Nutrient Enrichment of Agro–Industrial Waste Using Solid State Fermentation

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 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]. Sibtain Ahmed et al [19] used rice polishing as medium cultivated with Trichoderma harzianum, Maximum fungal biomass production was obtained at pH4 and 28° C. Tesfaye Alemu [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 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*.

Italic :[1EV]Comment

Italic :[2EV]Comment

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 Harold Eddleman [25].

2.4 Inoculum Preparation

The inoculum of ***Trichoderma reesei*** was prepared by the method described by Priscila Chaverri 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 107 spores per ml ,used to inoculate 100 ml of medium.

2.5 SSF Technique

SSF was conducted according to the method of Saxena and Singh [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 – 1g, NaCl- 1g, Yeast extract – 5g, Peptone – 1g]. [28] Triplicate sets of each substrate were autoclaved at 121℃ at 15 psi for 15 min, and then inoculated with 10ml of the prepared inoculum; all flasks were incubated at 28℃ ± 2 ℃ 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. Sibtain Ahmed et al [19] found maximum protein content for rice polishings with *Trichoderma harzianum* at pH4, *M. Arshad et al* [30] *use Candida utilis* to give a maximum biomass production at pH 6.5 from wheat bran, M. Irfan et al [31] found pH 5.5

is an optimum value to Produce maximum protein with *Chaetomimum* sp., Z. Y. Zhang 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 S. Ahmed et al [33]. The composition of wastes under our study at different pH values using ***Trichoderma reesei*** summarized in Figures (1-10).

3.1.1 Mango

The results represented in figure 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 Figure 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 Sonika et al. [34], they mentioned that improve of crude protein by different bio agents under pH5.

Missing comma **:[3EV]Comment**

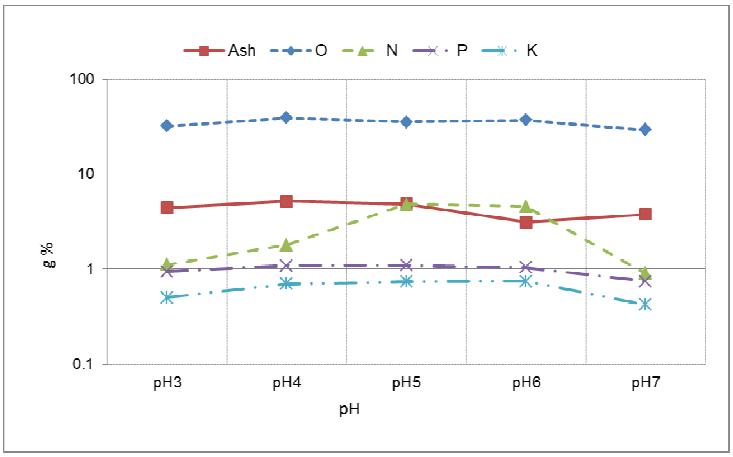

Fig. (1) Mineral Composition of Mango peels under different pHs by ***Trichoderma reesei***..

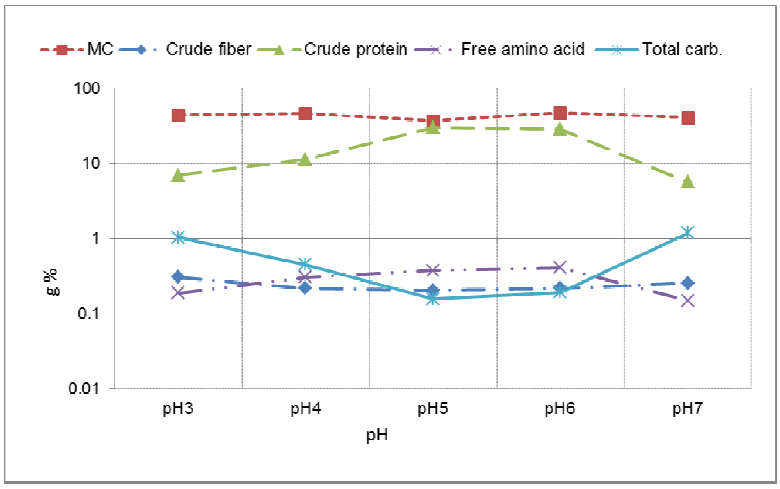

Fig. (2) Biochemical Composition of Apple peels under different pHs by ***Trichoderma reesei.***

3.1.2 Apple

As Represented in figure 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 figure 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 U. Bacha et al. [35] they stated that enrichment of crude protein for different agro-industrial waste by SSF under optimum pH 4.5.

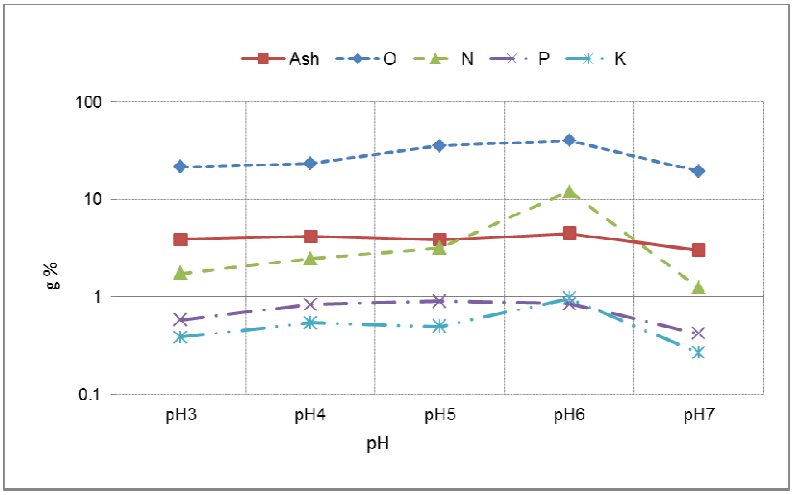

Fig. (3) Mineral Composition of Apple peels under different pHs by ***Trichoderma reesei.***.

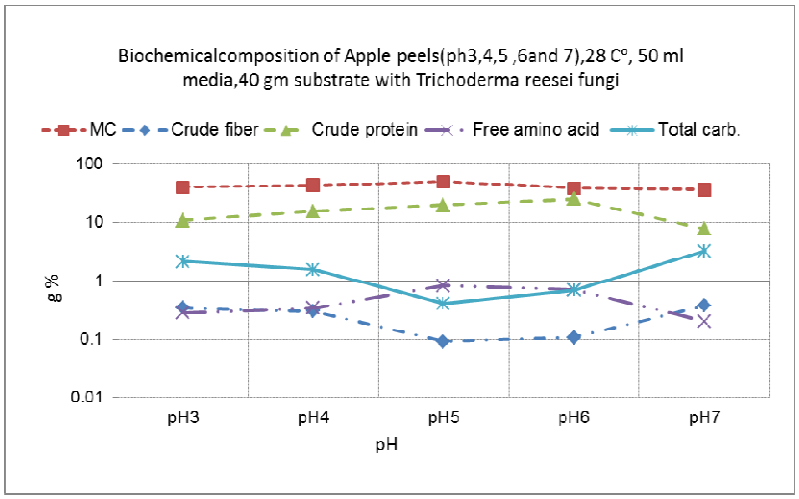

Fig. (4) Biochemical Composition of Apple peels under different pHs by ***Trichoderma reesei.***

3.1.3 Banana

The effect of different pH values by ***Trichoderma reesei***, on mineral composition of banana peels samples are represented in figure 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 figure 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 was 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 Karina M Rompato et al. [36].They stated that under pH 5 at different fermented times, they are found that the protein concentration increase after fermentation periods at pH5 .

Incorrect verb **:[4EV]Comment**

`

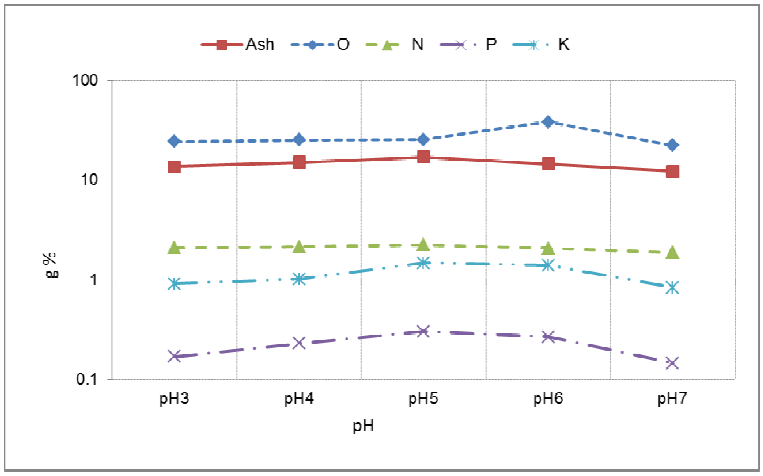

Fig. (5) Mineral Composition of Banana peels under different pHs by ***Trichoderma reesei.***.

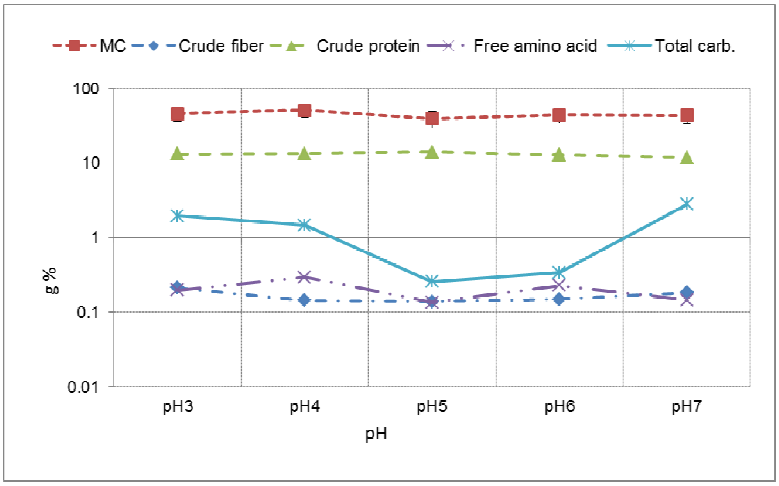

Fig. (6) Biochemical Composition of Banana peels under different pHs by ***Trichoderma reesei.***

3.1.4 Orange

The results represented in figure 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 figure 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, Hossam S. Hamdy, [37] .The researcher found that orange peel reached to maximum percentage of protein after treated using SSF at pH6 .

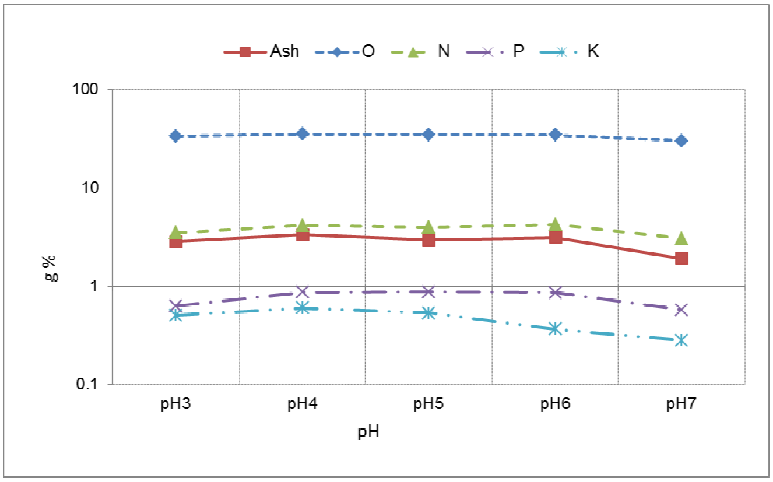

Fig. (7) Mineral Composition of Orange peels under different pHs by ***Trichoderma reesei.***.

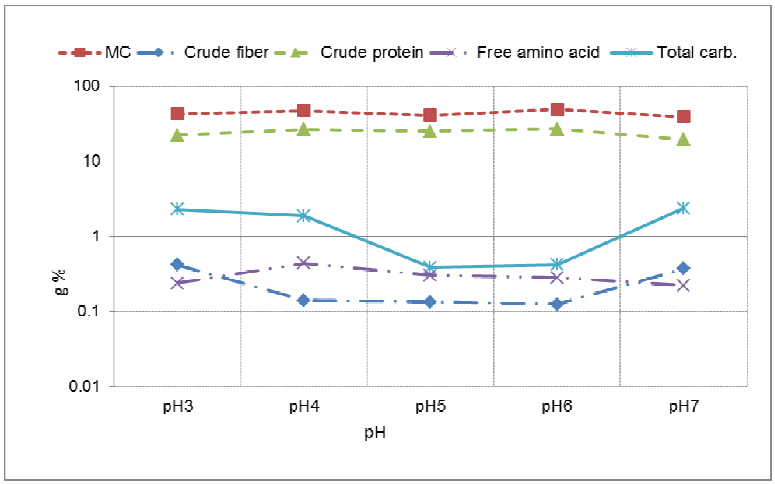

Fig. (8) Biochemical Composition of Orange peels under different pHs by ***Trichoderma reesei.***

3.1.5 Tomato

The effect of different pH values by ***Trichoderma reesei***, on mineral composition of Tomato peels samples are represented in figure 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 figure 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***. Hayet Boukhalfa-Lezzar [38] reported low significant difference in optimum pH, the researcher recoded pH6 is an optimum value for tomato peel by *Aspergillus oryzae* 2220 using SSF.

Confused word **:[5EV]Comment**

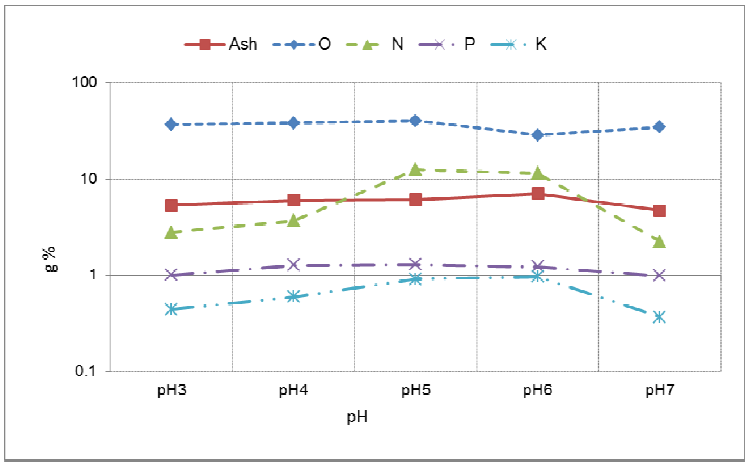

Fig. (9) Mineral Composition of tomato peels under different pHs by ***Trichoderma reesei.***.

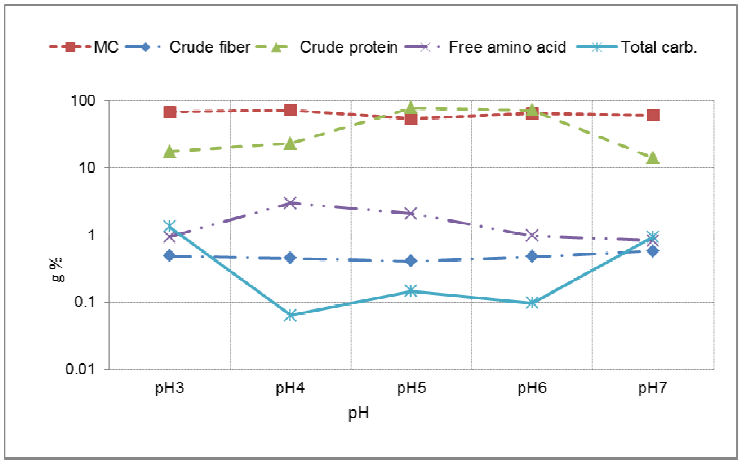

Fig. (10) Biochemical Composition of tomato peels under different pHs by ***Trichoderma reesei.***

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 Table1. 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 Hayet Boukhalfa-Lezzar 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 Y. P. A. Silva et al.[39],

Missing comma :[6EV]Comment

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

2. 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 peels were found to be 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.

Incorrect verb **:[7EV]Comment**

References

1- Rodreguez-Couto, S., 2008. Exploitation of biological wastes for the production of value-added products under solid-state fermentation conditions. Biotechnol. J. 3, 859–870.

2- Göğüş N, Evcan E, Tarı C, Cavalitto SF. 2015 Evaluation of agro-industrial wastes, their state, and mixing ratio for maximum polygalacturonase and biomass production in submerged fermentation. Environ Technol.; 36 (20):2657-67.

3- Robinson T, Singh D, Nigam P. 2001 Solid-state fermentation: A promising microbial technology for secondary metabolite production. Appl Microbiol Biotechnol. 55, 284-289.

4- Nasir, M. and M. S. Butt. 2011. Maize Germ: A Nutrient Dense Substance for Food Value- Addition. LAP Lambert Academic Publishing, Germany, 240p.

5- Raghavarao, K. S. M. S., Ranganathan, T. V., Karanth, N. G. 2003, Some engineering aspects of solid-state fermentation. Biochem. Eng. 13, 127–135.

6- Bhanu Prakash, G.V.S., Padmaja, V., Siva Kiran, R.R., 2008. Statistical optimization of process variabes for the large-scale production of Metarhizium anisopliae conidiospores in solid-state fermentation. Bioresour. Technol. 99, 1530–1537.

7- Talebnia, F. 2008. Ethanol production from cellulosic biomass by encapsulated Saccharomyces cerevisiae. PhD. Thesis. Chalmers Univ. Technol. Gothenburg (Sweden).

8- Ugwuanyi J.O., McNeil B., Harvey L.M. 2009. Production of Protein-Enriched Feed Using Agro-Industrial Residues as Substrates. In: Singh nee' Nigam P.,

Correct the word **:[8EV]Comment**

Pandey A. (eds) Biotechnology for Agro-Industrial Residues Utilisation. Springer, Dordrecht pp 77-103

9- Sherman- Huntoon R, 2000. Latest developments in mid-to-large-scale vermicomposting, Biocycle, 41 (11), 51-54.

10- Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A. 2010 Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol.; 46(7):541–549.

11- Bhargav, S., Panda, B., Ali, M., 2008. Solid-state Fermentation: An Overview. Chemical and Biochemical Engineering, 22(1), 49–70.

12- Thomas L, Larroche C, Pandey A. 2013. Current developments in solid-state fermentation. Biochem Eng J.; 81:146–161.

13- Pandey S, Shahid M, Srivastava M, Sharma Antima, Singh Anuradha, 2014 Isolation purification and characterization of glucanase enzyme isolated from antagonistic fungus Trichoderma species. International Journal of Scientific & Engineering Research. 5: 646-649

14- Vitikainen M, Arvas M, Pakula T, Oja M, Penttilä M, 2010. Array comparative genomic hybridization analysis of Trichoderma reesei strains with enhanced cellulase production properties. BMC Genomics 11: 441.

15- Bampidis VA, Robinson PH 2006. Citrus by-products as ruminant feeds: A review. Animal Feed Science and Technology. 128: 175–217

16- Mahawar M, Singh A, Kumbhar BK, Sahgal M, Chand K (2012). Solid state fermentation of Apple pomace as affected by combinations of enzymatic treatment and yeast strains. Journal of Progressive Agriculture. 3 (1): 59-62.

17- Volanis M, Zoiopoulos P, Panagou E, Tzerakis C (2006) Utilization of an ensiled citrus pulp mixture in the feeding of lactating dairy ewes. Small Ruminant Research 64: 190–195

18- C.M. Ajila, S.K. Brar, M. Verma, R.D. Tyagi, S. Godbout, and J.R. Valéro.2012. Bio-processing of agro-byproducts to animal feed. Critical Reviews in Biotechnology. 32(4):382-400.

19- Sibtain Ahmed, Ghulam Mustafa, Muhammad Arshad, and Muhammad Ibrahim Rajoka. 2017 Fungal Biomass Protein Production from *Trichoderma harzianum* Using Rice Polishing. Hindawi BioMed Research International Volume, Article ID 6232793.

20- Tesfaye Alemu , 2013. Process of Optimization and Characterization of Protein Enrichment of Orange Wastes through Solid State Fermentation by *Aspergillus niger* Isolate No.5. Journal of Biological Sciences,13 (5): 341-348.

21- Eyini, M, Babitha, S, Lee, MW. 2002. Cellulose utilization and protein productivity of some cellulolytic fungal co-cultures. Mycobiology.30 (3):166–169.

22- Gurpreet S Dhillon,Satinder K Brar,Surinder Kaur Mausam Verma. 2013. Screening of agro-industrial wastes for citric acid bioproduction by Aspergillus niger NRRL 2001 through solid state fermentation. J Sci Food Agric.; 93(7):1560-7.

23- AOAC (The Association of Official Analytical Chemist) 2006. The official methods of analysis of AOAC international, 18th ed. The Assoc. Official Ana. Chem. Arlington, U.S.A.

24- Benko Z, Drahos E, Szengyel Z, Puranen T, Vehmaanpera J, Reczey K. 2007 Thermoascus aurantiacus CBHI/Cel7A production in Trichoderma reesei on alternative carbon sources. Applied Biochemistry and Biotechnology. 137:195–204.

25- Harold Eddleman, Ph. D 1998. .Making Bacteria Media from Potato. Indiana Biolab. disknet.com. Retrieved 2011-03-04

26- Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R, Degenkolb T, Samuels GJ .2015. Samuels Systematics of the Trichoderma harzianum species complex and the re-identification of commercial biocontrol strains. Mycologia. 107(3): 558–590.

27- Rajshree Saxena and Rajni Singh. 2011. Amylase production by solid-state fermentation of agro-industrial wastes using Bacillus sp. Braz J Microbiol. 42(4): 1334–1342.

28- Tiina M. Pakula, Katri Salonen, Jaana Uusitalo and Merja Penttila¨ 2005. The effect of specific growth rate on protein synthesis and secretion in the filamentous fungus Trichoderma reesei. Microbiology. 151, 135-143

29- Romero, C.M., L.M. Pera, F. Loto, C. Vallejos, G. Castro and M.D. Baigori 2012. Purification of an organic solvent-tolerant lipase from *Aspergillus niger* MYA 135 and its application in ester synthesis. Biocat. Agri. Biotechnol. 1: 25-31

30- M. Arshad, S. Ahmed, M. A. Zia, and M. I. Rajoka, 2014. Kinetics and thermodynamics of ethanol production by Saccharomyces cerevisiae MLD10 using molasses. Applied Biochemistry and Biotechnology, 172 (5),2455–2464.

31- M. Irfan, M. I. Nazir, M. Nadeem, M. Gulsher, Q. Syed, and S. Baig, 2011. Optimization of process parameters for the production of single cell biomass of Candida utilis in solid state fermentation. American-Eurasian Journal of Agricultural and Environmental Sciences, 10. 264–270.

32- Z. Y. Zhang, B. Jin, Z. H. Bai, and X. Y. Wang, 2008. Production of fungal biomass protein using micro fungi from winery wastewater treatment. Biore source Technology, 99 (9):3871– 3876.

33- S. Ahmed, S. Riaz, and A. Jamil, , 2009. Molecular cloning of fungal xylanases: an overview," Applied Microbiology and Biotechnology, 84 (1):19–35.

34- Sonika Pandey, Mukesh Srivastava, Mohammad Shahid, Vipul Kumar, Anuradha Singh, Shubha Trivedi and Y.K. Srivastava 2015. Trichoderma species Cellulases Produced by Solid State Fermentation. Data Mining Genomics Proteomics, 6:2.

35- U. Bacha, M. Nasir, A. Khalique, A. A. Anjum* and M. A. Jabbar. 2011. Comparative assessment of various agro-industrial wastes for saccharomyces cerevisiae biomass production and its quality evaluation as single cell protein. The Journal of Animal & Plant Sciences, 21(4) : 844-849.

36- Karina M Rompato, Susana N Somoza. 2015. Protein enrichment of fruit processing byproducts using solid state fermentation with Saccharomyces cerevisiae and Bacillus subtilis. Biotecnología Aplicada.;32:4221-4227

37- Hossam S. Hamdy 2013. Production of mini-food by Aspergillus niger, Rhizopus oryzae and Saccharomyces cerevisiae using orange peels. Romanian Biotechnological Letters 18,(1): 7929 – 7949.

Italic **:[9EV]Comment**

Italic **:[10EV]Comment**

Uppercase and Italic **:[11EV]Comment**

Italic **:[12EV]Comment**

38- Hayet *Boukhalfa-Lezzar, Hind Leghlimi, Estelle Copinet, Francis Duchiron, Aicha Mechakra-Maza 2014. Utilization of tomato pomace as a substrate for neutral protease production by Aspergillus oryzae 2220 on solid-state fermentation. International Journal of Advanced Research. 2 (11):338-346.*

39- Y. P. A. Silva, B. C. Borba, M. G. Reis, M. Caliari, T. A. P. C. Ferreira. 2016. Tomato industrial waste as potential source of nutrients. International technical symposium food: the tree that sustains life. 51: 2108 – 3111.