International Journal of Recycling of Organic Waste in Agriculture (2018) 7:345–355
https://doi.org/10.1007/s40093-018-0220-z

ORIGINAL RESEARCH

Utilization of wheat straw for fungal phytase production

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Received: 20 March 2018 / Accepted: 8 September 2018 / Published online: 17 September 2018

Abstract

Purpose Wheat straw is an agricultural waste which can be used as a cost effective animal feed. However, high hemicellulose and phytic acid content in wheat straw prevents it as a primary feed choice. Utilization of wheat straw in solid-state fermentation may result in wheat straw valorization and enzyme production. In this study, phytase production in solid-state fermentation of wheat straw using *Aspergillus ficuum* and valorization of wheat straw were evaluated.

Methods A two-step experimental design procedure was employed for screening and optimization of influencing factors on phytase production. Effects of different nutritional and environmental factors were investigated by one factor at the time method (OFATM). To reach higher amounts of phytase, response surface methodology (RSM) was employed to optimize phytase production as a function of three of the most effective factors.

Results Optimization of the significant parameters resulted in an increase in the phytase activity from 0.74 ± 0.12 to a maximum of 16.46 ± 0.56 Units per gram dry substrate (U gds^{-1}). The high degree of the fungal phytase activity on wheat straw resulted in the decrease in phytic acid content by 57.4%, as compared to the untreated sample. Scanning electron microscopy (SEM) and FTIR structural analysis showed intensive fungal growth on wheat straw, and partial removal of hemicelluloses, lignin and phytic acid.

Conclusion The study demonstrated the feasibility of wheat straw utilization in solid-state fermentation using *Aspergillus ficuum* toward the production of phytase and valorization of wheat straw as an animal feed.

Keywords Wheat straw · Phytase · *Aspergillus ficuum* · Valorization · Solid state fermentation

Introduction

Wheat straw is the second most abundant lignocellulosic raw material in the world (Pensupa et al. 2013). The international grain council forecasted the annual world wheat production of 754 million tons in 2016 (ICG 2017) with a straw to grain ratio of 1.3 (for most wheat varieties). Surplus amount of straw has resulted in environmental and public health concerns attributing to the inefficiency of the conventional straw disposal or utilization methods. Currently, wheat straw is used as animal feed, as supporting materials (Panthapulakkal et al. 2006), as raw material for pulp and paper production (Nasser et al. 2015), and as a substrate for biogas, bioethanol, and mushroom production. Wheat straw is also burnt as a fuel and is added to soil for its maintenance (Ferreira et al. 2014; Huang et al. 2017; Mahboubi et al. 2017; Tomás-Pejó et al. 2017). Wheat straw contains 350–450 g kg^{-1} cellulose, making it an excellent potential source of energy for ruminants. However, intact straw is

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not an ideal feed for ruminants because of its high lignocellulose (200–300 g kg^{-1} hemicellulose and 80–150 g kg^{-1} lignin) and phytic acid content. Phytic acid, the main source of phosphorous in cereals, is not absorbed by monogastric animals, and due to its very strong chelating agent properties, it binds to metal ions (i.e., Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}) and proteins, making them unavailable for absorption. Furthermore, it inhibits the activity of a number of enzymes such as α-amylase, acid phosphatase and tyrosinase (Singh and Satyanarayana 2006a). Hence, phytic acid acts as an antinutritional factor in straw-derived feed components (Hurrell et al. 2003). On the other hand, phytic acid can find its way into waterways and consequently causes rapid algal growth and reduction in water oxygen content. This contributes to death of fish and hypoxia among other problems (Roopesh et al. 2006). These problems can be effectively addressed if the straw-derived feed diets are supplemented with phytase, an enzyme that degrades phytic acid. Phytase is produced naturally by several strains of bacteria, yeast, and filamentous fungi. Filamentous fungi have been widely used for the production of a wide range of extracellular enzymes and value-added supplements (Souza et al. 2015). Fungi-derived phytase has obtained considerable attention due to the ease of cultivation and high production yields (Roopesh et al. 2006). Various filamentous fungi such as *Aspergillus* sp. (Bhavsar et al. 2011; Moreira et al. 2014), *Mucor racemosus* (Bogar et al. 2003b), and *Sporotrichum thermophile* (Singh and Satyanarayana 2006b, Maurya et al. 2017), *Thermomyces lanuginosus* (Makolomakwa et al. 2017), and *Rhizopus oryzae* (Arora et al. 2017) have been reported to produce phytase. Several filamentous fungi and their enzyme products are used to break down the bond between the polysaccharides (cellulose and hemicellulose) and lignin in wheat straw to improve its digestibility through the process referred to as biological-pretreatment (Sindhu et al. 2016, Choudhary et al. 2016, Bagewadi et al. 2017). Many previous studies have also reported the ability of filamentous fungi to produce various commercial enzymes from wheat straw (Table 1).

However, only limited reports are available regarding the production of phytase from wheat straw using filamentous fungi (Singh 2014). The cultivation of filamentous fungi on wheat straw for phytase production could lead to the direct use of the crude product as a value-added supplement in feed mixtures (Bogar et al. 2003b). The aim of the present work is to evaluate phytase production through solid-state fermentation of wheat straw using filamentous fungi *Aspergillus ficuum*. Attempts were made to optimize the effective parameters on phytase production using response surface methodology. The effects of microbial growth on the physical and chemical characteristic of wheat straw were also investigated.

Table 1 Previous reports on wheat straw-derived fungal enzymes

Microorganism/s	Produced enzyme/s	Time (days)	Enzyme activity	Refs.
Aspergillus fumigatus	Endoglucanase	3	4.54 U mL^{-1}	Mehboob et al. (2014)
	Exoglucanase		3.81 U mL^{-1}	
	β-glucosidase		4.30 U mL^{-1}	
Schizophyllum commune	β-glucosidase	7	0.9 U mL^{-1}	Musatti et al. (2017)
	Xylanase	7	19 U mL^{-1}	
Aspergillus niger ADH-11	Cellulose	8	0.15 FPU[a] mL^{-1}	Patel et al. (2017)
	Xylanase	8	34.49 U mL^{-1}	
Penicillium janthinellum EMS-UV-8	Cellulase	8	1.2 FPU mL^{-1}	Sharma et al. (2015)
	CMCase	8	26.6 U mL^{-1}	
	β-Glucosidase	8	1.4 U mL^{-1}	
	Xylanase	8	76 U mL^{-1}	
Aspergillus niger	Cellulase	5	24 U g^{-1}	Pensupa et al. (2013)
Pleurotus ostreatus HP-1	Laccase	8	14.189 U g^{-1}	Thakur et al. (2013)
	Manganes peroxidase	8	562.80 U g^{-1}	
	Xylanase	8	10 U g^{-1}	
Fusarium concolor	Laccase	10	31.14U g^{-1}	Chang et al. (2012)
	Cellubiose	5	19.93U g^{-1}	
	Dehydrogenase	2	4.99 U g^{-1}	
	Xylanase	10	14 U g^{-1}	
	CMCase	5	15.64 U g^{-1}	
	FPase			

[a]Filter paper unit

Materials and methods

Materials

Wheat straw was obtained from a local farm in Shiraz, Iran. It was dried in a forced air oven at 60 °C for 24 h, milled and sieved (0.21–0.84 mm). All the chemicals used in this research work were of analytical grade and purchased from Sigma-Aldrich, Germany.

Microorganism

The fungus *Aspergillus ficuum* PTCC 5288 was maintained on Sabouraud Dextrose Agar (SDA) slants at 30 °C for 4 days and stored at 4 °C. Inoculum was prepared by adding 10 mL of sterile distilled water with Tween 80 (1 mL L^{-1}), to fully sporulated slant using a sterile pipette. The spores were gently agitated using a needle under strict aseptic conditions. The total spore count was determined by the Neubauer counting method.

Solid state fermentation of wheat straw

Solid-state fermentation of wheat straw (5 g dry weight) was carried out in 250 mL Erlenmeyer flasks. Flasks were sterilized at 120 ± 1 °C and 15 psi for 20 min. After cooling, the substrate was moistened (under aseptic conditions) with a sterilized solution containing the additives (carbon source, nitrogen source, mineral salt) mentioned below, inoculated with the spore suspension of *A. ficuum* (10^6 spores gds^{-1}) and incubated for 86 h (the time needed for the fully growth of the fungus on straw). The moisture level of 600 g kg^{-1}, the substrate particle size of 0.70 ± 0.14 mm, and the temperature of 30 °C were used for all the tests, except otherwise specified. After fermentation, the fermented straw was mixed with 120 mL of 0.1 M sodium acetate buffer (pH 5.5) at room temperature and agitated for 120 min at 180 rpm to extract the crude enzyme. Finally, the enzyme extract was centrifuged at 13000*g* and 4 °C for 15 min and the supernatant was collected for phytase activity determination. All the experiments described below have been performed in duplicate (except otherwise specified), and all the data points presented are the mean of duplicate runs.

Screening of the significant factors affecting phytase production

Fermentation experiments were carried out using one factor at the time (OFAT) experimental approach to screen among the effective factors on phytase production. Various carbon and nitrogen sources and mineral salts were examined to find out the required additives to the straw. The effects of variation in temperature, moisture, and substrate particle size on phytase production were also investigated.

Comparison of different carbon sources as inducers for the fungal growth and phytase production

An experiment was conducted to select the best carbon source to supplement wheat straw for rapid growth of the fungi. Mannitol, glucose, lactose, maltose, fructose, and sucrose were tested for this purpose. Each of the materials was added to the straw to the concentration of 0.1 g gds^{-1}. The fermentation was conducted as described in Sec.2.3. A control run (without carbon source) was also included for this experiment.

Comparison of different nitrogen sources as supplements for the fungal growth and phytase production

Malt, ammonium sulfate, yeast extract, tryptone, sodium nitrate, ammonium nitrate, peptone and ammonium chloride were compared to find out the best nitrogen sources among them. Each of the materials was added to the wheat straw to the concentration of 0.05 g gds^{-1}. Glucose (as the best carbon source) was added to the straw (0.1 g gds^{-1}). The fermentation was conducted as described in "Solid state fermentation of wheat straw". A control run (without nitrogen source) was also included for this experiment.

Comparison of different mineral salts as supplements for the fungal growth and phytase production

KCl, KH_2PO_4, K_2HPO_4, $MgSO_4$, $ZnCl_2$, $MnSO_4$, NaCl, and $FeSO_4$ were added to the straw to the concentration of 0.005 g gds^{-1}. Glucose (0.1 g gds^{-1}) and ammonium sulfate (0.05 g gds^{-1}) were also added to the straw. The fermentation was conducted as described in "Solid state fermentation of wheat straw". A control run (without any mineral salts) was also included for this experiment.

The effects of temperature, moisture, and particle size on the fungal growth and phytase production

Three sets of experiments were conducted to evaluate the effects of temperature (in the range of 25–35 °C), moisture (in the range of 400–750 g kg^{-1}), and particle size (in the range of 0.25–0.70 mm) on the fungal growth and phytase production. The fermentation process was carried out as described in "Solid-state fermentation of wheat straw". All the samples contained 0.1 g gds^{-1} glucose, 0.05 g gds^{-1} ammonium sulfate, and 0.005 g gds^{-1} manganese sulfate.

Response surface approach for optimizing phytase production

Response surface method (RSM) was applied to optimize phytase production as a function of moisture level, and glucose and ammonium sulfate concentrations, which were identified as the most effective factors on phytase production by the OFAT screening process. Each factor was studied at three different levels following the Box–Behnken design. The fermentation process was conducted as described in "Solid-state fermentation of wheat straw". The experimental design for this part is depicted in Table 2.

A total of three replicates were carried out for each experimental set, and the average values are presented with standard deviation.

Analytical methods

Spectrophotometric (PG Instrument Ltd., UK) enzyme assay method was performed as described by Coban et al. (2015). One unit of phytase (U) was defined as the amount of enzyme that released 1 µmol/min of inorganic phosphorus from 1.5 mM sodium phytate under the assay conditions. The enzyme activity was expressed in units per gram of dry substrate (U gds^{-1}), unless otherwise specified.

Structural analysis

Scanning electron microscope (SEM) (Tescanvega 3, Czech Republic) was used for the structural analysis of wheat straw. Fermented wheat straw was oven dried at 50 °C for 72 h. Dry fermented wheat straw was fixed on the aluminum stub and SEM image was captured. The investigation of the structural changes in the fermented wheat straw was also carried out using a Fourier Transform Infrared (FTIR) spectrometer (Impact 410 iS10, Nicolet Instrument Corp., Madison, WI, USA). The spectra of the fermented and untreated wheat straw were obtained with an average of 64 scans and resolution of 4 cm^{-1} from 600 to 4000 cm^{-1}. The spectrum data were developed by Nicolet OMNIC 4.1 software (Nair et al. 2017).

Table 2 Experimental design matrix and the results from Box–Behnken design for phytase production

Run	Glucose (g gds^{-1})	Ammonium sulfate (g gds^{-1})	Moisture (g kg^{-1})	Phytase activity (U gds^{-1})	
				Observed	Predicted
1	0.00	0.00	600	0.75 ± 0.11	1.58
2	0.30	0.00	600	3.70 ± 0.21	3.73
3	0.00	0.10	600	2.55 ± 0.13	2.52
4	0.30	0.10	600	14.37 ± 0.57	13.54
5	0.00	0.05	400	0.15 ± 0.07	-0.34
6	0.30	0.05	400	3.27 ± 0.24	3.58
7	0.00	0.05	800	1.05 ± 0.10	0.74
8	0.30	0.05	800	9.50 ± 0.32	9.99
9	0.15	0.00	400	0.29 ± 0.05	-0.05
10	0.15	0.10	400	2.77 ± 0.18	3.29
11	0.15	0.00	800	2.17 ± 0.12	1.65
12	0.15	0.10	800	8.73 ± 0.36	9.07
13	0.15	0.05	600	13.22 ± 0.69	13.16

Determination of phytic acid

A modified protocol of Harland and Oberleas (1977) was followed for the extraction of phytate from the untreated and fungal fermented wheat straw. The phytate content was measured based on the reaction between ferric ion and sulfosalicylic acid (Wade reagent) following the rapid colorimetric method described by Latta and Eskin (1980).

Results and discussion

Solid-state fermentation (SSF) of wheat straw using *Aspergillus ficuum* was carried out to produce phytase. Phytase production from wheat straw was found to improve its digestibility as observed with its structural analysis. To reach the maximum phytase production, efforts were made in this study to optimize the physical and nutritional parameters affecting the fungal phytase production, which is explained below.

Fungal phytase from wheat straw: screening for culture parameters

The effects of nutrient parameters such as carbon source inducers, nitrogen sources and mineral salts together with the physical parameters such as temperature, moisture content and wheat straw particle size, on phytase production were evaluated. Among the different carbon sources, glucose was found to yield the maximum phytase production (Table 3). Glucose has previously been reported as the most effective carbon source for phytase production from various microorganisms such as *Myceliophthora thermophile* (Hassouni et al. 2006), *Pseudomonas aeruginosa* (Sasirekha et al. 2012), and *Aspergillus flavus* (Gaind and Singh 2015). The optimum nitrogen source was selected by supplementation of the media with 0.1 g gds^{-1} of glucose together with 0.05 g gds^{-1} of different nitrogen sources. Compared to other nitrogen sources, ammonium sulfate showed considerable improvement in the phytase production (Table 3). Similar observations were made by Bogar et al. (2003a), Singh and Satyanarayana (2006b), and Bala et al. (2014), demonstrating the significance of ammonium sulfate as

Table 3 Effect of different nutritional parameters on phytase production

Carbon source (0.1 g gds^{-1})	Phytase activity (U gds^{-1})	Nitrogen source (0.05 g gds^{-1})	Phytase activity (U gds^{-1})	Mineral salts (0.005 g gds^{-1})	Phytase activity (U gds^{-1})
Manitol	1.602 ± 0.11	Malt extract	4.044 ± 0.18	KCl	7.252 ± 0.34
Glucose	**3.242 ± 0.24**	**$(NH_4)_2SO_4$**	**8.912 ± 0.05**	KH_2PO_4	2.285 ± 0.19
Lactose	0.886 ± 0.16	Yeast extract	2.936 ± 0.13	K_2HPO_4	3.057 ± 0.27
Maltose	1.795 ± 0.21	Tryptone	1.690 ± 0.06	$MgSO_4$	8.679 ± 0.23
Fructose	2.948 ± 0.19	$NaNO_3$	4.042 ± 0.20	$ZnCl_2$	5.930 ± 0.48
Sucrose	2.255 ± 0.30	NH_4NO_3	7.805 ± 0.28	**$MnSO_4$**	**10.200 ± 0.24**
Control	0.739 ± 0.12	Peptone	4.672 ± 0.28	NaCl	9.041 ± 0.11
		NH_4Cl	6.923 ± 0.10	$Fe_2(SO_4)_3$	9.024 ± 0.43
		Control	3.210 ± 0.07	Control	8.900 ± 0.25

the most favorable nitrogen source for phytase production. Among various mineral salts added to the cultivation media, $MnSO_4$ exhibited maximum increase in phytase production.

Both the phosphate salts (KH_2PO_4 and K_2HPO_4) had an inhibitory effect on phytase production, which could be attributed to the phosphate existence in media and lack of need for phytate hydrolyzing and phytase production. Similar observations on the inhibitory effect of phosphate on phytase production have been reported previously. Exclusion of phosphate from the medium enhanced phytase production by *A. niger* (Żyła and Gogol 2002) and *Sporotrichum thermophile* (Singh and Satyanarayana 2006a).

Concerning the physical culture conditions, it was observed that the temperature variation in the range of 27–35 °C had no significant effect ($p \geq 0.05$) on phytase production (Table 4).

Similarly, variation in wheat straw particle sizes in the range of 0.297 to 0.840 mm showed no significant effect on phytase production. However, reduction in particle sizes lower than 0.297 mm led to a decrease in the phytase production (Table 4). This drop might be due to compaction and consequent formation of agglomerates that contribute to a lower oxygen transfer (Schmidt and Furlong 2012). Table 4 shows that moisture variation significantly affects phytase production by the fungus. In the investigated range of 400–750 g kg^{-1}, phytase production increased with the moisture content of the substrate and reached an optimum value at the moisture content of 650 g kg^{-1}.

Response surface optimization of the effective process parameters

The significant factors influencing phytase production were optimized using response surface method. The predicted and observed responses are in Table 2.

The experimental data were fitted to a quadratic equation with phytase activity (U gds^{-1}) as a function of the concentration of glucose (A), ammonium sulfate (B), and moisture content (C). The model is:

$$\begin{aligned}\text{Phytase activity} = &-48.83 + 32.64A + 104.70B \\ &+ 0.170C + 295.66AB + 0.044AC \\ &+ 0.102BC - 173.75A^2 \\ &- 1564.80B^2 - 0.0001C^2\end{aligned} \quad (1)$$

Table 5 shows the analysis of variance for the model. P value for the model was less than 0.0001, indicating that phytase activity is affected significantly by the model parameters. The test of lack of fit was insignificant (P

Table 4 Effect of different physical parameters on phytase production

Temperature (°C)	Phytase activity (U gds^{-1})	Moisture content (g kg^{-1})	Phytase activity (U gds^{-1})	Substrate particle size (mm)	Phytase activity (U gds^{-1})
25	9.400 ± 0.11	400	2.086 ± 0.15	0.707–0.840	10.192 ± 0.29
27	9.871 ± 0.16	450	5.534 ± 0.38	0.595–0.707	10.127 ± 0.15
30	10.220 ± 0.39	500	8.028 ± 0.53	0.420–0.595	10.024 ± 0.12
32	10.418 ± 0.13	550	9.859 ± 0.44	0.297–0.420	10.159 ± 0.34
35	8.533 ± 0.42	600	10.213 ± 0.36	0.250–0.297	9.184 ± 0.27
		650	10.318 ± 0.47	0.210–0.250	8.327 ± 0.19
		700	9.215 ± 0.17		
		750	8.517 ± 0.73		

Table 5 Analysis of variance (ANOVA) for the quadratic model

Source	Sum of squares	*df*	Mean square	*F* value	Prob > *F*
Model	502.07	9	55.79	84.44	< 0.0001
A	86.72	1	86.72	131.27	< 0.0001
B	57.84	1	57.84	87.54	< 0.0001
C	28.01	1	28.01	42.40	0.0003
AB	19.67	1	19.67	29.77	0.0009
AC	7.10	1	7.10	10.75	0.0135
BC	4.16	1	4.16	6.30	0.0404
A^2	64.35	1	64.35	97.41	< 0.0001
B^2	64.44	1	64.44	97.54	< 0.0001
C^2	139.79	1	139.79	211.60	< 0.0001
Residual	4.62	7	0.66		
Lack of fit	2.81	3	0.94	2.07	0.2467
Pure error	1.81	4	0.45		
Total	506.70	16			
$R^2 = 0.9909$	Adj $R^2 = 0.9791$		Pred$R^2 = 0.9056$		

value = 0.2467) indicating good fitness of the model with the experimental data. The values of coefficients of determination ($R^2 = 0.9909$, Pred $R^2 = 0.9056$, Adj $R^2 = 0.9791$) indicated the suitability of the model to predict phytase activity as the function of the model parameters (Ghoshoon et al. 2015; Żyła and Gogol 2002).

Using the model surface plots were generated showing interactions between factor pairs (Fig. 1). All the plots show a maximum value of phytase activity as the function of model parameters. The plots indicate that there is a unique optimum value of phytase activity as the function of moisture, and glucose and ammonium sulfate concentrations.

Response surface optimization predicted the maximum phytase activity of 14.51 U gds^{-1} in the medium containing 0.17 g gds^{-1} glucose, 0.068 g gds^{-1} ammonium sulfate and 655 g kg^{-1} moisture content. To verify the accuracy of the model for the prediction of the optimum value, an additional experiment was conducted with the mentioned values of moisture, and glucose and ammonium sulfate concentrations. The phytase activity of 14 U gds^{-1} was obtained which is reasonably close to the value predicted by the model (3.64% deviation).

To find if extension of the incubation time could improve the maximum phytase production, solid-state fermentation was performed for varying incubation time, with the culture parameters being constant at their optimum value.

The results (shown in Fig. 2) suggested that phytase production increased with the increase in incubation time, with the maximum enzyme production (16.4 U gds^{-1}) at 144 h.

The enzyme activity, however, declined with further incubation, which could be attributed to the reduced nutrient level and enzyme deactivation. The straw-derived fungal phytase obtained in the present study was higher than phytase produced from other crop residues such as sesame oil cake (0.34 U gds^{-1}) (Singh and Satyanarayana 2008), Groundnut oilcake (24.3 U gds^{-1}) (Roopesh et al. 2006), soybean meal (16 U gds^{-1}), wheat bran (4.5 U gds^{-1}) (Chantasartrasamee et al. 2005) and cotton seed cake (1.10 U gds^{-1}) (Singh 2014). Furthermore, *Aspergillus ficuum* strain (in the present study) showed higher phytase production in the solid-state fermentation of wheat straw, as compared to other filamentous fungal strains such as *Aspergillus oryzae* (Singh 2014).

To determine the effect of fungal fermentation on wheat straw, phytic acid was extracted from untreated and fermented wheat straw using hydrochloric acid. Analysis of the crude extract indicated a sharp decrease in phytic acid content from 23.5 ± 0.67 mg kg^{-1} in the untreated straw to 10 ± 0.83 mg kg^{-1} in fungal fermented straw, which indicates that the treatment reduces the phytic acid content of the straw effectively.

Structural analysis of fermented wheat straw

Scanning electron microscopy (SEM) analysis was used to characterize the structural and surface morphology of the fermented wheat straw (Fig. 3).

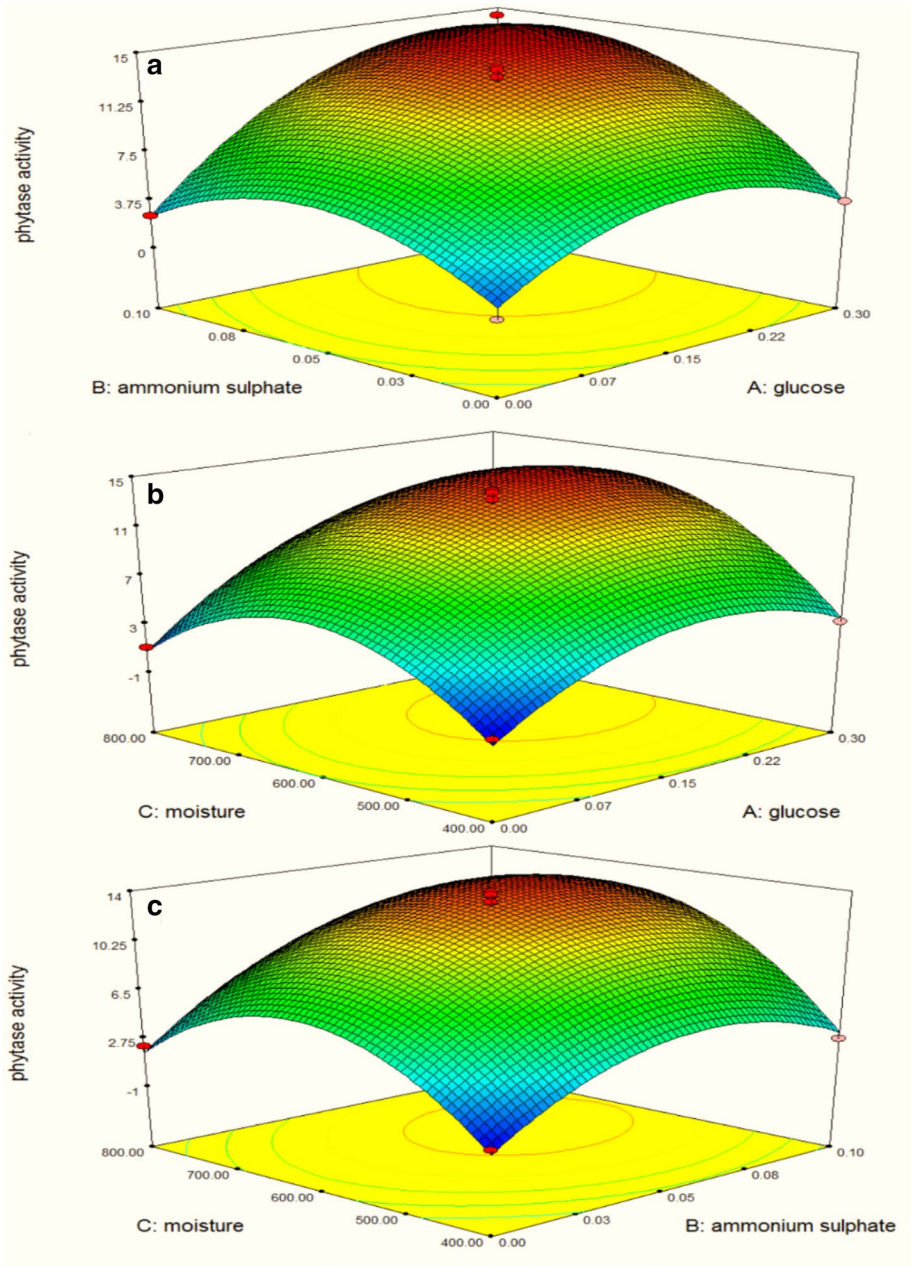

Fig. 1 Factorial interaction of **a** ammonium sulfate (g gds^{-1}) and glucose (g gds^{-1}), **b** moisture (g kg^{-1}) and glucose (g gds^{-1}) and **c** moisture (g kg^{-1}) and ammonium sulfate (g $gds^{-1)}$ on phytase production (U gds^{-1})

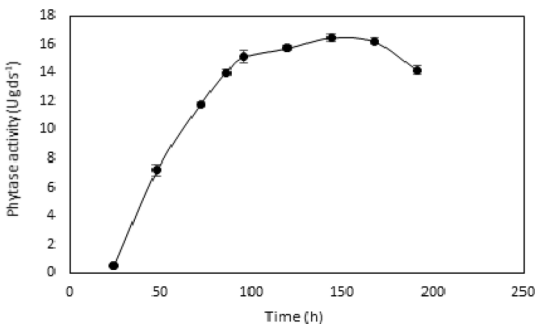

Fig. 2 Phytase production during incubation time

The SEM image showed intense fungal growth on wheat straw and hyphae penetration in straw pores and channels (Fig. 3, bottom). The untreated wheat straw (control) image, however, showed the packed and inaccessible surface structures (Fig. 3, top). The fungal growth on wheat straw resulted in the destruction and modification of the fibrous structure and increase in the porosity. Fermented wheat straw also showed more accessible surfaces compared to the unfermented wheat straw (Fig. 3). The changes in the functional groups of wheat straw after fermentation were analyzed based on the FTIR spectra of the untreated and fermented wheat straw (Fig. 4). Peak assignments are summarized in

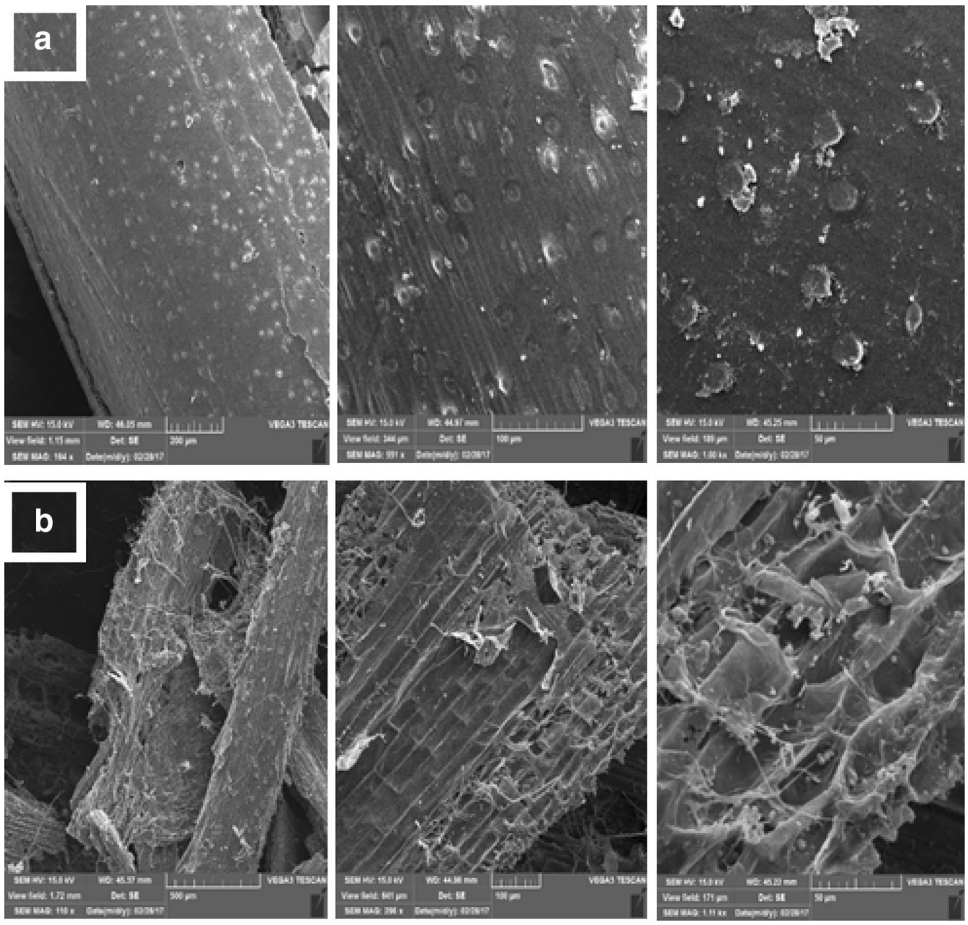

Fig. 3 Scanning electron microscopy (SEM) image indicating the inaccessible and packed structure of untreated wheat straw (top), and fungal hyphae penetration together with germinated spores in the channels and pores on fermented wheat straw (bottom)

Table 6, based on the previous literature reports (Kaushik et al. 2010; Shen et al. 2011).

The analysis of the FTIR spectra depicts significant changes in the structure of the fermented wheat straw compared to untreated wheat straw. Decrease in the polymerization of the cellulose chains was clearly indicated in the fermented wheat straw by the decrease in the absorbance of hydrogen bonds (O–H), represented at peak 3300 cm^{-1} (Oh et al. 2005) (Fig. 4). The absorbance intensity of the peak at 1730 cm^{-1} (that corresponds to the acetyl group) was reduced from 0.036 for untreated wheat straw to 0.032 for fermented wheat straw. Hemicelluloses bind to lignin via acetyl group. Therefore, this can be an indication of cleavage between hemicelluloses and lignin. A sharp peak at 1030 cm^{-1} corresponds to the phytate content (He et al. 2007; Ishiguro et al. 2003) and has significantly decreased in fermented wheat straw, indicating the partial removal of phytic acid (Fig. 4).

Conclusion

Solid-state fermentation of wheat straw using *Aspergillus ficcum* is a feasible way to valorize the straw as an animal feed. Addition of glucose as an inducer and ammonium sulfate enhance phytase production considerably. Maximum phytase activity of 16.4 U gds^{-1} was achieved at the optimum medium conditions using 0.17 g gds^{-1} glucose, 0.068 g gds^{-1} ammonium sulfate and 655 g kg^{-1} moisture after 144 h of incubation at 30 °C. Scanning electron microscopy (SEM) analysis of the fermented wheat straw showed high intensity of fungal growth resulting in the structural destruction and larger accessible surface area in the wheat straw. Chemical analysis and FTIR measurements of the wheat straw revealed the partial removal of phytic acid (57.4% reduction), hemicelluloses and lignin.

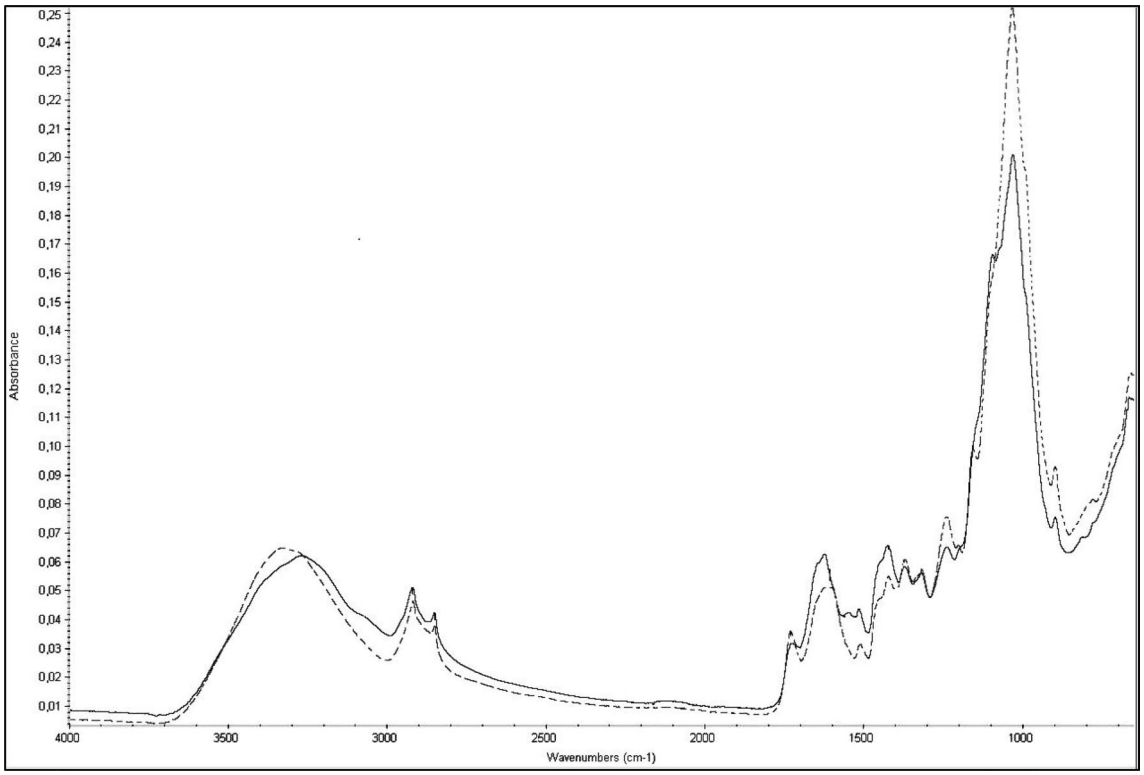

Fig. 4 FTIR spectra of untreated (dashed line), and fermented wheat straw (continuous line)

Table 6 Characteristics and variations in FTIR spectra of untreated and fermented wheat straw

Wavenumber (cm^{-1})	Functional group	Untreated wheat straw	Fermented wheat straw
3300	–OH stretching intramolecular hydrogen band	0.065	0.063
2919	C–H stretching band	0.047	0.052
1730	C = O stretching of acetyl or carboxylic acid	0.036	0.032
1620	C = C stretching of the aromatic ring	0.052	0.063
1510	C = C stretching of the aromatic ring	0.033	0.044
1422	C–H2 symmetric bending	0.056	0.066
1370	C–H asymmetric bending	0.061	0.059
1317	C–H2 wagging	0.058	0.056
1200	C–O–C asymmetric stretching bond	0.076	0.065
1030	C–O stretching band	0.252	0.2
897	C–O–C stretching at β-glucosidic linkage	0.094	0.077

Acknowledgements This study was financially supported by Grant no.: 950508 of the Biotechnology Development Council of the Islamic Republic of Iran. Zohre Shahryari is grateful to the Pharmaceutical Sciences, Research Center, Shiraz University of Medical Science for providing the facility for experiments and Vahid Niknezhad for his technical assistance and valuable discussions.

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