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Evaluation of some Basidiomycetes fruit bodies and cultivation conditions of most efficient fungus, *Pleurotus ostreatus* for phytase production

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Pleurotus ostreatus, phytase, supplementation, Basidiomycets.

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ABSTRACT

Among ten mushroom species, the local isolate ,*Pleurotus ostreatus* (11L) was superior in production of endo-phytase which produce 0.66 unit/ml compare to other mushrooms ranged between 0.16 and 0.61 unit/ml. The optimum conditions for highest phytase activity from *P. ostreatus* (11L) were studied, wheat straw was the best substrate which gave highest Biological efficiency (BE) and phytase activity resulting in 71.07% and 0.63 unit/ml, respectively. The optimum spawn rates were 4 and 5% of the basis substrate dry weight, resulting in 0.68 unit/ml for each rates. the optimum temperature for fruit bodies development and phytase activity was 17℃ with highest activity which was 0.71 u/ml and decrease in phytase activity at 21 and 23℃ which reached to 0.57 and 0.51unit/ml, respectively. Biological efficiency of *P. ostreatus* (11L) and phytase activity was increased when wheat straw supplemented with (3%) of wheat bran which was 81.38% and 0.81unit/ml compared to71.07% and 0.63 unit/ml in control, respectively. In addition to these conditions phytase activity increased to 0.88 unit/ml when fruit bodies of *P. ostreatus* harvested at mature stage (8 days from the pinning stage).

تقييم الاجسام الثمرية لبعض الفطريات البازيدية والظروف الزراعية للفطر الاكفأ *Pleurotus ostreatus* لانتاج انزيم الفايتيز

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الكلمات المفتاحية :
Pleurotus ostreatus ، انزيم الفايتيز، اضافات غذائية، فطريات بازيدية .

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الخلاصــة

اظهرت العزلة المحلية للفطر *Pleurotus ostreatus* (11L) من بين عشرة انواع من الفطريات تفوقها بانتاج الفايتيز الداخل خلوي اذ بلغت الفعالية الانزيمية 0.66 وحدة/مل مقارنة بمدى 0.16−0.61 وحدة/مل للفطريات الاخرى. درست الظروف المثلى لانتاج اعلى فعالية انزيمية من الفطر *P.ostreatus* (11L)، ابدى وسط تبن الحنطة اعلى انتاجية للفطر(كفاءة احيائية) واعلى فعالية انزيمية للاجسام الثمرية النامية فيه اذ بلغت 71.07% و 0.63 وحدة/مل، على التوالي. وسجل معدل اللقاح الفطري عند 4 و5% اعلى فعالية انزيمية بلغت لكليهما 0.68 وحدة/مل فيما كانت الدرجة الحرارية 17 م^0 المثلى لنمو ثمار الفطر وتسجيلها لاعلى فعالية انزيمية بلغت 0.71 وحدة/مل ثم انخفضت الى 0.57 و0.51 وحدة/مل عند درجتي 21 و23م على التوالي. ارتفعت الكفاءة الاحيائية والفعالية الانزيمية عند تدعيم وسط تبن الحنطة بـ 3% من نخالة الحنطة اذ بلغتا 81.38% و0.81 وحدة /مل ، مقارنة بـ 71.07% و 0.63 وحدة/مل على التوالي ، فضلا عن تلك الظروف فقد ارتفعت الفعالية الانزيمية الى 0.88 وحدة/مل عند حصاد الاجسام الثمرية عند مرحلة النضج (بعد 8 ايام من مرحلة التبرعم).

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INTRODUCTION

Phytate (*myo*-inositol hexakisphosphate) is the predominant form of storage of phosphorus in cereals, oilseeds and vegetables which are the major ingredients of animal feed (Reddy *et al*., 1982; Wodzinski and Ullah, 1996). Monogastric animals such as swine, poultry and fish are unable to utilize phytate due to the low levels of phytate-degrading enzyme activity in their digestive tracts and inorganic phosphate is added to the feed for the purpose of phosphorous supplementation (Bitar and Reinhold, 1972; Common, 1989). Hence non-metabolized phytate and unabsorbed phosphate pass through the intestinal tract and are excreted in manure causing phosphorous eutrophication. Furthermore, phytate also acts as an antinutritional factor in monogastric animals by chelating proteins and various metal ions needed by the animal, such as calcium, copper, zinc … etc. therefore decreasing the dietary availability of these nutrients (Applegate *et al*., 2003; Bohn *et al*., 2008; Veum *et al*., 2006). Phytase (*myo*-inositol hexaphosphate phosphohydrolase, EC 3.1.3.8 or EC 3.1.3.2.6) hydrolyzes phytate, thereby releasing inorganic phosphate (Liu *et al*., 1998; Wodzinski and Ullah, 1996). Supplemental microbial phytase in meal diets for swine, poultry and fish effectively improves phytate phosphorus utilization by these animals, The phytases enhances the bioavailibility of minerals, protein and phosphorus in monogastric animals decreasing minerals excretion pollution (Augspurger *et al*., 2003; Rutherfurd *etal*., 2002; Olukosi *et al*., 2007). In addition to animal feed, phytase was used in many industrial applications such as, food industry, preparation of myo-inositol phosphate, detoxified agent, Paper industry, soil improvement and the elimination of environmental pollution.(Soni, 2009). Phytase as exo-enzyme was purified from many filamentous fungi including especially *Aspergillus niger*, *A. terreus*, *A. oryzae, Neurospora crassa , Emericella nidulans* and *thermomyces lanuginosus*. In addition from several yeast species such as *Saccharomyces cerviceia*, *Candida tropicalis , Kluveromyces fragilis, Torulopsis candida, Debaryomyces castelli and Schwanniomyce scastelli*, but the first commercial phytase was produced from *Aspergillus niger* (Nayini and Markakiz, 1984, Greiner and Konietzny, 2006 , Zhou, *et al*., 2006). All these organisms produced phytase with submerged cultures using high cost fermenters, while other researches focused on the type of fungi called Mushrooms for production of phytase as endo-enzyme due to low cost- mushrooms cultivation technology. Phytase was purified and characterized from a few types of mushrooms, these mushrooms were edible and cultured, included *Agrocybe pediades*, *Cenporia sp., Peniophora lycii, Trametes pubenscence* and *Agaricus bisporus (*Lassen, *et al*., 2001; Collopy and Royse,2004), *Lentenus edodes*(Zhang, *et al*., 2013), *Volvariella volvacea* (Xu*, et al.,* 2011) and *Flammulina velotipes*(Zhu, *et al*., 2011). Whereas there was no any study in the country for the production, purification and characterization of phytase from fungi. In addition low cost mushrooms cultivation, edible mushrooms were a safe food source that didn't contain any toxin. For these reasons, the present study was achieved which aimed to:

1. Evaluate some edible mushrooms species for their phytase activity and select the more efficient one.
2. Optimize of solid state fermentation conditions for highest phytase activity.

MATERIALS AND METHODS

Mushroom Species

Number of local and imported Basidiomycetes and Ascomycetes were listed in table (1). The origin of *Agaricus bisporus* B62 was Le lion varrains, company France, while the origin of *Lentinus edodes* and *Pleurotus ostreatus*(White oyster-Whi), was Mushroom Box Company united kingdom. Other mushroom species were collected from local Iraqi environment. All mushroom species were provided from the mushroom of research unit in Tikrit university.

Table(1) mushroom species used in the present study

Mushroom species	Source	Origin of strain
Agaricus bisporus B62	Mushroom Research Unit/Tikrit University	Le lion Varrains ,company France
A. campestris	Mushroom Research Unit/Tikrit University	Local isolate
Coprinus comatus	Mushroom Research Unit/Tikrit University	Local isolate
Ganoderma lucidium	Mushroom Research Unit/Tikrit University	Local isolate
Lentinus edodes	Mushroom Research Unit/Tikrit University	Mushroom Box company,UK.
Pleurotus ostreatus (White oyster-Whi)	Mushroom Research Unit/Tikrit University	Mushroom Box company,UK.
Pleurotus ostreatus	Mushroom Research Unit/Tikrit University	Local isolate
Polyporus sp.	Mushroom Research Unit/Tikrit University	Local isolate
Terfezia claveryi (Black truffle)	Local market	Local isolate
T. hafizi(white truffle)	Local market	Local isolate

Phytase Extraction

100g of fruit bodies from each mushroom species were blended with ammonium carbonate mono hydrate NH_4HCO_3 buffer pH (8) with motor and pestle then filtrated and centrifuged at 5000 rpm for 25 min then the clear solution (supernatant) was collected as crude enzyme.

Phytase Assay

Assay of phytase activity was measured by incubating of enzyme extract solution (0.1 ml) with 0.9 ml of 2mM Sodium phytate in 0.1 M Tris-HCl buffer (pH 7.0).The enzyme reaction was carried out at 37^0 C for 15 min, the reaction terminated by add 0.75 ml 5% trichloro acetic acid. The released phosphate was measured at 700 nm after adding 1 ml of color reagent, then the phytase activity calculated depending on phosphate standard curve. One unit of phytase activity was defined as the amount of enzyme needed to liberate 1 µmol of phosphate per min under the assay condition. The protein concentration in enzyme solution was measured too (Xu *et al*., 2011) .

Phosphate Standard Curve

A Stock solution of potassium phosphate di hydrate was prepared by dissolving 0.22g in 500ml sterilized D.W to obtain final concentration 100µg/ml. Serial concentrations of KH_2PO_4 (0-20 µg/ml) were prepared from the stock solution by dilution with D.W .One ml of color reagent was added to 1ml of each concentration of KH_2PO_4 . The blank consisted of 1ml D.W and 1ml of Color reagent ; the tubes were incubated at 37^0 C for 15 min, the reaction terminated by addition of 0.75 ml 5% trichloroacetic acid. The optical density of the solutions were measured at 700nm after adding 1 ml of color reagent. The standard curve was plotted absorbance values against KH_2PO_4 concentration. Fig. (1) .

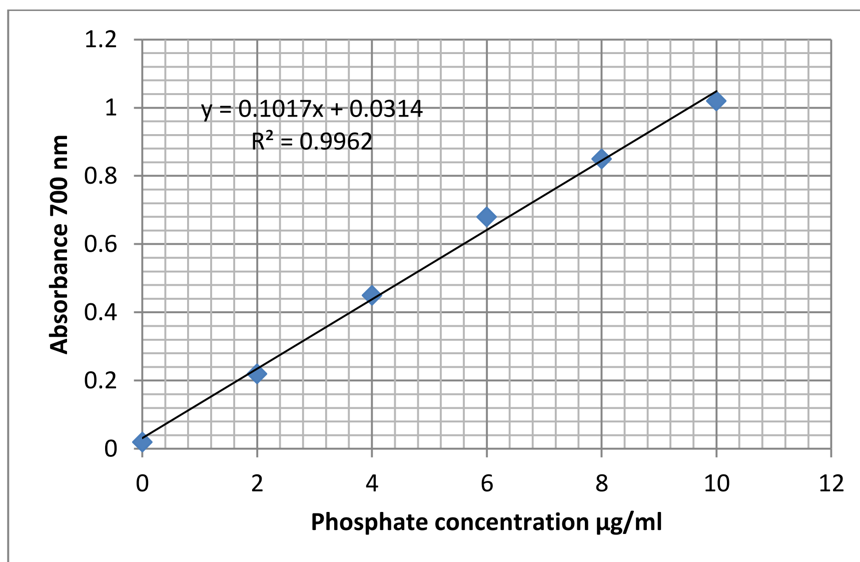


Figure (1): Phosphate standard curve

Assay of Protein Concentration

Protein concentration and the standard curve were determined as the method described by (Bradford, 1976).

The specific activity was calculated as the equation below (Berg, *et al*., 2002).

Specific activity(U/mg protein) = Enzyme activity(U/ml)/protein concentration (mg/ml)

Activation of Mushroom Species

After selecting the most efficient fungus for its ability in highest phytase activity, *P. ostreatus* (11L)was activated by transmitted part of the mother culture, to the fresh PDA by the cork porer, and incubated at 25°C for 7 days .

Preparation of *Pleurotus ostereatus* (L11) Spawn

Modified Elliot's method was used to *P. ostreatus* spawn wheat grains which were cooked for one hour. The excess water was then drained off, 2% calcium sulfate and 5% calcium carbonate was mixed with cooked grains, Flasks(500ml volume) were filled with 200g grains/flasks, and plugged with cotton wool. flasks with their contents were sterilized in an autoclave at 121°C, pressure 15 pound/ench^2 for one hour after cooling grains were inoculated with active *P. ostreatus* culture by transferring a pies of 0.5 cm diameter by cork porer. The flasks were incubated at 25°C for 7days and shaked every 48 hours in order not to be accumulated (Hassan, *et al*., 1996).

Cultivation of *P. ostreatus* (11L)

Wheat straw was wetted overnight in water. The excess water then drained off and pasteurized at 70^{0}c for 6 hours, after cooling, wheat straw was inoculated with spawn at a rate of 2%. The mixture was filled in polyethylene bags (30×50cm) and incubated at 25^{0}c, for fruit bodies formation. After 21days (or after full growth of mycelium), temperature lowered at 16^{0}c with raising the humidity to 90% and applying photo cycle for 6 hours (light, darkness) by normal fluorescent tube which had cool white light. After fruit bodies were appeared the period time for appearing fruit bodies, protein concentration in fruit bodies and biological efficiency were determined :

Biological efficiency =Wt. of fresh fruit bodies / Wt. of dry medium (substrate)×100% (Hassan, 1997).

Determination of Protein Content in Fruit Bodies

Protein content was determined according to kjeldahl's method (A.O.A.C. , 2004).

Determenation of Optimal Conditions for Phytese Production They include screening media, inoculation rate, organic a supplementation type (addition of meals and brans) rate of supplementation, fruiting temperature, pH of production media , and the maturity of fruit bodies.

Evaluation of Various Substrates for Phytase Production

Different solid state culture media were used for *P. ostreatus* growth and phytase production including (Wheat straw, Barley straw ,Wild Reed residues , and Corn cobs) These substrates were cultivated separately by putting them in water for 12 hours. Fruit bodies were produced as the above description. The incubated period, protein concentrations, and biological efficiency were determined.

Spawn Rate

The best substrate (wheat straw) was inoculated with different rates of spawn (1,2,3,4,5)% in three replicates after the cultivation and fruit bodies production, the phytase was extracted and its activity was measured.

Organic Supplementation

Organic supplementation includes, wheat bran , Corn powder, Wheat powder ,barley powder, and Soy bean powder, which were mixed with wheat straw at 2% then pasteurized and inoculated. After producing fruiting body, the enzyme was extracted and its activity was measured.

Supplementation rate

Different rates of best supplementation (wheat bran) was mixed at (1,2,3,4, and 5%) with wheat straw then cultivated, and enzyme estimated.

Effect of Temperature

The effect of temperature was studied by incubating the inoculated production medium with spawn of *P. ostreatus* (11L) at (17,19,21,23)°C In each temperature, three replicates media were studied. After incubation period (21) days, The enzyme was extracted and its activity was estimated.

Effect of pH

The production medium of *P. ostreatus* (11L) was made by different values of pH (4-8) by wetting it with different buffers (Chang and Miles, 2004). The media were inoculated with *P. ostreatus* (11L). After extracting the enzyme, its activity was estimated.

Evaluation of Fruitbodies Maturity

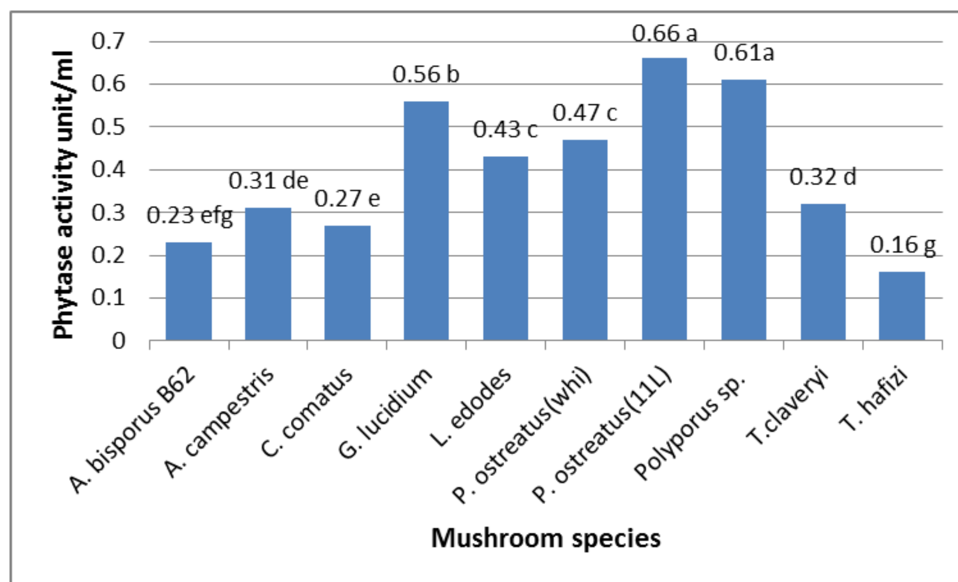
Phytase activity mature fruitbodies (8) days from the pinning stage, and non-mature fruitbodies (4) days from the pinning stage were determined.

RESULTS AND DISCUSSIONS

Screening of Mushroom species for producing phytase

Figure(2) showed that fungus *P. ostreatus* (11L) superiority in production of phytase (according to Duncans multiple range tests in the probability <0.01) which produces 0.66u/ml, followed by the fungus *Polyporus sp*. which produces 0.61unit/ml (without significant differences between them). The lowest phytase activity was 0.16 unit/ml in *T. hafizi* , whereas the phytase activity in other mushrooms ranged between 0.23 and 0.56 unit/ml, according to these results, *P. ostreatus* was selected for other phytase optimization experiments. Similar studies recorded difference in phytase activity depending on each of the fungal species (Muhammad *et al*., 2010; vihudas, *et al*., 2012). Fungi are different in their genotype, thus phytase as any biological product is encoded by some genes. So fungi that have the phytase genes are encoded whereas those that do not have its genes do not produce it. It is noticed from the results that all the studied mushrooms produced the enzyme. Thus all of them have enzyme encoding genes but the genes expression differs according to the type of fungi. It is noticed that the highest genes expression was in *P. ostreatus*(11L), *polyporus sp.*, and *G. leucidium*. It can also be attributed to the difference in phytase production from the studied

fungi to their fruit bodies production conditions such as medium, pH, temperature, …etc in addition to nutrition ingredients of these media.



Figure(2) Evaluation of some mushrooms species for phytase

Determination of optimum conditions for phytase production

Solid media(substrates)

Five production media (substrates) phytase production, were tested in an attempt to improve the phytase production from *P.ostreatus* (11L) under solid-state cultivation included; Wheat straw, Corn straw, Rice hulls, Wild reed, Barley straw, Table(2)showed the effect of these substrates and their protein content (%), on time of fruiting, biological efficiency (%), and phytase activity, the results showed the earlier fruiting of *P. ostreatus*(11L) was 24 day(from spawning) when cultivated in Wheat straw, Corn straw, Wiled reed, and Barley straw, maximum protein content of Wheat straw andCorn straw was 0.72, 0.70% whereas the lowest protein content was 0.51% in Wild reed, and Barley straw, the productivity of fruit bodies of *P.ostreatus*(11L) determined as biological efficiency was superior in fruit bodies raised from Wheat straw resulting in 71.07%, Minimum Biological efficiency was 66% in fruit bodies grown on Rice hulls, the highest phytase activity was 0.63 unit/ml in fruit bodies raised from Wheat straw followed by Corn straw 0.60u/ml. (without significant differences between them). Whereas the lowest phytase activity was 0.43unit/ml in *P. ostreatus*(11L) fruit bodies grown on Wild reed. Wheat straw medium was given highest biological efficiency and highest enzyme activity, and this was proved that the culture media compounds play important role in fungus growth and enzyme production. Substrates are different in chemical composition e.g. proteins, carbohydrates, lipids, and minerals….etc. Substrates tested in this study were basically carbon sources and it's the preferred in commercial production of *P. ostreatus* (Hassan, *et al.*, 1996). Thus they are also different contents of cellulose, hemicellulose, and lignin, according to this, variation was reflected on the time of the appearance of the fruit bodies, Biological efficiency, and amount of production enzyme, according to this results, Wheat straw was the best medium not only in developing the fruit bodies with highest phytase activity but productivity (Biological efficiency) superior on the rest substrates as increasing productivity of mushroom fruit bodies means that there is a larger amount of enzyme production.

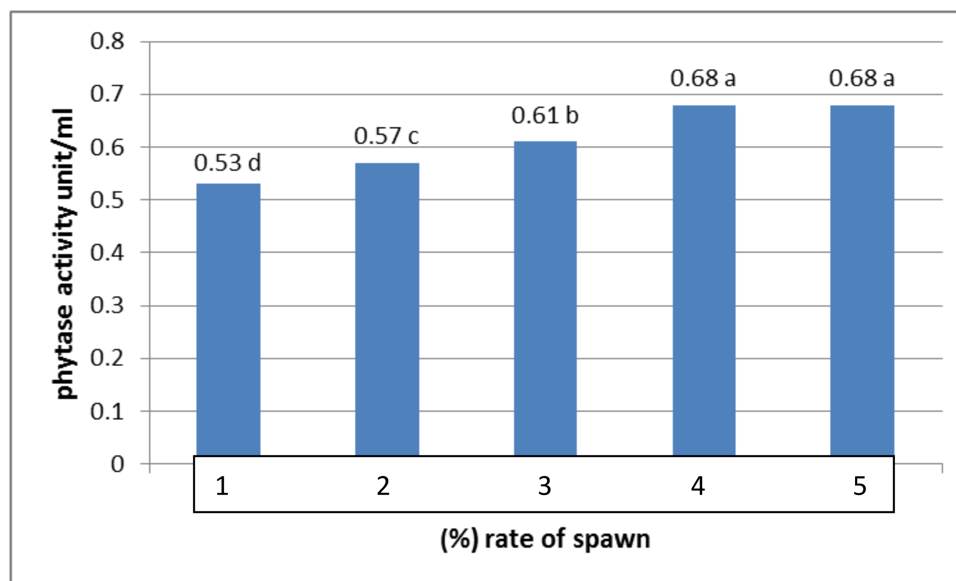
Table (2)Evaluation of different media(substrates) for time of fruitbodies production, biological efficiency and phytase activity of *P. ostreatus* (11L)

Substrate	Protein content (%)	Time of fruit bodies production(days)	biological efficiency(%)	Phytase activity(unit/ml)
Wheatstraw	0.72 a	24 b	71.07 a	0.63 a
Corn straw	0.70 a	24 b	68.12 b	0.60 a
Rice hulls	0.64 c	26 a	66.0 c	0.51 b
Wild Reed	0.51 b	24 a	68.42 b	0.43 c
Barley stra	0.51 d	24 b	68.0 b	0.50 b

•Values within column that not sharing the same common letters are significantly different at P> 0.05 as determined by Duncan multiple range test.

The optimum rate of spawn

The effect of different spawn rates (1, 2, 3, 4 and 5%) on phytase activity were studied and the results in fig. (3) showed that the optimum spawn rates were 4 and 5% of the basis substrate dry weight, resulting in 0.68 unit/ml for each rates, followed by the spawn rate 3%, in which phytase activity was 0.61 unit/ml. Spawn rate was affected on speed of mycelium growth and utilized nutrients from substrate in high spawn rate, mycelium growth and nutrient utilization were faster than low spawn rate. Thus, full growth substrate in mycelium gave full growth substrate in fruit bodies. Full growth substrate in mycelium was achieved at 4% spawn rate so any increase in spawn rate isn't affected on formation of fruit bodies and phytase activity.



Figure(3) effect of spawn rate of *P. ostreatus* (p. 11) grown on wheat straw on phytase activity

Optimum temperature for phytase production

The effect of *P. ostreatus*(11L) fruiting temperature on phytase activity was studiedin the temperature range of 17-23℃, the results in fig.(4) showed increase in phytase activity with decrease of fruiting temperature, the optimum temperature for fruitbodies development and phytase activity was 17℃ with highest activity which was 0.71 unit/ml and decrease in phytase activity at 21 and 23℃ which reached to 0.57 and 0.51unit/ml, respectively. Temperature is one of the most critical parameters to be controlled in any bioprocess (Sasirekha, 2012).

All the metabolism processes for producing any biological product are affected by temperature for this reason, and according to the results, it seems that the highest production of phytase from the fungus *P. ostreatus* with low temperature (less than 20^{0}C). We think that the pathway of producing

this enzyme is committed with the catalysis of fruiting production. Previous studies (Chang and Miles, 2004; Hassan, 1996) recorded that the best catalysis for producing fruitbodies was between 16-19^{0}C.

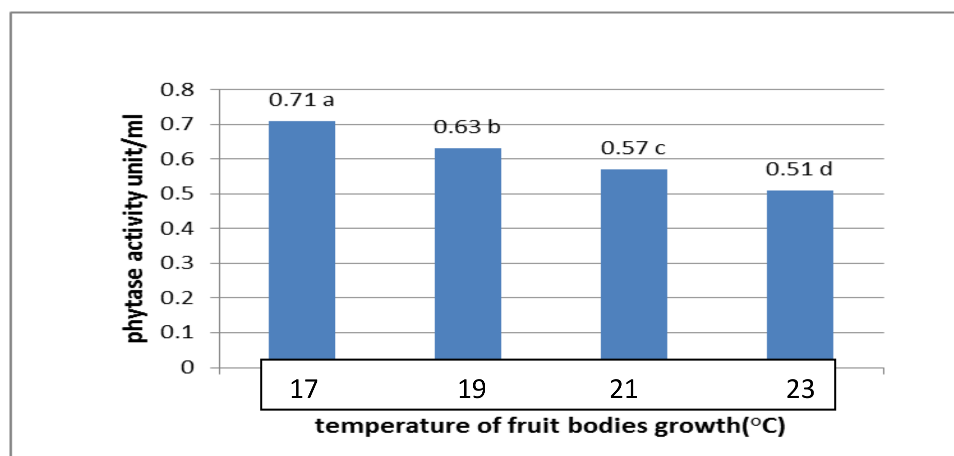


Fig. (4) Effect of temperature on phytase production from *P. ostreatus*(11L)

Effect of supplements on culture media for phytase production

The impact of number of supplementation on phytase enzyme activity which was an addition to the culture substrates (Wheat bran, Corn powder, Wheat powder, Barley powder and Soy bean powder) with rate of 2% of the dry weight of the substrate. Table(4)showed the effect of adding these organic materials and their protein content (%) in the time of fruitbodies production, Biological efficiency and phytase activity**.** Time of Fruit bodies production was earlier (22days) when adding the wheat bran as a supplement followed by the addition of Corn powder and Wheat powder resulting in 24 days, compared with control (without any addition) which was 27days. This can be attributed to the nature of the supplement and its contents that if there are some complicated materials , there is a need for more time to decompose them and to get benefit of mushroom. Wheat bran and soy bean powder showed highest Biological efficiency which were 76.76 and 74.42 %, respectively, (without significant differences between both treatments) table(4). The results showed the optimum activity of enzyme in wheat straw supplemented with wheat bran with activity of 0.74 unit/ml, followed by the addition of soy bean powder with activity 0.73 unit/ml (without significant differences between both treatments). The lowest phytase activity was 0.42u/ml with the addition of wheat powder whereas the phytase activity in the other organic materials (corn powder and Barley powder) were 0.67 and 0.62 unit/ml respectively. The results showed no relationship between protein content of supplemented substrate (wheat straw) with phytase activity, the protein content was higher (9.82 and 5.78%) in present of Soybean powder and Corn powder than Wheat bran (5.4%) but phytase activity in Wheat bran was higher than Soybean and Corn powder table (4) according to these results, wheat bran was selected for other phytase experiments. The addition of wheat bran in this study was a significant effect to increase the phytase enzyme production which is perhaps the causes attributed the fact that this protein source suitable as a catalyst for production of enzyme from the fungus *P. ostreatus* and this result is consistent with studies demonstrated the production of phytase enzyme from some fungi in supplemented media.This result was agree with(Muhammad, 2010)used many product as a Different carbon sources like wheat bran, rice husk, rice bran, fish meal, corn seed and corn gluten were explored for the production of phytase from thermophilic fungus *Sporotrichum thermophile* and found the wheat bran as carbon source are best as compared to other carbon sources used which gave 2.21 unit/ml of phytase .

table(4) Effect of addition supplements in wheat straw on phytase production from *P. ostreatus* (11L)

Supplementation Type(2%)	Protein content(%)	Time of Fruit bodies production (days)	Biological Efficiency (%)	Phytase activity (unit/ml)
Wheat bran	5.4 bc	22 c	76.76 a	0.74 a
Corn powder	5.78 b	24 b	71.30 b	0.67 b
Wheat powder	5.31 bc	24 b	69.16 b	0.65 bc
Barley powder	4.06 c	26 a	65.50 c	0.64 c
Soy bean powder	9.82 a	27 a	74.42 a	0.73 a
Control	0.72 d	24 b	71.07 b	0.63 c

• Values within column that not sharing the same common letters are significantly different at $P<0.05$ as determined by Duncan multiple range test.

Optimum rate of wheat bran for phytase activity

In studying the effect of the addition the supplement wheat bran in different rates after to maintain that it's the best supplement to producing phytase from fungus *P. ostreatus* (11L) and table(5)showed that time of fruit bodies production was later in the high wheat bran rates, resulting in 24 days in 5% and 23days in both rates 3 and 4%, in low wheat bran rates (1 and 2%) time of fruit bodies production was earlier than other rates that take off 22 days. Results also showed the biological efficiency was 81.38 , 82.5 and 82.5% at the rates 3,4 and 5% , respectively, without significant differences among these values. Phytase activity in all tested wheat bran rates was superior on control as shown in table(5) the best phytase activity with (3%) of wheat bran which was 0.81u/ml and followed by the rate (4,5 and 2%) which enzyme activity was 0.78,0.76 and 0.74unit/ml, respectively (without significant differences among these values).Protein content in wheat straw increased with the increase of wheat bran rates, biological efficiency was also increased with the increasing of wheat bran rates until a rate of 4%, whereas phytase activity was increased with the increase of wheat bran rates until a rate of 3%.It might be due to the reason that wheat bran provided adequate amounts of nutrients like carbohydrates, proteins, fats, calcium, phosphorus, potassium and amino acids. These nutrients are necessary for the adequate production of phytase. These findings are of particular importance because the use of agricultural residues are more feasible than that of purified substrates(Lynd, *et al*., 2002). Results agree with (Vihudas, *et al*., 2012)who found the of seven thermophilic fungal strains screened for phytase production, *Sporotrichum thermophile* was found to produce higher extracellular phytase when grown on solid state of wheat bran. Muhammed, *et al*., (2010) found that the wheat bran as carbon source are the best as compared to other carbon sources. It is noticed from the results that the increase of Wheat bran leads to an increase in phytase activity up to 4% or more as the enzyme is decreased. This can be attributed to the idea that for each organism there are certain nutrient levels that it can use them to grow and get energy. If there is a larger quantity of these nutrients the fungus will not be able to consumpted it because of its limited enzyme system to get benefit of these nutrients. There is no relationship between protein concentration produced by adding various rates of Wheat bran and the quantity of the enzyme can be attributed to protein type and its content from amino acids as well as the benefit of these proteins in adding other biological factors especially the main extracellular enzymes for fungus growth like cellulase, hemicellulose, ligase, phenol oxidase, protase, and lipase (Wood, 1985; Daniel, 2014).

Table(5)Effect of various wheat bran rates on phytaseproduction from *P. ostreatus* (11L)

Wheat bran (%)	Protein content(%)	Time of Fruit bodies production (days)	Biological Efficiency (%)	Phytase activity (unit/ml)
0	0.72 d	24 a	71.07 d	0.63 d
1	3.88 e	22 c	73.30 c	0.66 c
2	5.43 d	22 c	76.76 b	0.74 b
3	6.38 c	23 b	81.38 a	0.81 a
4	8.65 b	23 b	82.50 a	0.78 b
5	10.21 a	24 a	82.50 a	0.76 b

• **Values within column that not sharing the same common letters are significantly different at P<0.05 as determined by Duncan multiplerange test.**

Effect of fruit bodies maturity stages of *P. ostreatus*(11L) on phytase enzyme production.

Fig (4) showed that the highest phytase activity was 0.88 unit/ml in mature fruit bodies of *P. ostreatus* (11L) (harvested at 8 days from the pinning stage)compared with 0.67unit/ml in immature fruit bodies (harvested at 4 days from the pinning stage) fig (4).

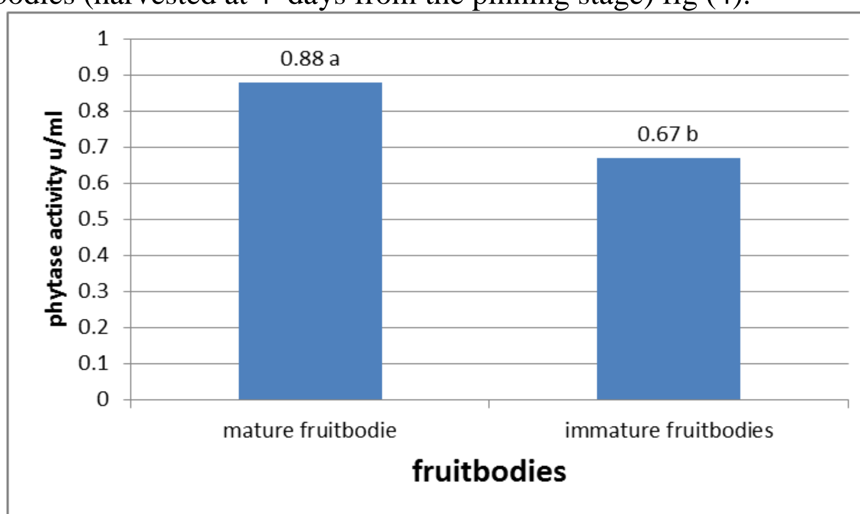


Fig.(4) Effect of fruit bodies maturity stages of *P. ostreatus*(11L) on phytase enzyme production

Evaluation of Fruitbodies Maturity

Phytase activity mature fruitbodies (8) days from the pinning stage, and non-mature fruitbodies (4) days from the pinning stage were determined.

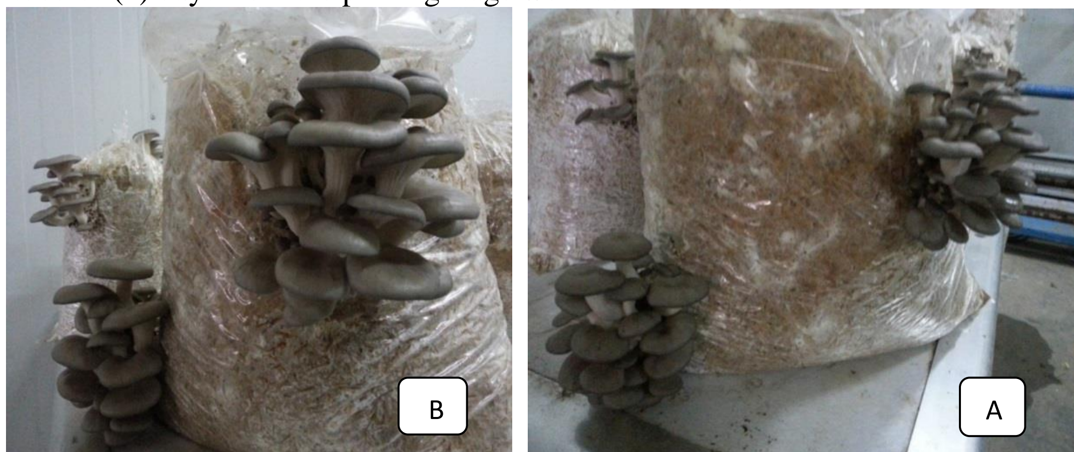


Fig.(5) Fruitbodies of *P. ostreatus* (P. 11) ,Immature fruit bodies (4 days from the pinning stage)-A, mature fruit bodies (8 days from the pinning stage)-B.

The reason of the differences in increasing phytase activity in mature fruit bodies can be attributed to period of phytase biosynthesis inside fungus cells. The complete synthesis of phytase in mature fruitbodies may be regarded as defence factor against infections of pathogenic agents like fungi, Bacteria, nematodes..etc. Some mushrooms enzymes such as ribonucleases (Wang and Ng, 2000), deoxyribonuclease (Wang and Ng, 2001), chitinases, glucanase and peroxidase (Ye and Ng, 2002), have antimicrobial and antifungal activity. This is supported by the study of (Zhu, *et al*., 2011) proves that the phytase inhibits the fungi growth that causes the infection to mushroom *f. velutipes*.

REFERENCES

AOAC (2004). Official method of Analysis of the Association of official Analytical chemists. 15th Ed., Washington. USA.

Applegate, T. J., Webel D. M., and Lei X. G. (2003). Efficacy of a phytase derived from *Escherichia coli* and expressed in yeast on phosphorus utilization and bone mineralization in turkey poults. Poult. Sci. 82:1726 –1732.

Augspurger, N. R., Webel D. M., Lei X. G., and Baker D. H. (2003). Efficacy of an E. coli phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. J. Anim. Sci. 81:474-483.

Berg, J. M. ; Tymoczk, J. K. and Stryer, L. (2002). Biochemistry. (5th Ed.) pP:196-219. W. H. Freeman and Company. New York.

Bitar K. Reinhold J.G. (1972). Phytase and alkaline phosphatase activities in intestinal mucosae of rat, chicken, calf and man. Biochim, Biophys. Acta ; 268: 442-452.

Bohn L, Meyer A. S. and Rasussen S K (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J Zhejiang Univ. Sci B 9, 165-191.

Bradford, M.M. 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Chang, S. T. and P. G. Miles.(2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact (Second Edition).CRC Press. Boca Raton, FL, USA: CRC Press. 451pp.

Collopy P. D. and Royse D. J. (2004). Characterization of Phytase Activity from Cultivated Edible Mushrooms and Their Production Substrates. J. Agric. Food Chem. 52: 7518-7524.

Common F. H. (1989). Biological availability of phosphorus for pigs. Nature 143: 370- 380.

Daniel, J. R. (2014). A global Perspective on the high five : *Agaricus, Pleurotus, Lentinula, Aurcularia and Flammulina*.Proceeding of the 8 th International conference on Mushroom Biology p. 1-6. USA.

Greiner, R., and Konietzny, U. (2006). Phytase for food application. Food Technology and Biotechnology, 44(2):125–140.

Hassan, A. A.(1996).Production of Pleurotus spp. for human consumption on agricultural wastes and utilization its by-products for animal feed, MS.C. thesis University of Baghdad, Iraq.

Lassen SF, Breinholt J, Ostergaard PR, Brugger R, Bischoff A, Wyss M, Fuglsang C.C. (2001). Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycii, agrocybe pediades, Ceriporia* sp. and *Trametes pubescens*. Appl. Environ. Microbiol. 67: 4701-4707.

Liu, B-L. Rafiq, A. T. Y-M and Abdul Rob. (1998). The induction characterization of phytase and beyond., Enzyme and Microbial Technology 22:415–424.

Lynd, L.R., P.J. Weimer, Z.W. Van and I.S. Pretorius. 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev., 66: 506-577

Muhammad M. J., Waseem A., Sana Z. AND Ikram U. (2010).Solid State Culturing of the thermophilic fungi for phytase production. Pak. J. Bot., 42(5): 3605-3611.

Nayini N.R.. Markakis P. (1984) . the phytase of yeast. Lebensm Wiss Technol. 17:24–26.

Olukosi, O.A., A.J. Cowieson, and O. Adeola. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult Sci. 86: 77 – 86.

Reddy NR, Sathe SK, Salunkhe DK (1982). Phytates in legumes and cereals. Adv. Food Res. 28: 1-92.

Rutherfurd, S. M., T.K. Chung, and P.J. Moughan. (2002). Theeffect microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Brit. Poult.Sci. 43: 598-606.

Sasirekha B., Bedashree T., Champa K. (2012) . Optimization and partial purification of extracellular phytase from *Pseudomonas aeruginosa* p6. Eur J ExpBiol; 2(1): 95-104.

Soni, S. K., (2009). Phytase from *Aspergillus niger* NCIM 563: Isolation, Purification, Characterization and its Applications. PH. D thesis, University of Pune

Veum, T.L., Bollinger, D.W., Buff, C.E. and Bedford, M.R. (2006). A genetically engineered Escherichia coli phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. J. Anim. Sci. 84: 1147-1158.

Vihnudas J., Jojula M. and Singaracharya M. A.(2012). A Culturing of Fungi for Phytase production by solid state from different sources. Current World Environment. 7(1): 187- 190.

Wang H, Ng TB (2001). Isolation of a novel deoxyribonuclease with antifungal activity from asparagus officinalis seeds. Biochem. Biophys. Res Commun. 289: 120-124.

Wang HX, Ng TB (2000). Flammulin: a novel ribosome-inactivating protein from fruiting bodies of the winter mushroom *Flammulina velutipes*. Biochem. Cell Biol. 78: 699-702.

Wood, D. A. (1985). Production and roles of extracellular enzymes during morphogenesis of basidiomycetes fungi, in Developmental biology of *Agaricus*, Moore, D.M., Ed., Symposium Series , British mycological Society, Cambridge University Press, Cambridge, U.K., 375-388.

Woodzinski RJ, Ullah A. (1996) Biochemical characterization of cloned *Aspergillus fumigatus* phytase (phyA). Adv.ApplMicrobiol 42:263–302.

Xu L., Zhang G., Hexiang W., and Tzi B. Ng .(2011). Purification and characterization of phytase with a wide pH adaptation from common mushroom *Volvariella volvacea*(straw mushroom). Indian journal of Biochemistry and Biophysica Vol, 49, pp 49-54.

Ye XY, Ng TB (2002). Isolation of a novel peroxidase from French bean legumes and first demonstration of antifungal activity of a non- milk peroxidase. Life Sci. 71: 1667-1680.

Zhang, G-Q., Wu, Y-Y., Ng, T-B., Chen, Q-J and Wang, H-X.(2013). A Phytase Characterized by Relatively High pH Tolerance and Thermostability from the Shiitake Mushroom *Lentinus edodes* . Hindawi Publishing Corporation BioMed Research International Volume 2013, Article ID 540239.

Zhou X-L., Shen W., Zhuge J. and Wang Z-X. (2006). Biochemical properties of a thermostable phytase from *Neurospora crassa*. Biotechnology, Southern Yangtze University, Wuxi, China FEMS Microbiol Lett 258:61–66 .

Zhu M. J. Wang H. X. and Ng T. B. (2011) .Purification and identification of a phytase from fruity bodies of the winter mushroom, *Flammulina velutipes* African Journal of Biotechnology Vol. 10(77) : 17845-17852.