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Single cell protein production from some food wastes using yeasts

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Abstract

Bioconversion of various food wastes into specific valuable products such as single cell protein (SCP) has the simultaneous potential to solve the worldwide dietary protein deficiency by obtaining an economical food and feed product and to significant mitigation of environmental pollutants by using these wastes as substrates for the production of high nutritional value products. Therefore, the present study aimed to evaluate the feasibility of using potato and orange peels for the production of SCP using yeast isolates *Saccharomyces cerevisiae* and *Debaromyces hansenii* and to evaluate the protein quality of the produced SCP. The results showed that potato peel medium used for the growth of yeast isolates is the best medium for the production of SCP, and *S. cerevisiae* was better than *D. hansenii* for the production of higher amount of biomass, crude protein, total amino acids, and riboflavin. The bioconversion of various wastes into specific valuable SCP represents a promising prospect to resolve protein deficiency problem and reducing environmental pollutants via utilizing food wastes as substrates.

Keywords: single cell protein, food wastes, yeast liquid-state fermentation, biomass, amino acids, riboflavin.

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1. Introduction

Bioconversion of fruit waste into single cell protein (SCP) has the potential to solve the problem of dietary protein deficiency worldwide by obtaining economical food and feed products. Using food processing residues in SCP production as a substrate can mitigate environmental contamination. Microbial protein also has many properties such as high efficiency in substrate conversion, high productivity due to the rapid growth rate of microorganisms and does not depend on seasonality or climate. Furthermore, SCP can be produced using a wide range of raw materials (Mondal *et al*., 2012; Nasseri *et al*., 2011; Punniamoorthy *et al*., 2022). Microbial products are considered to be natural and have high safety profiles compared to chemical products, which produce harmful byproducts that may be hazardous to the ecosystem (Vassileva *et al*., 2022). Fungi existed in diverse habitats and associated with chemoheterotrophic pattern afford several metabolomics comprising primary and secondary metabolites boosted in numerous bioactivities and biotechnological applications (Mohamed *et al*., 2021). These metabolites encompass polysaccharides (Giavasis, 2014), lipids and fatty acids (Hassane *et al*., 2024; Mohamed *et al*., 2022), and enzymes and peptides (Al Mousa *et al*., 2022a,b; Khalaf *et al*., 2023; 2024), as well as, low molecular weight secondary products including mainly phenolic acids, alkaloids, saponins, flavonoids, and terpenoids (Abdelrahem *et al*., 2023; 2024; Al Mousa *et al*., 2022c, 2024; Hassane *et al*., 2022a,b; Pimentel *et al*., 2011). Moreover, different mycotoxins with negative economic importance are produced by fungi (Abo Dahab *et al*., 2016; Saber *et al*., 2016). In the last century, microorganisms in the form of fermented foods such as bread, wine, alcoholic beverages, beer, sake, cheese, yogurt, and soy sauce have been consumed as a food source. Recently, research has turned into utilizing microorganisms to ferment fruit wastes to produce materials of economic importance, such as organic acids, enzymes, vibrant colors, flavors, aroma esters, cellulose, pectin, antibiotics, biocides, biohydrogen, plant growth regulators, bioethanol, biogas, single-cell oils and single cell protein (SCP) (Panda *et al*., 2018; Riesute *et al*., 2021). Single cell protein derived from agricultural waste has recently attracted increasing attention as an alternative source of protein for improving human and animal nutrition. Agricultural waste used to produce single cell protein has also proven to be a cheap and economical substrate and also helps in waste management and may serve as a permanent food source in the event of disasters along with solving the global shortage of protein-rich diets (Ahmed *et al*., 2024; Garcia *et al*., 2022; Khan *et al*., 2022). Microorganism-derived SCP is eco-friendly alternative source to animal-derived proteins. Due to rising protein demand worldwide, food processing sector advances are expected to increase. Many microbes. Bacteria, algae, yeasts, and other fungi have been studied for SCP synthesis (Haris *et al*., 2022; Salgado *et al*., 2021). Therefore, the aim of the present study was to investigate the possibility of manufacturing SCP using liquid-state fermentation of some yeasts with easily accessible food peels (potato

and orange peels) as inexpensive sources. In addition, the evaluation of protein quality and amino acid composition of the single cell protein was carried out.

2. Materials and methods

2.1 Collection and preparation of substrates

Agricultural samples including potatoes (*Solanum tuberosum*) and oranges (*Citrus sinensis*) were purchased from local markets in Assiut city, Egypt. Peel samples were obtained on a laboratory scale by traditional manual peeling. Peels were dried in oven (at 50±2 ºC overnight), then grinded and sieved through a 1 mm mesh sieve according to the method described by Collins and Past (1981). Samples were packed in transparent polythene bags and stored at room temperature until further study.

2.2 Microorganism

Saccharomyces cerevisiae AUMC 10203 and *Debaromyces hansenii* AUMC 14029, obtained from Mycology Laboratory, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut Branch, Egypt were used in this study.

2.3 Preparation of fermentation media

Forty grams of each type of potato and orange peels powder were hydrolyzed with 50 mL of HCl (10%) and incubated in a water bath at 100 °C for an hour. The mixture was cooled and filtered through Whatman no.1 filter paper. Finally, the volume was completed to one liter and the following ingredients were added (ammonium sulphate 2 g, monopotassium phosphate 1 g, magnesium sulphate hepthydrate 0.5 g, sodium chloride 0.1 g, calcium chloride 0.1 g and glucose 2 g). The pH was adjusted to 5.5. and the medium was autoclaved at 121 °C for 15 minutes (Chandak *et al*., 2014).

2.4 Activation of yeasts before fermentation process

Saccharomyces cerevisiae and *D. hansenii* were incubated at 28±2 °C for 24 hours after inoculation into a sterilized broth composed of yeast extract 5 g/L, peptone 10g/L, glucose 10g/L and pH 5.5.

2.5 Fermentation process

The growth medium was divided into two groups*:* potato peel residue (P) and orange peel residue (OR). Growth media were inoculated with a volume of 4 mL *Sacchromyces* (S) and *Debaromyces* (D). They were divided into volumes of 500 mL and incubated for 2, 4, 6, and 8 days at 28±2 °C under aeration conditions. The results were 4 different growth media (P+S, OR+S, P+D and OR+D). Replicates of each growth medium were performed.

2.6 Determination of yeast biomass

At the end of incubation time, after 2, 4, 6 and 8 days, all cultures were centrifuged at 6000 rpm for 10 minutes. The supernatant was discarded, and the cell pellets were washed with sterile water.

The biomasses were oven dried at 50 °C for 16 h followed by cooling in desiccators and the mean dry weight of biomass was determined (Dharumadurai *et al.*, 2011).

2.7 Approximate composition

Moisture, proteins, fats, fibers and ash were determined according to official methods (AOAC, 2006). Total carbohydrates was calculated by differences.

2.8 Amino acids analysis

Amino acid composition of SCP was demonstrated using HPLC. One gram of sample was mixed with 5 mL n-hexane. The mixture was allowed to macerate for 24 h. Then, the mixture was filtered through Whatman no. 1 filter paper and the residue was transferred into a test tube where it was incubated in an oven with 10 mL 6 N HCl for 24 h at 110 °C. After the incubation, the sample was filtered on Whatman no. 1 filter paper, evaporated on rotary evaporator and dissolved completely in 100 mL dilution buffer, then diluted 1 ml in 10 ml volumetric flask, filtered using 0.22 µ m syringe filter and 100 µl was injected (Jajic *et al*., 2013).

2.9 Determination of riboflavin

Riboflavin content of SCP was determined by high performance liquid chromatography (HPLC) (Giorgi *et al.*, 2012).

2.10 Statistical analysis

The experimental data was evaluated using one-way analysis of variance (ANOVA), and Duncan's multiple comparison test was used to detect differences using the software SPSS 19.0 (IBM Corp., Armonk, NY).

3. Results and Discussion

3.1 Approximate composition of potato and orange peels

Table (1) showed the moisture, protein, fats, fibers, ash and total carbohydrate of potato and orange peels. Based on proximate composition, potato peel showed the highest values of the protein and carbohydrate, while it recorded the lowest values for fiber compared to orange peels. On the other hand, orange peels had the highest fiber content. Moreover, both peels, potato and orange, contained relatively small amounts of fats of 2.10% and 3.18% for potato and orange peels, respectively. Both potato and orange peel wastes included varying amounts of total carbohydrates and fibers that upon hydrolysis act as carbon source for yeasts development during fermentation. Our results at the same level as reported by Onoh *et al*. (2019) and Clement *et al*. (2023). Pathak *et al*. (2019) reported that proximate composition of fruit peels may vary with variety, origin, geographic location, seasonal variations, and maturity stage of the fruits.

Table (1): Approximate composition of potato and orange peels (based on dry weight).

Composition	Potato peel	Orange peel
Moisture	7.80±0.27[b] **	9.90±0.24[a]
Protein	14.04±0.36[a]	7.72±0.28[b]
Fats	2.10±0.61[b]	3.18±0.48[a]
Ash	5. 10 ±0.43[a,b]	5.18±0.33[a]
Fibers	5. 30±0.54[b]	13.00±0.42[a]
Total carbohydrates *	65.70±0.22[a]	61.02±0.16[b]

*Total carbohydrates were calculated by difference. **Results were shown as mean ± standard deviation of three replicates, and different superscript. Letters in the same row are significantly different at $p < 0.05$.

3.2 Crude protein content and riboflavin from dry biomass

Data presented in Table (2) showed the crude protein and riboflavin from biomass produced by yeast isolates (*S. cerevisiae* and *D. hansenii*) fermented on potato and orange peel substrates. The biomasses of 0.75, 0.55, 0.49, and 0.38 g/100 mL were recorded with S+P on the second day, S+OR on the fourth day, D+P on the six days, and on the eighth day for D+OR, respectively). It can be observed that *S. cerevisiae* afforded the highest amount of biomass, crude protein, and riboflavin with both potato and orange peels fermentation media. Variation in the protein yield could result in high sugar content produced by hydrolysis of the solid substrate containing cellulose, starch, and pectin by microbial enzymes (cellulase, amylase, and pectinase) (Yabaya and Ado, 2008). Riboflavin content found in the *S. cerevisiae* and *D. hansenii* biomass produced from potato and orange peel medium was low. This may be due to the traditional method used for drying the biomass, exposure to light. Riboflavin is a photosensitive molecule that can be triggered with exposure to light, showing photo degradation after exposure to short-wave radiation (< 400 nm) (De La Rochette *et al*., 2000). In general, *S. cerevisiae* was better than *D. hansenii* regarding protein content and produced biomass. On the other hand, potato peel substrate showed the highest percentage of protein and biomass production. This could be attributed to the ability of *S. cerevisiae* to reproduce quickly and adapt to different environments. *Saccharomyces cerevisiae* is an excellent microbial cell factory for producing valuable recombinant proteins because of its fast growth rate, robustness and biosafety (Yang *et al*., 2024). The type of substrate affects microbial biomass production due to the difference in nutrient utilization rate as microorganisms interact differently with each substrate (Punniamoorthy *et al*., 2022). Moreover, the type of substrate is one of the factors that influence the nutritional quality of microbial protein (Bratosin *et al*., 2021).

Table (2): Crude protein and riboflavin content of *S. cerevisiae and D. hansenii* biomass produced from potato and orange peels media (based on dry weight).

Groups	Fermentation (days)	Dry biomass (g/100 ml)	Riboflavin (mg/100 mg)	Crude protein (%)
S+P	2	0.75 ±0.24[a]	0. 008 ±0.15[b]	65.40 ±0.34[a]
S+OR	6	0.55 ±0.36[b]	0. 01 ±0.31[a]	51.10 ±0.25[c]
D +P	4	0. 49 ± 0.53[c]	0. 006 ±0.32[d]	55.50 ±0.32[b]
D+OR	8	0. 38 ±0.42[d]	0. 007±0.19[c]	37.75±0.18[d]

S+P: Potato peel + *S. cerevisiae*, S+OR: Orange peel + *S. cerevisiae*, D+P: Potato peel + *D. hansenii*, and D+OR: Orange peel + *D. hansenii*. Results are shown as mean ± standard deviation of three replicates, and different superscript letters in the same column are significantly different at $p < 0.05$.

3.3 Amino acid content

The amino acid profile of the biomass produced by *S. cerevisiae* and *D. hansenii* grown on potato and orange peel media was shown in Table (3) and Figures (1, 2, 3, and 4). The number of amino acids, essential and non-essential, in all biomasses produced was 14 amino acids, except for the potato peel medium used for the growth of *S. cerevisiae*, where 15 amino acids with an increase in the non-essential amino acid tyrosine. The essential amino acids were valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, and threonine. The detected non-essential amino acids were proline, alanine, glycine, glutamic acid, aspartic acid, serine, and tyrosine.

Table (3): Amino acid profile of SCP produced from *S. cerevisiae and D. hansenii* on potato and orange peels (g/100g dry biomass).

Amino acid	S+P	S+OR	D+P	D+OR
Essential				
Valine	0.515	0.307	0.196	0.353
Histidine	0.151	0.093	0.085	0.086
Isoleucine	0.097	0.013	0.010	0.006
Leucine	0.386	0.107	0.092	0.100
Lysine	0.292	0.060	0.340	0.024
Methionine	0.222	0.104	0.099	0.116
Phenylalanine	0.316	0.278	0.268	0.250
Threonine	6.019	1.299	2.992	0.859
Total essential	7.998	2.261	4.082	1.794
Non-essential				
Proline	0.379	0.060	0.211	0.156
Alanine	1.155	0.309	0.247	0.247
Glycine	0.921	0.268	0.222	0.247
Glutamic	1.705	0.580	0.361	0.250
Aspartic	0.689	0.175	0.145	0.197
Serine	0.649	0.214	0.225	0.225
Tyrosine	0.063	ND	ND	ND
Total non-essential	5.561	1.606	1.411	1.322
Total amino acids	13.559	3.867	5.493	3.116

S+P: *S. cerevisiae* + Potato peel, S+OR: *S. cerevisiae* + Orange peel, D+P: *Debaromyces hansenii* + Potato peel, and D+OR: *Debaromyces hansenii* + Orange peel. ND: Not detected.

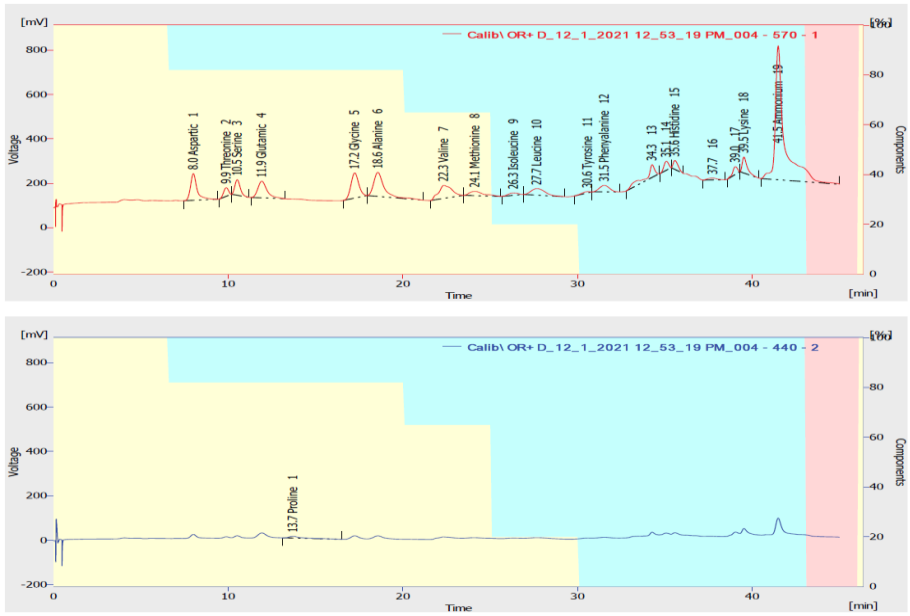

Figure (1): Estimation of amino acids produced in (P+S) sample.

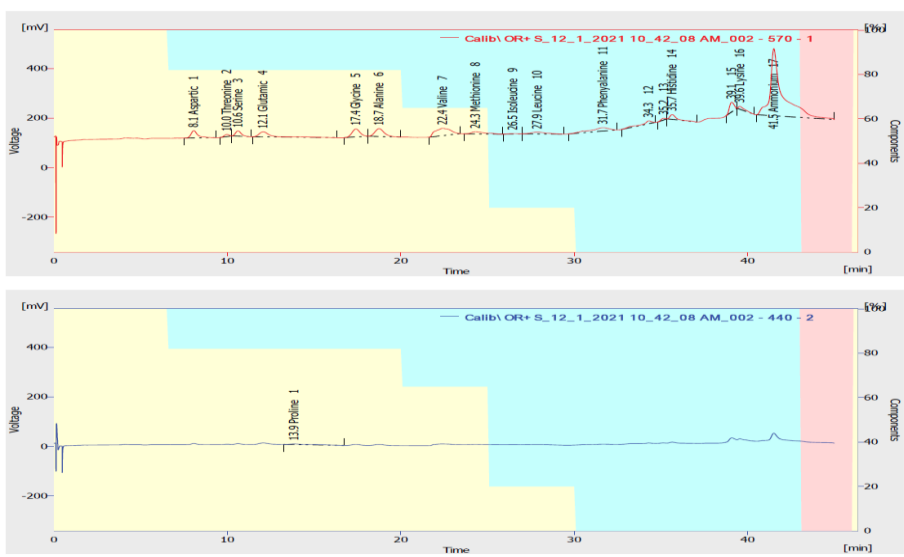

Figure (2): Estimation of amino acids produced in (OR+S) sample.

The *S. cerevisiae* grown on potato peel medium (S+P) recorded the highest percentage of total amino acids (13.559%) as well as the highest percentage of essential amino acids was 7.998%. While the *D. hansenii* grown on orange peel medium (OR+P) recorded the lowest percentage of total amino acids (3.116%),

as well as essential amino acids was 1.794%. The nutritive value of SCP varies with the microorganisms used and the substrate on which the microorganisms grow.

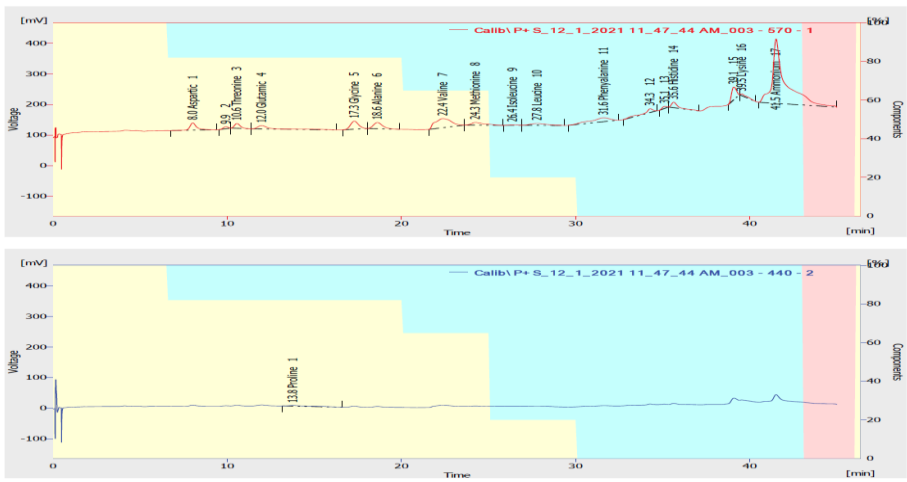

Figure (3): Estimation of amino acids produced in (P+D) sample.

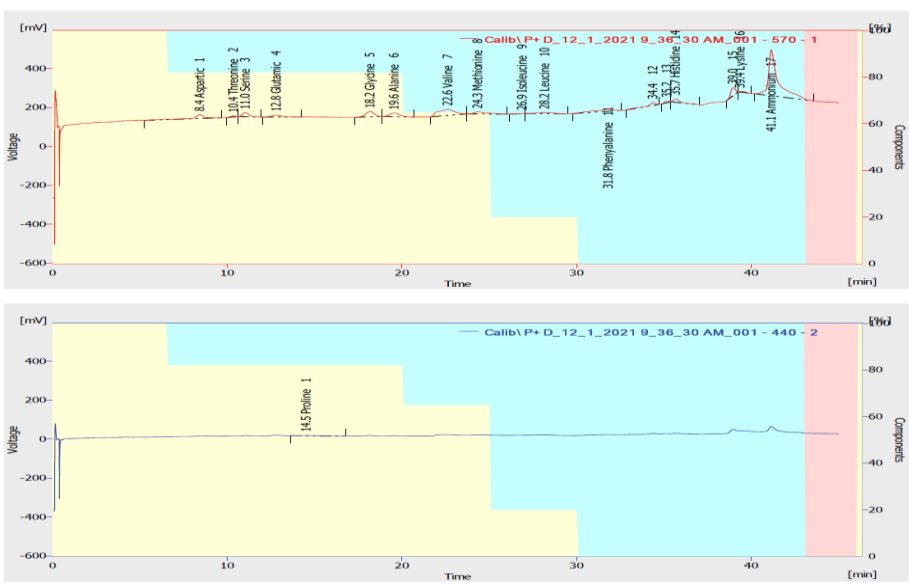

Figure (4): Estimation of amino acids produced in (OR+D) sample.

Adedayo *et al*. (2011) reported that the method of harvesting, drying, and processing conditions also affects the nutritive value of the finished product. In general, the percentages of amino acids were low in the produced biomass for all media. SCP with high nucleic acid content was only approved for animal nutrition

and recommended for animals with a short life span. Human consumption of more than 2 g of nucleic acid equivalent per day elevates the serum uric acid level from purine metabolism and may lead to gout and renal calculi (Mariana *et al*., 2020). Therefore, for human consumption, the nucleic acid contents of SCP must be reduced below 2% (Nasseri *et al*., 2011).

5. Conclusion

The results showed that the use of potato and orange peels as growth media for *S. cerevisiae* and *D. hansenii* through fermentation gave promising results for the production of SCP. From these results, we can say that further studies are needed to improve the protein quality of the produced biomass. Bioconversion of various wastes into specific value products such as SCP has the simultaneous potential to solve the worldwide nutritive protein deficiency. Moreover, obtaining an economical food and feed product and significantly reduce environmental pollutants by using these wastes as substrates for the production of a high nutritional value products.

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