

Production of Single Cell Protein of *Trichosporon Insectorum* AUMC13761 Using Wastewater of Potato Chips Manufacturing

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ABSTRACT

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Production of single-cell protein (SCP) from yeast cells is a highly promising process for providing adequate protein source for food manufacturing applications. This research designed to manufacture single-cell protein (SCP) from *Trichosporon insectorum* AUMC13761 that possess the ability to grow on wastewater of potato chip manufacturing. The selected yeast strain was isolated from the wastewater of potato chip manufacturing and subsequently identified using molecular techniques. *Trichosporon insectorum* AUMC13761 showed high biomass (24.92g dry cells/L) and protein yield (34.31g protein/100 g dry cells). Cytotoxicity SRB test was conducted, which confirmed its safety and suitability for use, whereas *Trichosporon insectorum* AUMC13761 biomass exhibited an IC₅₀ value greater than 100µg/ml. Furthermore, an analysis was conducted to determine the quantitative and qualitative composition of the *Trichosporon insectorum* AUMC13761 biomass in terms of amino acids. *Trichosporon insectorum* AUMC13761 biomass contained all the essential amino acids and exhibited high content of total amino acids, reaching 11.2% (w/w) It is best to write a recommendation for the research.

KEYWORDS: single cell protein, amino acids, cytotoxicity, ITS.

1. INTRODUCTION

The potato processing industry uses large quantities of water, it leads to the production of substantial quantities of liquid waste. The wastewater has a high level of chemical oxygen demand (COD) because it contains a significant amount of carbohydrates and protein (Gautam *et al.*, 017).

The potato washing process in the potato crisps industry necessitates a significant amount of water, resulting in a significant amount of liquid waste. Arapogluo *et al.*, (2009) determined that the rinsing of one ton of potatoes requires 4.78 m³ of water. This quantity is divided into a variety of phases, such as 0.57m³/tone for initial potato washing, 0.73m³/tone for peeling, 0.28m³/tone for water transport, 0.85m³/tone for slicing and soaking, 0.8m³/tone for the final wash, and 1.54m³/tone for machines and floors. A significant concentration of organic compounds and minerals was observed in the wastewater generated during the slicing and soaking of potato crisps.

Abdel-Raheam *et al.*, 2022. mentioned that by utilizing wastewater of potato chip manufacturing as a fermentation medium for the production of single-cell protein (SCP), they can be considered valuable by-products. This approach not only enhances the worth of subsequent goods but also helps offset the expenses associated with reprocessing.

Microorganism-derived SCP is eco-friendly alternative source to animal-derived proteins. Due to rising worldwide protein demand, food processing sector advances are expected to increase Single Cell Protein (SCP)'s importance, despite its low market share. Over \$18.5 billion will be spent on SCPs by 2030 (Global Market Insights, 2022). Many microbes have been studied for single-cell protein synthesis. Bacteria, algae, yeast, and other fungi (Alhomodi *et al.*, 2021, Salgado *et al.*, 2021, and Haris *et al.*, 2022) are among these microorganisms.

Among the yeast and fungal species studied, *Candida* (Kurcz *et al.*, 2018), *Kluyveromyces* (Yadav *et al.*, 2014), *Pichia*, *Galactomyces* (Zhou *et al.*, 2022), *Nectaromyces* (Zhang *et al.*, 2021), *Rhodotorula* (Myint *et al.*,

2020), *Saccharomyces*, *Meyerozyma* (Coimbra *et al.*, 2021), *Aureobasidium*, *Neurospora*, *Fusarium* (Ai-Farsi1 *et al.*, 2019), *Aspergillus* (Chakraborty *et al.*, 2022), and *Trichoderma* spp. (Alhomodi *et al.*, 2021) have demonstrated potential for SCP manufacture. These microorganisms have beneficial chemical compositions, nutrients (such as vitamin B-complex and folic acid), and amino acid profiles (particularly high in essential amino acids like lysine and threonine), which meet the standards established by the Food and Agriculture Organization (FAO) (Nasseri *et al.*, 2011 and Sharif *et al.*, 2021).

Fungi might potentially attain protein concentrations ranging from 30% to 50% with targeted fermentation optimization aimed at raising cellular protein levels (Nasseri *et al.*, 2011). Multiple studies have emphasized the appropriateness of fungi and yeasts for the synthesis of SCP, single-cell oil (Sarris *et al.*, 2019), and other useful substances (Tzirita *et al.*, 2019).

The production of single-cell protein (SCP) is characterized by its convenience, which is attributed to the rapid growth of microbial cells, high efficiency, and its ability to utilize a wide range of fermentation substrates (KV, 2022). SCP has extensive applications in both the agricultural and industrial domains. Notably, it has been employed as a protein source in fresh meals (Hansen *et al.*, 2021 and Agboola *et al.*, 2022), as a foam-stabilizing agent (Kupfer *et al.*, 2017), and in the production of paper and leather (Bratosin *et al.*, 2021). Moreover, SCP exhibits potential as a prospective packaging material (Singha *et al.*, 2021) and as a feasible contender for animal feed supplements (Uwineza *et al.*, 2021) owing to its nutritional worth and its capacity to diminish enteric CH₄ emissions in ruminant animals (Abbott *et al.*, 2020). Moreover, there have been proposals indicating that SCP might function as a durable food supply in the event of disasters (García Martínez *et al.*, 2022) or as a substitute food source for space missions (Alvarado *et al.*, 2021, and Alvarado *et al.*, 2023). The continuing inquiry focuses on customers' views and tolerance towards food products that include single-cell

protein (SCP). Limited investigations have been undertaken on this specific topic.

A number of recent studies (Khan *et al.*, 2022) have provided confirmation that the addition of SCP to food products improves their nutritional content without altering their sensory characteristics.

The objective of this research is the isolation of yeast that possesses the ability to utilize potato starch from potato chips manufacturing wastewater obtained at slicing and soaking potatoes and exploit its productivity in production of SCP during submerged fermentation.

2. MATERIALS AND METHODS

2.1. Waste material utilized

The wastewater collected during the cutting, slicing, and soaking operations at a potato chips factory in Assiut Governorate, Egypt "Egypt Chips" The specimens were enclosed in polyethylene pouches to "the laboratory of the department of food science, faculty of agriculture, Beni-Suef University" and preserved at a temperature of -18 °C until subsequent examination.

2.2. Analysis of the chemical composition of potato chip manufacturing wastewater

In our earlier study (Abdel-Raheem *et al.*, 2022), we studied the wastewater produced during the cutting, slicing, and soaking processes in a potato chips factory. Our findings showed that this wastewater contains a significant amount of organic substances that can be utilized in the fermentation industry, particularly for the production of single-cell protein.

2.3. Isolation of yeast from potato chips manufacturing wastewater:

The dilution plating method was employed to isolate yeast from selected wastewater. A volume of one milliliter of the potato wastewater was carefully put into sterile Petri dishes. Subsequently, around 20 milliliters of a suitable agar medium, after cooling the plates (5 plates for each kind of medium) were placed in an incubator at a temperature of 28 °C for 5 days.

Following this, the yeasts that had developed on the plates were separated and then preserved on yeast extract malt extract agar (YM). The preserved yeasts were then stored at a temperature of 5 °C until their identity was verified, followed the method Sukmawati *et al.* (2015)

2.3.1. Media used for isolation of yeasts:

Potato wastewater medium (PW) was used to isolate yeasts from the potato wastewater. Medium was prepared using solid material obtained after drying the wastewater, of the following composition (g/L) solid materials (after drying of the wastewater) 10.0, agar 15.0, to which rose bengal (25 µg/mL) as a bacteriostatic agent was added.

2.3.2. Genotypic identification of yeast strains

The yeast strains were classified according to their genotypes. The identification of yeast genera and species was conducted using molecular techniques. This involved amplifying the internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA using primers ITS1 and ITS4 from the SolGent Company in Daejeon, South Korea. The methodology used in this study was described by Abdel-Sater et al. in 2016. The selected strains of the identified species were subsequently stored in the Assiut University Moubasher Center for Mycological Science Culture Collection (AUMC). In addition, the ITS gene sequences of these yeast strains were submitted to the National Center for Biotechnological Information (NCBI), and unique accession numbers were provided to each strain.

2.4. Inoculum preparation and fermentation for SCP production

The chosen yeast isolates were freshly cultivated on yeast extract malt extract agar medium (YM) in a shaker incubator at 28°C, 120 rpm for 48 h. Single-cell protein was produced in 250 ml erlenmeyer flasks for every yeast with three duplicates using 100 ml sterile potato chip wastewater free of additives. The flasks were kept at 28 °C, 120 rpm in a shaking incubator for seven days

2.5. Biomass yield and its protein content of the yeast culture

Gravimetric method was used to detect the dry biomass yield. Each of the yeast cultures had 30 mL taken, centrifuged for 10 min, then twice washed using distilled water. Drying the wet cell biomass at 85 °C for 24 h resulted in a constant weight. Weighing the dried biomass produced values represented in grams of dry weight per liter of culture media (Kot *et al.*, 2015). Using the Biuret technique (Gornall *et al.*, 1949), dry yeast biomass was investigated for protein concentration.

2.6. Cytotoxicity assay

The evaluation of cell viability was carried out using the Sulforhodamine B (SRB) assay on Oral epithelial cells (OEC). Oral epithelial cells (OEC) were procured from Nawah Scientific Inc., (Mokatam, Cairo, Egypt), following the method described by (Skehan *et al.*, 1990; Allam *et al.*, 2018; Vichai and Kirtikara, 2006).

2.7. Amino Acids analysis

The analysis of the amino acid content involved weighing approximately 2.5 g of the basidioma sample into an extraction thimble. Fat was then extracted from the sample using a chloroform/methanol mixture (2:1, v/v) and a Soxhlet apparatus, following the method described by AOAC (2005). The extraction

process lasted for 5-6 hours. The stock solution was diluted by adding 60 µL to a 1.5 mL vial with buffer solution. The solution was then filtered using a 0.22 µm syringe filter. Subsequently, 100 µL of this filtrate was subjected to analysis using the Sykam Amino Acid Analyzer, manufactured by Sykam GmbH in Eresing, Germany.

3. RESULT AND DISCUSSION

3.1. Isolation and identification of yeasts capable of starch utilization:

A yeast isolate was successfully obtained from the waste water of the potato chips industry, capable of exploiting these wastes on the medium of potato starch, and it was identified as *Trichosporon insectorum* AUMC13761 (Table 1).

Many previous references mentioned many types of yeast that can be used as SCP, because their biomass contains amino acids and other important nutritional elements. Among the yeast species studied, *Candida* (Kurcz *et al.*, 2018), *Kluyveromyces* (Yadav *et al.*, 2014), *Pichia*, *Galactomyces* (Zhou *et al.*, 2022), *Nectaromyces* (Zhang *et al.*, 2021), *Rhodotorula* (Myint *et al.*, 2020), *Saccharomyces*, *Meyerozyma* (Coimbra *et al.*, 2021).

Table 1. Assiut University Moubasher Centre for Mycological Science accession number (AUMC) of yeast strains isolated from potato wastewater, along with the closest GenBank match and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC Number	Accession GenBank Number	Length (bp)	Closest Genbank match #		Sequencing similarity (%)	Species
			ITS	Accession no.		
AUMC 13761	OQ294043	513	CBS:10422 ^T	KY105746	513/513(100%)	<i>Trichosporon insectorum</i>

3.2. Cytotoxicity assay

Figure (1) displays the cytotoxic effects of several yeast strains on oral epithelium normal cell lines (OEC) exposed to *Trichosporon insectorum* AUMC13761 biomasses. The SRB

assay was used to investigate biomass cytotoxicity against OEC normal cell lines. The study found that biomass has no cytotoxic effects on normal oral epithelial cell lines at concentrations ranging from 0.01 to 100 µg/mL. The biomass has an IC₅₀ of >100 µg/mL and a viability range of 100.000 to

80.0602%. The biomass can be safely used in food systems.

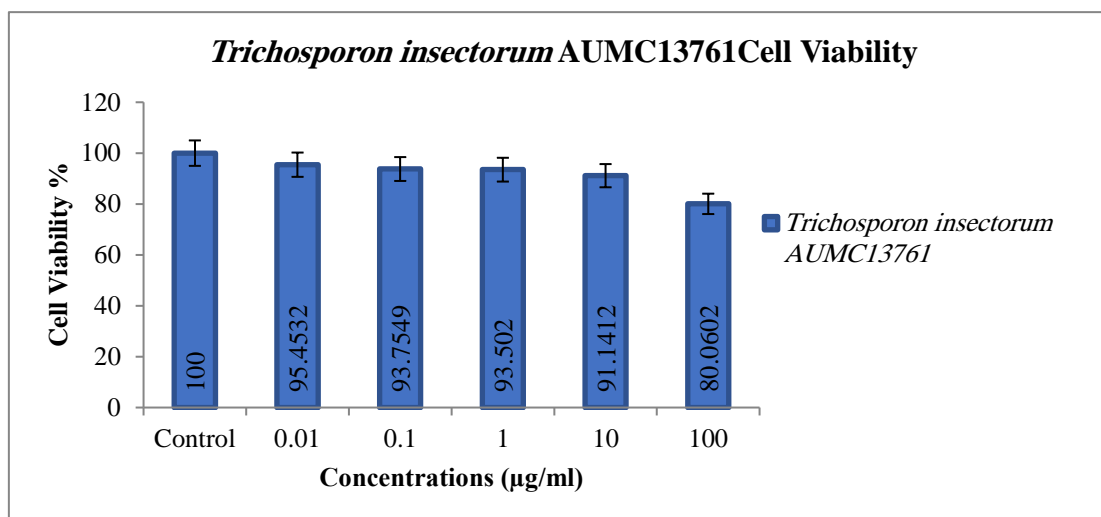


Figure 1. SRB cytotoxicity assay of selected yeast strains.

Interestingly, at a starting dosage of 100 µg/mL, *Trichosporon insectorum* AUMC13761 dry biomasses reduced cell viability by up to 19%. Tihauan *et al.*, (2023) tested pea, almond, yeast, *Spirulina*, and *Pleurotus* spp. flour for cytotoxicity. They used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test and lactate dehydrogenase (LDH) release to evaluate the cytotoxicity of these protein-rich dietary supplements. *Pleurotus* spp. flour reduced cell viability by 77% at 500mg/ml. Next, yeast powder lost up to 69% viability. Almond and pea powder lost up to 30% viability. Cytotoxicity was equivalent at 250 mg/mL, 62.5 mg/mL, 7.813 mg/mL, and 0.651 mg/mL.

3.3. Biomass yield of the yeast culture and its total protein Content

Trichosporon insectorum AUMC13761 was cultured in potato wastewater medium in a shaking incubator at 28 °C, 120 rpm for 7 days and we obtained 24.92 g dry cells/L and 8.22 g/L protein, yielding 34.31 g protein/100g dry cells (Figure, 2). Aggelopoulos *et al.* (2014) used solid-state fermentation (SSF) using whey, molasses, brewer's solid waste, orange and potato wastes to study the protein yield of Kefir sp, *Kluyveromyces marxianus*, and *Saccharomyces cerevisiae*. Their study discovered that the protein yields were 23%, 34% and 39%, respectively.

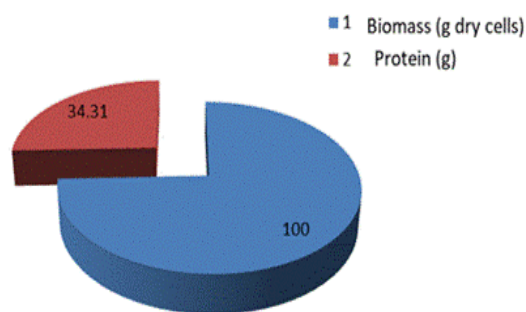
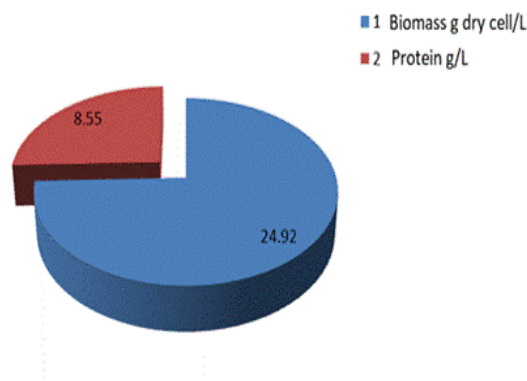
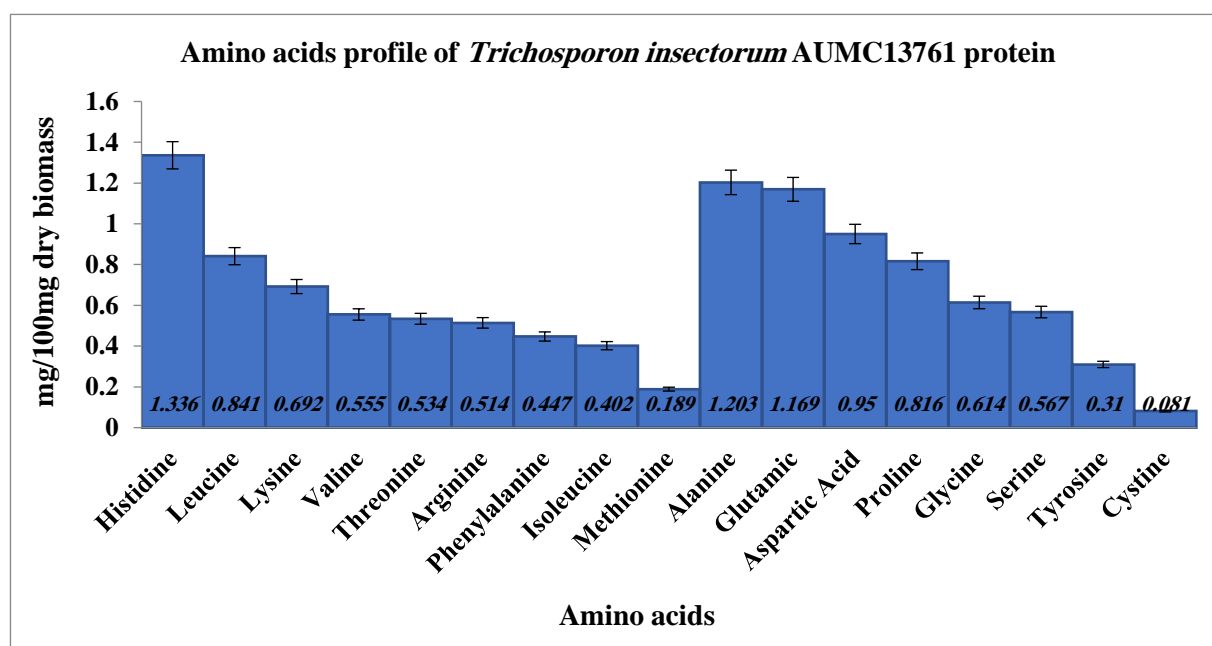


Figure 2. The biomass yield and protein content produced by *Trichosporon insectorum* AUMC13761**3.4. Amino acid analysis**

Figure. 3 revealed *Trichosporon insectorum* AUMC13761 amino acid profiles of obtained dry biomass. It show that *Trichosporon insectorum* AUMC13761 produced 11.220 mg/100 mg dry dells of all essential amino acids for human metabolism. Histidine (His) (1.336%), Leucine (Leu) (0.841%), Lysine (Lys) (0.692%),

Valine (Val) (0.555%), and Threonine (Thr) (0.534%) were the most abundant essential amino acids in *Trichosporon insectorum* AUMC13761's dry-weight biomass. Alanine (Ala), Glutamic Acid (Glu), Aspartic Acid (Asp), Proline (Pro), Glycine (GLy), and Serine (Ser) were dominant among the non-essential amino acids, comprising 1.203, 1.169, 0.95, 0.816, 0.614 and 0.567 % respectively.

**Figure 3. Amino acids profile of *Trichosporon insectorum* AUMC13761 protein.**

The *Trichosporon insectorum* AUMC13761 biomass contains Lysine (0.692%) and Threonine (0.534%), making it a good protein supply for cereal protein amino acid imbalance in the pastry industry. Methionine was detected in lower concentrations than other important amino acids.

All yeast proteins contain the nutritionally essential amino acids, according to Agboola *et al.*, (2022) observed 20 g/kg dry matter of leucine, lysine, aspartic acid, and glutamic acid in yeasts. When cultivated in hydrolysates of pre-treated wood and chicken products, *Cyberlindnera*

jadinii, *Blastobotrys adenivorans*, and *Wickerhamomyces anomalus* had amino acid compositions similar to fishmeal and soybean meal.

Additionally, Razzaq *et al.*, (2020) examined the nutritional properties of single-cell protein (SCP) from sugar-beet bagasse-grown *S. cerevisiae*. Researchers found that SCP includes a variety of important amino acids, including leucine (43.5 grams per kilogram), valine (38.3 grams), and lysine (31.4 grams). In another work, Samadi *et al.*, (2016) found 17 amino acids in sugarcane bagasse-derived SCP from *S.*

cerevisiae. Almost all essential amino acids were present, except threonine and tryptophan. The amino acid profiles of SCP and soya protein were identical.

4. CONCLUSION

It is best to write a conclusion for the research that contains a summary of the most important main points and ends with recommendations

5. ACKNOWLEDGMENT

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الملخص العربي

إنتاج البروتين وحيد الخلية من خميرة التريكوسبورم إنسكتوريم بواسطة إستخدام الماء الناتج من غسيل شرائح البطاطس أثناء التصنيع

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إنتاج البروتين وحيد الخلية من خلايا الخميرة كمصدر للبروتين يستخدم فى تدعيم تصنيع الأغذية حيث أنه فى هذا البحث تم إنتاج البروتين وحيد الخلية من خميرة التريكوسبورم إنسكتوريم التى تستطيع النمو على الماء الناتج من غسيل شرائح البطاطس أثناء التصنيع وتم عزل هذه السلالة من الماء الناتج من غسيل شرائح البطاطس وتعريفها بإستخدام طريقة التتبع الجينى حيث أنها أظهرت أعلى إنتاج من الكتلة الحيوية (٢٤.٩٢ جم خلايا جافة/التر) والبروتين (٣٤.٣١ جم بروتين/١٠٠ جم خلايا جافة) وبإستخدام إختبار السيتوكسيستى تم التأكد من إنها آمنة ومناسبة للإستخدام وذلك لأنه عند إستخدام السلالة بتركيز ١٠٠ ميكروجرام/ملتر كان عدد الخلايا الحية أكثر من ٥٠ % وهو الحد الآمن وعند تحليل مكونات الكتلة الحيوية لسلالة الخميرة وجد أنها تحتوى على جميع الأحماض الأمينية الأساسية وبالتالي فهو بروتين عالى الجودة فهو يحتوى على ١١.٢ % أحماض أمينية .

الكلمات المفتاحية : البروتين وحيد الخلية ، الأحماض الأمينية ، إختبار السيتوكسيستى ، تقنية التعريف إستخدام الحمض النووى