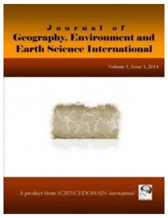

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Protein Enrichment of Agro–Industrial Waste by *Trichoderma harzianum* EMCC 540 through Solid State Fermentation for Use as Animal Feed

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Authors' contributions

This work was carried out in collaboration between all authors. All authors participated equally in the study idea, literature review, data collection and analyses, methodology, statistical analyses, tabulating the data, results validation, writing and revising the whole manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The disposal of agro-industrial wastes is a serious problem and their deposition poses health hazard for all the living beings. Agro-industrial residues constitute a major proportion (almost 30%) of worldwide agricultural production. SSF methods are widely used for the protein enrichment of agro–industrial waste. The main aim of the present study is to use the *Trichoderma harzianum* with five different types from agro-industrial wastes (peels of mango, orange, apple, banana and tomato)

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for the enrichment of protein for wastes by SSF to use as animal feed. Fermentation temperature is one of the most important factors in fermentation process. The agro-industrial waste samples were incubated at different temperatures to determine the ideal fermentation temperature. From results the optimum fermentation temperature used to obtained the maximum crude protein by using *Trichoderma harzianum* was 28°C. Crude protein content in fermented substrates increased from (23.35, 21.88, 24.13,16.19 and 9.5) g% to (84.46, 33.37, 28.60, 21.65 and 17.60) g% for peels of tomato, mango, orange, apple and banana respectively. Tomato peels is a good substrate for protein enrichment followed by mango peel, orange peel, apple peel and banana peel respectively.

Keywords: Solid state fermentation; nutrient enrichment; agro–industrial waste; Trichoderma harzianum; animal feed.

1. INTRODUCTION

Agricultural and food industry residues refuse and wastes constitute a significant proportion (estimated to amount to over 30%) of worldwide agricultural productivity [1]. These wastes include lignocellulose materials, fruit and vegetable wastes, sugar industry wastes [2]. They represent valuable biomass and potential solutions to problems of animal nutrition and world wide supply of protein and calories if appropriate technologies can be deployed for their valorization by protein enrichment [3]. Agro-industrial waste in Egypt, are major contributors to the economy; however, they could constitute a severe source of pollution for the environment due to the huge amount of agro-industrial waste generated. Management of agro-industrial biowaste is often practiced without proper planning and with potential high environmental and socio-economic risk. In recent times, researchers in food industries are making a great effort to reduce the amount of waste and find alternative uses by different treatment methods for agro-industrial wastes to reduce their quantity and their environmental impact [4]. Solid state fermentation is one of the most important technologies used to protein enrichment of agro-industrial waste and used as animal feed [5].

The safety concerns throughout production of protein must be addressed by proper selection of microorganisms to be those classified as GRAS (generally regarded as safe), appropriate suitable substrate selection to deliver products that are safe not just for animal feed [6].

Solid state fermentation (SSF) can be defined as a process, in which solid substrates are decomposed by known pure or mixed cultures of microorganisms (mainly fungi, which can grow on and through the substrate) under controlled conditions, with the aim of producing a high quality standardized product [7]. The substrate, a mixture of different particles, is characterized by a relatively low water content [8,9]. Solid state fermentation involves the growth of microorganisms on moist solid particles [10]. The protein produced by SSF generally use substrates that are byproducts from the agro-food industry, as for example peels of mango, banana, apple and citrus fruit bagasse [11].

Fermentation temperature is one of the most important factors in fermentation process [12]. The incubation temperature of the fermentation medium must be very well defined and optimized depending on the microorganism, substrate and production technique and initial pH value [13]. It is known that optimum temperatures for growth of cells and product formation may be different in some fermentation processes [14].

The filamentous fungi *Trichoderma* is an important fungus used to produce enzymes and protein by fermentation process [15]. Fungal genera like *Trichoderma* and *Aspergillus* are thought to be the good cellulase producers. Crude enzymes produced by these microorganisms are commercially available for agricultural and industrial uses [16]. *Trichoderma harzianum* is a filamentous fungus and is important in biotechnological point of view [17]. The major goal of this research was enrichment of protein for agro-industrial wastes by *Trichoderma harzianum* under solid state fermentation process in relation to effect of incubation temperature.

2. MATERIALS AND METHODS

2.1 Mold Culture

The Filamentous fungus used to ferment agro-industrial wastes was Trichoderma harzianum EMCC 540 obtained from the Egypt Microbial Culture Collection at Ain Shams University. Mold culture was grown on Potato Dextrose Agar

(PDA) containing 15.0 g/L starch, 20.0 g/L D-glucose, and 18.0 g/L agar according to the method described by Benko et al. [18] and Eddleman [19].

2.2 Substrate Preparation

Five types of agro-industrial wastes were used as a substrate. Peels of mango, orange, apple, banana and tomato were collected from agro-industrial factories in Egypt. All agro-industrial wastes were dried under sunlight till constant weight. Dried agro-industrial wastes were then ground and passed through sieves to obtain the desired particle size of 1.7–2 mm [20]. The samples were packed in polystyrene bags, and stored at room temperature for further studies.

2.3 Inoculum Preparation

The inoculum of *Trichoderma reesei* was prepared by the method described by [21] suspension of spores were prepared by the addition of 10 ml from sterile distilled water to slant and scratching the surface fungal mat with sterilized loop. The cultures were incubated at 28°C to seven days old. Fungal spore were filtered through sterilized glass wool. One ml of such suspension contains 1 x 10^7 spores per ml, used to inoculate 100 ml of medium. All isolated cultures were maintained on slants of Czepek's Dox Agar and routinely subculture whenever required. The suspended inocula were kept in chiller at 4°C for further use.

2.4 Fermentation Technique

SSF was conducted according to the method of [22] with some modifications. Substrate weight of 50 gm for was added to 250 ml Erlenmeyer flasks moistened with 50 ml of sterile liquid nutrient medium. with 10% glucose containing: [KH_2PO_4 – 1 g, NaCl- 1 g, Yeast extract – 5 g, Peptone – 1 g] [23]. Initial pH of the medium was adjusted to 5 using 1N-HCL and 1N-NaOH [24]. Triplicate sets of flasks were autoclaved at 121°C at 15 psi for 15 min. Each flask was then inoculated with 10 ml of the fungal culture using *Trichoderma harzianum* (10^7 spores / ml). Fermentation was carried out at pH5 under static condition by using different incubation temperatures (25, 28, 30, 35 and 45)°C for 7 days. All experiments were carried-out in triplicate and the results were presented as mean of the triplicate experiments. Mineral and biochemical parameters were measured to determine optimum temperature to produce high percentage of protein with suitable fungus inoculum.

2.5 Proximate Analysis

The waste samples were analyzed for crude protein, crude fiber, ash, free amino acids, total carbohydrate, nitrogen, phosphorus and potassium according to the method of [25].

3. RESULTS AND DISCUSSION

3.1 Enrichment the Nutritional Value of the Wastes by *Trichoderma harzianum* at Different Incubation Temperatures

The incubation temperature of fermentation process is known as one of the most critical parameter for protein enrichment by fungus strains. The effect of incubation Temperature reported in various researches study by using different fungus strain with a lot of agro-industrial wastes under different technical conditions. The main observation in all results obtained in our study the optimal temperature is 28±2°C with optimum pH5 with *Trichoderma harzianum* [26]. At lower temperatures, the decrease in protein content may be due to inactivation of cellular activities while at higher temperatures the enzymes of the cell may be denatured and Incubation at higher temperature affected the fungus harmfully [27,28]. In the next, the effect of change of temperature to improve the characteristics of fermented substrates using solid state fermentation process to optimize the incubation had been discussed temperature.

3.1.1 Mango (*Mangifera indica)*

The results represented in Fig. 1 show the mineral composition of mango peels waste under different incubation temperatures by *Trichoderma harzianum* using solid state fermentation. The results showed that the percentage of ash, oxygen, nitrogen, phosphorous and potassium reached to maximum at 28°C.

The influence of incubation temperature on biochemical compositions of mango peel show in Fig. 2. Maximum crude protein and free amino acids production was obtained at 28°C. The percentage of total carbohydrates and crude fibers were decreased to a minimum value under 28°C and 25°C respectively. *Trichoderma harzianum* more active to produce protein with mango peel waste at 28±2°C to increase of crude protein content and free amino acids after incubation period, low value of total carbohydrate and crude fibers indicating that it's used to feed the fungus. The results are quite similar to the finding by Garg and Ashfaque [29]. They are carried out SSF of mango peel waste and found that the optimum incubation temperature is 25°C. In the same context, Purnachandra and Saritha [30] reported that the temperature plays an important role on the growth of fungal species, under the optimal temperature the growth rate of fungi was increased, with the increase of grow rate of fungi the protein content was also increased. Mahnaaz et al. [31] reported the maximum content of crude protein in mango peel under SSF recorded at optimum temperature 27 ±2°C.

3.1.2 Apple (*Malus sp*)

The effect of different incubation temperatures by *Trichoderma harzianum*, on mineral composition of apple peel samples are represented in Fig. 3. The results showed that the percentage of ash, oxygen, nitrogen, phosphorous and potassium reached to peak value at 28°C.

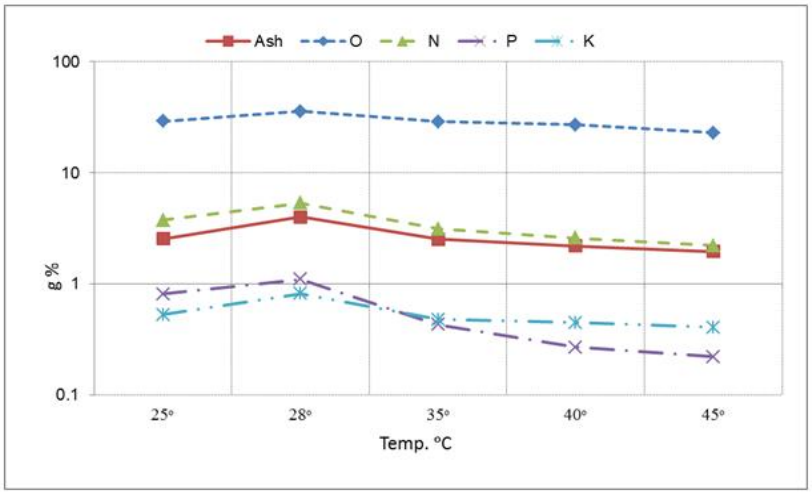

Fig. 1. Mineral Composition of Mango peels at different incubation temperatures by *Trichoderma harzianum*

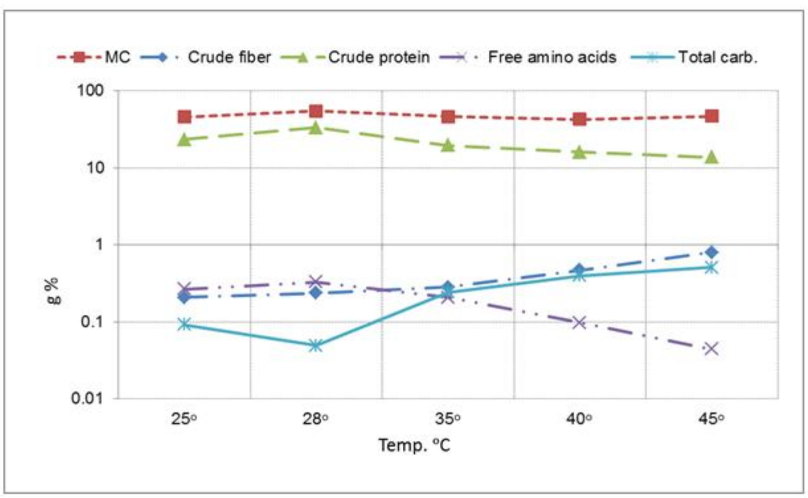

Fig. 2. Biochemical composition of mango peels at different incubation temperatures by *Trichoderma harzianum*

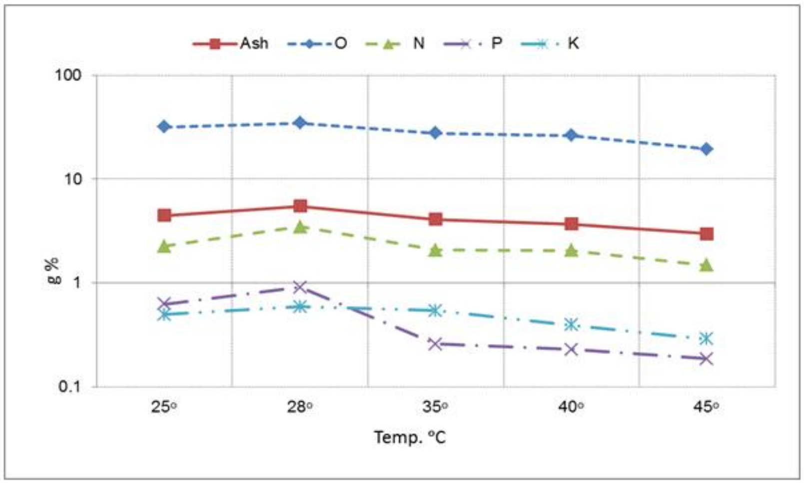

Fig. 3. Mineral composition of apple peels at different incubation temperatures by *Trichoderma harzianum*

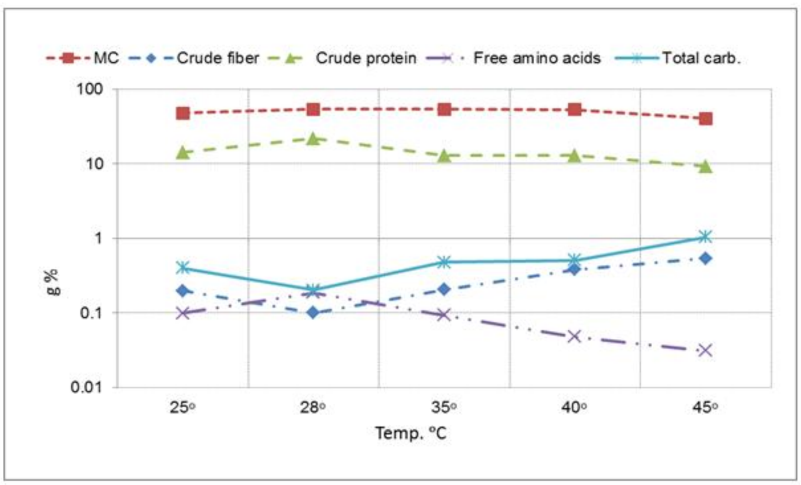

Fig. 4. Biochemical composition of apple peels at different incubation temperatures by *Trichoderma harzianum*

The results represented in Fig. 4 show the effect of temperature values on the percentage of biochemical composition in apple peel under SSF by *Trichoderma harzianum*. Crude protein and free amino acids reached maximum at 28°C. Decrease concentration of total carbohydrate and crude fibers acting due to produce high percentage of protein at 28°C. From the results, 28°C is an optimum value for apple peel enrichment by *Trichoderma harzianum*. This is in accordance with the results obtained by Mahnaaz et al. [31]. They used apple peel and other fruit wastes to production of protein by SSF. Apple peels reach to higher amount of crude protein at 27 ±2°C. Haiyan et al. [32] reported low significant difference in optimum incubation temperature; the researcher recoded 32°C is an optimum incubation temperature for apple waste for maximum protein content under SSF. Gurpreet et al. [20] used apple waste with *Aspergillus niger* NRRL 2001 through SSF, they concluded that 30±1°C is an optimum temperature for fermentation process.

3.1.3 Banana (*Musa sp)*

The effect of different incubation temperatures by *Trichoderma harzianum*, on mineral composition of banana peels samples are represented in Fig. 5. The results showed that the percentage of ash, oxygen, nitrogen, phosphorous and potassium reached to maximum values at 28°C.

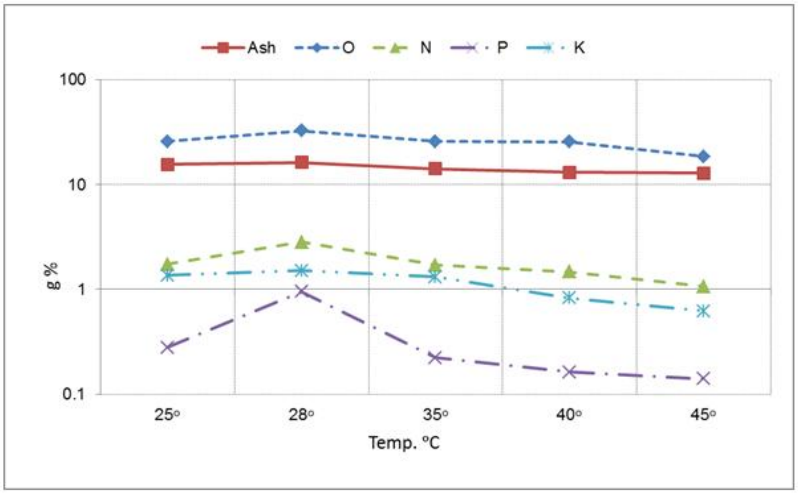

Fig. 5. Mineral composition of banana peels at different incubation temperatures by *Trichoderma harzianum*

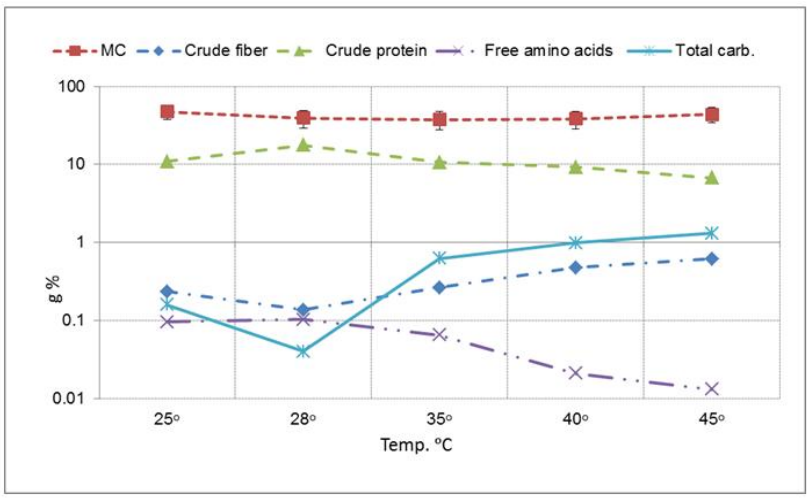

Fig. 6. Biochemical composition of banana peels at different incubation temperatures by *Trichoderma harzianum*

The influence of incubation temperature on biochemical compositions of banana peels show in Fig. 6. Maximum crude protein production and free amino acids was obtained at 28°C. At 28°C the percentage of total carbohydrates and crude fibers were decreased to a minimum value when crude protein reach to maximum value. So with banana peel waste 28°C its optimum condition to produce higher value of crude protein with *Trichoderma harzianum*. This is in accordance with the results obtained by Anbu et al. [33] they stated that enrichment of crude protein for banana by SSF under optimum incubation temperature 28±2ºC. Essien et al. [34] utilized banana peel as a substrate under SSF. They are reported that an increase of crude protein at optimum incubation temperature 28°C. Baig et al. [35] reported significant difference in optimum incubation temperature, they are recorded 40°C is an optimum temperature for banana waste substrate with *Trichoderma lignorum* by using SSF. The obtained results are quite similar to the finding by Amande and Itah [36], they are carried out SSF of banana peel using *Saccharomyces cerevisiae* and found that The increase in crude protein and a corresponding reduction in the carbohydrate content at 25°C.

3.1.4 Orange (*Citrus reticulata)*

The results represented in Fig. 7 show the effect of incubation temperatures on the percentage of mineral composition in orange peel under SSF by *Trichoderma harzianum*. The results showed that the percentage of ash recorded maximum value at 35°C, while the percentage of oxygen, nitrogen, phosphorous and potassium reached to peak at 25°C.

On the other hand the effect of different incubation temperature on the biochemical composition of orange peel samples under SSF by *Trichoderma harzianum* is represented in Fig. 8. The results show that, increase percentage of crude protein and free amino acids with decrease of crude fibers and total carbohydrate at 25°C. So the optimum incubation temperature for orange peel wastes. This accordance the results were obtained by Pranita and Anita [37]. They carried out SSF of orange peel and found that maximum value of crude protein after fermentation period recorded at 28°C. Mahnaaz et al. [31] reported the use of orange peel waste as a potential substrate with SSF for the production of protein at optimum temperature 27 ±2°C. Halina et al. [38] recorded a significant difference in optimum incubation temperature when orange peel was used as a substrate for SSF with *Penicillium chrysogenum*. They found that 37°C is the optimal temperature to achieve ideal results. In another study the results obtained by Amit et al., [39] reported that 28°C is an optimum incubation temperature for fermentation of orange peel by SSF for maximum crude protein production.

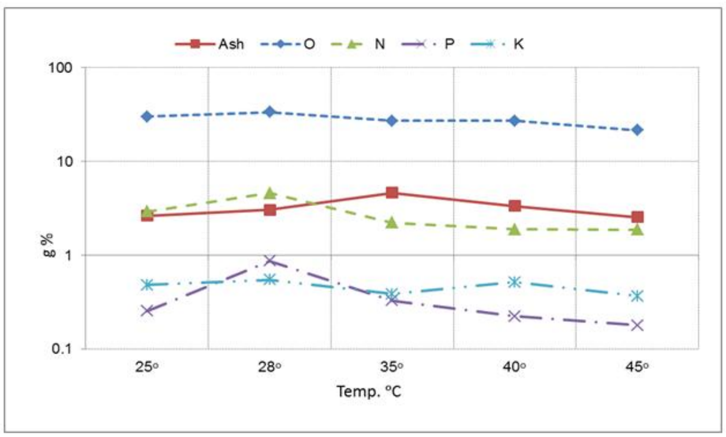

Fig. 7. Mineral composition of orange peels at different incubation temperatures by *Trichoderma harzianum*

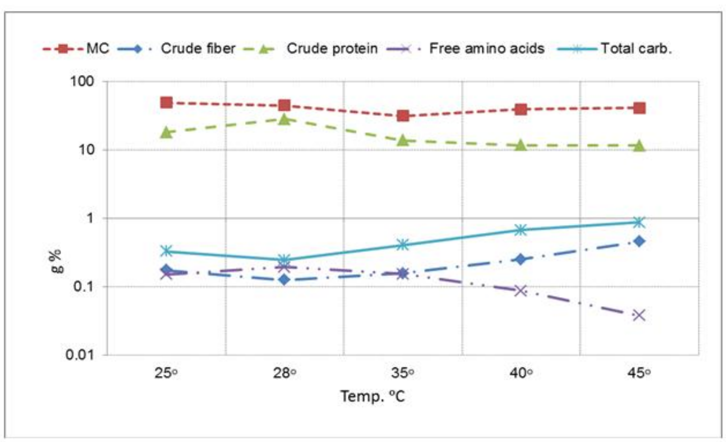

Fig. 8. Biochemical composition of orange peel at different incubation temperatures by *Trichoderma harzianum*

3.1.5 Tomato (*lycopersicon esculentum*)

The effect of different incubation temperatures by *Trichoderma harzianum*, on mineral composition of tomato peels samples are represented in Fig. 9. The results showed that the percentage of ash, oxygen, nitrogen and phosphorous reached to maximum at 28°C, While the percentage of potassium reached to peek at 25°C.

The results represented in Fig. 10 show the effect of temperature on the percentage of biochemical composition in tomato peels under SSF by *Trichoderma harzianum*. Crude protein and free amino acids reached maximum at 28°C. Minimum value of total carbohydrate and crude fibers act when crude protein increased at 28°C. From the results 28°C is an optimum incubation temperature for fermentation of tomato peels to production maximum amount of crude protein by *Trichoderma harzianum*. This is in accordance with the results obtained by Hayet et al. [40]. They used tomato waste as a substrate by SSF; the protein value recorded a maximum value at 30±4°C. Roja et al. [41] found a low difference when they used tomato waste to enhance protein content to use as animal feed. The protein content in tomato waste reached maximum at 37°C which was in agreement with the results obtained by Maria et al. [42]. They mentioned that improve of crude protein in tomato waste using SSF at incubation temperature of 25°C.

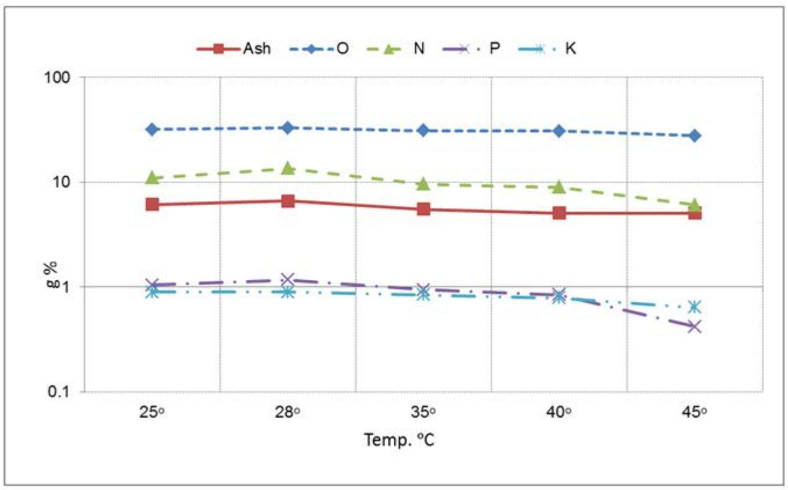

Fig. 9. Mineral composition of tomato peels at different incubation temperatures by *Trichoderma harzianum*

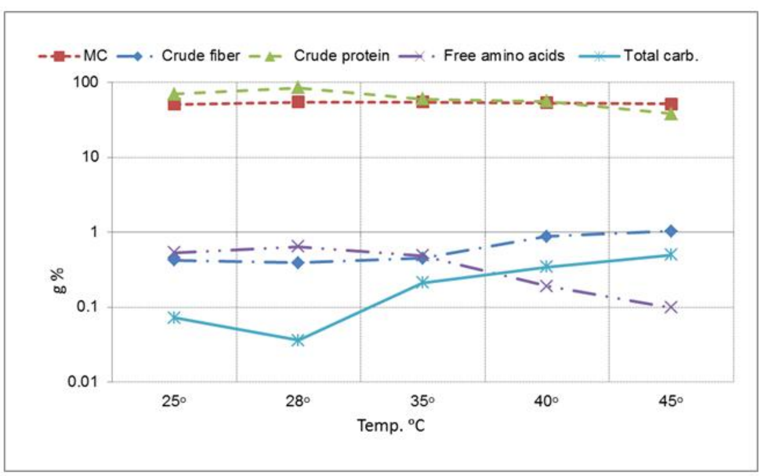

Fig. 10. Biochemical composition of tomato peels at different incubation temperatures by *Trichoderma harzianum*

Table 1. Composition of various agro-industrial wastes samples at optimum incubation temperature 28°C with *Trichoderma harzianum*

Waste	Parameter	Ash (g %)	O (g %)	N (g %)	P (g %)	K (g %)	MC %	Crude fibers (g %)	Crude protein (g %)	Free amino acids (g %)	Total carbohydrate (g %)
Mango	Treated	4.00	35.76	5.34	1.10	0.81	54.81	0.24	33.38	0.47	0.05
	control	4.08	8.33	3.5	0.24	0.86	75.92	16.37	21.88	0.09	6.85
Apple	Treated	5.50	34.48	3.46	0.91	0.59	53.77	0.10	21.65	0.60	0.20
	control	2.86	6.38	2.59	0.21	0.76	12.66	13.77	16.19	0.57	9.21
Banana	Treated	16.23	32.55	2.82	0.95	1.51	39.09	0.14	17.60	0.32	0.04
	control	8.93	11.42	1.52	0.32	6.58	11.50	14.93	9.5	0.057	5.12
Orange	Treated	3.03	33.47	4.58	0.87	0.55	44.84	0.13	28.60	0.36	0.25
	control	4.29	8.69	3.86	0.21	0.91	12.09	14.69	24.13	0.19	7.28
Tomato	Treated	6.60	32.79	13.51	1.17	0.90	54.20	0.39	84.46	2.55	0.04
	control	5.05	13.88	3.74	1.02	1.04	6.66	42.22	23.35	0.09	3.77

3.2 Ideal Agro-Industrial Wastes Used for Protein Enrichment by *Trichoderma harzianum* under SSF at Optimum Incubation Temperature

Table 1 represented the mineral and biochemical composition of five tested agro-industrial wastes at optimum fermentation conditions by *Trichoderma harzianum* under SSF. The results showed that the composition of different waste samples was significantly different under the tested parameters. Percentage of crude protein was 84.46, 33.37, 28.60, 21.65 and 17.60 for peels of tomato, mango, orange, apple and banana respectively. By comparing the results the variation in nutritional value among control and treated wastes was due to the difference in the mineral and biochemical compositions of each waste. The increase of crude protein in the wastes by SSF is very important in the enrichment of wastes as a source for growth. In the other hand the results also showed increase in free amino acids and decrease of total carbohydrate and crude fibers at the same time. Properties of treated wastes specially crude protein and free amino acids were increased after fermentation with *Trichoderma harzianum*. The results also showed that tomato is the most important one in enrichment of wastes with protein. In this regard, AboSiada et al. [43] stated that increase of crude protein and free amino acids among peels of tomato, mango, orange, apple and banana during fermentation at 28°C and conclude that tomato peel was a good substrate for protein enrichment. The results agree with Osama et al. [44] who used *Trichoderma harzianum* as strain with different agro-industrial wastes under SSF to use as animal feed. The researchers found that as increase of crude protein in the fermented substrate was due to *Trichoderma harzianum*. Sonika et al. [45] used eight *Tricoderma* species with agro-industrial wastes for SSF to reach to optimum condition and ideal fungal species was *Trichoderma harzianum*. With optimum temperature between 30 and 40°C

4. CONCLUSION

The present study explores an enrichment of protein in five different types of agro-industrial waste peels of orange, banana, mango, apple and tomato using SSF by *Trichoderma harzianum*. It is concluded that all the wastes used in this study could be used as a substrate for the growth of food for *Trichoderma harzianum* to use as animal feed. Optimum fermentation temperature used for maximum crude protein from *Trichoderma harzianum* was 28°C. From the results, it could be deduced that tomato peel is a good substrate for protein enrichment followed by mango peel, orange peel, apple peel and banana peel respectively. It is recommended to use the obtained results for the enrichment of protein in agro-industrial wastes by *Trichoderma harzianum* on a large scale at the factories site to maximize its economic return from this important technology and can be widely applied to other fungal fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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