtered through mesh size 40 to obtain fine particles and was then diluted with distilled water to produce microbial biomass through the process of fermentation.

Optimization of various growth parameters for yeast biomass production using response surface methodology

For microbial growth different parameters like pH, temperature, shaking and glucose concentration were optimized in the media containing 10% substrate, 1% yeast extract and 0.5% ammonium nitrate using the statistical approach of response surface methodology. In the study JMP 12.1.0 software was used for graphical and statistical analysis. For the said purpose $a2^4$ rotatable central composite design was adopted consisted of different sets of experiments with different combinations of variables. To confirm the optimum laboratory requirements for maximum production of microbial biomass, anew experiment was performed under the optimum conditions as predicted by the model. For determination of the significant parameters analysis of variance (ANOVA) was used.

Harvesting and drying of microbial biomass

After successful growth under optimized conditions microbial biomass was then harvested by centrifugation and vacuum filtration using a sterile 0.45 micron filter paper. Obtained biomass was then dried into analuminum pan to a constant weight in a hot air oven at 70ºC for 48 hours and was then analyzed for the presence of amino acids (Ojokoh & Uzeh, 2005).

Amino acids profile of produced single cell microbial protein

For evaluation of amino acid profile dried single cell microbial protein was crushed in a pestle and mortar. After acid hydrolysis microbial biomass was then evaluated for the presence of amino acids through High Performance Liquid Chromatography (Shimadzu LC-20A) using Ortho Phthalaldehyde (OPA)as fluorescence agent (Ishida, Fujita, & Asai, 1981).

Results and Discussions

Optimization of various parameters using response surface methodology

Using response surface methodology all mentioned variables are set to their minimum and maximum values as shown in table 1 while experimental plan is shown in table 2.Maximum microbial mass (5.86g/L ± 0.98 %) was produced at pH 5, glucose concentration 2.36%, shaking 150 rpm and temperature 32ºC which was higher than the biomass produced at pH 5, shaking of 150 rpm and temperature of 30ºC. Surface plots among dependent and independent variables is presented in figure 1.

For instance the presented model was observed with a p-value of 0.01which indicates its significance. For fitness the model was expressed as the coefficient of variations (R^2). The R^2 value of the model was 0.8240, which indicate that the model can explain 82.4% of the variability in the response. As the R^2 value closer to 1.0 the model will be strong and the better it will predict the responses (Haaland, 1989).

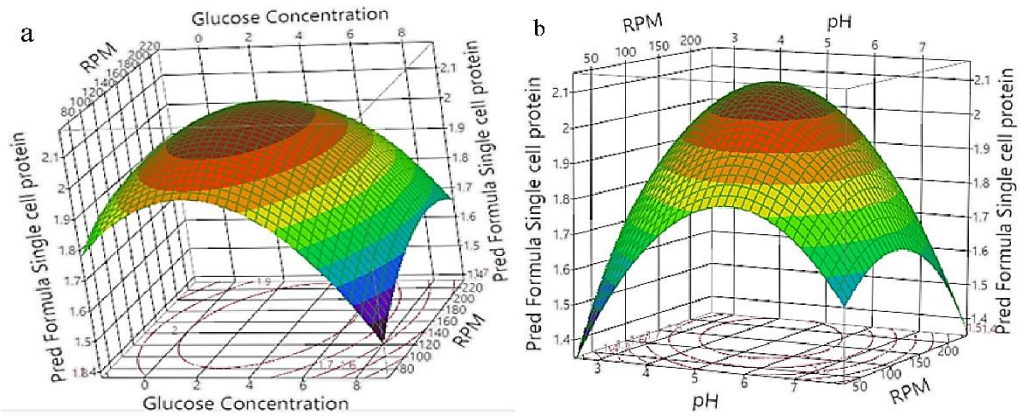
Table 1 Independent variables along with their minimum and maximum values

	pH	Glucose concentration	RPM	Temperature
Minimum value	3	0	100	25
Maximum value	7	5	200	35

Table 2 Experimental plan along with produced single cell microbial protein

Experiment	Glucose	pH	Shaking (RPM)	Temperature	SCP
1	0	3	100	25	1.4 ±0.26%
2	0	3	100	35	1.7 ±0.15%
3	0	3	200	25	1.7 ±0.2%
4	0	3	200	35	1.8 ±0.18%
5	0	5	150	30	2 ±0.46%
6	0	7	100	25	1.9 ±0.52%
7	0	7	100	35	1.95 ±0.34%
8	0	7	200	25	1.7 ±0.71%

9	0	7	200	35	1.65 ±0.32%
10	2.5	3	150	30	2.05 ±0.74%
11	2.5	5	100	30	2.05 ±1.21%
12	2.5	5	150	25	2 ±0.38%
13	2.5	5	150	30	2.1 ±0.59%
14	2.5	5	150	30	2.1 ±0.82%
15	2.5	5	150	35	2.1 ±0.65%
16	2.5	5	200	30	2.04 ±1.22%
17	2.5	7	150	30	1.75 ±0.42%
18	5	3	100	25	1.8 ±0.67%
19	5	3	100	35	1.8 ±0.37%
20	5	3	200	25	1.83 ±0.88%
21	5	3	200	35	1.9 ±0.93%
22	5	5	150	30	2.05 ±0.28%
23	5	7	100	25	1.7 ±0.39%
24	5	7	100	35	1.74 ±0.51%
25	5	7	200	25	1.72 ±0.36%
26	5	7	200	35	1.76 ±1.75%



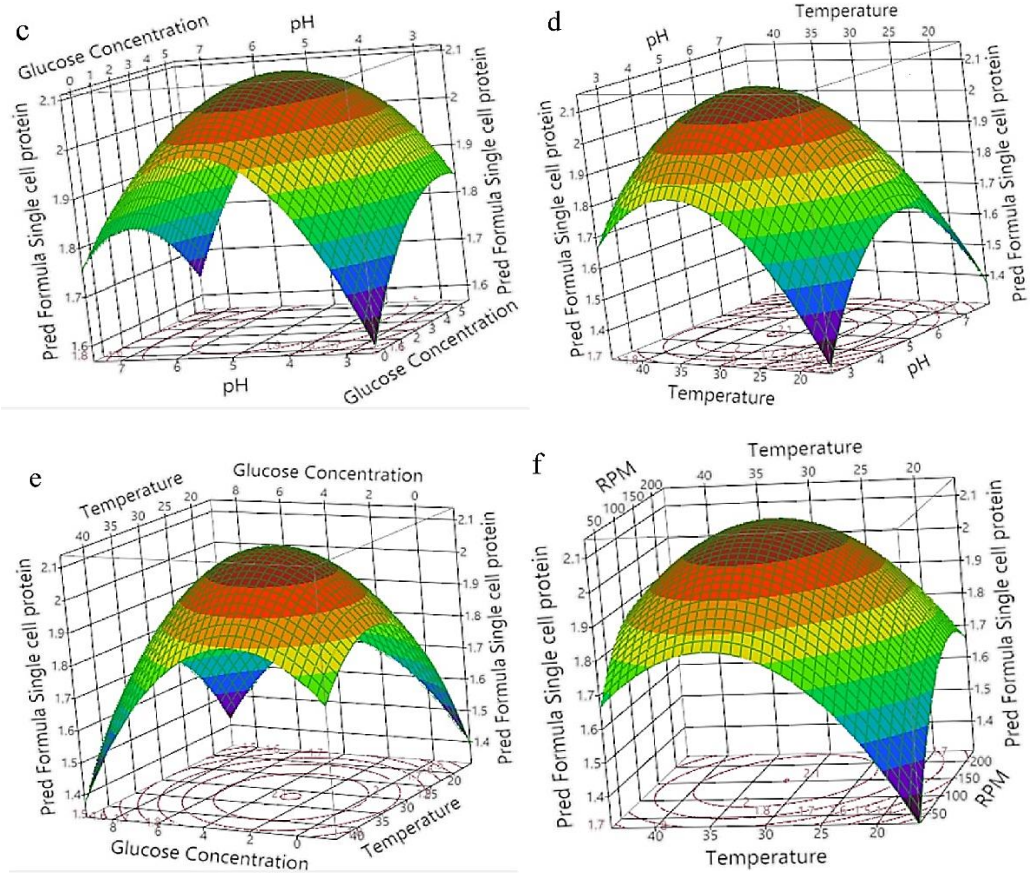


Fig.1.Surface plots along with contour plots showing the mutual effect of different factors on microbial growth. a) Glucose concentration and Shaking (rpm); b)Shaking (rpm)and pH;c) Glucose concentration and pH; d) Temperature and pH; e) Glucose concentration and Temperature; f) Shaking (rpm) and Temperature.

Amino acids profile of dry single cell microbial protein

Amino acids composition of dry single cell protein is presented in figure 2. It was observed that composition was different for different amino acids. Concentration of Aspartic acid was high followed by leucine i.e. 17.82±3.97% and 15.90±2.33% respectively. Kihlberg (1972), (Kihlberg, 1972) Fred & Peterson (1921) (Fred & Peterson, 1921) and Tannenbaum & Wang (1975) (Tannenbaum & Wang, 1975) conducted experiments to analyze single cell protein produced utilizing different microorganisms. They observed that produced single cell protein were deficient in methionine which corroborate the findings presented in this study.

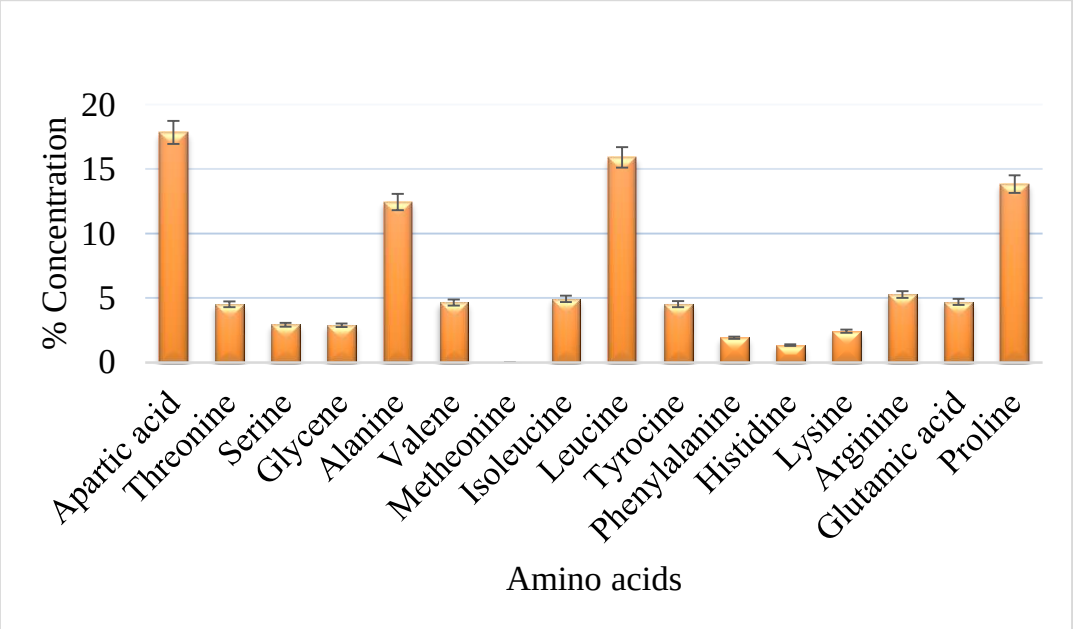


Fig.2. Amino acids profile of dry single cell protein

Conclusion

From the present study, it is concluded that using response surface methodology indigenous *S. cerevisiae* can effectively be grown on orange peels for maximum production of single cell protein which is a good source of amino acids. Single cell protein can effectively be used as supplementation in human food as well as animal and poultry feed.

Acknowledgement

Authors are thankful to Higher Education Commission Pakistan for their financial support throughout the study.

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