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CONTENT OF PROTEIN AND ORTHO-DIHYDRIC PHENOL IN *AGARICUS BISPORUS* X25 CULTIVATED ON DECOMPOSED AGROSUBSTRATES

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Abstract

This study investigates the nutritional value of white button mushroom *Agaricus bisporus* X25 which cultivated on six composts viz., wheat straw compost, reed (*Phragmites australis*) straw compost and the mixture (1:1) (wheat straw and reed straw) which decomposed with or without adding bacterial inoculum Streptomyces during substrates composting. In general, using bacterial inoculum raised values of proteins and ortho-dihydric phenol in fruiting bodies of *A. bisporus* X25, while the mixture compost raised protein content and reed compost raised ortho-dihydric phenol of fruiting bodies of *A. bisporus*. Thus, this mushroom strain has considered amounts of proteins and phenols.

Keywords: Compost, nutritional value, pharmaceutical value, *Phragmites australis*, *Streptomyces*.

FARKLI KOMPOSTLARDA ÜRETİLEN *AGARICUS BISPORUS* X25 MANTARLARININ PROTEİN VE ORTO-DIHIDRIK FENOL İÇERİKLERİ

Öz

Bu çalışmada *Streptomyces* kültürü kullanılarak veya kullanılmaksızın hazırlanmış beyaz buğday samanı, kamış samanı (*Phragmites australis*) ve bu ikisinin 1:1'lik karışımı olmak üzere altı farklı kompostta üretilen beyaz düğme mantarlarının (*Agaricus bisporus* X25) besleyici değeri incelenmiştir. Genel olarak bakteriyal aşılama işlemi *A. bisporus* X25 mantarlarının protein ve orto-dihidrik fenol içeriğini arttırırmıştır. Karışım kompost ve kamış kompost kullanımı *A. bisporus* mantarlarında sırasıyla protein ve orto-dihidrik fenol içeriğini arttırmıştır. Sonuçta bu mantar türünün protein ve fenolik bileşikler açısından değeri ortaya konulmuştur.

Anahtar kelimeler: Kompost, besleyici değer, farmasötik değer, *Phragmites australis*, *Streptomyces*.

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INTRODUCTION

The edible mushroom *Agaricus bisporus* belongs to *Basidiomycota* and it is called white button mushroom as a common name (1). Medicinally, *Agaricus bisporus* X25 has antioxidant activity (2) because of its content of phenols and ergothioneine (3). It has a high nutritional value due to its high amino acids/proteins content especially (4, 5). It has a high ability to bio-convert lignocelluloses matters to fresh edible mushroom, such as using composted wheat straw, common reed, sesaban plant and sunflower head for *A. bisporus* cultivation (4, 6, 7), whereas sawdust with wheat straw was used for its production globally (8). These composts supplemented with licorice extract and decomposed using the bacteria that led to increasing their proteins content (6, 9).

Agaricus bisporus has a lowest heavy metal concentrations K, Na, Co, Pb, Cr, Fe, Ni, Cu, Zn, Cd, Mn (3,5), P, K, S, Ca and Li, without starch and rich of essential vitamins A, B, C and D (10), that lead to encourage peoples for consuming healthy food. This mushroom was found to be good sources of microelements. Also, it has essential amino acids (4) and active compounds to decrease cholesterol of blood serum; therefore, it is a benefit to treating atherosclerosis and diabetes mellitus. It has low calories gain that useful for treatment the obesity (11). *A. bisporus* X25 has trace mineral elements such as Pb, Cd and Cr at concentrations 7.5-8.0, 0.2-0.3 and 24.6-14.1 mg/Kg respectively based on dry matter, but these levels were below the safety limits defined by FAO/ WHO for weekly Required Dietary Intake (RDI) (5).

Fruiting bodies which produced on commercial substrates are non-economic because of the high price of substrate especially wheat straw substrate. Thus must determining the nutritional value of mushroom which cultivated on local composts have low prices and available during the year from a side and to decrease the pollutions outcome from burning it from another side. However, no reference is found in the literature regarding the comparison of determining the nutritional value of *A. bisporus* fruiting bodies cultivated on local composts. Thus, the objective of this study is calculating the nutritional value of *A. bisporus* harvested from six composts containing wheat straw and reed straw.

MATERIALS AND METHODS

Mushroom samples

Fruiting bodies of white button mushroom *Agaricus bisporus* X25 obtained from Iraqi local farm related with the University of Anbar. Fruiting bodies which harvested from six composts containing wheat (*Triticum sativum*) straw, reed (*Phragmites australis*) straw and their mixture 1:1 with or without *Streptomyces* inoculum treatment during the composting process. Six treatments were used in this test including: wheat straw compost, reed straw compost, the mixture compost (1:1), the decomposed wheat straw compost using *Streptomyces* sp., the decomposed reed straw compost using *Streptomyces* sp., and the decomposed mixture compost using *Streptomyces* sp. Solid state fermentation was achieved outdoor and indoor during phase 1 and phase 2 respectively as mentioned by Muslat et al. (6). After pasteurization of compost and cooling, mushroom spawn was applied within bed at percent 2% based on dry weight using Ruffling Method until mycelial growth completion.

Protein determination using Kjeldahl method

The nitrogen content in fruiting bodies of **Agaricus bisporus** X25 was determined using Kjeldahl method by Gallenkamp Kjeldahl Apparatus in Plant Tissues Lab., Department of Biology, College of Science, University of Anbar. Protein content was calculated using this equation: Protein%=Nitrogen contentx6.25x100 (12).

Ortho-dihydric phenol determination using Arnow's method

In a water bath, one gram of fresh fruiting bodies extracted within 5-10 ml 80% Ethyl alcohol for 5-10 min. After cooling the samples, the mixture crushed in a porcelain mortar, then filtrated using filter paper Whatman No. 1. The residue was re-extracted again within 3 ml of Ethyl alcohol for 2-3 min and filtrated, then completed the volume to 10 ml using 80% Ethyl alcohol. The reagent was prepared by dissolution 10 g of Sodium Nitrite ($NaNO_2$) and 10 g of Sodium Molybdate ($NaMoO_2$) and then completed to volume 100ml using Distilled water (D.W). The reagent was stored in dark bottles until use. Add

one ml from each extracted alcohol solution, (0.5 N) HCl and the reagent within 10 ml of D.W then intermix the intermixture with 2 ml (1 N) NaOH. After mixing the mixture with NaOH, the color of intermixture changed to pink color. Read at 515 nm using Spectrophotometer and the result calculated using stander curve of ortho-dihydric phenols (1.88-16.92) g/L (13).

Statistical Analysis

The experimental data were subjected to an analysis of variance by two ways analysis (ANOVA) using GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK) to determine the least significant difference among means at the level least of 0.05 (P <0.05). Three replicates were examined in the experiments.

RESULTS AND DISCUSSION

The chemical analysis of six fruiting bodies of *Agaricus bisporus* X25 was observed in Table 1. Protein percentage was defined based on the dry mushroom. The higher protein percentage was 21.85% achieved using basidiocarps which cultivated on mixture compost with treatment by Streptomyces inoculum, significantly (P <0.05), followed 19.51% by fruits of wheat straw and mixture composts with and without treatment. Basidiocarps which harvested from reed straw compost with treatment recorded protein content 18.09%. The lower protein percentage was 16.38% for fruits of reed straw compost without treatment compared with 17.02% by basidiocarps cultivated on wheat straw compost (control).

The content of ortho-dihydric phenols was determined on the fresh mushroom basis (Table 1). The higher ortho-dihydric phenols content was 10.45 g/L achieved on basidiocarps of reed straw compost with treatment by bacterial inoculum significantly (P <0.05), followed 9.04% by fruits of same compost without treatment. Basidiocarps of the mixture compost with treatment, wheat straw (control) and reed straw composts without treatment recorded ortho-dihydric phenols content 8.97, 8.91 and 8.90 g/L, respectively. The lower ortho-dihydric phenols content was 8.60 g/L for fruits of reed straw compost with treatment.

From another side, fruiting bodies of *Agaricus bisporus* X25 which cultivated on the mixture compost 1:1 (wheat straw: reed straw) observed higher protein percentage 20.68% significantly (P <0.05). However, the control treatment (fruits from wheat straw compost) recorded percentage 18.26% followed 17.23% for the fruits from reed straw compost which was poor in protein level compared with others. That is because of low content of nitrogen (amino acids) in reed straw compost which had higher C:N ratio (1:22) than the mixture (1:20) and control (1:10) composts, thus must use the mixture compost to improve quality of these composts (14).

Also, the reason of low protein content in *A. bisporus* X25 fruits which harvested from reed straw compost may be related to high lignin content in this medium in comparison to cellulose and hemicellulose in wheat straw and mixture composts. Indeed, lignin of reed plant slower decomposition than cellulose and hemicellulose (15). The mixture compost observed high protein content for basidiocarps of *A. bisporus* X25 because of various and increasing soil microbes which had good growth in the aerobic condition in solid substrate fermentation (6).

Muslat et al., (6) mentioned that i ncreasing temperatures of the mixture compost during composting processes help to improve C:N ratio

Table 1 Nutritional content of fruiting bodies of *A. bisporus* X25

Fruiting bodies	Wheat straw	Reed wastes	Mixture 1:1	LSD (P <0.05)
	Protein percentage based on dry mushroom			
Without treatment	17.02	16.38	19.51	0.450
Treated with *Streptomyces*	19.51	18.09	21.85	
Decomposed substrates	Ortho-dihydric phenols (g/L) based on fresh mushroom			
Without treatment	8.91	9.04	8.90	0.420
Treated with *Streptomyces*	8.60	10.45	8.97	

Legend: Average of moisture content of fruiting bodies about 89%-90%.

to an optimal condition for white button mushroom (*A. bisporus*) cultivation. In solid substrate fermentation, using Streptomyces inoculum during composting processes also lead to increase protein content in compost because of the high biomass of lysed soil bacteria and fungi. That agrees with Benimelia et al., (16) who referred to increase amino acids and enzymes in compost from the outcome of lysed bacteria/microbes, thus, the growth of soil microbes in compost guides to concentrate nitrogen and minerals sources to support mushroom cultivation (17).

From another side, the content of ortho-dihydric phenols differed according to type of cellulosic waste used as a media for cultivation. The higher significant (P <0.05) ortho-dihydric phenols was 9.74 g/L (basis on fresh weight) for cultivated *A. bisporus* X25 on reed straw compost, followed 8.93 and 8.75 g/L for fruits of the mixture and wheat straw composts, respectively.

Indeed, *A. bisporus* is useful enzymatic source such as Laccase which oxidizes phenolic compounds (18). That demonstrates reason of low ortho-dihydric phenol content in some samples. Also, *A. bisporus* has a hydrolytic system (oxidization factors) to consume carbohydrates, and ligniolytic system to decomposed lignocellulosic matters and phenol rings (19).

The high ortho-dihydric phenols in fruiting bodies of reed straw compost may be returned to inhibit polyphenol oxidase because of the high lignin content in this compost (18). Although, fruits of reed straw compost had higher ortho-dihydric phenol content (Table 2) but this finding near from control (fruits which harvested from wheat straw compost). That agrees with Al-Kiasy (20) who referred to ortho-dihydric phenol content 9.39 g/L with fruiting bodies of *A. bisporus* harvested from wheat straw compost. That is important to inhibit pathogenic microbes because of its antioxidant and antimicrobial activity (21).

CONCLUSION

This study was investigated the nutritional value of white button mushroom *Agaricus bisporus* X25 harvested from six composts viz., wheat straw compost, reed (Phragmites australis) straw compost and the mixture compost (1:1) were treated with or without bacteria inoculum (*Streptomyces*) during the composting process. The mixture compost which decomposed using bacterium Streptomyces has the best protein content (21.85%) significantly (P <0.0).The higher content of ortho-dihydric phenols was 10.45 g/L with fruits of reed straw compost without treatment.

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