

## **IMPROVING THE NUTRITIVE VALUES OF WHEAT STRAW AND ARTICHOKE PETALS AND THEIR COMBINATIONS BY FUNGAL TREATMENTS**

**Baker, A. A.; Galila A.M.A. Darwish and Mervat, S.M. Hasanin**  
Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt.

### **ABSTRACT**

*Pleurotus ostreatus* and *Phanerochaete chrysosporium* successfully to upgrade nutritional value of wheat straw in combined mixture with artichoke petals at different ratios (1:1, 1:3 and 3:1), using solid state fermentation technique. Treatment with *Pleurotus ostreatus* succeeded to increase protein content of wheat straw from 3.30% to 10.85 when it was mixed with artichoke petals at ratio of 1:1 and fermented for 30 days, While *Phanerochaete chrysosporium* increased crude protein content of wheat straw to 11.10%. Pronounced degradation of hemicellulose and lignin was recorded, when wheat straw was mixed with artichoke petals at different ratios (1:1, 1:3 and 3:1). In addition, treatment of wheat straw white rot fungi improved *in vitro* digestibility when artichoke petals were added. The present study indicated that fungal treatment of wheat straw when mixed with artichoke petals improved the nutritive value and digestibility of poor quality roughages and produced a nutritionally valuable feed replacing good quality roughage for ruminants. Further studies are recommended to test the digestibility coefficients of the treated materials through metabolism trials. Also feed trial is needed to estimate their trend on palatability; feed intake, live body gain, feed conversion and economic efficiency.

### **INTRODUCTION**

Further studies are recommended to test the digestibility coefficients of the treated materials through metabolism trials. Also feed trial is needed to estimate their trend on palatability; feed intake, live body gain, feed conversion and economic efficiency. Crop residues represent a potential source of dietary energy to ruminants if the protein content can be enriched. As these residues are renewable and in an abundant supply and they represent a potential solution to feeding animals in developing countries.

Lignocellulosic crop residues may be characterised by being high in cellulose, hemicellulose and lignin, but low in protein. They tend to be difficult to be digested by ruminants and so are limited as potential animal feed (Pandey *et al.*, 2001).

World wide lignocellulosic residue generation every day results in pollution of the environment and in loss of valuable materials that can be bioconverted to several added-value products (Howard *et al.*, 2003).

Other authors have shown that some fungi, particularly some species of *Pleurotus* are able to colonize different types of vegetable wastes, increasing their digestibility (Villas-Boas *et al.*, 2002; Zhang *et al.*, 2002; Mukherjee and Nandi, 2004, Salmones *et al.*, 2005). Previous studies showed the feasibility of using these kinds of wastes to produce animal feed and as substrate for mushroom production (Yildiz *et al.*, 2002).

Shubhayu *et al.* (2002) studied the soil state bioconversion of wheat straw by *Phanerochaete chrysosporium* for the production of animal feed. A time profile study of the solid state bioconversion of wheat straw indicated that the highest lignin and lowest cellulose degradation levels occurred on the sixth day of cultivation.

In Egypt, about 21.6 million ton of agriculture residues are produced annually (Ministry of agric. Egypt, 2007), however, Shoukry (2013) estimated agricultural residues by about 25.5 million tons annually. These residues such as wheat straw contain considerable quantities of cellulose and hemicellulose, while, the availability of these components is controlled by lignin-carbohydrate complex, which limits the digestion of cellulose and hemicelluloses. The major limitation of using these agricultural residues as feed is low palatability, digestibility, protein and high fiber contents. Many attempts have been done to increase the digestibility and utilization of the agricultural residues, one of them is the biological treatment by using white rot fungi in order to degrade lignocellulose into lignin, cellulose and hemicelluloses and improve crude portion content, digestibility, nutritive value and feed intake, (Abedel -Aziz and Ismail, 2001).

Many reports showed that white rot fungi can degrade a variety of persistent aromatic or gamapollutants such as pentachlorophenol, polychlorinated biphenyls, dichlorodiphenyltrichloroethane and phenanthrene (Won *et al.*, 2000).

The primary purpose of this study was to evaluate the use of low quality roughages as wheat straw which was treated with white rot fungi, with or without addition of agro-industrial by-products (artichoke petals) to increase the nutritive value.

## **MATERIALS AND METHODS**

### **MATERIALS:**

This work was carried out at Regional center for food and feed, belongs into Agricultural Research Center, Ministry of Agriculture. Wheat straw was obtained from the experimental farm of Agricultural Research Center, Giza, Egypt. Wheat straw was rinsed with water, air dried, then cut into pieces (1-2 cm). The chopped straw was dried at 70° C for 24 hour.

White rot fungi *Pleurotus ostreatus* NRRL-2366 and *Phanerochaete chrysosporium* NRRL-6356 were obtained from the National Center of Agricultural Utilization Research Service, US., Department of Agriculture, Peoria, Illinois, USA. The strains were maintained on potato dextrose agar medium (Difco Manual, 1979), and then stored at 4°C until used.

Microbiological methods:

Preparation of fungal broth inoculum. Fungal inoculum (*Phanerochaete chrysosporium*) was prepared by inoculating conical flasks (250 ml. capacity) containing 50 ml of nutrient glucose broth medium (Fouda *et al.*, 1960). The flasks were inoculated with mycelial discs (5 mm diameter) which were born from the margins of 9 days old cultures. The inoculated flasks were incubated on rotary shaker (200 rpm) at 28° C for 7 days.

Preparation of grain spawn: To prepare grain master spawn, wheat seeds or sorghum seeds were used. Seeds were cleaned from debris, then soaked in water overnight. Dead seeds were removed, then boiled in water for 15 min. After cooling, the seeds were transferred to a round bottle (occupy 2/3 of its volume) and mixed with calcium carbonate 2% (w/w) and calcium sulphate 1% (w/w). Bottles were then sterilized, for 1 hr. at 121°C. After cooling, the sterilized bottles were inoculated with mycelial discs (5 mm diameter) which were born from the margins of 6 days old culture of *Pleurotus ostreatus*. The inoculated bottles were incubated at 25° C for 15-20 days. The grain master spawn was used to inoculate bags containing (100 gm) pasteurized wheat straw.

Preparation of food industrial waste: Artichoke petals were washed with tap water, then dried at 105 °C for 3 hrs. The dried waste was milled to pass from 50 mesh sieve.

Solid state cultivation technique: The nutritional up-grading trial of wheat straw was carried out in plastic bags containing 100 g of chopped straw (1-2 cm length), then, straw was pasteurized in hot water (90 °C for 2 hrs.). The moisture content of straw was adjusted at 70%. The bags were inoculated with 10-12 gm *Pleurotus ostreatus* spawn or mycelial preculture (10-15 ml) of *Phanerochaete chrysosporium*. Different combinations between wheat straw and artichoke petals, in ratio of 3:1, 1:1 and 1:3 were made to test their effect on nutritional upgrading of wheat straw.

In vitro DM and OM disappearance:

An experiment was carried out to determine the in vitro DM (IVDMD) and OM (IVOMD) disappearance using the method of (Tilly and Terry , 1963) with a slight modification made by (Ahmed , 1989). The rumen liquor was collected from ruminal fistulated rams. Animals were maintained on a basal diet of good Berseem hay given at a rate of 3% of body weight/ head/day.

Chemical analytical methods: Moisture content, ash, crude fiber (CF), crude protein (CP) and ether extract (EE) were determined as reported in AOAC (1990). Nitrogen free extract (NFE) was obtained by the difference. Total hydrolyzable carbohydrates were determined according to Montgomery (1961). On dry matter basis, lignocellulosic fractions were determined (Van Soest et al., 1980).

Statistical analysis of the data.

Statistical analysis for each of the collected data was done according to Gomez and Gomez (1984). The treatment means were compared using the least significant difference test (LSD) at the 5% level of probability as out lined by Waller and Dancan (1969).

## **RESULTES AND DISCUSSION**

### **Chemical composition of wheat straw and artichoke:**

Data presented in Table 1 show chemical composition of wheat straw. The data showed that holocellulose (cellulose plus hemicellulose) exhibited the most prevalent amount (62.70%) in wheat straw. Data also revealed that

wheat straw contains 39.70, 35.00 and 27.71% of crude fiber, cellulose and hemicellulose, respectively. Similar results were reported by Chahal (1991) lignin and crude protein were 12.70 and 3.30%, respectively. The data also indicated that wheat straw contains about 12.05% ash.

**Table (I): Chemical composition and fiber fractions of wheat straw and artichoke petals.**

Raw Material	EE	CP	CF	NFE	Ash	holocellulose	Hemicellulose	Cellulose	Lignin
Wheat straw	1.50	3.30	39.70	43.45	12.05	62.70	27.71	35.00	12.70
Artichoke petals	1.51	12.20	20.76	51.71	7.82	55.47	14.84	24.63	14.54

Wheat straw is more available for ruminants feeding because of its low content of hemicellulose, cellulose and lignin, but it is relatively poor in crude protein compared with other poor quality roughages.

Data presented in Table (1) show chemical composition of artichoke petals. Results revealed that artichoke petals contains abundant amounts of holocellulose being 55.47%. Cellulose and hemicellulose comprise 24.63 and 14.84%, respectively. Artichoke petals is protein rich substrate, whereas, crude protein content reaches 12.20%. These results agreed well with those reported by Salman et al.(2014) who reported that artichoke by-products had higher CP (16.6%) compared with kidney bean straw.

*Pleurotus ostreatus* and *Phanerochaete chrysosporium* have ability to degrade lignocellulosic material (wheat straw) and increase crude protein. Thus, fungal treatment of wheat straw in combined mixture with artichoke petals via solid state fermentation technique was achieved. The bioconversion of wheat straw in combined mixture with different ratios of artichoke petals (1:3, 1:1 and 3:1) into protein enriched product was done as shown in Tables 2 and 3.

Data presented in table (2) show potentiality of *Pleurotus ostreatus* to convert wheat straw in combined mixture with artichoke petals into protein enriched product. The results revealed pronounced increase of protein content whereas, it increased from 3.30% (on the basis of dry weight of untreated wheat straw) to 10.85, 13.15 and 16.60% of *Pleurotus ostreatus* treated wheat straw: artichoke petals (1:1), wheat straw: artichoke petals (1:3) and fungal treated artichoke petals, respectively. The corresponding figures for fungal treatment using *Phanerochaete chrysosporium* (as shown in Table 3) increased from 3.30% to 11.10, 13.05 and 17.00%, respectively. Similar trend was observed with fungal treatment using *Phanerochaete chrysosporium*. Ragunatham et al. (1996), Ragini et al. (1997), El-Ashry et al. (2002a) and Fazaeli et al. (2004). As reported in Tables 2 and 3 fungal treatments increased crude protein content and reduce fiber fraction of treated wheat straw and artichoke petals and their combinations mixed. The increase in CP content, could be the result of the decrease of CF (Chandra et al., 1991). In these respect, El-Ashry et al. (2003) indicated that the improvement of CP content could be attributed to fungus growth on the produced cellulolytic enzymes by the fungal enzymatic system. In addition,

Iconomou *et al.* (1997) indicated that the micro-organism used most of the fermentable sugars for protein synthesis.

Concerning chemical analyses of fiber fraction as shown in tables 2 and 3, data revealed potentialities of both *Pleurotus ostreatus* and *Phanerochaete chrysosporium* for degrading hemicellulose and lignin, therefore hemicellulose decreased from 27.71, 21.68 and 14.84% to 21.90, 12.33 and 9.59%, respectively, when wheat straw, combined mixture of wheat straw and artichoke petals (1:1) and artichoke only were treated with *Pleurotus ostreatus*. The corresponding figures for *Phanerochaete chrysosporium* were from 27.71, 21.68 and 14.84% to 22.53, 16.69 and 12.05%, respectively. Both *Pleurotus ostreatus* and *Phanerochaete chrysosporium* were succeeded to degrade lignocelluloses materials during 30 days of fermentation using solid state fermentation technique. Similar results were reported by Cruz *et al.* (2000), Cohen *et al.* (2002), Pradeep and Datta (2002), Shubhayu *et al.* (2002) Lorenzo *et al.* (2002) Rodriguez *et al.* (2003), Rodriguez and Sanroman (2005), El-Shafie *et al.*, (2007), Sallam *et al.* (2007), Akinfemi *et al.* (2008) and Akinfemi (2010). Thus, biological treatment of resulted in reducing cellulose, hemicellulose and lignin compared with untreated materials. These results might be due to the breakdown of lignocelluloses bonds where the cellulose can be hydrolyzed by fungi (El-Ashry *et al.*, 2002b). Mccarthy (1986) reported that fungus have a similar degradative mechanism, as they degrade cellulose and hemicellulose by oxidize and solublize the lignin component.

The results of the *in vitro* digestibility DMD (dry matter digestibility) and OMD (organic matter digestibility) are shown in Tables 2 and 3. The estimated dry matter digestibility and organic matter digestibility are increased from 34.71 and 32.66 % in the control to 61.88%, 57.80%, 65.24 % and 62.38%, when treated wheat straw with *Pleurotus ostreatus* and *Phanerochaete chrysosporium* incombined mixture with artichoke petals in ratio 1:1, respectively. Differences were significant ( $p < 0.05$ ). El- Sheikh (2007) reported a positive correlation between CP content in the diet and *in vitro* dry matter digestibility. These data imply that the microbes in the rumen and animal have high nutrient uptake (Chumpuwadee *et al.*, 2007). The high natural detergent fiber (NDF) and acid detergent lignin (ADL) contents in feedstuffs result in lower fiber degradation (Van Soest, 1988). This agrees with Hamza *et al.* (2005), Sallam (2005) and Albores *et al.* (2006). The results indicated also that fungal treatment decreased hemicellulose, cellulose, lignin and ash while, CP, IVDMD and IVOMD % were significantly increased compared with the control group. Similar results were reported by Subhash *et al.* (1991) and Mohamed *et al.* (1998). They indicated that the fungal treatment increased CP and decreased CF contents. Also, Surinder and Suman (1986) reported that the biological treatments of wheat straw produced an increase in IVDMD. In conclusion, there are considerable differences in the fermentation ability of wheat straw between different fungus. This study suggested that artichoke petals have a potential fermentation efficiency better than wheat straw only and therefore, artichoke petals could be incorporated in feed mixtures to replace conventional

roughage sources (berseem hay, silage) in ruminant diets similar results have been reported by Salman et al. (2014) who found that DM intake and nutrients digestibility of artichoke by-products were greater than kidney bean straw. In addition, due to the high digestibility of artichoke petals, it can be incorporated into the diet without major problem. Further work is needed to explore the possibility of using other poor quality roughages and use the end product to feeding medium producing ruminants.

**Table (2): effect of biological treatment with *Pleurotus ostreatus* on, chemical composition, fiber fractions and in-vitro digestibilities of wheat straw, artichoke petals and their combinations**

	Treatments	Chemical analysis					In vitro digestibility	
		Crude protein	Hemicellulose %	Cellulose %	Lignin %	Ash %	DMD %	OMD %
T1	* Wheat straw Fungal untreated	3.30	27.71	35.00	12.70	12.05	34.71 <sup>f</sup>	32.66 <sup>i</sup>
	**Wheat straw Fungal treated	5.80	21.90	30.00	10.50	10.60	50.66 <sup>d</sup>	45.61 <sup>de</sup>
T2	Wheat straw: artichoke petals 3:1 (untreated)	6.50	24.49	31.91	12.91	14.69	40.52 <sup>e</sup>	36.12 <sup>hi</sup>
	Wheat straw: artichoke petals 3:1 (treated)	8.80	18.09	21.76	9.17	11.40	52.15 <sup>d</sup>	48.05 <sup>ef</sup>
T3	Wheat straw: artichoke petals 1:1 (untreated)	8.20	21.68	28.81	13.12	11.25	43.22 <sup>e</sup>	38.02 <sup>gh</sup>
	Wheat straw: artichoke petals 1:1 (treated)	10.85	12.32	20.06	10.04	9.87	61.88 <sup>bc</sup>	57.80 <sup>bc</sup>
T4	Wheat straw: artichoke petals 1:3 (untreated)	8.80	18.06	25.73	14.33	7.80	43.48 <sup>e</sup>	37.68 <sup>fg</sup>
	Wheat straw: artichoke petals 1:3 (treated)	13.15	17.77	22.10	8.00	9.83	56.68 <sup>cd</sup>	52.95 <sup>cd</sup>
T5	Artichoke petals Fungal untreated	12.20	14.84	24.63	14.54	7.83	33.97 <sup>f</sup>	29.14 <sup>ij</sup>
	Artichoke petals Fungal treated	16.60	9.59	17.10	8.12	8.09	68.68 <sup>a</sup>	66.18 <sup>a</sup>

\* Fungal untreated wheat straw (raw substrate control).

\*\* Fungal wheat straw (*Pleurotus ostreatus*).

IV DMD- in-vitro dry matter disappearance.

IV OMD- in-vitro organic matter disappearance.

**Table (3):effect of biological treatment with Phanerochaete chrysosporium on, chemical composition, fiber fractions and in-vitro digestibilities of wheat straw, artichoke petals and their combinations**

	Treatments	Chemical analysis					In vitro digestibility	
		Crude protein	Hemicellulose %	Cellulose %	Lignin %	Ash %	DMD %	OMD %
T6	* Wheat straw Fungal untreated	3.30	27.71	35.00	12.70	12.05	34.71 <sup>f</sup>	32.66 <sup>l</sup>
	**Wheat straw Fungal treated	5.60	22.53	29.95	9.19	9.80	55.11 <sup>d</sup>	51.18 <sup>ef</sup>
T7	Wheat straw: artichoke petals 3:1 (untreated)	5.60	24.49	31.91	12.91	11.40	40.52 <sup>e</sup>	36.12 <sup>hi</sup>
	Wheat straw: artichoke petals 3:1 (treated)	9.06	20.15	24.64	8.34	10.69	66.40 <sup>ab</sup>	61.73 <sup>b</sup>
T8	Wheat straw: artichoke petals 1:1 (untreated)	8.20	21.68	28.81	13.12	11.25	43.22 <sup>e</sup>	38.02 <sup>gh</sup>
	Wheat straw: artichoke petals 1:1 (treated)	11.10	16.69	23.43	8.99	11.87	65.24 <sup>ab</sup>	62.38 <sup>b</sup>
T9	Wheat straw: artichoke petals 1:3 (untreated)	8.80	18.06	25.73	14.00	9.80	43.48 <sup>e</sup>	37.68 <sup>g</sup>
	Wheat straw: artichoke petals 1:3(treated)	13.05	11.66	20.57	7.98	9.83	52.77 <sup>d</sup>	48.94 <sup>cd</sup>
T10	Artichoke petals Fungal untreated	12.20	14.84	22.63	14.54	8.83	33.97 <sup>f</sup>	29.14 <sup>l</sup>
	Artichoke petals Fungal treated	17.00	12.05	20.98	7.48	8.20	52.58 <sup>d</sup>	50.49 <sup>cd</sup>

\* Fungal untreated wheat straw (raw substrate control).

\*\* Fungal wheat straw (Phanerochaete chrysosporium).

IV DMD- in-vitro dry matter disappearance.

IV OMD- in-vitro organic matter disappearance.

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### تحسين القيم الغذائية لتبن القمح وبتلات الخرشوف وخليطهما بالمعاملات الفطرية جليلة علي محمد علي درويش ، عادل احمد بكر و مرفت سيد حسنين المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية - الجيزة - جمهورية مصر العربية

نجحت فطريات العفن الأبيض ومنها فطري *Pleurotus ostreatus* و *Phanerochaete chrysosporium* في رفع القيمة الغذائية للمخلفات الزراعية المستخدمة كأعلاف خشنة والتي ينقصها المحتوى البروتيني وتزداد فيها المواد اللجنوسليلوزية ، لذا تستخدم هذه الفطريات لتحسين القيمة الغذائية ورفع المحتوى البروتيني . وفي هذه الدراسة استخدام تبن القمح كمنتج ثانوي قليل القيمة الغذائية مع مخلف زراعي صناعي وهو بتلات الخرشوف بنسبة مختلفة (١:٣ ، ١:١ ، أو ٣:١) باستخدام تكتيك التخمر للمواد الصلبة. فقد نجح فطر الـ *Pleurotus ostreatus* في زيادة المحتوى البروتيني لتبن القمح من ٣,٣٠% إلي ١٠,٨٥ عند خلطه بنسبة ١:١ مع بتلات الخرشوف والتخمير لمدة ٣٠ يوم ، بينما أدى استخدام فطر *Phanerochaete chrysosporium* إلي زيادة المحتوى البروتيني الي ١١,١٠% .

كما حدث تكسر واضح في كل من الهميسليلوز واللجنين عند خلط تبن القمح مع بتلات الخرشوف بنسب مختلفة (١:١ ، ٣:١ ، ٣:٣) . بالإضافة الي ذلك ، أدت المعاملة الفطرية إلي تحسين المعاملات الهضمية. لذا اظهرت هذه النتائج الي ان المعاملة الفطرية لتبن القمح وعند اضافة بتلات الخرشوف الي حدوث تحسن واضح في كل من القيم الغذائية ومعاملات الهضم و إنتاج منتج غذائي ذات قيمة تحسن من المخلف ويمكن استخدامه كبديل للأعلاف الخشنة الجيدة المجترات. ونوصي بمزيد من الدراسة لتقدير معاملات الهضم من خلال تجارب هضم علي الاغنام للمواد المعاملة وإجراء تجارب نمو لدراسة تقدير الإستساغة و الغذاء المأكول و معدلات النمو و التحويل الغذائي و كذلك الكفاءة الإقتصادية.