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IMPROVED SUBMERGED *ASPERGILLUS FICUUM* PHYTASE PRODUCTION IN BENCH-TOP BIOREACTORS BY OPTIMIZATION OF FERMENTATION MEDIUM

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Phytase is an important feed and food additive, which is used in diets to increase the absorption of divalent ions, amino acids, and proteins in the bodies and to decrease the excessive phosphorus release in the manure to prevent negative effects on the environment. To date, phytase has been mostly produced in solid state fermentations with insignificant production volumes. Thus, there is a need to produce phytase in submerged fermentations, which can be scaled-up for commercial productions. Additionally, optimization of fermentation medium has not been studied well in the literature. Therefore, this study has been undertaken to improve *Aspergillus ficuum* phytase production in submerged fermentations by optimizing important nutrients in the fermentation medium (glucose, Na-phytate, and $CaSO_4$) using Box-Behnken design of Response Surface Methodology. Also, effects of pH and temperature on phytase activity were studied. Optimum glucose, Na-phytate, and $CaSO_4$ concentrations were determined as 126, 14, and 1.1 g l^{-1}, respectively. Additionally, pH 5.5 and 55 ºC were determined as optimum for the produced *A. ficuum* phytase activity. Under these conditions, phytase activity was increased to 3.45 U ml^{-1}, which is about 50% higher than the previous results. Furthermore, the lowest activity loss was observed under 4 ºC storage conditions during 1 week of storage.

Keywords: phytase, *Aspergillus ficuum,* medium optimization, fermentation, submerged

Phytate is the major phosphate source in plants. Especially legumes, cereals, pollens, and nuts contain high amount of phytate in their structure (Vohra & Satyanarayana, 2003). Nevertheless, phytate chelates proteins, amino acids, and divalent ions, such as Ca^{+2}, Mg^{+2}, Zn^{+2}, Cu^{+2}, Fe^{+2}, and Mn^{+2} and creates insoluble salt forms in animals and human body (Haefner et al., 2005). Therefore, phytate consumption in the diets may cause some several health problems, such as iron deficiency, bone weakness, tooth decay, and digestion problems (Sanson et al., 1981; Hurrell et al., 2003). Additionally, there are several environmental issues reported about phytate consumption. Monogastric animals are not able to utilize phytate, since they do not have the necessary microflora in their digestion systems (Mullaney et al., 2000). Since these animals are generally fed with phytate rich compounds, such as wheat, rice, and corn, excessive amount of phosphorus accumulates in their manure. This causes environmental problems, such as water pollution, algal blooms, fish kills, and changing of fauna and flora in the environment (Mullaney et al., 2000). However, farmers and industry can overcome this problem if the diets are supplemented with phytase enzyme, which decreases phytate content in the feed. Phytase application in the feed formulation can save feed industry around $2 billion per year by reducing nutritional inputs in the diets (Cowieson et al., 2012). Bacteria and yeasts, but most commonly moulds were used for phytase production in several studies. First generation of commercial phytase was produced in 1991

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by using *Aspergillus niger*, which reduced phytate content in the diets by 35–40% (Cowieson et al., 2012). Nelson and co-workers (1968) used phytase, which is produced by *A. niger* to pre-treat a corn–soybean diet for broils. They showed that the phosphorus availability increased by 60% and phosphorus content decreased by 50% in the manure (Nelson et al., 1968). Furthermore, positive effect of phytase application on absorption of minor nutrients was shown in the literature (Sebastian et al., 1996). They demonstrated that phytase supplementation increased the relative retention of total P^{-3}, Ca^{+2}, Cu^{+2}, and Zn^{+2} by 12.5, 12.2., 19.3, and 62.3%, respectively, in broils.

To date, phytase production has been mostly performed in solid state fermentation. However, solid state phytase production is not very flexible to scale-up for commercial production and it needs costly and complex extraction steps. Additionally, solid-state fermentations have very low homogeneity comparing to submerged systems, which causes heterogeneity in phytase properties (Shuler & Kargi, 1992). Overall, there is a need to produce phytase in submerged fermentations to increase the uniformity of the enzyme and to optimize fermentation medium composition to enhance phytase activity. In our previous study, *Aspergillus ficuum* phytase activity was obtained as 1.02 U ml^{-1} in shake-flask productions. After scaling up to 1-liter (working volume) bioreactors and optimizing of the growth parameters, phytase activity increased to 2.27 U ml^{-1} (Coban & Demirci, 2014). There are also several other studies performed in the literature to optimize fermentation medium composition for phytase production with different microorganisms. For example, Lan and co-workers (2002) reported that addition of rice bran, which has high phytate content, to the fermentation medium, increased phytase production by *Mitsuokella jalaludinii* significantly. They measured phytase activity as 5.08 U ml^{-1} when 5% rice bran was used in the medium and activity rose to 12.93 U ml^{-1} when rice bran was increased to 20%. They also showed that glucose can be utilized more rapidly, compared to other carbon sources, in their fermentations (Lan et al., 2002). Additionally, Sasirekha and co-workers (2012) studied the effect of several carbon sources on phytase production by *Pseudomonas* spp. They reported that phytase productions were very similar when glucose (0.727 U ml^{-1}), sucrose (0.739 U ml^{-1}) or lactose (0.724 U ml^{-1}) were used as carbon source. However, slightly lower phytase production was obtained (0.704 U ml^{-1}) when maltose was used as carbon source in the fermentation medium. Moreover, the effect of phosphorus concentration on phytase production was studied by Dahiya and co-workers (2009). In their study, phytase activity was measured as around 18 U ml^{-1} when 0.05% inorganic phosphate was used in the fermentation medium. However, phytase activity decreased to almost 0.35 U ml^{-1} when inorganic phosphate concentration was increased to 0.20%. Also, various agricultural wastes were used as phytate sources in fermentation medium in several studies. For example, orange peel (0.062–0.082% phytate) was used as phytate source in phytase production by *Klebsiella* sp. DB3 (Mittal et al., 2011). In this study, it was reported that phytase activity increased from 0.6 to 3.15 U ml^{-1} when 2% orange peel bran was used in the fermentation medium. Liu and co-workers (2011) studied the effect of K^+, Ca^{2+}, and Mg^{2+} concentrations in the fermentation medium on phytase production by *Pichia pastoris*. They reported that 13.25 g l^{-1} of K_2SO_4, 1.03 g l^{-1} of $CaSO_4 \cdot 2H_2O$, and 17.94 g l^{-1} of $MgSO_4 \cdot 7H_2O$ concentrations are the optimum concentrations to maximize phytase production.

This study is undertaken in order to shed some light on phytase fermentation medium optimization, enzyme assay, and storage conditions. The main goal is to improve phytase production by *A. ficuum* in submerged fermentation by optimization of the medium as well as determination of the optimal pH and temperature for the enzyme activity assay and optimum temperature for storage.

1. Materials and methods

1.1. Microorganism and medium

There are several microorganisms including moulds, yeasts, and bacteria, that have been reported as phytase producers. *Aspergillus ficuum* (NRRL 3135) was used in this study as suggested by our previous study (Coban & Demirci, 2014). *A. ficuum* was obtained from Agricultural Research Service Culture Collection (Peoria, IL) and grown on potato dextrose agar (PDA) (Difco, Sparks, MD) slants for 6 days at 30 °C and stored at 4 °C as the working culture. In order to maintain viability, *A. ficuum* was transferred to sterile fresh agar slant biweekly.

Base phytase fermentation medium included 100 g of glucose, 0.5 g of KCl, 0.1 g of $FeSO_4(7H_2O)$, 0.5 g of $MgSO_4(7H_2O)$, 0.01 g of $MnSO_4(7H_2O)$, 8.6 g of $NaNO_3$, 3 g $(NH_4)_2SO_4$, and 10 g of Na-phytate (A&Z Food Additives Co. Ltd., Zhejiang, China) per liter of deionised water.

1.2. Inoculum preparation

A. ficuum spores were grown on 25 PDA plates for 6 days at 30 °C. After incubation, spores were suspended by adding 7 ml of sterile 0.1% peptone water to each plate and the resulting solution (~10^6 spores ml^{-1}) has been collected and used as the inoculum.

1.3. Batch fermentation

Based on Box-Behnken design, phytase production was performed in 15 different media compositions. Samples were collected from the reactors every 24 h to determine phytase activity and glucose residuals during 6 days. All runs were performed in Sartorius Biostat B Plus bioreactor (Allentown, PA) equipped with a 2-liter vessel and 1-liter working volume. Reactors were inoculated with 3% (v/v) prepared inoculum. Fermentation runs were performed at 33 °C, 4.5 pH, and 0.9 vvm aeration as suggested by our previous studies (Coban & Demirci, 2014). Agitation was maintained at 300 r.p.m. for all fermentation runs.

1.4. Optimization of fermentation medium

In the fermentation base medium, glucose, Na-phytate, and $CaSO_4$ concentrations seemed to be important based on the literature review. In order to find optimum concentrations for these nutrients, Box-Behnken design of Response Surface Methodology (RSM) was used between the concentration ranges of 100–200, 5–20, and 0–1.36 g l^{-1} for glucose, Na-phytate, and $CaSO_4$, respectively, which were selected based on the literature (Lan et al., 2002; Sasirekha et al., 2012) and our preliminary studies (Table 1). Observed phytase activities were statistically analysed by using RSM optimizer module of MINITAB statistical software (Version 15, State College, PA).

1.5. Validation of the model

After determining the optimum concentrations, three fermentation runs were performed at the determined optimum conditions to validate results. Observed and predicted values were compared to each other and root mean square error (RMSE) and mean absolute error (MAE) values were determined to validate the RSM model.

Table 1. Effect of different growth parameter combinations on batch phytase production with *A. ficuum*

Run order	Glucose (g l^{-1})	Na-phytate (g l^{-1})	$CaSO_4$ (g l^{-1})	Measured phytase activity (U ml^{-1})	Predicted phytase activity (U ml^{-1})	Glucose residual (g l^{-1})	Glucose consumption percentages (%)
1	100	12.50	1.36	3.40	3.30	56	44
2	200	5.00	0.68	2.30	2.36	130	35
3	200	20.00	0.68	2.47	2.31	136	32
4	150	12.50	0.68	3.28	3.26	91	39
5	200	12.50	0.00	1.88	1.98	120	40
6	200	12.50	1.36	2.62	2.61	142	29
7	100	20.00	0.68	3.02	2.96	50	50
8	150	12.50	0.68	3.25	3.26	86	43
9	150	5.00	1.36	2.85	2.79	95	37
10	150	5.00	0.00	2.09	1.93	86	43
11	100	12.50	0.00	2.12	2.12	62	38
12	100	5.00	0.68	2.38	2.54	52	48
13	150	20.00	1.36	2.85	3.01	76	49
14	150	20.00	0.00	2.01	2.06	104	31
15	150	12.50	0.68	3.25	3.26	88	41

1.6. Effect of storage temperature on phytase activity

Samples were collected at the highest activity under optimum conditions and centrifuged (VWR, Galaxy, Radnor, PA) at 5200×*g* for 15 min to remove the biomass. Then, 1 ml of supernatant was transferred into glass vials and stored at –20, 4, 25, and 33 °C. One sample from each storage condition was taken to measure phytase activity daily until the 10th day of storage, then every other day. Samples were kept at room temperature for an hour before the analyses were performed. Activity losses from each sample were compared to each other to determine the best temperature condition for *A. ficuum* phytase storage.

1.7. Analysis

Samples (2 ml) were collected from the reactors every 24 h to determine phytase activity and glucose concentration during 6 days. Collected samples were centrifuged at 5200×*g* for 15 min to remove the biomass. Then, supernatant was used for phytase activity and glucose concentration analyses. Biomass concentration could not be measured in the collected samples due to bulk microbial growth in the reactors.

1.7.1. Phytase activity. Phytase, which was produced under optimum conditions, was collected from the reactor and used to determine optimum pH and temperature values in the enzyme assay. In order to determine the optimum temperature for the enzyme assay, samples were incubated with Na-phytate solution in water bath, which was set up to 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 °C. Similarly, to determine the optimum pH for the enzyme assay, pH values of all assay solutions and samples were adjusted to 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, or 9. Enzyme assay was performed under the determined optimum

temperature and pH as described by KIM and co-workers (1998) with minor modifications. Briefly, cell-free broth (0.125 ml) was mixed with 0.125 ml of 1.5 mM Na-phytate in 0.1 M sodium acetate solution and mixtures were incubated in the water bath at 55 °C for 30 min. After incubation, the reaction was stopped by adding 0.25 ml of 15% trichloroacetic acid (TCA) solution into the tubes. Then, 2 ml of colour reagent was added, which was prepared freshly with 2:1:1:1 ratio of water: 2.5% ammonium molybdate: 6 N H_2SO_4: 10% ascorbic acid and tubes were incubated at 55 °C for 30 min. After cooling down to room temperature, absorbances were measured at 700 nm by using a spectrophotometer (Beckman Coulter, Fullerton, CA). Uninoculated fermentation medium was used as the blank for the measurement. The obtained data was used to calculate the activity unit of phytase (U ml^{-1}), which was defined as the μmole of phosphorus liberated from 1.5 mM phytate per min under the set assay conditions.

1.7.2. Residual glucose concentration. Glucose concentrations were measured using high performance liquid chromatography (HPLC) with a refractive index detector (Waters, Milford, MA). Glucose determination was performed by using Aminex HPX-87H column (Bio-Rad, Richmond, CA) with 0.8 ml min^{-1} isocratic flow of 0.012 N sulphuric acid. The detector and column temperature were maintained at 35 and 65 ºC, respectively. The cell-free samples were filtered by using 13 mm diameter, 0.2 μm pore sized filters (PALL Life Sciences, Port Washington, NY).

1.7.3. Statistical analysis. MINITAB Statistical Software package was used for statistical analyses. Analysis of variance (ANOVA) performed to investigate significant differences between phytase activities at different medium compositions. The terms with P-value lower than 0.05 were considered as significant. Also, R^2, RMSE, and MAE values were validated when the model represented the process successfully.

2. Results and discussion

2.1. Optimum temperature and pH of enzyme assay

In order to determine the optimum reaction temperature of *A. ficuum* phytase, activities were measured at a temperature range between 25 and 80 °C. Figure 1 shows that *A. ficuum* phytase activity increased as temperature increased until 55 °C and remained almost the same at 60 °C. However, a sharp decrease in the activity was observed when the assay was performed at 65 °C or above. Therefore, optimum pH was determined as 55 °C for the produced phytase. Similarly, effect of pH was evaluated for the enzyme activity at a pH range between 2.0 and 9.0. It was observed that *A. ficuum* phytase was active in a very wide pH spectrum. However, the highest phytase activity was obtained when the pH was adjusted to 5.5 (3.45 U ml^{-1}). Moreover, *A. ficuum* phytase showed a second high activity point when the pH was 2.5, which is an acidic condition (Fig. 2). These results were very similar the ones reported by ULLAH and GIBSON (1987). They also reported that optimum catalytic pH of phytase, which is produced by *A. ficuum* NRLL 3135, was 2.5 and 5–5.5 and optimum temperature was 58 °C. These characteristics of *A. ficuum* phytase are promising for the food industry, the phytase can maintain its activity in a wide spectrum, so it can benefit animals and humans in every phase of gastrointestinal metabolism.

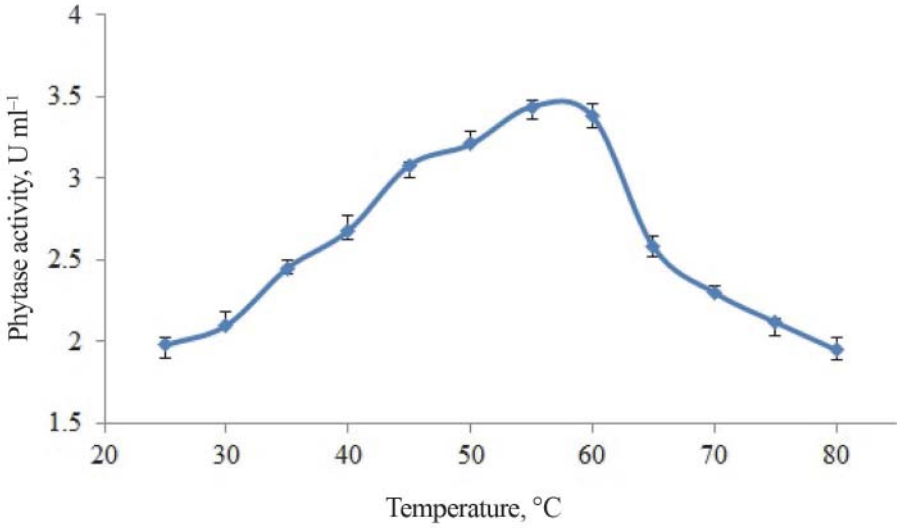

Fig. 1. Determination of optimum temperature for phytase activity

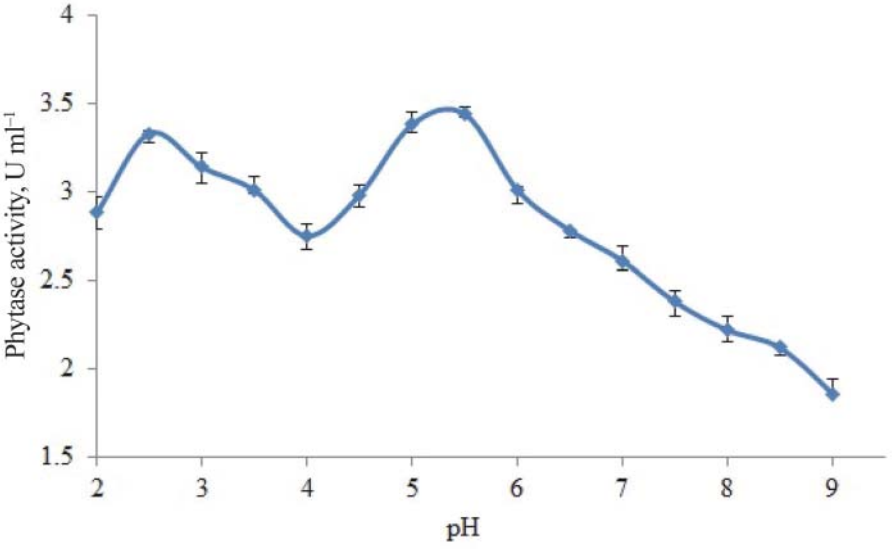

Fig. 2. Determination of optimum pH for phytase activity

2.2. Optimization of fermentation medium

In this study, glucose, Na-phytate, and $CaSO_4$ concentrations were studied to optimize in the fermentation medium for *A. ficuum* phytase production. Glucose serves as carbon source, Na-phytate is the substrate of phytase, which plays an important role as inducer, and $CaSO_4$ was used, since Ca^{2+} is considered as cofactor of phytase (Qian et al., 1997). Table 1 shows

the overall Box-Behnken design, experimental, predicted phytase activity values, glucose residuals, and glucose consumption percentages for different medium compositions. Phytase activity was always less than 3 U ml^{-1} when glucose concentration in the fermentation medium was 200 g l^{-1} (run #2, 3, 5, and 6). Under these conditions, it was also observed that the medium became viscous and thick. This may cause inefficient agitation and aeration conditions and the decreasing of nutrient mass transfer rates, and consequently resulted in lower phytase activities. Among all runs, phytase activity was measured the lowest as 1.88 U ml^{-1} when glucose was used in high levels (200 g l^{-1}) in the medium (run #5). On the other hand, the lowest phytase activities were measured as 2.12 and 2.01 U ml^{-1} when lower glucose amount was used in the medium, 100 g l^{-1} and 150 g l^{-1}, respectively. When Na-phytate was used in low concentrations in the reactors (5 g l^{-1}), phytase activity was always below 2.85 U ml^{-1} (run #2, 9, 10, and 12). However, when the fermentation medium had 20 g l^{-1} of Na-phytate, occurrence of a dark colour was observed in the reactors compared to other runs under the same conditions, phytase activities were less than 3.02 U ml^{-1} (run #3, 7, 13, and 14). Additionally, it was observed that $CaSO_4$ has an important effect on phytase activity. Phytase activity was only up to 2.12 U ml^{-1} when there was no $CaSO_4$ added in the medium (run #5, 10, 11, and 14). However, phytase activity increased to 3.40 U ml^{-1} when 1.36 g $l^{-1}$of $CaSO_4$ was added to the fermentation medium (run #1). *A. ficuum* resulted in the highest glucose consumption by 50% at 100 g l^{-1} of glucose, 20 g l^{-1} of Na-phytate, and 0.68 g l^{-1} of $CaSO_4$ concentrations, but phytase activity was not the highest (3.02 U ml^{-1}) (Table 1). The reason for this might be that glucose might be used to produce more biomass than phytase. Unfortunately, biomass was not measured during the experiments due to bulk of *A. ficuum* in the reactor medium. In the literature, there are several studies showing that glucose is one of the most commonly used carbon source for phytase productions (Hosseinkhani et al., 2009; Sasirekha et al., 2012). Similarly, agroindustrial wastes, which include high phytate concentration, were used in the fermentation media as phytate sources for phytase productions in several studies (Lan et al., 2002; Mittal et al., 2011). Only study that available about optimization of $CaSO_4.2H_2O$ concentration for phytase production was made by Liu and co-workers (2011), and they reported the optimum concentration as 1.03 g l^{-1} in the phytase fermentation medium for *Pichia pastoris*, which is very close to our results.

2.3. Response surface model

ANOVA table (Table 2) and a second order polynomial equation (Equation 1) were created by MINITAB software to show the effects of glucose, Na-phytate, and $CaSO_4$ on batch phytase production with *A. ficuum*.

$$\text{Phytase activity (U ml}^{-1}) = -2.16292 + 0.04224 \times (G) + 0.22817 \times (P) + 2.45581 \times (C) - 0.00013 \times (G \times G) - 0.00687 \times (P \times P) - 0.91453 \times (C \times C) - 0.00031 \times (G \times P) - 0.00397 \times (G \times C) + 0.00392 \times (P \times C) \quad (1)$$

where "G" is glucose, "P" is Na-phytate, and "C" is $CaSO_4$ concentrations.

Regression coefficient (R^2) was determined as 0.9622 for the model, whereas R^2_{adj} was given as 0.8942. To show the good fit, experimental and predicted values were plotted (not shown) and the slope of the best fitted line was determined as 0.9645, which is very close to "1". Additionally, RMSE and MAE values were calculated as 0.095 and 0.075, respectively, which are very low and show the model represents the process successfully. Additionally,

ANOVA showed that glucose, Na-phytate, and $CaSO_4$ concentrations are all have significant effects on phytase activity (Table 2). Among the main effects, $CaSO_4$ had the most effect among the medium ingredients on phytase activity with the lowest P-value by 0.005. Application of optimization in MINITAB suggested that the maximum phytase activity can be obtained as 3.45 U ml^{-1}, if the fermentation is performed with 126 g l^{-1} of glucose, 14 g l^{-1} of Na-phytate, and 1.1 g l^{-1} of $CaSO_4$ concentration under the evaluated conditions.

Table 2. ANOVA table for phytase production by *A. ficuum* in submerged fermentation bioreactor

Terms	Coefficients	Standard error coefficient	P-values
Constant	–2.16292	0.959119	0.0074
Glucose	0.04224	0.011035	0.012
Na-phytate	0.22817	0.053086	0.008
$CaSO_4$	2.45581	0.502154	0.005
Glucose × Glucose	–0.00013	0.000035	0.013
Na-phytate × Na-phytate	–0.00687	0.001552	0.007
$CaSO_4$× $CaSO_4$	–0.91453	0.188392	0.005
Glucose × Na-phytate	–0.00031	0.000224	0.220
Glucose × $CaSO_4$	–0.00397	0.002464	0.168
Na-phytate × $CaSO_4$	0.00392	0.016428	0.821

Figure 3 shows phytase activity trends by varying medium composition. These graphs especially help to design economic fermentation media, which is an important concern especially at industrial scales. Phytase was produced with high activity between 100–150 g l^{-1} of glucose concentrations. However, activity decreased remarkably above 150 g l^{-1} of glucose concentration (Fig. 3A). Figure 3B shows that phytase activity was increased by adding Na-phytate into the fermentation medium until around 15 g l^{-1}. Above this level, the phytase activity continuously decreased. Therefore, 14 g l^{-1} of phytate was determined as optimum phytate concentration. However, it can be seen in Figure 3B that phytase can still be produced around 3 U ml^{-1} even when 5 g l^{-1} phytate and more than 1 g l^{-1} $CaSO_4$ were used in the fermentation medium. Figure 3C shows the effect of $CaSO_4$ on phytase activity. Above 1.1 g l^{-1} of $CaSO_4$, phytase activity stays the same. Therefore, there is no need to use $CaSO_4$ above 1.1 g l^{-1} in the fermentation medium.

2.4. Validation of the optimum conditions

The model was validated under the optimized conditions. Batch fermentations were conducted in triplicate at the determined optimum conditions, which were 126 g l^{-1} of glucose, 14 g l^{-1} of Na-phytate, and 1.1 g l^{-1} of $CaSO_4$ in the fermentation medium. Under these conditions, the highest phytase activity was measured as 3.39±0.10 U ml^{-1} (Fig. 4), which was very similar compared to the predicted value from the model, which clearly demonstrated the model represents the fermentation successfully. Additionally, maximum glucose consumption rate and maximum phytase activity production rate were calculated as 0.70 g glucose ml^{-1} h^{-1} and 0.0426 U ml^{-1} h^{-1}, respectively, under optimized conditions, which were both around 2-fold more compared to base medium production results (Coban & Demirci, 2014).

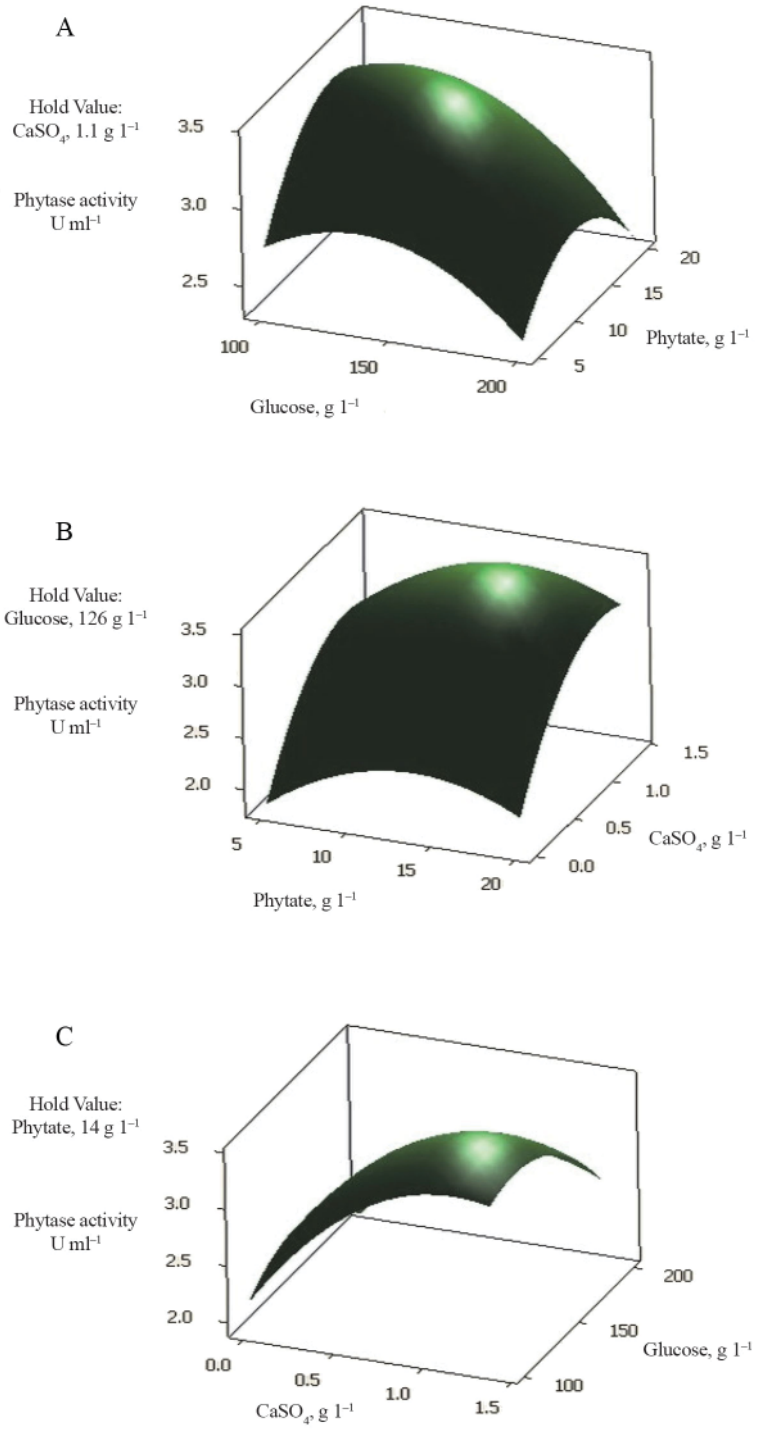

Fig. 3. Phytase activity profiles with various medium compositions

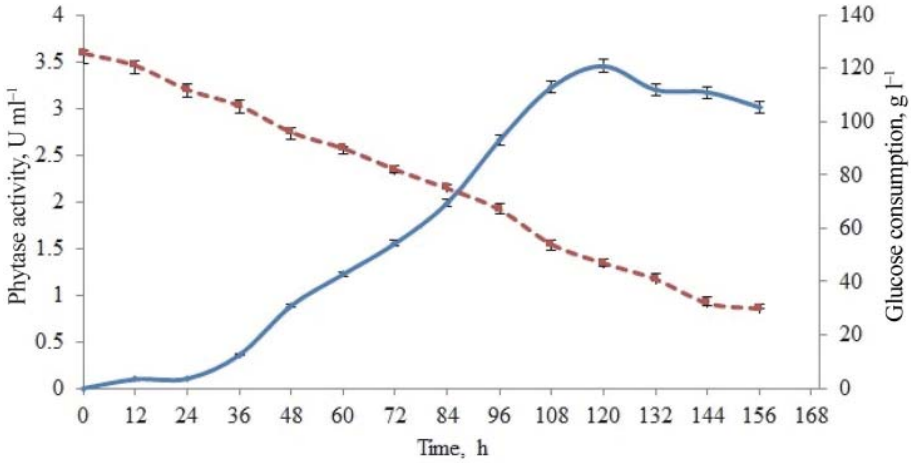

Fig. 4. Glucose consumption and phytase activity under optimum conditions
Phytase activity: —◆—; Glucose consumption: – ■ –

2.5. Effect of storage temperature on phytase activity

Effect of various storage temperatures on phytase activity was studied and results were shown in Fig. 5. The lowest phytase activity loss was observed under 4 °C during the first week of the storage. Phytase activity decreased from 3.45 to 3.32 U ml^{-1} (3.76% loss) after 7 days storage at 4 °C. However, after 7 days, it was observed that samples that were stored at

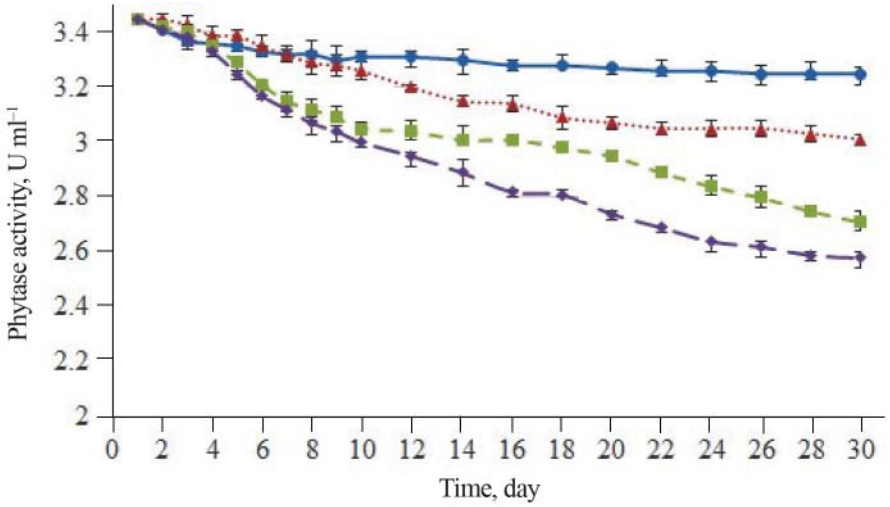

Fig. 5. Effect of storage temperature on *A. ficuum* phytase activity
–20 ºC: —■—; 4 ºC: · · ▲ · ·; 25 ºC: – ■ –; 33 ºC: – ◆ –

–20 °C rather than 4 °C retained their activity more. Therefore, it is concluded that samples that will be used in one week must be stored at 4 °C, and –20 °C conditions should be preferred for further storage periods. The reason that more activity losses were measured during the first week of storage in the samples stored at –20 °C, may be the negative effect of occurrence of ice crystals in the vial. These ice crystals may damage enzyme structure partially and decrease the activity. Further microbial growth and activity in the sample vials might be a reason for high activity loss in these conditions. After 30 days of storage at –20, 4, 25, and 33 °C phytase activities were measured as 3.25, 3.01, 2.71, and 2.58 U ml^{-1}, respectively.

3. Conclusions

In conclusion, this study showed that optimum pH and temperature were 5.5 and 55 °C for *A. ficuum* phytase. Also, 126 g l^{-1} of glucose, 14 g l^{-1} of Na-phytate, and 1.1 g l^{-1} of $CaSO_4$ were determined as optimum concentrations in the fermentation medium composition. Under these conditions, phytase activity increased to 3.45 from 2.27 U ml^{-1}, which is about 50% increase compared to the base fermentation medium results. A mathematical model created by MINITAB successfully represented the process, can be used for future studies and scale-up purposes. Also, it was determined that pH 5.5 and 55 °C are the optimum conditions for the produced *A. ficuum* phytase activity. Additionally, it was shown that 4 °C was the best condition for less than 1 week storage of *A. ficuum* phytase.

Overall, this study shows that fungal phytase can be produced in submerged fermentation successfully and enzyme activity can be further increased by improving the media and process conditions.

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