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Nutrient Enrichment of Agro–Industrial Waste Using Solid State Fermentation

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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

Agro-industrial wastes are generated during the industrial processing of agricultural crops. Agro-industrial wastes are estimated to over 30% of worldwide agricultural productivity every year. The main aim of this study is improve of protein content and nutritional value of agro-industrial wastes by solid state fermentation to use as animal fodder. Fungal strain *Trichoderma reesei* were used with

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five different substrates from agro-industrial wastes, namely; peels of mango, orange, apple, banana and tomato. pH5 is the optimum condition for protein enrichment in different waste types. Crude protein content in fermented substrates with *Trichoderma reesei* increased from 23.35%, 21.88%, 24.13%, 16.19%, 9.5% to 78.17%, 30.05%, 28.84%, 19.82% and 14.06% for peels of tomato, mango, orange, apple and banana respectively. Tomato peel had the highest value of crude protein, so could be a good substrate for production of crude protein by *Trichoderma reesei.*

Keywords: Nutrient enrichment; Agro–industrial waste; solid state fermentation; Trichoderma reesei.

1. INTRODUCTION

Worldwide food, agricultural and forestry industries produce annually large volumes of wastes, which cause a serious disposal problem [1]. Egypt is not far from this as Agro-industries are major contributors to the Egyptian economy. Agricultural and food industry residues constitute over 30% of worldwide agricultural productivity [2]. Developing and deploying appropriate technologies for the reprocessing and reuse of these abundant energy rich resources in human or animal feeding will go a long way in reducing the pressure on agricultural productivity [3]. Technologies available for protein enrichment of agricultural wastes include solid substrate fermentation, ensiling and high solid or slurry processes [4]. Recently, academic and industrial researchers are putting more and more efforts to reduce the amount of these wastes by finding alternative uses [5]. Due to the composition rich in sugars, these wastes are easily assimilated by microorganisms; and could be appropriate for use as raw materials in the production of industrially relevant compounds under solid-state fermentation (SSF) conditions [1]. In fact, SSF has emerged as an appropriate technology for the management of agro industrial residues and for their value addition [6]. Wastes have come to be seen more as resources in the wrong location and form than as a problem to be safely disposed of. Aside from their capacity to cause pollution, most food processing wastes are potentially of good enough quality to be recycled [7].

In particular such technologies need to deliver products that are safe not just for animal feed use but also from the perspective of human feeding. The use of organisms that are generally recognized as safe (GRAS) for the protein enrichment and reprocessing of waste will enhance user confidence [8].

SSF is generally defined as the growth of the micro-organisms on (moist) solid material in the absence or near absence of free water [9]. SSF methods are widely used for the industrial production of microbial enzymes and metabolites [10]. SSF processes performance can be varied and controlled by changing physical and chemical factors [11]. SSF has been utilized for the conversion of cellulose to ethanol by yeast [12]. *Trichoderma* spp is an important fungus used to produce enzymes by fermentation process [13]. This genus secretes large amounts of cellulose and hemicellulose enzymes capable of degrading carbohydrate polymer [14].

Growing international production of fruits has led to increasing accumulation of fruit wastes such as citrus pulp, seeds, peels, grape pomace [15] and apple pomace among others [16]. Fruit wastes have only minimal protein content which limits their value in animal nutrition [17]. Thus, exploitation of these wastes in animal nutrition will depend on the deployment of processes for their protein enrichment by biotechnological means [18]. Ahmed S. et al. [19] used rice polishing as medium cultivated with *Trichoderma harzianum*, Maximum fungal biomass production was obtained at pH4 and 28°C. Alemu T. [20] improved the protein content of sweet orange and orange pulp wastes was cultivated with *Aspergillus niger*, resulting in increase in protein content to 50.84% at pH7 with sweet orange substrate. Eyini M. et al. [21] fermented cashewnut bran with *Trichoderma viride* using SSF, the protein content increase from 40 to 78 g/kg after 15 days. The main aim of this study is using solid state fermentation process to nutrient enrichment of some selected agro-industrial wastes by using *Trichoderma reesei EMCC 532*.

2. MATERIALS AND METHODS

2.1 Substrate Preparation

Peels of orange, banana, mango, apple and tomato were collected from different agro-industrial factories in Egypt. All agro-industrial wastes were dried under sunlight to constant weight, then grinded and passed through sieves to obtain the desired particle size of 1.7–2 mm [22]. The prepared samples were packed in transparent Zip-lock polythene bags and stored at room temperature for further study.

2.2 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 AOAC, (2006) [23].

2.3 Strains Used

Trichoderma reesei EMCC 532 was obtained from the Egypt Microbial Culture Collection at Ain Shams University. then 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 Z. et al. [24] and Eddleman H. [25].

2.4 Inoculum Preparation

The inoculum of *Trichoderma reesei* was prepared by the method described by Chaverri P. et al. [26] 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 gradually seven days old. Fungal spore were filtered through sterilized glass wool. One ml of such suspension contains 4 x 10^7 spores per ml used to inoculate 100 ml of medium.

2.5 SSF Technique

SSF was conducted according to the method of Saxena R. and Singh R. [27] with some modifications. Fifty grams of each sample was added in 250 ml Erlenmeyer flasks moistened with 50 ml of sterile liquid nutrient medium with 10% glucose containing: [KH_2PO_4 – 1 g, NaCl-1g, Yeast extract – 5g, Peptone – 1 g]. [28] Triplicate sets of each substrate were autoclaved at 121°C at 15 psi for 15 min, and then inoculated with 10ml of the prepared inoculum; all flasks were incubated at 28°C ± 2°C for 5 days at pH5. After the end of incubation, samples were aseptically withdrawn and assayed for crude protein, crude fiber, ash, free amino acids, total carbohydrate, nitrogen, phosphorus and potassium. Different pH values were also investigated.

3. RESULTS AND DISCUSSION

3.1 Effect of Different pH Values on Nutrient Enrichment of Agro–Industrial Wastes by *Trichoderma reesei*

pH value is the most important factor, which markedly influence nutrient enrichment by using solid state fermentation. [29] pH had a significant effect on crude protein for different agro-industrial wastes, the results agree with previous studies which show the effect of pH. Ahmed S. et al. [19] found maximum protein content for rice polishings with *Trichoderma harzianum* at pH4, Arshad M.et a, [30] *use Candida utilis* to give a maximum biomass production at pH 6.5 from wheat bran, Irfan M. et al. [31] found pH 5.5 is an optimum value to Produce maximum protein with *Chaetomimum* sp., Zhang Z. Y. et al. [32] found the highest amount of Crude protein was produced by *T. viride* WEBL0702 at pH 4.5. A significant increase in the protein from *Aspergillus niger* and *Rhizopus oryzae* was achieved when the initial pH was increased from 5.5 to 6 by Ahmed S. et al. [33]. The composition of wastes under our study at different pH values using *Trichoderma reesei* summarized in Figs. (1-10).

<u>3.1.1 Mango</u>

The results represented in Fig. 1 Show the effect of pH values on the percentage of mineral composition in mango peels under SSF by *Trichoderma reesei.* The results showed that the percentage of phosphorus and nitrogen were maximum at pH 5, while the percentage of ash and oxygen was maximum under pH4.

On the other hand, the effect of different pH values on the biochemical composition of mango peels sample under SSF by *Trichoderma reesei* is represented in Fig. 2 The results show that, increase percentage of crude protein to 30.05%. The results are also shown that the crude fiber and total carbohydrate was decreased to 0.21% and 0.16% respectively at pH5 the highest value of free amino acids is 0.41% at pH 6. Our results are agreed with the results of Pandey S. et al. [34], they mentioned that improve of crude protein by different bio agents under pH5.

<u>3.1.2 Apple</u>

As Represented in Fig. 3 the mineral composition of apple peels waste under different values of pH by *Trichoderma reesei* using solid state fermentation. Percentage of ash, oxygen, nitrogen and potassium reached to maximum under pH6, percentage of phosphorus reached to maximum value under pH5.

The influence of pH on biochemical compositions of Apple peel show in Fig. 4. Maximum crude protein production was obtained at pH6. Under pH5 free amino acids reached to maximum. The percentage of total carbohydrates and crude fibers were decreased to a minimum value under pH5. This is in accordance with the results obtained by Bacha U. et al. [35] they stated that enrichment of crude protein for different agro-industrial waste by SSF under optimum pH 4.5.

3.1.3 Banana

The effect of different pH values by *Trichoderma reesei*, on mineral composition of banana peels samples are represented in Fig. 5. The results showed that the percentage of ash, nitrogen, phosphorus and potassium reached to peek under pH 5. But the percentage of oxygen records the maximum at pH6.

The results represented in Fig. 6. Show the effect of pH values on the percentage of biochemical composition in banana peels under SSF by *Trichoderma reesei*. Crude protein reached maximum at pH5. The results are also showed that the total carbohydrate and crude fibers decreased as the crude protein increase at pH5. Percentages of free amino acids were increased at pH4. From the results pH5 is an optimum value for banana peels to nutrient enrichment by *Trichoderma reesei* . This is in accordance with the results obtained by Rompato K. et al. [36]. They used two strains in the fermentation of banana peel, the protein value was increased in more than 115% at pH5.5.

3.1.4 Orange

The results represented in Fig. 7. Represented the effect of pH values on the percentage of mineral composition in orange peels under SSF by *Trichoderma reesei* the results showed that the percentage of oxygen and phosphorus reached peaks at pH5, but the Percentage of ash and nitrogen reached to maximum under pH6.

The effect of different pH values under SSF by *Trichoderma reesei,* on biochemical composition of orange samples are represented in Fig. 8. The results showed that, crude protein reached to maximum at pH6, crude fibers decreased to lowest value under pH6. The highest value of free amino acids recorded at pH4. The *Trichoderma reesei* was most active at pH6 when using orange peels. In this respect, Hamdy H. [37] .The researcher found that orange peel reached to maximum percentage of protein after treated using SSF at pH6.

3.1.5 Tomato

The effect of different pH values by *Trichoderma reesei*, on mineral composition of Tomato peels samples are represented in Fig. 9. The results showed that the percentage of ash and potassium reached to maximum at pH6, while nitrogen, phosphorus and oxygen reached to peek under pH 5.

The results represented in Fig. 10. Show the effect of pH values on the percentage of biochemical composition in tomato peels under SSF by *Trichoderma reesei*. Crude protein reached maximum at pH5. Percentages of free amino acids were increased at pH4. From the results pH5 is an optimum value for tomato peels enrichment by *Trichoderma reesei*. Boukhalfa H. et al. [38] reported low significant difference in optimum pH, the researcher found that pH6 is an optimum value for tomato peel by *Aspergillus oryzae* 2220 using SSF.

3.2 Ideal Agro-industrial Wastes Used for Protein Enrichment by *Trichoderma reesei* under SSF at Optimum pH5

Mineral and biochemical composition of agro-industrial wastes at optimum pH5 with *Trichoderma reesei* under SSF are summarized in Table 1. The results showed that the composition of different waste samples was significantly different under the tested parameters. The results show that the amount of protein was (78.17, 30.05, 28.84, 19.82 and 14.06)% for peels of tomato, mango, orange, apple and banana respectively. The results are shown variation in the nutritional value of all wastes than control which is due to the difference in the chemical composition 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 this regard, U. Bacha et al. [35] stated that the composition of different agro-industrial wastes are enhanced after treated by SSF, the increase of protein in waste treatment by SSF considers an important factor in wastes enrichment. In our results it was showed, that tomato is the most important one in enrichment of wastes with protein. Our results are agreed with Boukhalfa H. et al. [38].The researchers found an increase of protein content in tomato waste at different pH. The composition of raw material for tomato peels agree with Silva Y. P. A. et al. [39].

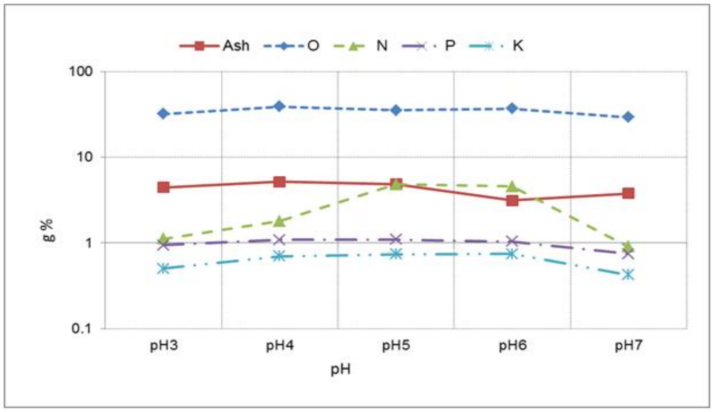

Fig. 1. Mineral composition of mango peels under different pHs by *Trichoderma reesei*

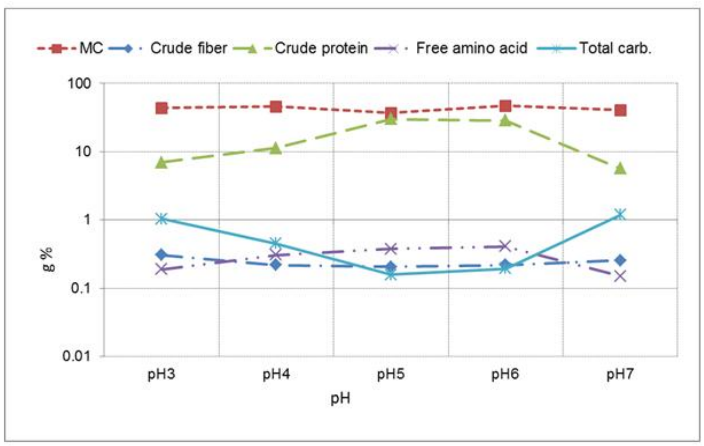

Fig. 2. Biochemical composition of mango peels under different pHs by *Trichoderma reesei*

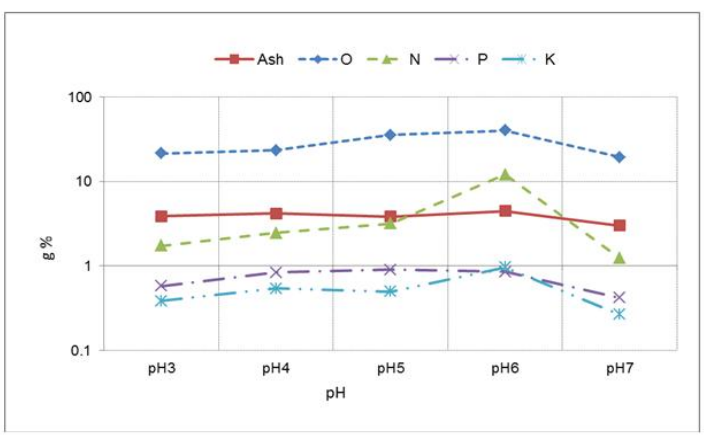

Fig. 3. Mineral composition of apple peels under different pHs by *Trichoderma reesei*

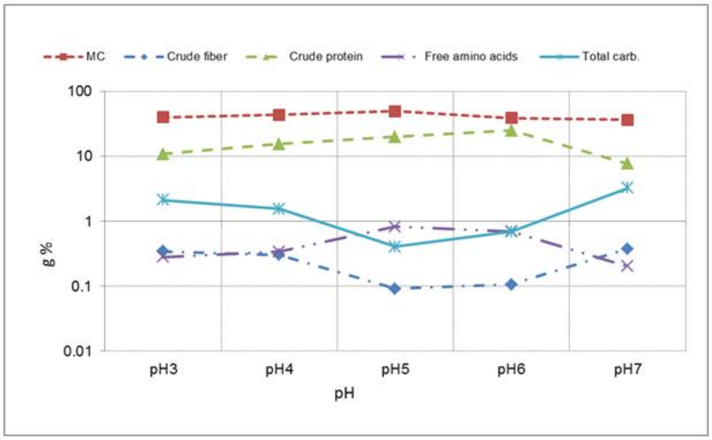

Fig. 4. Biochemical composition of apple peels under different pHs by *Trichoderma reesei*

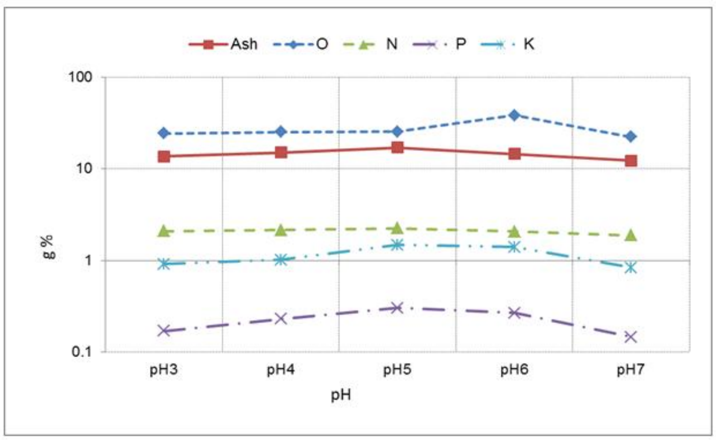

Fig. 5. Mineral composition of banana peels under different pHs by *Trichoderma reesei*

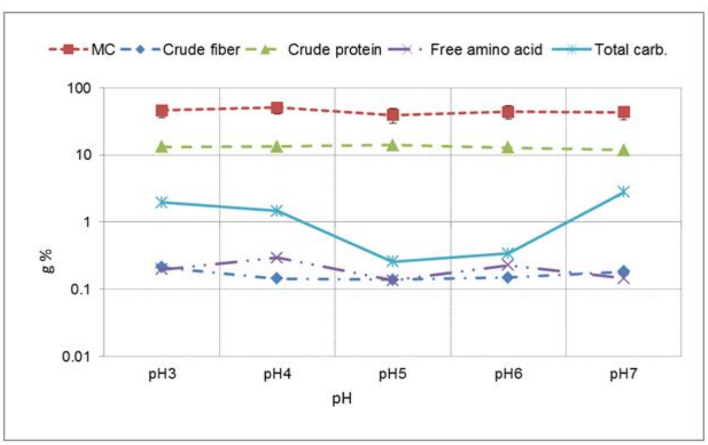

Fig. 6. Biochemical composition of banana peels under different pHs by *Trichoderma reesei*

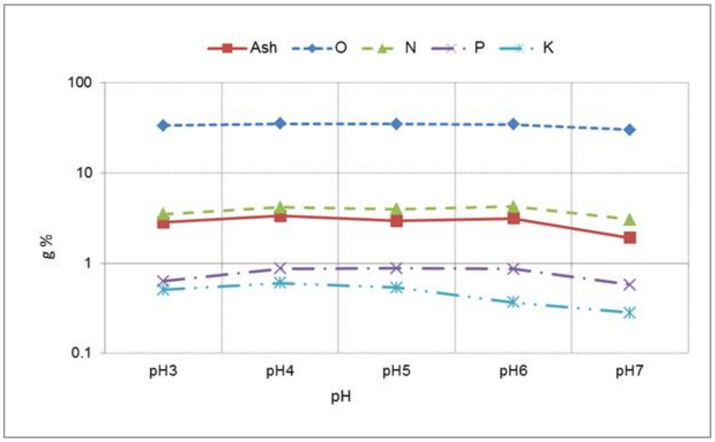

Fig. 7. Mineral composition of orange peels under different pHs by *Trichoderma reesei*

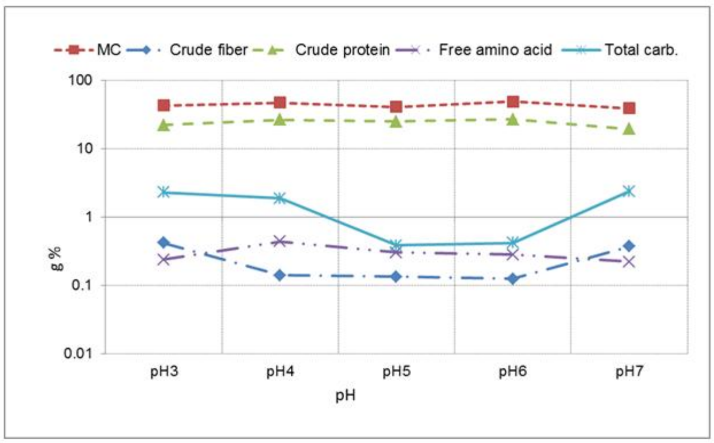

Fig. 8. Biochemical composition of orange peels under different pHs by *Trichoderma reesei*

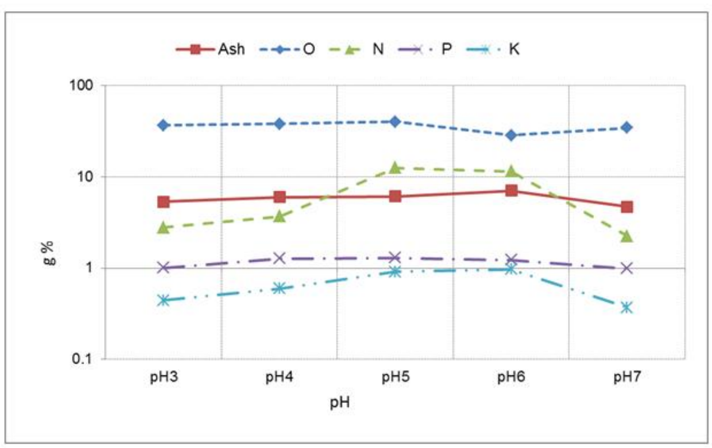

Fig. 9. Mineral composition of tomato peels under different pHs by *Trichoderma reesei*

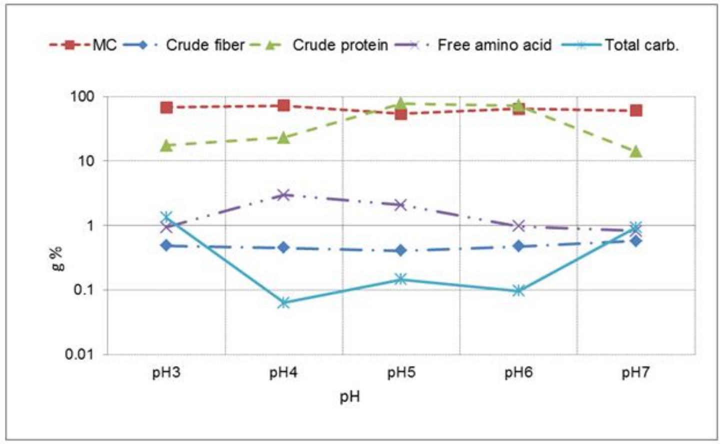

Fig. 10. Biochemical composition of tomato peels under different pHs by *Trichoderma reesei*

Table 1. composition of various wastes samples (pH5, 28 C°, 50 ml media, 50 gm substrate) by *Trichoderma reesei*

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**	4.85	37.01	4.81	1.10	0.73	37.01	0.40	30.05	0.38	0.16
	Control	4.08	8.33	3.5	0.24	0.86	75.92	16.37	21.88	0.09	6.85
Apple	**Treated**	3.86	35.45	3.17	0.90	0.50	49.45	0.09	19.83	0.82	0.41
	Control	2.86	6.38	2.59	0.21	0.76	12.66	13.77	16.19	0.57	9.21
Banana	**Treated**	17.14	25.63	2.25	0.30	1.48	39.47	0.14	14.06	0.14	0.26
	Control	8.93	11.42	1.52	0.32	6.58	11.50	14.93	9.5	0.057	5.12
Orange	**Treated**	2.95	35.04	3.97	0.88	0.54	40.51	0.13	24.84	0.30	0.38
	Control	4.29	8.69	3.86	0.21	0.91	12.09	14.69	24.13	0.189	7.28
Tomato	**Treated**	6.12	40.16	12.51	1.30	0.91	54.05	0.21	78.17	2.06	0.15
	Control	5.05	13.88	3.74	1.02	1.04	6.66	42.22	23.35	0.09	3.77

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 a promising fungal strain of *Trichoderma reesei*. It is concluded from the results that crude protein were maximum by *Trichoderma reesei* at pH5, Tomato peel is a good substrate for protein enrichment followed by mango peel, orange peel, apple peel and banana peel respectively. Our results need further studies to detect the most important one according to nutritional values.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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