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Production of single cell protein by fungi from different food wastes

Marwa Gamal Ahmed[1] · Shaymaa Ahmed Gouda[1] · Samah Donia[1] · Naziha Mohamed Hassanein[1]

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Abstract
Single-cell protein (SCP) which is derived from agricultural waste has recently drawn increased interest as a substitute source of protein to improve both human and animal nutrition. In this study, pineapple, orange, banana, sugarcane, and garlic wastes were prepared as substrates for SCP production using fungi under a liquid fermentation system. The fermentation conditions (temperature, pH, and nitrogen sources) of the most promising fungal isolates were optimized for maximum SCP production. Results obtained showed that *Aspergillus niger* with pineapple waste after 10 days gave the highest protein content (9.79 ± 0.11 g/L), followed by *Penicillium citrinum* with orange waste after 8 days (9.41 ± 0.15 g/L) and *Penicillium crustosum* with banana waste after 6 days (7.75 ± 0.11 g/L).The optimum fermentation temperature, pH value, and nitrogen source for SCP production were recorded at 30 °C, pH 4.3, and ammonium sulphate with *Aspergillus niger*; at 30 °C, pH 5.0, and ammonium sulphate with *Penicillium citrinum*; and at 20 °C, pH 5.0, and ammonium sulphate with *Penicillium crustosum*. Based on proximate analysis of SCP produced, fat content ranged from 2.74 ± 0.08~10.73 ± 0.1%, fibers from 4.8 ± 0.18~9.45 ± 0.15%, carbohydrates from 14.1 ± 0.06~20.81 ± 0.06%, ash from 4.05 ± 0.05~16.1 ± 0.2%, total phenols 0.14 ± 0.03~0.64 ± 0.04%, and total flavonoids 0.09 ± 0.01~0.55 ± 0.15%. The amino acid profile of SCP showed that all essential amino acids were present in significant amounts. This study recorded some fungal species as SCP producers for the first time and provided a significant amount of protein. Briefly, fungal SCP is a great protein source and other essential nutrients that may be used in food products.

Keywords Biomass · Food waste · Fungi · Optimization · Proximate analysis · Single cell protein

1 Introduction

Proteins are described as macromolecules by the Food and Agriculture Organization (FAO) of the United Nations as structural components of cells, tissues, muscles, and organs. Proteins are necessary for metabolic processes and serve as both a nitrogen supply and a building unit for the both functional and structural elements needed for life [1]. Among the major issues, particularly in developing nations, is its deficiency. Children that suffer from protein-calorie malnutrition (PCM) typically have insufficient immunity and delayed mental development [2]. According to the present state, 12.5% of people worldwide suffer from nutritional deficiency, chronic hunger, and an inadequate supply of nutrient-rich food [3].

The demand for animal and human food has increased due to the continued population growth. The world population might raise 9.3 billion by 2050, according to Pihlajaniemi [4], and at present consumption rates, there would be a 1250 million tons annual rise in the need for animal-derived protein [5, 6]. The need for protein-rich foods in the world has increased, which has prompted researchers to search for new protein formulation source alternative to conventional ones. One of the most significant steps towards accomplishing this objective is the development of single cell protein (SCP), which provides an alternative and valuable solution to the global food issue [7].

The definition of SCP is the dried and dead biomass of microorganisms, which includes algae, bacteria, filamentous fungi, and yeast that have been grown in a large scale [8, 9]. This form of protein has several benefits, such as being reasonably priced, using waste products as the primary substrate, and having an adequate nutritional value based on the amino acid composition in addition to the presence of lipids, carbs, nucleic acids, vitamins, and minerals. Furthermore, SCP provides a

✉ Marwa Gamal Ahmed
marwagamal@sci.asu.edu.eg

[1] Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt

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variety of essential amino acids that are deficient in most plant and animal meals, including lysine and methionine. It can replace costly identified sources as soya bean and others as an additive source for the primary diet [9, 10]. It is advantageous to concentrate on single cell protein from fungus rather than bacteria and algae since fungi are among microorganisms that are increasingly accepted and employed for single cell protein due to their bigger size, ease of harvesting, and nutritional value [11].

In recent years, the global fruit and vegetable consumption has increased by an average of 4.5% per annum, which is higher than the world population rate [12, 13], so several food wastes have been produced because of the global expansion of food production [14]. In Egypt, the volume of agricultural waste is estimated at about 35 million tons per year, of which 7 million tons are utilized as organic fertilizers, 7 million tons are utilized as animal feed, and about 21 million tons are left without use. These wastes accumulate annually without treatment. The improper disposal of these wastes pollutes air, water, and soil and compromises human and animal health, so it is necessary to focus on increasing the use of these wastes in order to increase their value and reduce the dangerous pollution of the environment [15, 16]. Carbohydrates and other essential elements found in food wastes are regarded as natural substrates for microbial development [17]. The utilization of food wastes in the creation of SCP will aid in reducing pollution besides solving the global lack of protein-rich diets [18]. SCP production is affected by the kind of substrate and microorganisms utilized; the availability of nitrogen and carbon sources; and the concentration of substrate, pH, airflow, temperature, agitation rate, and inoculum size [19].

Due to the consumption of animal proteins on a global scale, we have focused on finding substitute sources of protein to solve the global food problem as well as ways to manage unwanted food wastes that accumulated in huge amounts by serving as substrates for the growth of fungi.

The goal of the current study was to examine the potential for manufacturing SCP from fungi via liquid state fermentation while employing a variety of readily accessible food peels (pineapple, orange, banana, sugarcane, and garlic) as inexpensive energy sources. Moreover, the medium conditions (fermentation temperature, pH, and nitrogen source) of the selected strains were optimized for maximum protein production. Finally, the proximate analysis and amino acid composition of SCP produced was also studied.

2 Materials and methods

2.1 Fungal isolation

The fungi utilized for our research were isolated from soil, water, and air in four Governorates of Egypt (Cairo, Qalyubia, Sharkia, and Menoufia Governorate). The serial dilution technique was utilized to isolate the fungi from soil [20], the plating technique was used for the isolation of fungi from water [21], and the settle plate technique was utilized to isolate the fungi from air (indoor and outdoor), according to Valentina and Umadevi [22]. Fungi were isolated using three types of media: (i) Sabourad's yeast extract agar (SYEA) (HiMedia, India); (ii) potato dextrose agar (PDA g/L), potato slices 200, glucose 20, and agar 20 gm; and (iii) Czapek's dox agar (CDA) (HiMedia, India). The plates were maintained at 28 ± 2 °C and monitored every day for a week. The fungal growth was subcultured onto PDA fresh plates until pure isolates were obtained. The pure cultures were preserved on PDA slants and maintained by subculturing every 4 weeks for further study.

2.2 Wastes collection and preparation of fermentation medium

Fresh pineapple, orange, banana, sugarcane, and garlic peels were obtained from regional markets in Egypt. The food peels were extensively rinsed with distilled, sterile water before being dried for 2 days at 60 °C [23]. The peels were ground and weighted then blended with distilled water in the ratio 1:4 then filtrated. A total of 50 mL of each food waste filtrate was transferred into 250-mL Erlenmeyer flasks and autoclaved for 15 min at 121 °C [11].

2.3 Identification of fungal isolates

Fungal isolates were determined to the genus level according to morphological features (color and texture, etc.) and microscopic identification (slide culture technique) according to Pitt [24] for the genus *Penicillium*, Gilman [25] for the genus *Fusarium*, Raper and Fennell [26] for the genus *Aspergillus*, Ellis [27] for Dematiaceous, and Hesseltine [28] for Mucorales. Molecular identification was used to the most interesting fungi.

Molecular identification was performed according to manufacture's protocol. Total genomic DNA was isolated by using Zymo-Spin™ IICR Column. PCR was performed to amplify D1/D2 regions of the 28S rRNA large ribosomal subunit by using NL-1 (5′-GCATATCAATAAGCGGAG GAAAAG-3′) and NL-4 primers (5′-GGTCCGTGTTTC AAGACGG-3′). PCR amplicons were sequenced at GATC Company, Germany, by using ABI 3730×1 DNA sequences. Sequence data were blasted using nucleotide BLAST search at National Center for Biotechnology Information (NCBI) (blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was established with the neighboring join (NJ) method in MEGA software version 11 with 1000 bootstrap [29, 30]. The nucleotide sequences data of isolated fungi were entered into the

NCBI GenBank nucleotide sequences database under accession numbers.

2.4 Inoculum preparation and fermentation process

To separate the spores from the hyphae, 10 mL of sterile 1% v/v tween 80 solution was flooded over 7-day-old fungal culture plates (PDA). The mycelium debris was filtered, and the concentration of spores was adjusted to 10^6 spore/mL using a hemocytometer [11]. Food peel filtrate (50 mL) was transferred into Erlenmeyer flasks (250 mL) and sterilized at 121 °C for 15 min. Pineapple waste media (PWM), orange waste media (OWM), banana waste media (BWM), sugarcane bagasse waste media (SWM), and garlic waste media (GWM) were the designations given to the media. The natural pH values of PWM, OWM, BWM, SWM, and GWM were 4.33, 4.35, 5.62, 5.92, and 6.3, respectively. Each sterilized medium flask received 500 µL of fungal inoculum (10^6 spores/mL) as an inoculum. The fermentation was then performed for 10 days at 28 ± 2 °C in a shaking incubator (HYSC, Korea) at 120 rpm, and the fungal biomass was obtained every 2 days to determine the cell protein content [11].

2.5 Analytical methods

Measurements of the fungal biomass and protein content were made every 48 h for 10 days. Briefly, 50 mL of sterile distilled water was used to wash the fungal mycelia twice after they had been gathered via filtering by Whatman No. 1 filter paper [11]. The collected fungal biomass was oven dried (PURI VEN, Korea) at 65 °C for 24 h, and the weight of the dried biomass was recorded and ground into a powder known as SCP [31]. The protein content was assessed by lowery method utilizing bovine serum albumin (BSA) as a standard [32]. Proximate analyses of fungal biomass are as follows: carbohydrates, ash, moisture, fat, and fiber contents were determined according to A.O.A.C. [33] and total phenols and flavonoids according to Singleton and Rossi [34] and Zhishen [35], respectively.

2.6 Optimization of SCP production parameters

To optimize the process of fermentation, various experiments were performed in which various factors such as incubation temperature (20 °C, 30 °C, and 40 °C), pH values (3.0, 5.0, and 7.0), and 0.2% nitrogen salts (ammonium chloride, ammonium sulphate, and urea) [36]. The five fungal isolates that gave the highest SCP production on each waste were selected for this experiment. Dried biomass and total protein content were determined for each experiment. All assessments were conducted in three duplicates in a shaking incubator at 120 rpm for the optimum incubation period for high protein production under the above specified fermentation conditions.

2.7 Amino acid analysis of SCP

Amino acid composition of SCP was explored by high performance liquid chromatography (HPLC). In which, 0.1 g of the sample was mixed with 5 mL H_2O and 5 mL of 6 M HCl and heated at 100 °C for 24 h and then filtered. Finally, 1 mL of the filtrate was dried and resuspended in 0.1 M HCl and injected into HPLC (Agilent 1260, USA). The separation was carried out using Eclipse Plus C18 column (4.6 mm × 250 mm internal diameter, 5 µm). The mobile phase consisted of buffer (sodium phosphate dibasic and sodium borate) with pH 8.2 and acetonitrile:methanol:water (45:45:10, vol.%) at a flow rate 1.5 mL/min. The fluorescence detector was adjusted at 340/450 nm from 0 to 27 min and at 266/306 nm from 27 to 35 min [37, 38]. The chemicals HCL, sodium phosphate dibasic, sodium borate, acetonitrile, and methanol were of analytical grade (Sigma-Aldrich, USA).

2.8 Statistical analysis

The statistical analysis was carried out by the Minitab statistical program V17 for Windows (Minitab Inc., USA), and the findings were presented as the mean ± standard deviation of three repetitions in all experiments. One-way ANOVA was performed to analyze the data, and Tukey's multiple comparison test was employed to identify differences that were significant at $p < 0.05$.

3 Results

3.1 Fungal isolates

Thirty-five fungal isolates were isolated from various sources (soil, 8 isolates; water, 3 isolates; outdoor air, 16 isolates; and indoor air, 8 isolates) of different areas. The isolated fungi were subjected to preliminary morphological identification to the genus level. The results showed that out of 35 isolates, 7 belong to the genus *Penicillium*, 15 to the genus *Aspergillus*, 2 to the genus *Scopulariopsis*, 1 to the genus *Fusarium*, 2 to the genus *Rhizopus*, 1 to the genus *Curvularia*, 1 to the genus *Cladosporium*, 1 to genus *Monodictys*, 1 to the genus *Stachybotrys*, 1 to the genus *chaetomium*, 1 to the genus *Cephalosporium*, and 2 unknowns (Table 1).

3.2 Screening of fungal isolates for the highest biomass protein content of SCP

Thirty-five fungal isolates were tested in the fermentation process with five different food waste media (pineapple, orange, banana, sugarcane bagasse, and garlic) as the natural fermentation media for the highest biomass protein content production.

Figures 1 and 2 show the fungal isolates that gave the highest biomass and protein content with PWM as a fermentation medium. The isolate IA37 gave the most significant ($p < 0.05$) biomass of 21.3 ± 0.1 g/L and protein content (9.79 ± 0.11 g/L) after 10 days of incubation, followed by isolates OA43, OA25, and OA3, which gave protein contents of 9.22 ± 0.12, 8.53 ± 0.2, and 8.42 ± 0.11 g/L, respectively. However, after 8 days of incubation, isolate IA7 gave a dry biomass value of 17.9 ± 0.35 g/L and a protein content of 8.62 ± 0.12 g/L.

Concerning OWM, isolate OA22 had vigorous protein content 9.41 ± 0.15 g/L which is significantly ($p < 0.05$) higher than other isolates with biomass value of 20.4 ± 0.29 g/L after 8 days of incubation while isolate OA36 gave protein content of 8.53 ± 0.18 g/L and biomass value of 21.14 ± 0.05 g/L after the same period of incubation. The other isolates (IA37, W35, and OA3) showed the highest protein content after 10 days of incubation (Figs. 1 and 3).

Figures 1 and 4 indicate that the isolates IA24, S6, and OA29 produced the highest significant ($p < 0.05$) yield of biomass (15.6 ± 0.2, 13.4 ± 0.11, and 10.61 ± 0.21) and protein content (7.75 ± 0.11 g/L, 6.37 ± 0.17 g/L, and 5.31 ± 0.18 g/L), with BWM after 6 days of incubation, respectively. As opposed to that, isolate IA7 had the greatest

Table 1 Fungal genera isolated from various sources

Serial number	Genus	Source				Total no of isolates
		Soil	Water	Indoor air	Outdoor air	
1	*Aspergillus*	2	1	3	9	15
2	*Cephalosporium*	1	-	-	-	1
3	*Chaetomium*	-	-	-	1	1
4	*Cladosporium*	-	-	1	-	1
5	*Curvularia*	-	-	-	1	1
6	*Fusarium*	1	-	-	-	1
7	*Monodictys*	-	-	-	1	1
8	*Penicillium*	3	-	2	2	7
9	*Rhizopus*	-	-	1	1	2
10	*Scopulariopsis*	-	1	1	-	2
11	*Stachybotrys*	1	-	-	-	1
	Unknown 1	-	1	-	-	1
	Unknown 2	-	-	-	1	1
Total		8	3	8	16	35

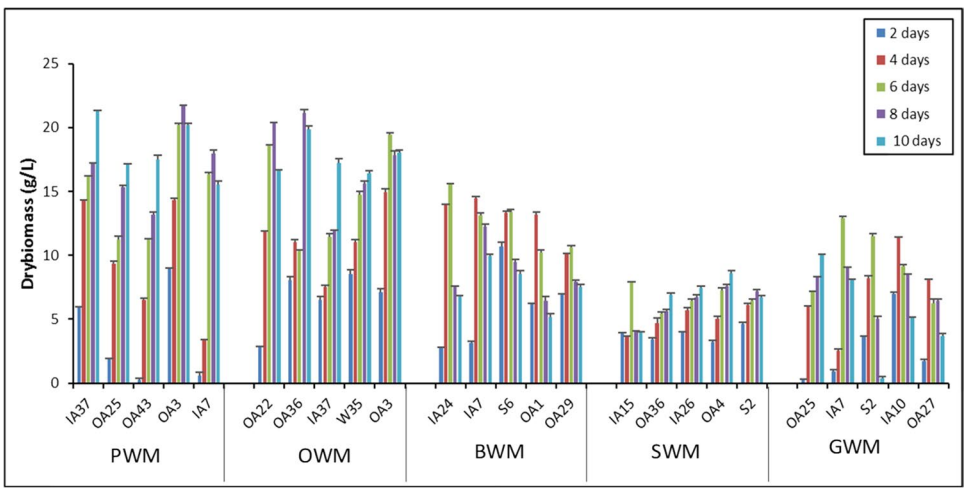

Fig. 1 Dried biomass of the promising fungi on a time course basis on PWM, OWM, BWM, SWM, and GWM

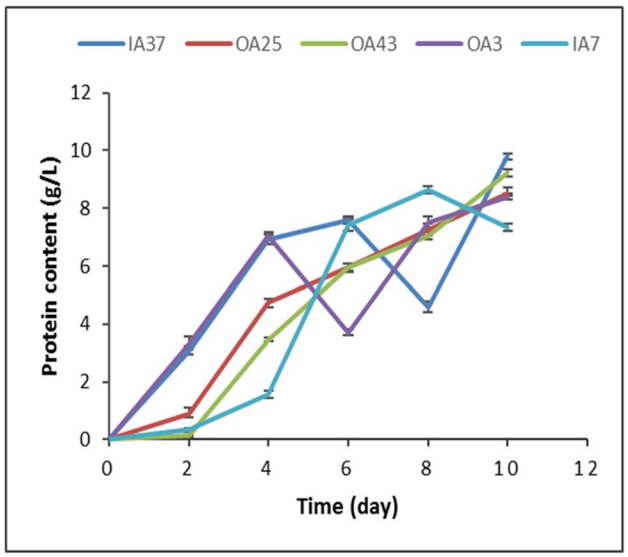

Fig. 2 Protein content of the promising fungi on a time course basis on PWM

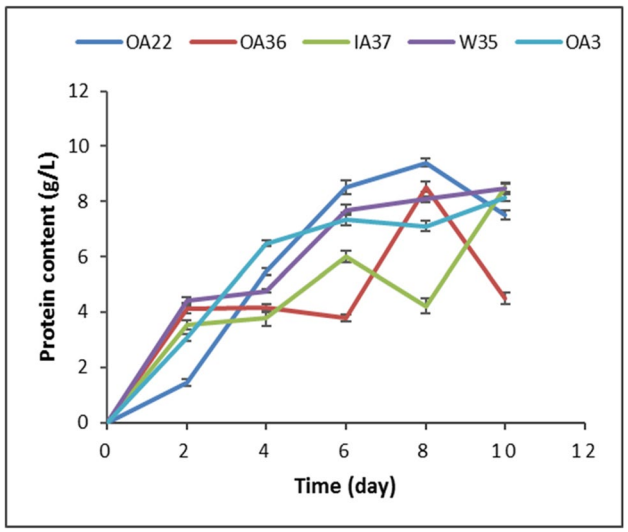

Fig. 3 Protein content of the promising fungi on a time course basis on OWM

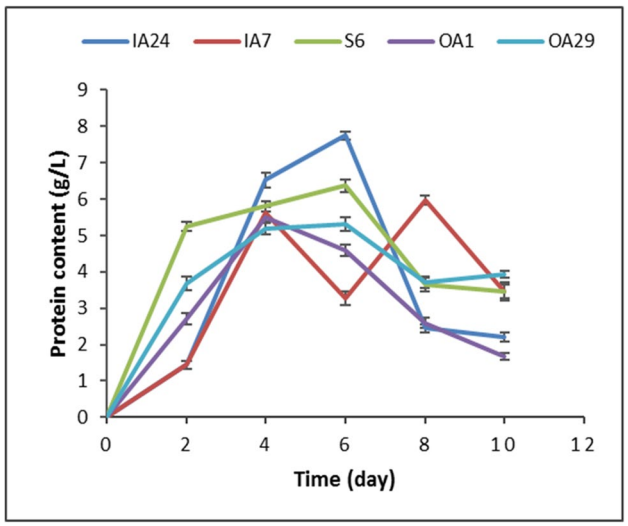

Fig. 4 Protein content of the promising fungi on a time course basis on BWM

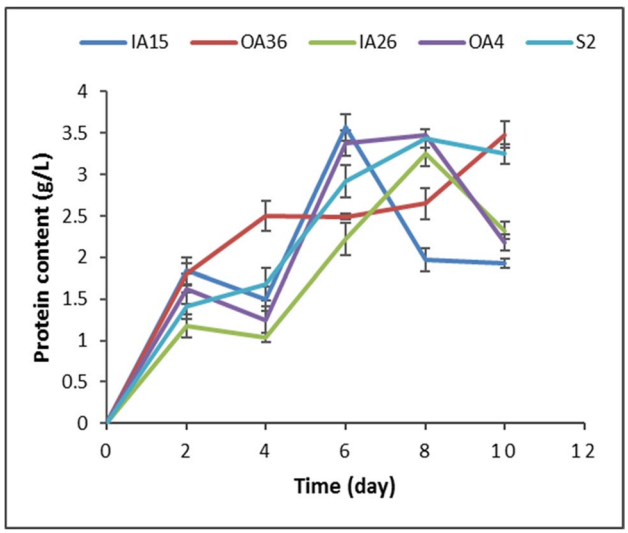

Fig. 5 Protein content of the promising fungi on a time course basis on SWM

significant ($p < 0.05$) protein content (5.97 ± 0.12 g/L) after 8 days of incubation while isolate OA1 produced a high protein content after 4 days of incubation.

Concerning SWM, Figs. 1 and 5 show that the isolate IA15 gave the highest significant ($p < 0.05$) protein content (3.57 ± 0.16 g/L) after 6 days of incubation followed by isolate OA36 with protein content of 3.48 ± 0.16 g/L after 10 days. On the other hand, the isolates IA26, OA4, and S2 gave the highest significant protein content (3.26 ± 0.16, 3.47 ± 0.07, and 3.44 ± 0.11 g/L) after 8 days of incubation, respectively.

The most significant ($p < 0.05$) protein content was attained by isolate OA25 (5.22 ± 0.1 g/L) after 10 days of incubation. Moreover, the isolates that recorded higher biomass were IA7 (12.8 ± 0.22) and S2 (11.5 ± 0.16) after 6 days of incubation, while isolates IA10 and OA27 gave the highest values of biomass and protein content after 4 days of incubation (Figs. 1 and 6).

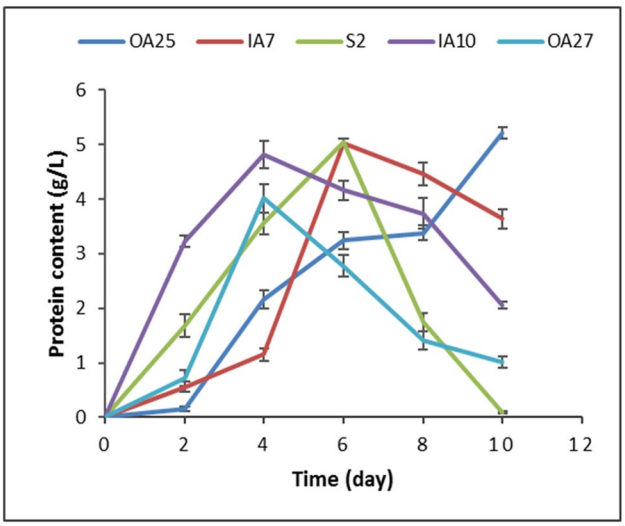

Fig. 6 Protein content of the promising fungi on a time course basis on GWM

3.3 Identification of the most promising fungi

3.3.1 Morphological identification

Based on screening of fungal isolates for the highest significant SCP synthesis, five fungal isolates were selected among the 35 isolates for further studies and identified morphologically as members of the genus *Aspergillus* for isolates IA37 and IA15 and *Penicillium* for isolates OA22, IA24, and OA25.

Figure 7a shows that the colonies of isolate IA37 initially appeared white, then quickly became black. The conidiophores are unbranched, carrying rounded to ovoid conidia. While the isolate IA15 appeared white, velvety, and raised colonies with a brown reverse, conidiophores smooth carrying chains of globose to sub-globose smooth walled and colorless conidia, as shown in Fig. 7b.

The colonies of isolate OA22 were velvety blue green with a white border and produced yellow droplets on the surface and yellow reverse, the conidiophores biverticillated, carrying globose-shaped conidia as shown in Fig. 7c. On the other hand, the isolate IA24 appeared as bluish green colonies, and the conidiophores terverticillated with bottle-shape philalide that carrying chains of oval-shaped smooth walled conidia, as shown in Fig. 7d. In addition, for the isolate OA25, colonies greyish in color, tinted lightly with pink on the surface and reddish-brown on the reverse, and the conidiophores monoverticillated, bearing ampulliform philalides, carrying sub-spherical, ellipsoidal shaped conidia with smooth walls as depicted in Fig. 7e.

3.3.2 Molecular identification

The molecular identification was carried out using rDNA of LSU D1/D2 region sequencing. In blast similarity analysis, the five selected isolated fungal sequences were BLAST searched in the database of GenBank (NCBI). Based on the sequence obtained from molecular identification together with their morphological features, the isolates IA37, IA15, OA22, IA24, and OA25 were identified as *Aspergillus niger*,

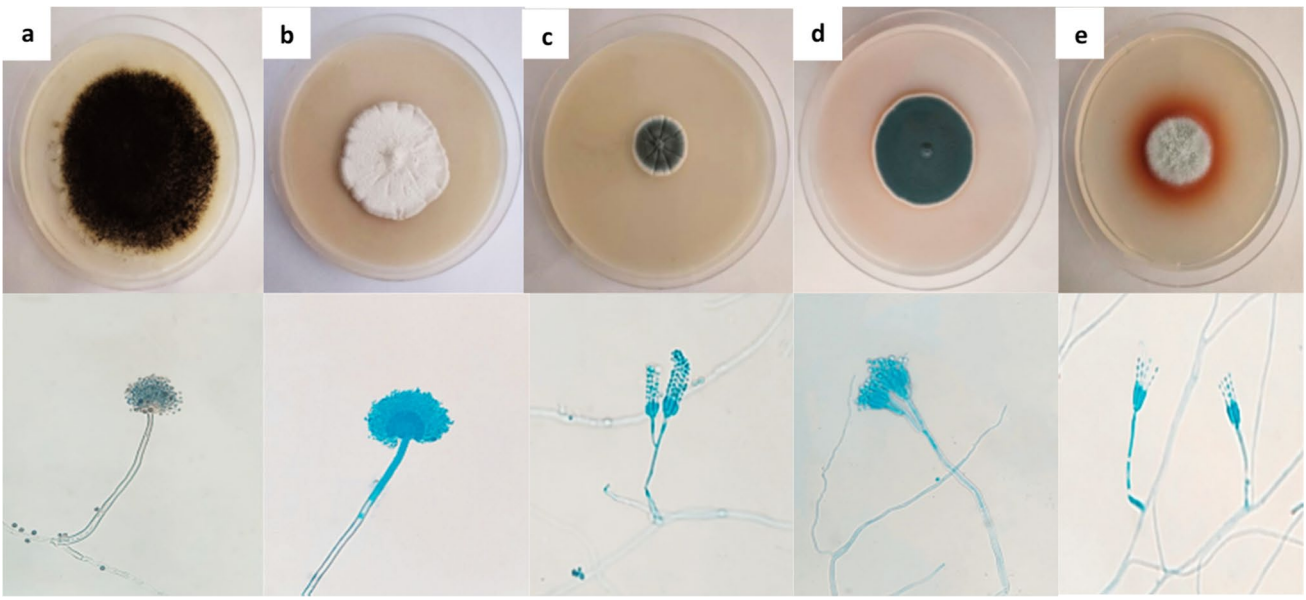

Fig. 7 Cultural and morphological features of the promising fungi on PDA at 28 ℃ for 7 days. **a** *Aspergillus* IA37. **b** *Aspergillus* IA15. **c** *Penicillium* OA22. **d** *Penicillium* IA24. **e** *Penicillium* OA25

Aspergillus allahabadii, *Penicillium citrinum*, *Penicillium crustosum*, and *Penicillium chermesinum,* respectively. The sequences of the five identified fungi were submitted to GenBank with accession number of OR229938 for *A. niger*, OR229935 for *A. allahabadii*, OR229941 for *P. citrinum*, OR229939 for *P. crustosum*, and OR230013 for *P. chermesinum*, and phylogenetic trees were constructed utilizing the neighboring join (NJ) technique to study the evolutionary relatedness of each fungus with similar GenBank sequences, as shown in Fig. 8.

3.4 Optimization conditions of the promising isolates on various waste media

According to the above results, the most significant protein-producing isolates were *A. niger* with PWM after 10 days of incubation, *P. citrinum* with OWM after 8 days of incubation, *P. crustosum* with BWM after 6 days of incubation, *A. allahabadii* with SWM after 6 days of incubation, and *P. chermesinum* with GWM after 10 days of incubation. Therefore, these isolates were selected to optimize the conditions for increasing the protein content of their biomass. The effect of various temperatures (20, 30, and 40 °C) at natural pH of each medium and 120 rpm of shaking incubator, pH values (3, 5, and 7), and nitrogen sources (urea, ammonium chloride, and ammonium sulphate at concentration of 0.2%) on the growth and SCP production of promising isolates in shaking incubator was investigated.

As revealed in Fig. 9, the significant optimum biomass and protein content produced by *A. niger* were 20.12 ± 0.32 g/L and 9.16 ± 0.33 g/L, respectively, at 30 °C and decreased to biomass of 10.36 ± 0.6 g/L and protein content of 4.13 ± 0.35 g/L at 40 °C. On the other hand, at natural medium pH (4.3), the biomass and protein content attained maximum yields of 20.67 ± 0.46 g/L and 9.13 ± 0.51 g/L, respectively, for 10 days at 30 °C. In the meanwhile, lower biomass and protein content (14.77 ± 0.63 g/L and 5.99 ± 0.72 g/L) were recorded at pH 7. The effect of different nitrogen sources was also studied on PWM with an initial natural pH of 4.3 after 10 days in a shaking incubator at 30 °C. The results indicated that the addition of 0.2% ammonium sulphate to PWM significantly increased the protein content from 9.3 ± 0.37 to 11.5 ± 0.23 g/L and the dried biomass from 20.6 ± 0.32 to 21.6 ± 0.65 g/L.

As depicted in Fig. 10, the maximum yield of dried biomass (7.06 ± 0.08 g/L) and protein content (3.23 ± 0.16 g/L) by *A. allahabadii* with SWM were attained when the fermentation temperature was 30 °C and the impact of initial pH on SCP production by *A. allahabadii* was investigated at various initial pH values (3, 5, and 7) as well as a control with SWM's natural pH of 5.9, which were incubated at 30 °C for 6 days. The results showed that the maximum yields for protein content (4.06 ± 0.04 g/L) and dried biomass (7.74 ± 0.15 g/L) were attained when the medium's initial pH value was 7 at which different nitrogen sources were tested and incubated in a shaking incubator at 30 °C for 6 days. The results showed that the protein content significantly increased from 4.09 ± 0.09 to 6.14 ± 0.48 and the dried biomass from 7.54 ± 0.30 to 11.11 ± 0.44 g/L when ammonium sulphate was added to SWM. The control without nitrogen supplement had the lowest biomass (7.54 ± 0.30 g/L) and protein content (4.09 ± 0.09 g/L).

As shown in Fig. 11, the dry biomass (18.5 ± 0.3 g/L) and protein content (9.14 ± 0.46) of *P. citrinum* on OWM were significantly high at an incubation temperature of 30 °C, whereas there is no yield at 40 °C. Different initial pH values (3, 5, and 7) were utilized to investigate the maximum pH level of OWM for optimum biomass and protein content values at 30 °C after 8 days. Results revealed that at pH 5, a significant increase in dry biomass (17.9 ± 0.6 g/L) and protein content (9.64 ± 0.24 g/L) was reported. On the other hand, a lower biomass (16.2 ± 0.32 g/L) and protein content (7.57 ± 0.2 g/L) were observed at pH 7. Furthermore, when different nitrogen sources (urea, ammonium chloride, and ammonium sulphate) were used at an initial pH 5 and at 30 °C for 8 days, data revealed that the ammonium sulphate was the best source of nitrogen for the production of the highest significant protein content (10.4 ± 0.31g/L). On the other hand, the medium's control had the lowest biomass and protein content (17.06 ± 0.79 and 8.06 ± 0.57 g/L, respectively).

As shown in Fig. 12, when the fermentation temperature of *P. crustosum* on BWM was raised from 20 to 40 °C, the protein content and yield of biomass significantly decreased. The *P. crustosum* had the significant highest biomass (16.24 ± 0.16 g/L) and protein content (7.55 ± 0.10 g/L) at 20 °C. As the temperature rose further to 30 °C, the yield of biomass and protein content decreased until they disappeared at 40 °C. As opposed to that, the impact of the medium's pH changes from its natural pH of 5.6 to 3, 5, and 7 on SCP production of *P. crustosum*. After 6 days of fermentation, dried biomass and protein content reached their significant maximum yields (17.04 ± 0.33 and 8.09 ± 0.38 g/L, respectively), when the initial pH value of the medium was established to 5. Additionally, when the three nitrogen sources were used at an initial pH of 5 and incubated in a shaking incubator at 20 °C for 6 days, the addition of 0.2% ammonium sulphate to BWM caused increase in protein content from 7.95 ± 0.18 to 9.41 ± 0.2 g/L and dried biomass from 16.78 ± 0.4 to 18.27 ± 0.37 g/L.

As observed in Fig. 13, the maximum yield of dried biomass (9.50 ± 0.52 g/L) and protein content (4.43 ± 0.44 g/L) by *P. chermesinum* grown on GWM were obtained when the fermentation temperature was maintained at 30 °C, whereas at lower temperatures, less biomass was observed. On the other hand, different initial pH values were studied during incubation at 30 °C for 10 days. The

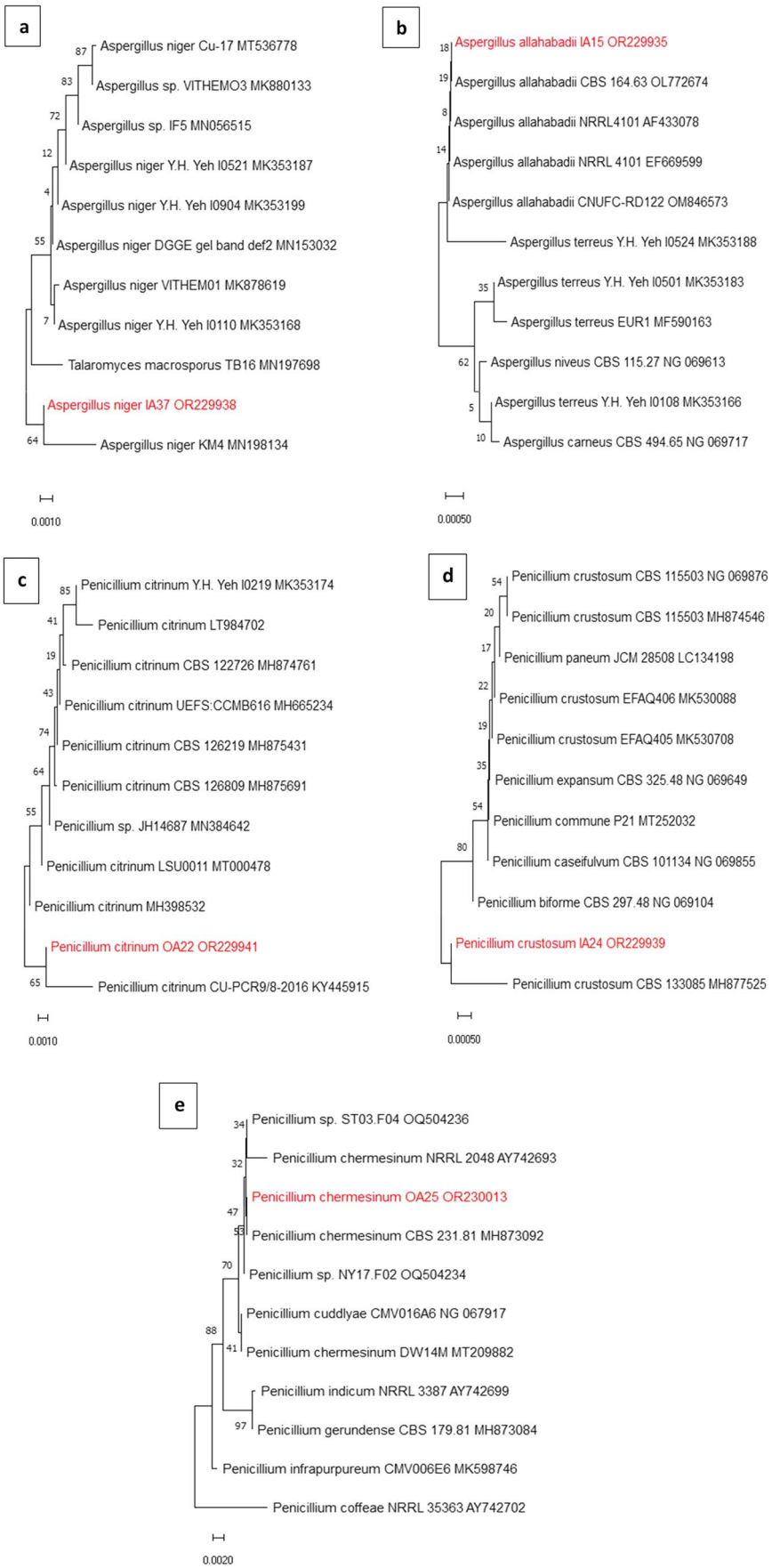

Fig. 8 Phylogenetic tree based on the neighboring-join analysis of D1/D2 region sequence database for five fungi (in red color). Bootstrap values (1000 replicates) were presented at each branch. **a** *Aspergillus niger*. **b** *Aspergillus allahabadii*. **c** *Penicillium citrinum*. **d** *Penicillium crustosum*. **e** *Penicillium chermesinum*

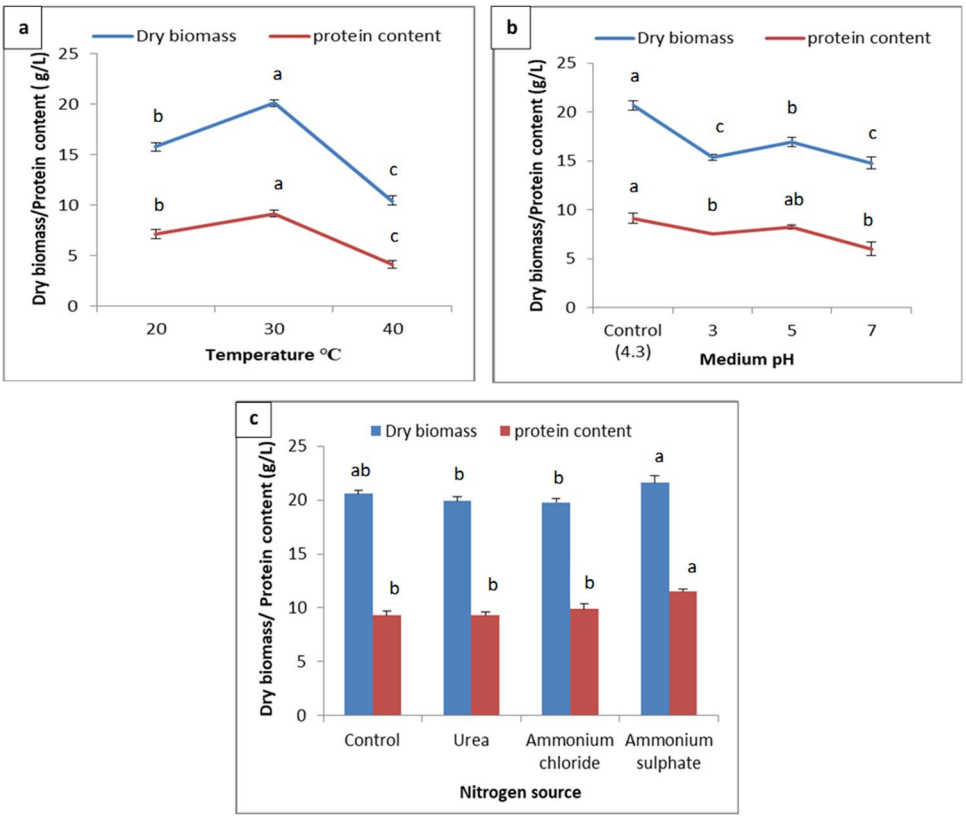

Fig. 9 Effect of various factors on dry biomass and protein content of *Aspergillus niger*. **a** Incubation temperature, **b** medium pH, and **c** nitrogen source. Data are expressed as means of 3 replicates ± SD. Different letters indicate significant difference at $p < 0.05$

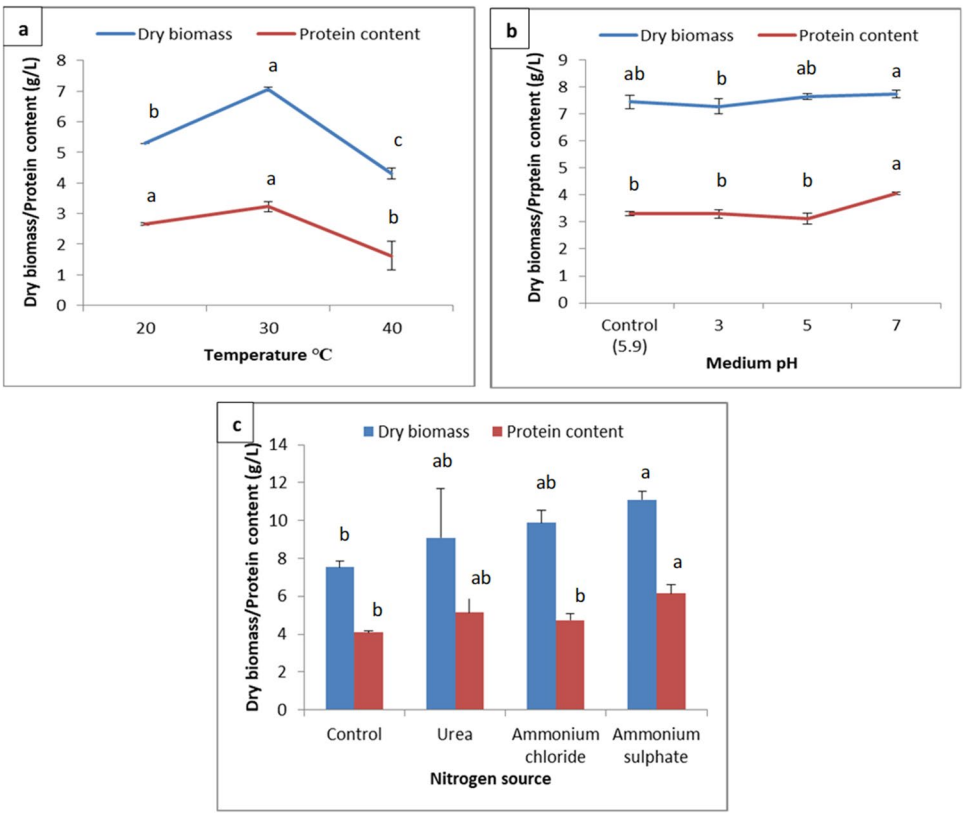

Fig. 10 Effect of various factors on dry biomass and protein content of *Aspergillus allahabadii*. **a** Incubation temperature, **b** medium pH, and **c** nitrogen source. Data are expressed as means of 3 replicates ± SD. Different letters indicate significant difference at $p < 0.05$

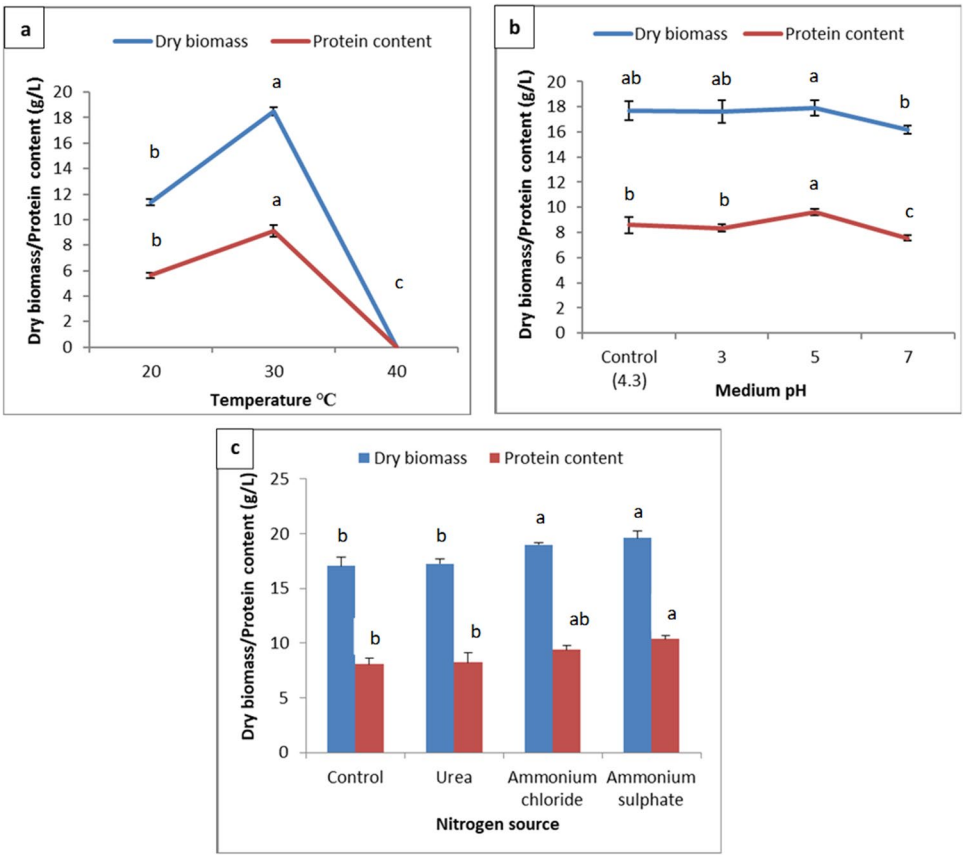

Fig. 11 Effect of various factors on dry biomass and protein content of *Penicillium citrinum*. **a** Incubation temperature, **b** medium pH, and **c** nitrogen source. Data are expressed as means of 3 replicates ± SD. Different letters indicate significant difference at $p < 0.05$

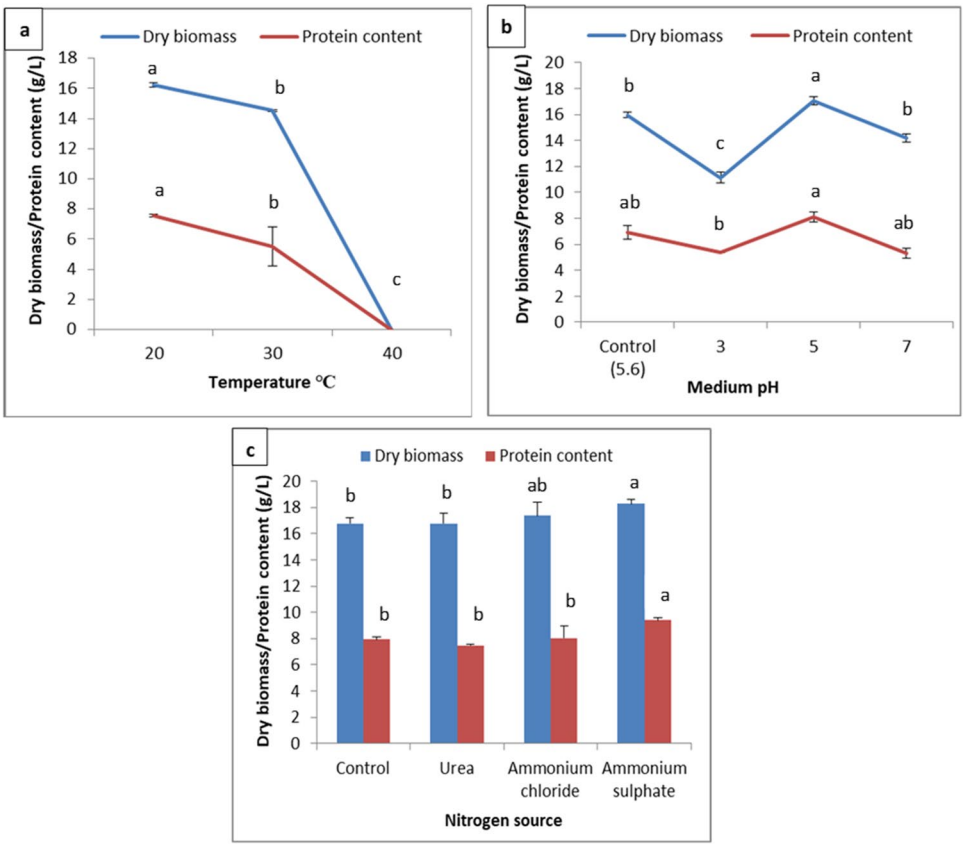

Fig. 12 Effect of various factors on dry biomass and protein content of *Penicillium crustosum*. **a** Incubation temperature, **b** medium pH, and **c** nitrogen source. Data are expressed as means of 3 replicates ± SD. Different letters indicate significant difference at $p < 0.05$

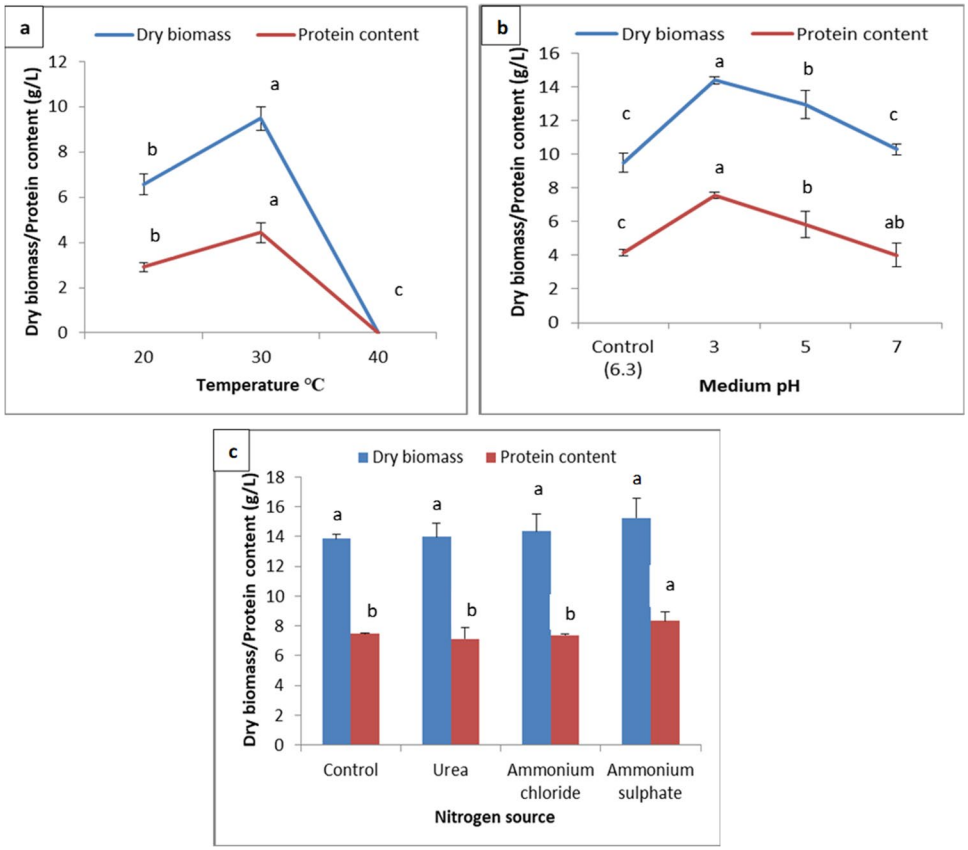

Fig. 13 Effect of various factors on dry biomass and protein content of *Penicillium chermesinum*. **a** Incubation temperature, **b** medium pH, and **c** nitrogen source. Data are expressed as means of 3 replicates ± SD. Different letters in the same column indicate significant difference at $p < 0.05$

protein content and biomass elevated significantly at pH 3, and then reduced with raising pH. At the initial pH of 3, the protein content and dried biomass attained maximum yields of 7.57 ± 0.19 and 14.38 ± 0.23 g/L, respectively. The impact of various sources of nitrogen on SCP synthesis by *P. chermesinum* grown on GWM was also investigated at an initial pH of 3 and incubated in a shaking incubator at 30 °C for 10 days. Results indicated that the protein content significantly increased from 7.46 ± 0.04 to 8.35 ± 0.59 g/L when ammonium sulphate was added to GWM. However, the addition of the other nitrogen sources had non-significant impact on protein content and dried biomass.

3.5 Proximate analysis of single cell protein of the promising fungi

The proximate composition of SCP was analyzed to characterize its potential as food ingredient (Table 2). The results indicated that SCP of *A. niger* gave the most significant amount of carbohydrates (20.81 ± 0.06%) followed by *P. crustosum* and *A. allahabadii* with a carbohydrates content of 17.79 ± 0.16 and 16.55 ± 0.11%, respectively. *A. allahabadii* contained the highest significant percentage of crude fats (10.73 ± 0.1%) compared to *A. niger* (6.72 ± 0.09%), *P. chermesinum* (3.93 ± 0.05%), *P. citrinum* (3.11 ± 0.06%), and *P. crustosum* (2.74 ± 0.08%). For

Table 2 Proximate composition of SCP of the promising fungi

Fungi producing SCP	Proximate composition (%)						
	Moisture	Ash	Crude fat	Carbohydrates	Crude fiber	Total phenols	Total flavonoids
Aspergillus niger	$8.26^{a} \pm 0.25$	$4.05^{e} \pm 0.05$	$6.72^{b} \pm 0.09$	$20.81^{a} \pm 0.06$	$9.45^{a} \pm 0.15$	$0.2^{bc} \pm 0.01$	$0.12^{b} \pm 0.035$
Aspergillus allahabadii	$6.01^{b} \pm 0.2$	$4.46^{d} \pm 0.15$	$10.73^{a} \pm 0.1$	$16.55^{c} \pm 0.11$	$6.23^{c} \pm 0.07$	$0.64^{a} \pm 0.04$	$0.55^{a} \pm 0.15$
Penicillium citrinum	$5.82^{b} \pm 0.2$	$6.46^{c} \pm 0.1$	$3.11^{d} \pm 0.06$	$14.1^{e} \pm 0.06$	$4.8^{e} \pm 0.18$	$0.23^{b} \pm 0.02$	$0.13^{b} \pm 0.02$
Penicillium crustosum	$6.06^{b} \pm 0.07$	$16.1^{a} \pm 0.2$	$2.74^{e} \pm 0.08$	$17.79^{b} \pm 0.16$	$7.1^{b} \pm 0.04$	$0.26^{b} \pm 0.031$	$0.14^{b} \pm 0.04$
Penicillium chermesinum	$1.91^{c} \pm 0.1$	$10.22^{b} \pm 0.16$	$3.93^{c} \pm 0.05$	$15.36^{d} \pm 0.07$	$5.44^{d} \pm 0.08$	$0.14^{c} \pm 0.035$	$0.09^{b} \pm 0.01$

Data are expressed as means of 3 replicates ± SD. Different letters in the same column indicate significant difference at $p < 0.05$

crude fiber content, *A. niger* gave the maximum significant amount of fiber (9.45 ± 0.15%) followed by *P. crustosum* (7.1 ± 0.04%), and *A. allahabadii* (6.23 ± 0.07%). The highest total phenols and flavonoids were obtained by *A. allahabadii* (0.64 ± 0.04% and 0.55 ± 0.15%) followed by *P. crustosum* (0.26 ± 0.031% and 0.14 ± 0.04%) and *P. citrinum* (0.23 ± 0.02% and 0.13 ± 0.02%), respectively. The highest significant ash content was observed in *P. crustosum* (16.1 ± 0.2%) followed by *P. chermesinum* (10.22 ± 0.16%) and *P. citrinum* (6.46 ± 0.1%). On the other hand, *A. niger*, *P. crustosum*, and *A. allahabadii* had higher moisture content (8.26 ± 0.25, 6.06 ± 0.07, and 6.01 ± 0.2%, respectively) compared to other tested fungi.

3.6 Amino acid profile of SCP

The amino acid profile of SCP of the five promising fungi showed high amount of essential amino acids (EAAs) (Table 3). Among EAAs, the highest amount of leucine was observed with *P. chermesinum* (1.56 g/100 g) followed by *P. crustosum* (1.35 g/100 g) and *P. citrinum* (1.24 g/100 g). Furthermore, *P. crustosum* gave the highest amount of threonine (1.32 g/100 g) followed by *P. chermesinum* and *P. citrinum* (1.2 and 1.08 g/100 g, respectively), while *P. chermesium* gave the highest amount of lysine (1.02 g/100 g). In addition to EAAs, other amino acids such as aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, and proline were recorded with high concentration by HPLC analysis (Table 3).

4 Discussion

In the present study, 35 fungal isolates belonging to 11 genera including *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Monodictys*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Stachybotrys*, and 2 unknowns were isolated from various sources, the results which confirmed by [39, 40].

Filamentous fungi, which are strong and effective factories of cells for producing protein at an industrial level, generated more than half of the commercially available proteins [41, 42]. In the present work, 35 fungal isolates were tested for their capacity to grow on agriculture waste as fermentation media and produce single cell protein. The most promising fungal isolates were identified depending on their molecular and morphological features as *A. niger*, *A. allahabadii*, *P. citrinum*, *P. crustosum,* and *P. chermesinum*, respectively. In the current study, primers targeting the rDNA large subunit D1/D2 regions were used for molecular identification of fungi due to their specificity and sufficient length compared to ITS regions [43].

It is appeared that *A. niger*, *A. allahabadii, P. citrinum*, *P. crustosum*, and *P. chermesinum* can be used as good sources for SCP production with a high protein content on PWM, SWM, OWM, BWM, and GWM, respectively. Numerous researches have revealed that some *Aspergillus* and *Penicillium* species have a high protein content and serve as excellent sources for the creation of SCP [36, 44–48].

It is well known that the incubation temperature is important factor influencing growth of cell and protein synthesis.

Table 3 Amino acid composition of SCP of the promising fungi

Amino acid (g/100 g)	Fungi				
	Aspergillus niger	*Aspergillus allahabadii*	*Penicillium citrinum*	*Penicillium crustosum*	*Penicillium chermesinum*
Aspartic acid	1.07	1.18	1.90	1.91	2.01
Glutamic acid	1.72	1.83	3.29	3.62	2.75
Serine	0.73	0.74	1.22	1.15	1.24
Histidine*	0.27	0.33	0.38	0.59	0.62
Glycine	0.61	0.68	1.06	1.09	1.31
Threonine*	0.64	0.78	1.08	1.32	1.20
Alanine	3.40	3.26	4.74	2.57	4.17
Tyrosine	0.45	0.38	0.65	0.83	0.77
Valine*	0.54	0.54	1.01	0.84	0.92
Methionine*	0.17	0.15	0.39	0.25	0.60
Phenylalanine*	0.48	0.48	0.78	1.00	0.92
Isoleucine*	0.45	0.45	0.84	0.71	0.79
Leucine*	0.82	0.82	1.24	1.35	1.56
Lysine*	0.60	0.74	0.89	0.96	1.02
Proline	0.16	0.25	0.56	0.27	0.15

*Essential amino acids

The highest yield of biomass and protein content of *A. niger, P. citrinum*, *P. chermesinum*, and *A. allahabadii* was recorded when the incubation temperature reached 30 ℃ on PWM, OWM, GWM, and SWM, respectively. Our findings agreed with many researches [45, 49–52]. These come from the fact that room temperature is the most prevalent temperature for microorganisms [19]. However, the microorganism's activity was hampered by the rise in temperature to 40 °C, which had a negative effect on cell activity because of the partial inactivation of metabolic pathway enzymes and also decreased the level of moisture of the substrate [53]. As opposed to that, *P. crustosum* gave the highest biomass and protein content on BWM at 20 °C which may be due to the maximum growth of some *Penicillium* spp. at this temperature in vitro [54].

The medium's initial pH value is known to have a significant impact on the synthesis of SCP. Our results revealed that the greatest biomass production and protein content from *A. niger*, *P. citrinum*, and *P. crustosum* were recorded at pH of 4.3, 5.0 and 5.0 on PWM, OWM, and BWM, respectively. The pH of the fermentation processes used to produce SCP typically ranges from 4.5 to 5.5 due to the fact that yeasts and filamentous fungi are frequently acidophiles [46, 49, 55, 56]. In contrast, *A. allahabadii* gave the highest protein content at pH 7.0, the result which is in harmony that of many researches [57, 58]. However, at an initial pH of 3.0, *P. chermesinum* produced the most amount of protein on GWM, the result which agreed with work of [59].

The fungi need an inorganic nitrogen source to grow because they are heterotrophic. According to the data above, the highest biomass and protein content of *A. niger*, *A. allhabadii, P. citrinum*, *P. crustosum*, and *P. chermesinum* were observed by adding ammonium sulphate to their best fermentation medium compared to ammonium chloride and urea. These findings are in line with that of [52, 57], it might be because ammonium sulphate contains more growth factors than other sources of nitrogen [60].

The proximate composition of fungal SCP was analyzed to characterize its potential as a food ingredient. It indicated that *A. niger* SCP is a wealthy source of carbohydrate and crude fiber content. Carbohydrates are important in dietary foods because they can enhance intestinal peristalsis and help with the therapy of several illnesses and ailments, like diabetes, hypertension, and obesity [61, 62]. The present results of carbohydrates were confirmed by that of [63]. Dietary fiber has been ranked as the seventh most important nutrient following protein, fat, saccharides, cellulose, minerals, and water [64]. Fiber is also called the "gut scavenger." Hence, consuming more fungi that are high in fiber can aid in the prevention of a number of illnesses [65]. On the other hand, the SCP of *P. crustosum* has the highest ash content and *A. allahabadii* SCP is rich in fat content the results which confirmed by [48]. Ash content is a good indicator of the amount of minerals in edible fungi that help the body maintain its normal physiological function [66]. Additionally, fats are a necessary component of life and have a critical role in diets by facilitating the absorption of fat-soluble vitamins [44]. Furthermore, our results of total phenols and flavonoids in fungal SCP are close to those priory recorded by [67].

The nutritious value of SCP is based on its composition and should be analyzed for amino acid composition before used as food and feed supplementation. Results indicated that the five SCP gave high amount of the essential amino acid leucine and *P. citrinum* gave the highest amount of valine. On the other hand, *P. chermesinum* gave the highest amount of lysine while *P. crustosum* contain the highest amount of threonine. These results are in parallel with those recorded by [36, 68, 69]**.**

To our knowledge, this is the first report indicated that *A. allahabadii* and *P. chermesinum* as SCP producers. However, they were isolated from available sources (air, soil, and water) and yielded the highest protein content from cheap sources.

5 Conclusions

This study proved that pineapple, sugarcane bagasse, orange, banana, and garlic wastes are good substrates for *A. niger*, *A. allahabadii*, *P. citrinum*, *P. crustosum*, and *P. chermesinum* to produce valuable SCP, respectively. Additionally, the fungal biomass can be enhanced by adding ammonium sulphate to the food waste medium and adjusting temperature and pH for optimal yield. Utilizing these food wastes for SCP production can end the protein deficiency globally and help in waste management. Keeping in view the nutritional composition and amino acid analysis, SCP can be utilized as a food and feed supplement. The future of SCP production is related to decreasing the cost of production, increasing the quality and quantity of SCP through the fermentation process, also using genetically modified species of fungi to be applied and scaling up for human food and animal feed.

Author contribution Conceptualization and conceived of the presented idea: N.M.H.; methodology: N.M.H., M.G.A., and S.A.G.; writing—original draft preparation: N.M.H., S.A.G., and M.G.A.; writing—review and editing: N.M.H., S.A.G., and M.G.A.; discussed the results and contributed to the final manuscript: N.M.H., S.A.G., and M.G.A.; supervision: N.M.H. and S.A.Z., and All authors revised the final manuscript.

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Data availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This work has been approved by the Faculty of Science at Ain Shams University in Cairo, Egypt (Approval Code: ASU-SCI/MICR/2023/5/2).

Competing interests The authors declare no competing interests.

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