Int J Recycl Org Waste Agricult (2015) 4:261–271
DOI 10.1007/s40093-015-0105-3

ORIGINAL RESEARCH

Biological fungal treatment of olive cake for better utilization in ruminants nutrition in Egypt

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Received: 26 March 2015 / Accepted: 31 August 2015 / Published online: 8 September 2015

Abstract
Background Crop residues and agro-industrial by-products, available in appreciable quantities, can play a significant role in the nutrition of ruminants. The appropriate utilization of by-products in animal nutrition can improve the economy and the efficiency of agricultural, industrial and animal production. The present work investigates the bio-conversion of olive cake (OC), generated by the olive oil industries in Egypt, using locally isolated filamentous fungi in solid state fermentation, so as to upgrade its nutritional values and digestibility for its use as ruminants feed.
Methods Seven non-mycotoxin producing fungal strains namely *Trichoderma reesei* F-418, *T. harzianum* F-416, *T. virdi*e F-520, *T. koningii* F-322, *Aspergillus oryzae* FK-923, *A. fumigatus* F-993, and *A. awamori* F-524 were cultured on OC for 7 days at 36 °C. Subsequently, the chemical composition and lignocellulolytic enzyme activities of the resultant substrate were determined.
Results The most promising result was obtained by *A. oryzae* FK-923, whereas, an increase in crude protein content ranging from 9.5 % (untreated) to 17.4 % (treated) was detected, while phenols were decreased from 3.1 to 0.92 % and fibers declined from 33 to 22.2 %. A reduction in the values of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were reported. The addition of sugar cane molasses at 2 % showed an increase in crude protein to 18.9 % with a reduction in phenols and fibers to 0.69 and 21.8 %, respectively. Furthermore, the addition of active dry yeast (*Saccharomyces cerevisiae*) at 1.5 % to the fermentation medium raised the crude protein to 20.2 % (w/w), while phenols and fibers were declined to 0.55 and 19.2 %, respectively.
Conclusions Therefore, the present findings revealed *A. oryzae* FK-923 to be an efficient organism for lignocellulolytic enzymes production and simultaneous enhancement in crude protein and in vitro digestibility of OC.

Keywords Olive cake · Biological treatment · Solid state fermentation · Ruminants feed

Introduction

In many developing countries, there is a gap between available and required ruminants feed. The productivity of the animals is very low, although experimental data suggest that their production potential is higher. The greatest constraint to livestock productivity is the shortage of feeds and forages. Therefore, some countries import cereal grains in an effort to increase livestock production and meet the ever-increasing demand for animal products. The Middle East countries raise some 99 million sheep and goats, 104 million beef cattle and 202 million dairy cattle (FAO 1981). Higher livestock productivity, however, should be sought through better use of locally available feed resources. Crop residues and agro-industrial by-products, which are available in appreciable quantities from processing of fruits and vegetables, can play a significant role in the nutrition of ruminants (Economides et al. 1981). In Egypt, although there are about 30 million tons of agricultural by-products produced annually, only 4.15 million tons of crop residues are used for feeding ruminants (Ministry of

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Agriculture 2006). The main factors limiting the utilization of crop residues in animal ration are the high crude fiber content, low crude protein, low digestibility and poor feed palatability (Abd El-Rahman et al. 2014). The gap between the availability and requirements of animal feeds in Egypt is about nine million tons of dry matter, equivalent to almost four million tons of total digestible nutrients per year (Bendray et al. 2006). Therefore, efforts should be done to efficiently use all the available by-products and wastes to decrease the ruminants feed shortage in Egypt (AboSalim and Bendary 2005).

Olive cake (OC) is the solid residue obtained after olive oil extraction. It is one of the most abundant agro-industrial by-products in the Mediterranean area constituting a source of environmental problems resulting from its accumulation and disposal (Azkar et al. 2008; Hachicha et al. 2008). In Egypt, there are about 118,382 feddan planted with olive trees, the fruitful area 96,810 feddan, produce about 314,450 ton of olive, representing 3.25 ton/feddan (Ministry of Agriculture and Land Reclamation, 2012). Basically, 100 kg of olives produced approximately 35 kg of crude OC. The utilization of OC as ruminants feed is a good way of recycling this by-product. However, the OC contains a large proportion of cell wall constituents, which renders it unpalatable and poorly digestible to the ruminants. Therefore, attempts have been made to improve the nutritive value of OC through different chemical agents, but results have been more or less satisfactory (Rowghani et al. 2008). Apart from the chemicals, the biological treatment is drawing much attention due to its potential advantages over chemical/physical treatments such as greater substrate and reaction specificity, lower energy requirements, lower pollution generation and higher yields of desired products (Misra et al. 2007; Canet et al. 2008).

Solid-state fermentation (SSF) technique using filamentous fungi has been reported to be a relatively low-cost appropriate technology for the upgrading of lignocellulosic materials in animal feeding (Ugwuanyi et al. 2009). Additionally, SSF has proven to be an efficient way to produce lignocellulolytic enzymes since it provides the filamentous fungi with environmental conditions similar to those of their natural habitat. Besides, the restricted water availability reduces the possibilities of contamination by bacteria and yeast (Neifar et al. 2009). It is well documented that the selection of a lignin degrading fungus is preferable for improving the animal feed quality without compromising much the cellulose and hemicellulose constituents. Therefore, the aim of the present study was to apply farm scaling up using biological fungal treatment of OC in SSF using *Aspergillus oryzae* FK-923 to reduce the anti-nutritional components like phenols and fibers. Besides, an increase in protein and gross energy contents in a short period of time was reported which improved the nutritive value of olive cake for its investment as animal feeds to overcome nutritional Gabe in Egypt. To the best of our knowledge, this is the first report on the nutritional evaluation of SSF olive cake with *A. oryzae* FK-923.

Materials and methods

Chemicals

All chemicals were purchased from Sigma–Aldrich or Fluka and were certified reagent grade.

Microorganism

Seven non-mycotoxin producing fungal strains namely *Trichoderma reesei* F-418 (T1), *T. harzianum* F-416 (T2), *T. virdie* F-520 (T3), *T. koningii* F-322 (T4), *Aspergillus fumigatus* F-993 (A1), *A. oryzae* FK-923 (A2), *A. awamori* F-524 (A3) and *Saccharomyces cerevisiae* F-707, obtained from the culture collection of Microbial Chemistry Department, National Research Centre (NRC), were grown for 5 days on potato dextrose agar slants (PDA) at 28 °C, and maintained at 4 °C. The slants were subcultured routinely every 3–4 weeks interval.

Preparation of spore suspension

Ten mL of sterilized water with 0.1 % Tween-80 solution was added to 5-day-old culture slants and agitated thoroughly using a shaker to suspend the spores. The number of the spore was adjusted to 1×10^6 spores/mL and was used as an inoculum throughout the study.

Screening medium

One mL aliquot (v/v) of inoculum size (1×10^6 spores/mL) was used to inoculate 500 mL Erlenmeyer flasks each containing 50 g of sugar beet pulp (SBP) moistened with distilled water to a moisture level of 65 % (v/w). The inoculated flasks were incubated for 4 days at 32 °C in a static condition. For fungal inoculum propagation, each flask from the above step was transferred to 10 L capacity Erlenmeyer flask contained 1 kg sterilized moistened SBP, and then incubated statically at 32 °C for 4 days.

Preparation of *A. oryzae* FK-923 inoculum

Three days old slants cultures of *A. oryzae* FK-923 was crushed into flasks containing 20 mL of sterilized water. The fungal spores suspensions were used at 10 % (v/w) to inoculate 500 mL capacity flasks containing 20 g of the ground waste moistened at a solid: liquid ratio of 1:2 with a basal

medium composed of (g/L): sugar cane-molasses, 40; urea, 2.0; K_2HPO_4, 1.5 and $MgSO_4 \cdot 7H_2O$, 0.5. The inoculated flasks were then incubated at 36 °C for 72 h under static solid state fermentation system. After fermentation period, the above-prepared inocula of *A. oryzae* FK-923 were employed to inoculate 200 g of wastes under study moistened by the above basal medium at a solid: liquid ratio of 1:2 and packed in polyethylene bags (40 × 60 cm). The inoculated bags were incubated under static conditions for 7 days at 36 °C. At the end of incubation period, such bags were opened and oven dried at 70 °C for chemical analysis.

Scaling up using fungal treatments

The above-fermented bags were employed to inoculate polyethylene bags (150 × 225 cm) containing 10 kg of ground wastes moistened with the basal medium at a solid: liquid ratio of 1:2. The bags were incubated in a 3 × 3 m room maintained at 36 °C for 7 days. After the incubation period, the fermented wastes were air-dried to 12 % moisture then packed and stored until fed to lactating goats.

Agro-industrial by-products

Olive by-product

The olive cake (OC), resulted from a continuous cold process of olive oil production, were packed in polyethylene bags and kept in a freezer at −20 °C. Before drying, olive cake samples were thawed during 24 h under refrigeration conditions at 5 °C. Drying process was carried out at 90 °C in a convective dryer, at Microbial Chemistry Department, National Research Centre. The dehydrated samples were packed and kept in dark until analysis.

Molasses

Molasses were obtained from Egyptian Sugar and Integrated Company El-Hawmdia, Giza, Egypt.

Sugar beet pulp

Sugar beet pulp (SBP), a by-product of beet sugar industry, was obtained from Abokorkas Sugar Factory, El-Minia, Egypt.

Physico-chemical analysis of olive cake

Protein content

Crude protein determination involved the use of routine Kjeldhal nitrogen assay method with a conversion factor of 6.25 (AOAC 1990).

Lipid content

The lipid content was determined by exhaustively extracting samples in a Soxhlet apparatus, using petroleum ether (Merck, p.a.) as a solvent according to the method described in AOAC method no. 920.39. The crude fiber content was estimated by Weende method through an acid/alkaline hydrolysis of insoluble residues in accordance with AOAC (1990). The ash content was estimated by incineration in a muffle furnace (Felisa, FE-341, Jalisco, Mexico) at 550 °C. All methodologies allowed the recommendations of the official methods.

Determination of total phenolic content

Total phenolic content (TPC) was determined colorimetrically using Folin–Ciocalteu reagent (FC) as described by Chuah et al. (2008) with modifications. 40 mL of absolute ethanol was added to 3–5 g dried and finely crushed sample. The mixture was homogenized during 24 h on a magnetic stirrer then filtered through a Whatman filter paper No 1. The filtrate was washed twice with 40 mL of absolute ethanol then evaporated under reduced pressure at 40 °C on a rotary evaporator (Büchi RE 121, Switzerland). The obtained extract was then dissolved in 100 mL of absolute ethanol and kept refrigerated for further analysis. 0.5 mL aliquot of the OC extract was transferred to a glass tube containing 0.5 mL of reactive FC, after 5 min 2 mL of Na_2CO_3 solution (200 mg/mL) was added. The sample was then mixed thoroughly on a vortex mixer and the reaction proceeded for 15 min at ambient temperature. A total of 10 mL of ultra-pure water was then added and the precipitate formed was removed by centrifugation for 5 min at $4000 \times g$. Finally, absorbance was measured at 725 nm in a spectrophotometer (Spectronic TM 20 Genesys TM131, IL, USA) and compared to a gallic acid (GA) calibration curve. Results were expressed as mg GA 100/g-dry matter. All measurements were conducted in triplicate and the data were expressed as means.

Chemical analysis

Dry matter (DM) loss was determined by calculating the difference between dry weight before and after fermentation and described as a percentage of initial weight. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the detergent system method, while acid detergent lignin (ADL) was measured according to the method described by AOAC (1990). Hemicellulose contents were estimated as the difference between NDF and ADF, while cellulose content was the difference between ADF and ADL. Ash was carried out on dried sample at 105 °C by ignition three samples each 50 g in muffle

furnace at 800 °C for 5 h, and the residual ash was calculated as a percentage (%) from the dried initial weight according to AOAC (1970).

Enzyme extraction

After incubation period, the enzyme was extracted with sodium citrate buffer (0.05 M, pH 5.0) (10 mL buffer g^{-1} substrate), by shaking for 1 h at 180 rpm at room temperature. The extract was filtered through Whatman filter paper No. 1 and centrifuged for 20 min at 5000 rpm (Neifar et al. 2009).

Enzymes assay

Filter paper activity

According to the method described by Bai et al. (2012); filter paper activity (FP-ase) was determined photometrically by measuring the increase in absorbance at 540 nm, after 30 min incubation of 1.0 mL of properly diluted enzyme with 50 mg Whatman filter paper No.1 (1 × 1 cm) and 1.0 mL sodium citrate buffer (0.05 M, pH 5.0) at 50 °C. The enzyme reaction was stopped by the addition of 3 mL of 3, 5-dinitrosalicylic acid (DNS) reagent (Miller 1959). One mL of 40 % Rochelle salt solution was added so as to maintain the color. Absorbance was measured at 575 nm and compared with a standard curve using 0.10–1.0 mg of glucose/mL. One unit of enzyme activity is defined as the amount of enzyme which releases 1 µmol of glucose per min, under the standard assay conditions.

Xylanase activity

Xylanase activity was estimated according to Bailey et al. (1992) using 1 % xylan from oat as the substrate. Xylan was dissolved in 0.05 M Glycine NaOH buffer (pH 9.0). The reaction mixture, containing 0.1 mL of an appropriate dilution of the enzyme and 0.5 mL of the substrate, was incubated for 30 min at 37 °C. The reaction was terminated by placing tubes in a boiling water bath for 10 min. The supernatant was analyzed for reducing sugar with DNS reagent and absorbance was read at 575 nm. A standard curve of D-xylose was used as reference. Each unit of xylanase activity (U) was that amount of activity which released 1 µmol D-xylose min^{-1}.

Glucoamylase activity

Glucoamylase activity was determined according to the method reported by Miller (1959), by incubating 1 % (w/v) maltose with 0.9 mL sodium citrate buffer (0.05 M, pH 5.0) and 0.1 mL of diluted enzyme solution at 50 °C for 30 min. The reaction was terminated by placing tubes in a boiling water bath for 10 min. The released reducing sugars were measured with DNS reagent using glucose as a standard. Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing 1 µmol of glucose per min under assay conditions.

Alpha-mylase activity

Alpha-mylase activity was determined in the culture filtrate by measuring the amount of starch hydrolyzed in the reaction mixture by the iodine method (Manning and Campbell 1961). One unit of enzyme activity has been defined as the amount of enzyme that hydrolyses 1 µmol of starch min^{-1} under assay conditions.

Protease activity

Protease activity was determined as described by Agrawal et al. (2005). 1 mL of suitably diluted enzyme was mixed with 5 mL of (2 %) casein solution (0.05 M Tris–HCl buffer, pH 8.0), and incubated at 40 °C on a gyratory shaker (200 rpm) for 30 min. An aliquot (0.5 mL) of the reaction mixture was withdrawn and the reaction was quenched by adding 1.5 mL pre-chilled trichloroacetic acid (10 %). The reaction tube was immersed in an ice bath (5 min) to completely precipitate the protein. The supernatant was recovered by centrifugation (10,000×*g*, 10 min). Tyrosine liberated during casein hydrolysis was measured in the supernatant using the method of Lowry et al. (1951). One unit of protease activity was defined as the amount of enzyme liberating 1 µmol of tyrosine per min under the standard assay conditions. The protease activity is reported per gram of dry solids used in the initial extraction.

Phytase activity

Phytase production was measured using a colorimetric method by following the release of inorganic phosphate from phytic acid according to the method presented by Dhiman et al. (2002). For this purpose, 150 µL of enzyme solution were mixed with 600 µL Tris–HCl (0.1 M, pH 7.0) supplemented with 2 mM sodium phytate and 2 mM of $CaCl_2$ and incubated at 37 °C for 30 min. The reaction was terminated by the addition of 750 µL of 5 % trichloroacetic acid, after which 1.5 µL of the color reagent were added to generate phosphomolybdate. The concentration of inorganic orthophosphate (pi) in this mixture was determined colorimetrically by measuring the absorbance of the solution at 700 nm using a Beckman Coulter DU640 Spectrophotometer (Fullerton, CA, USA). The color reagent was freshly prepared by mixing four volumes of 1.5 % (w/v) ammonium molybdate solution supplemented with 5.5 % (v/v) sulfuric

acid and one volume of 2.7 % (w/v) ferrous sulfate solution. The results were compared to a standard curve prepared using K_2HPO_4 as a source of inorganic phosphate at a concentration ranging from 0.0448 to 2.8706 μM. One unit (U) of phytase activity was defined as the concentration of inorganic phosphate, in μmol, released per min under the defined reaction conditions.

Laccase assay

Laccase activity was measured by monitoring the oxidation of 2,2′-azinodi-3-ethyl-benzothiazoline-6-sulfuric acid (ABTS) at 436 nm (Bourbonnais et al. 1998). The non-phenolic dye ABTS is oxidized by laccase to the more stable and preferred state of the cation radical. The concentration of the cation radical responsible for the intense blue-green color can be correlated to the enzyme activity (Majcherczyk et al. 1998). The reaction mixture contained 0.5 mM substrate (ABTS), 2.8 mL sodium acetate buffer (0.1 M, pH 4.5), and 100 μL of suitably diluted enzyme and incubated for 5 min at 30 °C. Absorbance was read at 436 nm in a spectrophotometer against a suitable blank. One unit of enzyme activity was defined as the amount of the laccase that oxidized 1 μmol of ABTS substrate per min under assay conditions.

Polygalacturinase assay

To measure the activity of polygalacturinase, the assay mixture (1 mL) containing an equal volume of enzyme and 1 % (w/v) pectin dissolved in 0.1 M phosphate buffer (pH 7.0) was incubated at 50 °C for 10 min. The reducing sugar released was measured by the dinitrosalicylic acid method. Control samples were prepared with inactivated enzymes. One unit of enzymatic activity (U) was defined as one μmol of galacturoric acid released per min. All the experiments were conducted in triplicate and the data were expressed as a mean. The enzyme activity is expressed in terms of units per gram dry fermented substrate (U/g-ds).

Protein determination

Estimation of total protein was done by using the Bradford method and bovine serum albumin (BSA) as standard (Bradford 1976).

Amino acids analysis

Amino acid analyzer was used for amino acids analysis by hydrolyzing 0.5 g of protein with 10 mL of 6 N HCl under vacuum at 110 °C for 24 h. While, cystine and cysteine were determined after pretreatment with performic acid (Christias et al. 1975).

Results

Chemical analysis of the olive cake

Data presented in Table 1 revealed that, the olive waste has many valid part used in animal and poultry nutrition; it has 9.5 % protein, 5.7 % olive oil, which consider as a good source of energy and antioxidants, and low ash content (7 %). In spite of the previous advantages, there are other components that limit its supplementation in rations by conquerable amounts resample essentially in phenols which characterized by its low biodegradability that eliminate its nutritional value as it inhibits the microflora in the ruminant and the present lignin restricts the utilization of cell plant content by microflora. Besides, the olive cake contains a high crude fiber (33 %) which may be a good carbon source for many microorganisms. So we select different fungal strains known in biological treatments by producing no mycotoxins and applied in food fermentation and used as a probiotic in feeding field.

Effect of biological fungal treatment on the chemical composition of olive cake

OC bio-conversion into a better quality feed by biological treatment in solid state cultures could be an alternative to chemical and physical treatment for enhancing the OC quality for microbial fermentation in rumen. In the current work, it was obvious that the chemical composition of olive cake was affected according to the fungal strain used. Pointed to our aim, *A. oryzae* FK-923 (A2) gave the most promising results concerning the enhancement of the nutritive value of the OC (Table 2), as the following were achieved according to the importance for nutrition: (1) reduce the phenols to less than one. (2) Increase total protein

Table 1 Chemical composition of the olive cake

Content	Value
Dry matter (%)	88.0
Crude protein (%)	9.5
Ether extract (%)	5.7
Crude fiber (%)	33.0
ADF (%)	48.0
NDF (%)	62.0
Phenols (%)	3.1
Ash (%)	7.0
Lignin (%)	21.0
Gross energy (kcal/kg)	4200
TDN (%)	34.0

ADF acid detergent fiber, *NDF* neutral detergent fiber, *TDN* total digestible nutrient

Table 2 Effect of biological fungal treatment on the chemical composition of olive cake after 8 days of fermentation at 36 °C under solid state fermentation

Analysis	Control	*T1*	*T2*	*T3*	*T4*	*A1*	*A2*	*A3*
Dry matter (%)	88	88.0	88.0	88.0	88.0	88.0	88.0	88.0
Total protein (%)	9.5	12.8	13.9	14.8	12.5	15.2	17.4	16.6
Ether extract (%)	5.7	6.1	5.9	6.0	7.2	6.0	6.2	6.0
Crude fibers (%)	33	26.8	25.5	27.2	27.6	24.4	23.1	23.8
ADF (%)	48	38.2	37.6	39.1	38.9	35.8	34.6	36.2
NDF (%)	62	52.8	45.4	52.2	51.4	49.2	48.2	50.4
Phenols (%)	3.1	2.42	1.86	1.88	1.12	0.96	0.92	1.72
Ash (%)	7	9.8	9.8	10.6	9.6	9.8	9.4	10.2
Lignin (%)	21	18.6	20.6	17.6	18.2	16.4	14.6	11.5
Gross energy (kcal/kg)	4200	4220	4410	4260	4320	4520	4620	4420

to 17.4 %. (3) Decrease the crude fibers. (4) Decrease lignin. (5) Increasing gross energy. These results indicated that, the phenolic compounds, which are the more obstacles towards the use of OC by more amounts in rations, was decreased to a limit that did not affect the microflora. Besides, the increased amount of total protein by about 90 kg/ton can be used to substitute other expensive ingredients in feed rations. Moreover, the reduction in crude fibers content makes free to accomplish different feed rations.

Influence of biological fungal treatment on the fermented olive cake recovery

From the data illustrated in Fig. 1, it was clear that the treated OC recovery is correlated to the fungal strain applied. The percent of the recovery obtained with the application of *Aspergillus* sp. was higher than that with *Trichoderma* sp., since, *A. oryzae* FK-923 and *A. fumigatus* achieved the high recovery percent with 92.2 %.

Effect of biological fungal treatment on the total protein yield of olive cake

Results illustrated in Fig. 2 clearly indicated that, the total protein yield (kg/ton) of the treated OC was varied according to the fungus used and the percent of recovery. Whereas, the highest total protein yield (174 kg/ton) was obtained when *A. oryzae* FK-923 was used in biological treatment of olive waste, followed by *A. awamori* F-524 (161 kg/ton). On the other hand, lowest total protein yield was obtained with *T. koningii* F-322 treatment.

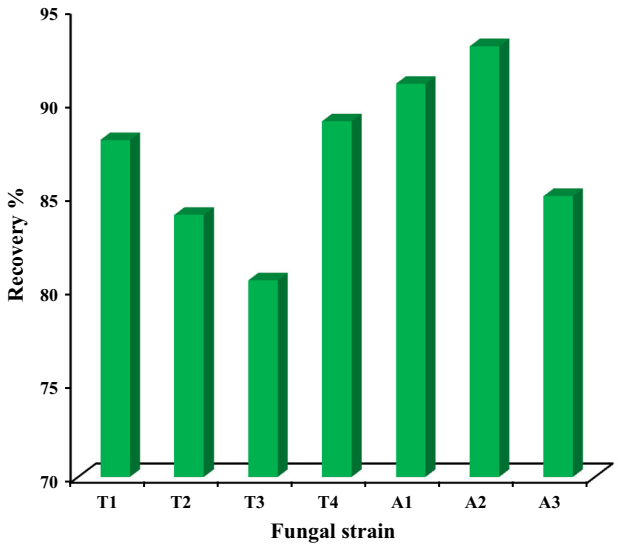

Fig. 1 Influence of biological treatment on the percent of olive waste recovery by different fungal strains after 8 days of incubation at 36 °C

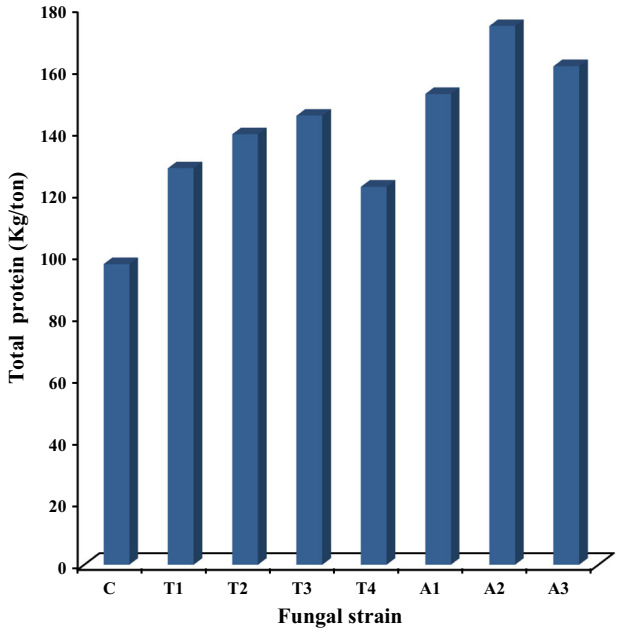

Fig. 2 Effect of biological treatment on the total protein yield in olive cake by different fungal strains

Table 3 Effect of sugar cane molasses supplementation on the chemical composition of olive cake treated by *A. oryzae* FK-923

Molasses amount (kg/ton)	Crude protein (%)	Dry matter recovery (%)	Total protein (%)	Organic matter (%)	Total fibers (%)	Phenols (%)
0	17.4	92.2	160.4	90.6	23.1	0.92
10	17.7	91.3	161.6	90.4	22.8	0.84
15	18.3	89.2	163.2	89.2	22.1	0.78
20	18.9	87.9	166.1	88.8	21.8	0.69
25	18.9	86.6	163.7	88.8	21.6	0.68

Table 4 Effect active dry yeast *S. cerevisiae* supplementation on the chemical composition of olive cake treated by *A. oryzae* FK-923

Yeast amount (kg/ton)	Crude protein (%)	Dry matter recovery (%)	Total protein (%)	Organic matter (%)	Total fibers (%)	Phenols (%)
0	18.9	87.8	166.1	88.8	21.8	0.69
0.5	19.2	87.2	167.4	88.8	20.6	0.65
1.0	20.0	86.8	175.3	88.4	19.8	0.61
1.5	20.2	85.4	170.8	88.1	19.2	0.55
2.0	19.6	85.4	167.4	88.1	19.2	0.55

Effect of sugarcane molasses supplementation on the chemical composition of treated olive cake

Form the data presented in Table 3, it was found that the addition of sugar cane molasses has an advantage effect in enhancement of feed quality of OC biologically treated by *A. oryzae* Fk-923, owing to the increase in protein content. The addition of sugar cane molasses at 2 % (w/w) was more suitable than other tested amounts, as the protein percent raised from 17.4 to 19 % in the fermented olive waste with total protein 166.1 kg/ton. On the other hand, phenols and fibers were reduced to 0.69 and 21.8 %, respectively.

Effect of active dry yeast *S. cerevisiae* supplementation on treated olive cake

Data presented in Table 4 showed that, the addition of active dry yeast has an advantage in the enhancement of feed quality of OC biologically treated by *A. oryzae* FK-923, as protein content was increased. On other hand, both phenols and fibers were decreased. Addition of dry yeast at 1.5 kg/ton olive waste was more suitable than other tested amounts, as the protein percent raised to 20.2 % in the fermented olive waste with total protein 170.8 kg/ton. On the other hand, phenols and fibers were reduced to 0.55 and 19.2 %, respectively.

Effect of biological treatment on the chemical composition of olive cake

The fermented OC showed a much lower cell wall component value than that of the untreated OC (Table 5). Whereas, organic matter, crude fiber, nitrogen free extract and total fiber contents in the biological treated olive cake were decreased than that of untreated OC (raw), while crude protein was increased from 9.5 to 20.2 % in the treated OC.

Table 5 Chemical composition, cell wall constituents and gross energy for olive cake before and after biological treatment by *A. oryzae* FK-923

Item	Olive cake	
	Raw (%)	Treated (%)
Chemical composition (%)		
Moisture	11.97	11.97
Component, % on DM basis		
OM	93.0	88.10
CP	9.5	20.2
CF	33.0	19.2
EE	5.7	6.1
NFE	76.42	42.46
Ash	7.0	11.90
Cell wall constituents, % on DM basis		
NDF	62	44.6
ADF	48	32.6
Total fibers	33	19.2
Gross energy, kcal/kg DM	4200	4320

OM organic matter, *CP* crude protein, *CF* crude fiber, *EE* ether extract, *NFE* nitrogen free extract

Enzymes involved in fermentation of olive cake by *A. oryzae* FK-923

The reduction of OC fiber content through the SSF process, using *A. oryzae* FK-923, is not surprising considering that this fungus possesses an efficient lignino-hemicellulolytic

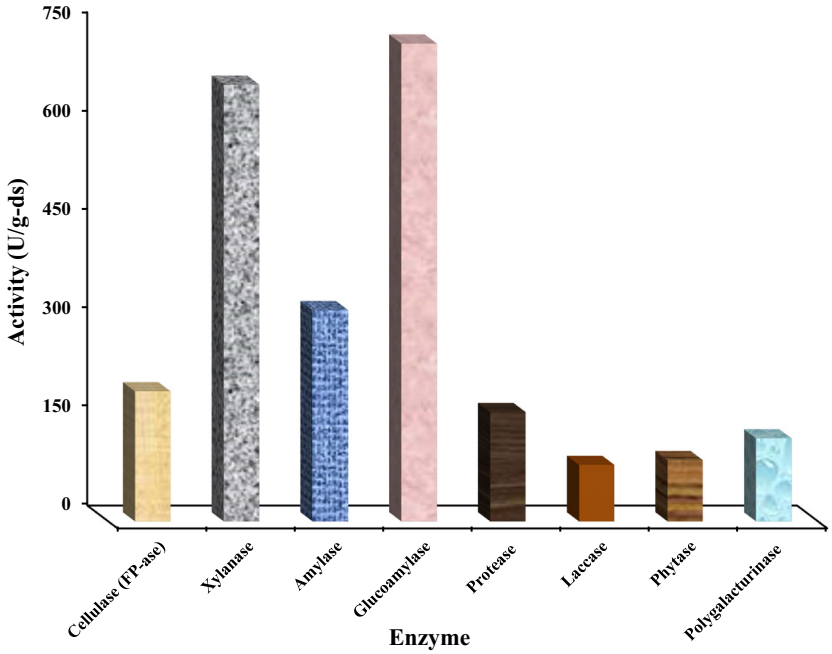

Fig. 3 Enzymes involved in fermentation of olive cake by *A. oryzae* FK-923 for 7 days at 36 °C

system as shown in Fig. 3. Since livestock and poultry cannot produce sufficient hydrolases enzymes in alimentary tracts, the presence of such enzymes add high nutritive value as well as save adding exogenous enzymes to feeds, thus increasing feed intake and production performance of livestock and poultry; increasing digestibility and absorptive of nutrients; enhancing the digestibility and utilization efficiency of feeds, and thus reducing feed costs.

The amino acid composition of microbial compared to alfalfa protein

The data presented in Table 6 showed that the amino acid profiles of *A. oryzae* FK-923 culture grown on olive cake have advantage in lysine, arginine, glutamic acid, proline, alanine, methionine and less in cysteine when compared with amino acid composition of alfalfa protein.

Table 6 Amino acid composition of microbial protein of *A. oryzae* FK-923 (g/100 g of protein) compared to Alfalfa protein

Amino acid	*A. oryzae* FK-923	Alfalfa
Lysine	9.2	6.70
Histidine	1.67	2.53
Arginine	7.12	5.54
Aspartic acid	12.8	12.54
Threonine	6.6	5.12
Serine	5.16	5.25
Glutamic acid	22.11	11.33
Proline	7.22	5.10
Glycine	6.10	5.73
Alanine	9.12	6.33
Valine	5.79	6.70
Methionine	2.05	1.36
Isoleucine	4.82	5.54
Leucine	8.86	8.43
Tyrosine	3.42	3.72
Phenylalanine	4.64	5.75
Cystine/cysteine	0.91	1.40

Discussion

In Egypt, large quantities of agriculture and agro-industrial by-products are generated and most of them are regarded as waste and non-conventional feedstuffs. The majority of researchers suggest that these lignocellulosic substrates when used as a source of protein, they are equivalent to soybean meal and other sources of protein for beef cattle diets (National Research Council (NRC) 2001; Loy and Wright 2003). The crude OC, used in this study is characterized by low protein content (9.5 %) and relatively high fat content (5.7 %). The OC is rich in fiber with NDF and ADF values of 62 and 48 %, respectively (Table 1). Since OC is rich in fiber, it has a low degradability in the rumen. OC bio-conversion into a better quality feed by *A. oryzae* FK-923 in SSF could be an alternative to chemical and physical treatment for enhancing the OC quality for microbial fermentation in rumen. The reduction in OC phenolic contents during SSF (Table 2) is an important

aspect in the adaptation of *A. oryzae* FK-923, since the capacity of a substrate to resist to degradation has been partially attributed to its array of phenolic compounds (Fermor and Macauley 1991). These results were in agreement with Salmones et al. (2005) who reported a significant negative relationship between phenolic concentration in coffee pulp and laccase activity.

An increase in protein content from 9.5 to 17.4 % was observed on OC fungal treatment with *A. oryzae* FK-923 over the untreated sample (Table 2). This result is consistent with the findings of Iyayi and Aderolu (2004) that showed an increase in CP contents *Trichoderma viride* treated agro-wastes. In addition, Dhanda et al. (1994) reported that the CP content of spent straw was increased from 3.42 to 6.19 % after biological treatment. The CP increase could be the result of increased fungal biomass (Chen et al. 1995) suggesting that the treated substrate is a good protein source for livestock. The improvement of CP contents could also be due to secretion of certain extracellular enzymes into the waste during their breakdown and its subsequent metabolism (Kinfemi et al. 2009). The addition of sugar cane molasses at 2 % (w/w) showed an increase in crude protein to 18.9 % with a reduction in phenols and fibers to 0.69 and 21.8 %, respectively (Table 3). Furthermore, *S. cerevisiae* supplementation at 1.5 % (w/w) to the fermentation medium raised the crude protein to 20.2 % (w/w), while phenols and fibers were declined to 0.55 and 19.2 %, respectively (Table 4). *S. cerevisiae* possesses several properties, such as its resistance to high sugar and alcohol concentrations and a high growth rate at increasing temperatures (Dhiman et al. 2002; Jung et al. 2008).

In most bioconversion studies, compositional changes, i.e. neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) followed by estimation of digestibility have been carried out to estimate the nutritive value of the final fermented feed (Shrivastava et al. 2012). In the present study, the fermented OC showed a much lower cell wall component value than that of the untreated OC, due to the decrease of NDF and ADF contents by 44.6 and 32.6 %, respectively (Table 5). These results might be due to the breakdown of lignocellulosic bonds by the fungal enzymes (Bilal 2008). The reduction in the total fibers content from 33 to 19.2 % confirms and proves the ability of the selected fungal strain in producing hemicellulases, cellulases, and polygalacturinase enzymes. In this concern Baraghit et al. (2009) reported that the biological treatments of different crop residues with various fungal and bacteria strains decreased cell wall total fibers content.

The present study revealed that, the phenolic compounds which are the more obstacles towards the use of OC decreased to a limit that not affected the microflora and this confirmed that the fungus could produce laccases under the culture conditions. Laccases (EC 1.10.3.2; benzenediol: oxygen oxidoreductases) are multicopper enzymes belonging to the group of blue oxidases that catalyses oxidation of a wide variety of organic and inorganic compounds, including diphenols, polyphenols, diamines and aromatic amines. One electron at a time is removed from the substrate, and molecular oxygen is used as the electron acceptor (Gianfreda et al. 1999). Some fungal laccases degrade toxic fungal metabolites, such as aflatoxin B1 (Alberts et al. 2009) and are also useful in the field of food microbiology. Furthermore, the chemical analysis indicated that, the gross energy was increased from 4200 to 4320 kcal/kg DM.

The degradation of polysaccharide compounds was associated with the increase in reducing sugars. Enhancing the concentration of these sugars is an additional advantage besides the reduction of lignocelluloses in the improvement of biomass quality through biological treatment. Enzymes assay showed that the residual fermented substrate loaded with glucoamylase 756, alpha-amylase 422.2, xylanase 664.4, celullases (FP-ase) 198.2 and polygalagturinase 76.8 U/g-ds (Fig. 3). Since livestock and poultry can't produce sufficient xylanase and cellulases in alimentary tracts, the presence of such enzymes add high nutritive value as well as save adding exogenous xylanase and cellulases to ruminants feed, thus increasing feed intake and production performance of livestock and poultry; increasing digestibility and absorptive of nutrients; enhancing the digestibility and utilization efficiency of feeds, and thus reducing feed costs (Yang and Wyman 2004).

Cellulases and xylanases have been used in the feed of mono gastric animals to hydrolyze non starch polysaccharides such as β-glucans and arabinoxylans. Cellulases, used as feed additives alone or with proteases, can significantly improve the quality of pork meat (Kumar and Wyman 2009). Glucanases and xylanases reduce viscosity of high fiber rye- and barley-based feeds in poultry and pig. These enzymes can also cause weight gain in chickens and piglets by improving digestion and absorption of feed materials (Taniguchi et al. 2005; Shrivastava et al. 2011). The detection of phytase in the fermented olive waste may be due to the addition of *S. cerevisiae*. Phytase (myoinositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyzes the hydrolysis of phytate to myoinositol pentakisphosphate and orthophosphate. Improving the digestibility of proteins and increasing the availability of phosphorus and other minerals, which are usually chelated by phytic acid (Konietzny and Greiner 2002) diminishes the anti-nutritive properties of phytate and prevents environmental pollution. Phytases have been studied in different yeast strains; an extracellular acid phytase was recently purified and characterized from *S. cerevisiae* by Jin-In et al. (2009).

Therefore, it can be concluded from this study that, untreated olive cake is poor in crude protein but rich in NDF and ADF. The biodegradation of OC using *A. oryzae* FK-923 resulted in an increase of crude protein and a decrease of fiber fractions and thus an increased digestibility of the resultant substrate. This suggests that SSF–*A. oryzae* FK-923 system is suitable for conversion of solid waste from olive processing industry into a value added animal feed. However, further research work in these areas is necessary to verify the effectiveness of this degraded substrate on the performance of live animals.

Acknowledgments The authors gratefully acknowledge the National Research Centre (NRC), 33 El-Bohouth St., Giza, Egypt, for the facilities and financial support that enable the authors to accomplish this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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