Bioconversion of the Olive Oil Mills By-Product (Jift) by Mushroom Fungi in Jordan

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Abstract: Olive oil mills by-product (Jift) and wheat straw (4:1, v/v) at 55% moisture was subjected to solid-state fermentation, and spawned (10% w/w) with *Pleurotus sajor-caju, Pleurotus ostreatus* and *Phanerochaete chrysosporium*. Biological conversion was monitored after 25, 50 and 75 days of incubation at 25°C and 30°C. *P. sajor-caju, P. ostreatus* and *P. chrysosporium* degraded 45.3, 32.5 and 50.4% of the original lignin content respectively. A reduction in the total phenolic compounds from 219 mg/g to 62.3 mg/g was caused by *P. sajor-caju* accompanied by 12.3% increase in crude protein. The pH of aqueous extracts decreased from 7.8 to 5.08. This is the first record of P. sajor-caju producing lignin peroxidase and manganese peroxidase. *P. ostreatus* and *P. sajor-caju* caused significant bleaching to olive seed fraction. Fungal protein extracted from *P. sajor-caju* colonized mix was investigated as a protein supplement in animal feed preparations.

Key words: Bioconversion, olive oil mills, Jift, *Pleurotus sajor-caju, Pleurotus ostreatus, Phanerochaete chrysosporium*, ligninolytic enzymes

1 Introduction

Olive oil mill byproducts are recognized as solid fraction, the olive pulp (olive pomace or Jift) and the olive wastewater (zebar) both of them constitute important agro-industrial wastes. The annual production of Jift is huge. Hamdi[1] reported that 1.4-1.8 million tons of Jift is produced in the Mediterranean area and those figures could be doubled or tripled due to increased olive oil production. In Jordan, it was estimated that over 100,000 tons of Jift is produced annually. This byproduct (Jift) has enormous potential to be recycled as animal foodstuff,[2] fuel, fertilizer, herbicide[3, 4] and as a source of various industrial chemicals.[5] Jift can be a beneficial resource for various economically valid uses that might be achieved after composting by fermentation techniques. Therefore, the present investigation is aimed to follow-up the gradual and continuous changes on Jift by the mushroom fungi P. *sajor-caju* and *P. ostreatus* during solid-state fermentation, and the assessment of ligninolytic enzyme activity during this process.

2 Materials and Methods

The olive pulp (Jift) was obtained from olive mills in northern Jordan, stock piled in a shed protected from outdoor environmental conditions, and used in the solid-state fermentation (SSF) experiments. The following fungi were utilized in the SSF process: *P.sajor-caju* and *P. ostreatus* (Mycology Research Laboratory, Jordan University of Science and Technology), and the white rot fungus *P. chrysosporium* (Suncrest Laboratories, Notasulga, AL, USA).

The inoculum (fungal spawn) was prepared as fungal growth on millet (*Sorghum bicolor*) seed contained in wide mouth glass jars (500 ml). The metal lid of each jar was made with central holes (2 cm diameter) plugged with cotton. Washed millet seeds were soaked overnight (at least 12 hours), autoclaved for 30 min. (121°C and 15 psi) and inoculated with agar cubes (0.5 cm^2) cut from actively growing fungal cultures. Inoculated jars were

incubated in darkness at 27°C until complete colonization of the substrate was achieved.[6]

The Jift was mixed with wheat straw in the ratio of 4:1 (v/v), moisturized (55 %) and distributed into jars similar as described above (150g per jar). Jars containing Jift-straw mix were autoclaved and inoculated with spawn (10% w/w) prepared above.[7] The treatments of Jift-straw mix with *P. sajor-caju, P. ostreatus*, and *P. chrysosporium*, and the controls (un-inoculated Jift alone and Jift-straw mix alone) were incubated under two temperature regimes, 25 and 30°C, and the process of SSF was monitored starting from day zero (t_0) throughout 75 days. Three jars from each treatment were pulled out at day zero (t_0), 25, 50 and 75 days of incubation. All treatments were replicated three times and experiments were repeated twice. Culture jars were housed inside the incubator and distributed as a randomized complete block for each temperature treatment.

At harvest time, colonized Jift-straw mixes were mixed with an equivalent amount of water (w/v) and filtered through several layers of cheesecloth. The collected liquid was kept frozen (-72°C) for further analyses. The solid fraction was re-suspended in a large amount of water inside a deep container in order to be separated into a floating portion, which is mainly mycelium and colonized olive pulp and straw and referred to as fungus protein (FP), and sediment of seed fragments (SF) in the bottom of the container. The three fractions, liquid, FP and SF, were subjected to the following chemical analysis. The protein and lipid contents were determined according to the micro-Kjeldahl and diethylether mixture extraction methods, respectively, and the acid detergent lignin was done by H_2SO_4 (72%) hydrolysis.[8] Total phenolic contents of solid Jift, Jift-straw mixes and their aqueous extracts were estimated by the Folin method.[9] The total condensed tannins were determined according to the method of Porter *et al.*[10] Qualitative analyses for total carbohydrates were determined by the anthrone method.[11] The amount of reducing sugars were estimated using the Somogyi-Nelson method.[12] The pH values of those fractions were also measured and recorded. Laccase, lignin peroxidase, and Mn-peroxidase activities were estimated in the crude aqueous extracts. Laccase activity was determined at pH 5.0 by monitoring the oxidation of 2,2-azinobis3-ethylbenzathiazoline-6-sulfonic acid (ABTS) at 405 nm, and lignin peroxidase was determined at pH 7.0 by monitoring the oxidation of ABTS at 405 nm.[13] The peroxidase activity was always corrected for laccase activity. Manganese peroxidase activity and laccase activity were estimated on the basis of the oxidation of 2,6-dimethoxyphenol (DMP).[14]

The seed fraction of Jift and Jift-straw mix were ground into small size with a hammer-mill to pass a 0.5 mm sieve. Samples from the ground seed fraction were evaluated for their degree of color brightness and yellowness indices, using a PREMIER COLORSCAN instrument. The color difference was measured using a CIELAB 1976 computer program, while the yellowness index was assessed using the ASTM D1925 computer program. The brightness index was assessed after the TAPPI45/ISO2470 computer program.[15]

The nutritional value of the FP was evaluated through partial supplementation of the commercial protein concentrate, AGRIMIX, (Broiler 10% EEC Belgium) the usual ingredient in animal food recipes. The FP was introduced in the animal food recipe as partial replacement to the commercial protein concentrate on the basis of the equivalent protein. Hence, the animal food was prepared with three different partial supplementary concentrations with the FP, namely 8.75%, 4.33%, and 2.17%, and utilized in a designed feeding experiment on male and female rats. The volume of FP precluded the preparation of animal food with higher FP concentrations, because it was introduced in its raw form. Also, Jift-straw mix, as a whole was utilized in the chicken food replacing corn ingredient by 25% and 50%.

3 Results

The jar culture experiment showed heavy colonization of the Jift-straw mix causing extensive biodegradation. The number of seed fragments, as well as their relative size and weight, were significantly reduced (Table 1).

Table 1. Properties of the olive seed fractions in the prepared Jift-mix, before and after colonization by *P. sajor-caju*

Properties of seed fragments per 10g of Jift-mix				
Kind of Product	**Number of Fragments**	**Average Diameter (mm)**	**Total Weigh (mg)**	**Average Particle(mg)**
Original (JIFT)	186*	2.7*	3220*	17.80*
Raw mix	126**	2.0*	2150*	17.28*
Original mix	181.3*	1.18*	1900*	10.65**

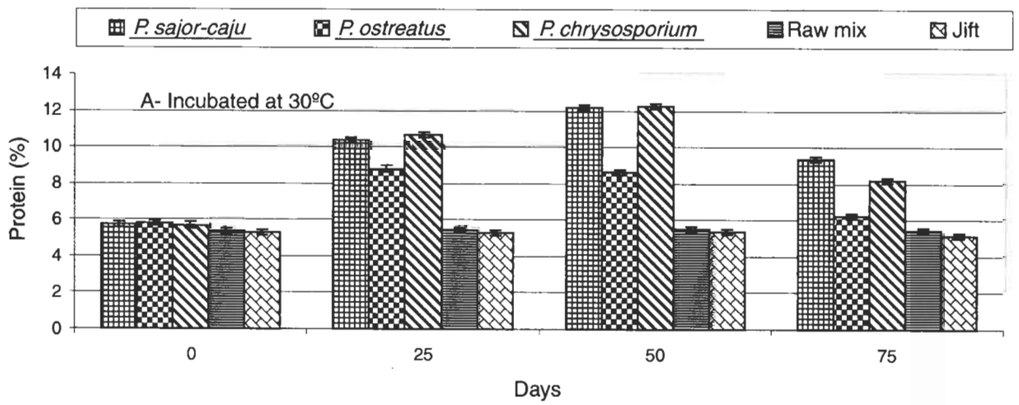
All values are averages of four replicates. * Significant at 0.05, ** at 0.01

3.1 Chemical changes in Jift-straw mix

3.1.1 Changes in protein content

Protein content values in the bio-converted Jift-straw mix shown in (Figure 1A) represent the extent of colonization by living fungal mycelium in the substrate. The highest protein content values (12.2%) was accomplished by *P. chrysosporium* and *P. sajor-caju*, after 50 days of incubation at 30°C, and lower values of 8.0% were found by *P. ostreatus*. The crude protein content of the substrate after 75 days of incubation declined to 8.2, 9.3 and 6.2% for the three fungi, respectively. In the second treatment at 25°C, *P. sajor-caju, P. ostreatus*, and *P. chrysosporium* accomplished crude protein percentages of 9.2%, 9.1% and 12% after 50 days incubation, respectively. However, after 75 days, those values were 7.1%, 9.2 %, and 8.4 %, respectively (Figure 1B). The percentages of protein content in the control were constant throughout all experiments at 5.4% for the Jift-straw mix control and 5.2% for the Jift alone.

The amount of protein passing into the aqueous extract from the bio-converted Jift was equivalent to 0.46%, 0.61% and 0.44% for *P. sajor-caju, P. ostreatus* and *P. chrysosporium*, respectively after 25 days at 30°C. At 25°C after the same period, the corresponding values were 0.41%, 0.50% and 0.28%. The value for aqueous extracts of the Jift-straw mix control and the Jift alone was 0.13% (Figures 2A and 2B).



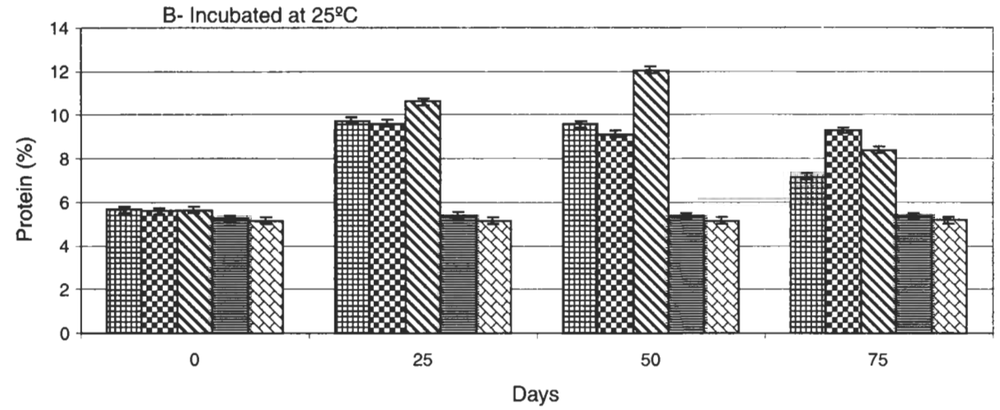
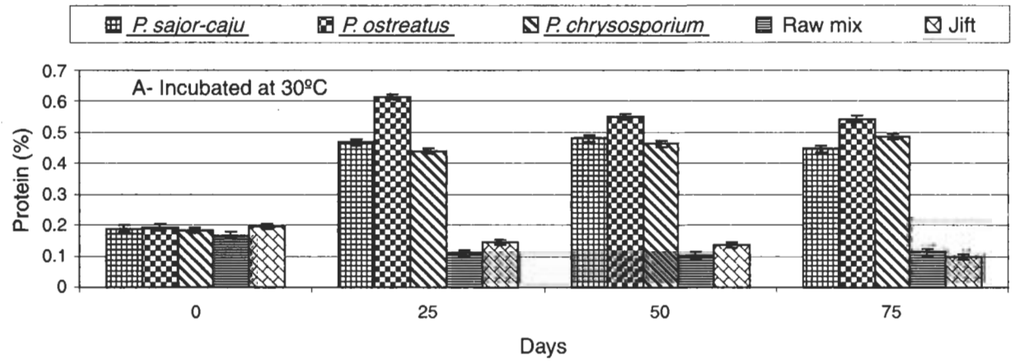


Figure 1. Percent protein content in the biologically converted solid part of the Jift-straw mix during the solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* after 0, 25, 50 and 75 days of incubation at 30°C (A) and 25°C (B)



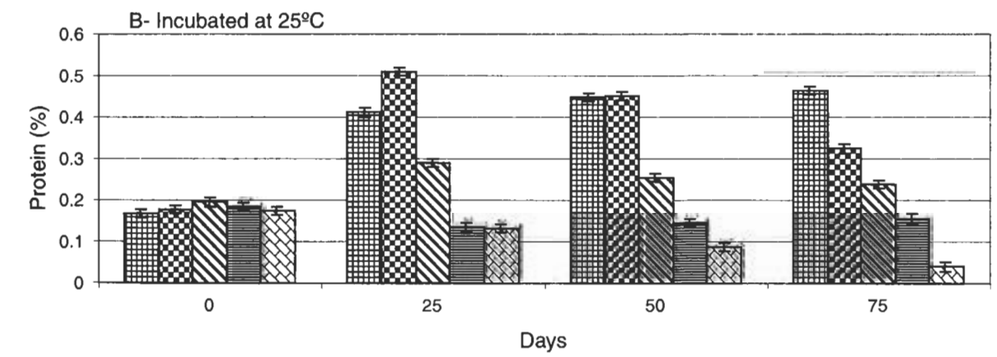


Figure 2. Percent protein content in the aqueous extract of the biologically converted Jift-mix during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* after 0, 25, 50 and 75 days of incubation at 30°C (A) and 25°C (B)

3.1.2 Changes in fat content

Results in Figures 3A and 3B show that Jift-mixes treated with *P. sajor-caju, P. ostreatus*, and *P. chrysosporium* underwent a progressive decrease in their percent content of fat throughout the 75 day incubation period. This significant reduction in the fat content of the bed mix occurred at both incubation temperatures (25°C and 30°C). However, percent of fat content in the controls, (2.8% and 2.3%) remained constant throughout the incubation period at both temperatures. After 75 days, the fat content decreased to 1.3%, 1.1% and 0.99% at 30°C, and 1.1%, 0.87% and 0.84% at 25°C, for *P. sajor-caju, P. ostreatus*, and *P. chrysosporium*,, respectively.

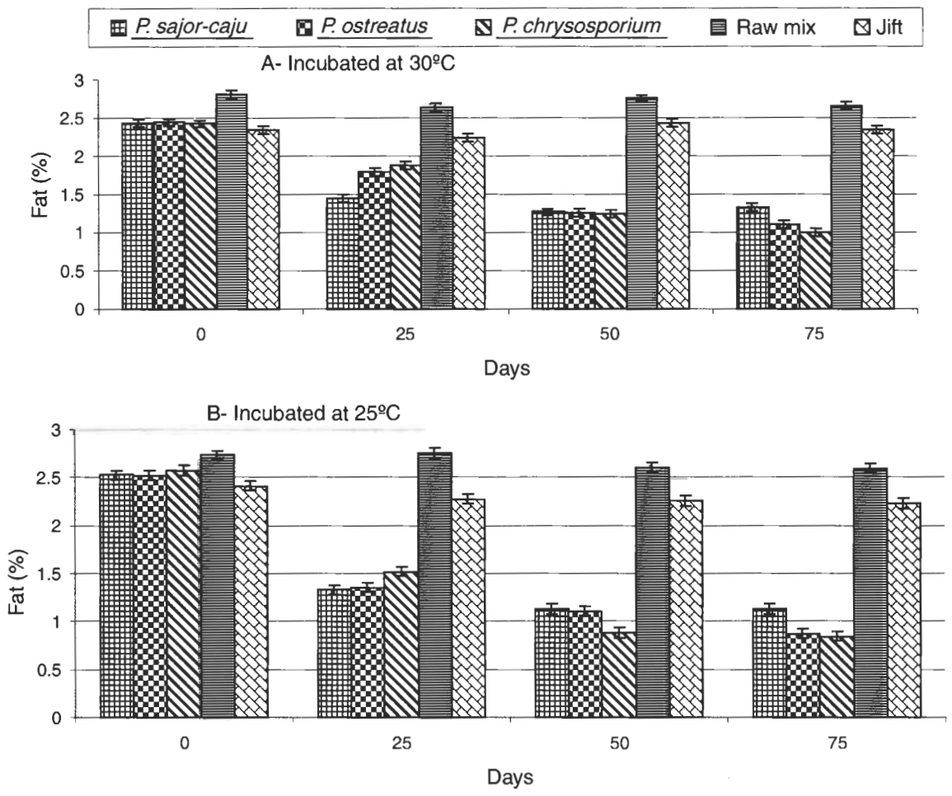


Figure 3. Fat (%) of Jift-mixes during solid-state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30°C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation
All analysis were replicated three times and repeated twice.

3.1.3 Changes in total phenolics and condensed tannins

The phenolic concentration was significantly reduced in the Jift-mixes by all three fungi over time at both incubation temperatures (Figures 4Aand 4B). The greatest decrease in phenolic concentration was achieved by *P. sajor-caju*, where it reached 62.3mg/g after 75 days at 30°C, followed by *P. chrysosporium* (77.3mg/g). *P. ostreatus*, reduced phenolics to 80.4 mg/g after 75 days at 25°C. Conversely, an increase in the total phenolics was observed in the bio-converted jift-straw mix aqueous extract with the highest levels observed in extracts of the *P. sajor-caju* converted mix (30.8mg/150ml), followed by *P. ostreatus* (30.5mg/150ml) and *P. chrysosporium* (29.8 mg/150 ml) (Figures 5A and 5B). Percentages of condensed tannins exhibited maximum reduction with *P. ostreatus* (0.50%) after 75 days at 25°C. Tannin reduction was 0.68% and 0.88% in the case of *P. sajor-caju* and *P. chrysosporium*, respectively after 75 days at 30°C.

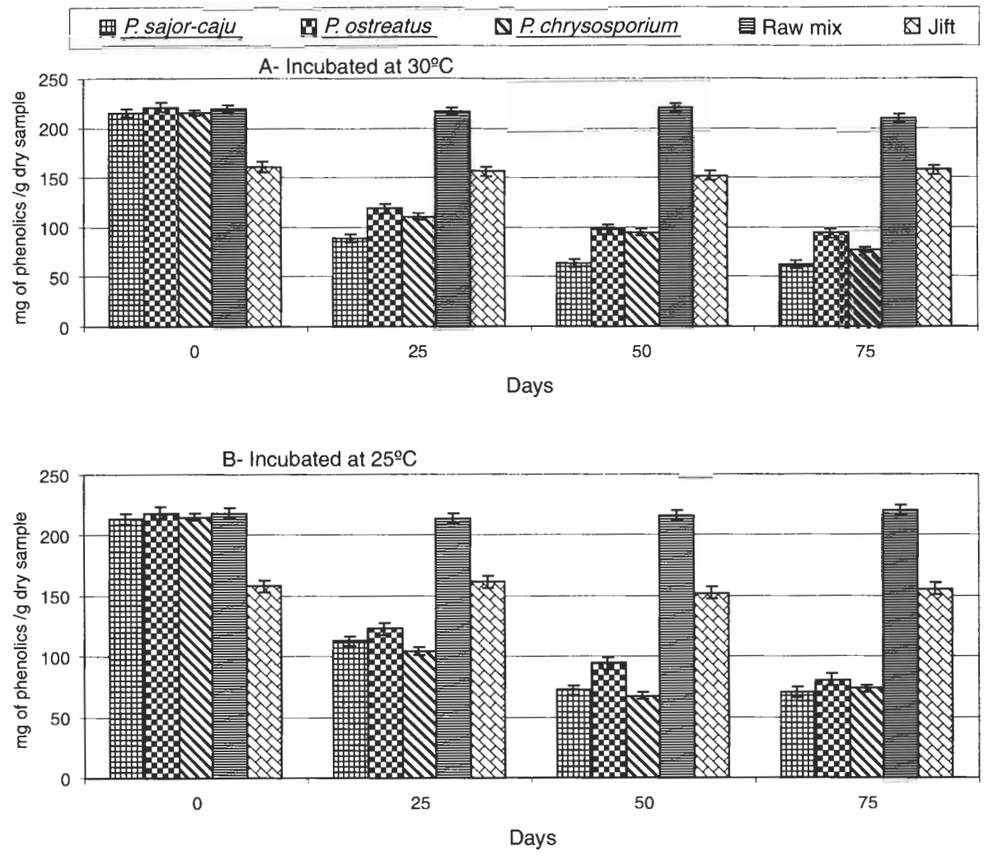
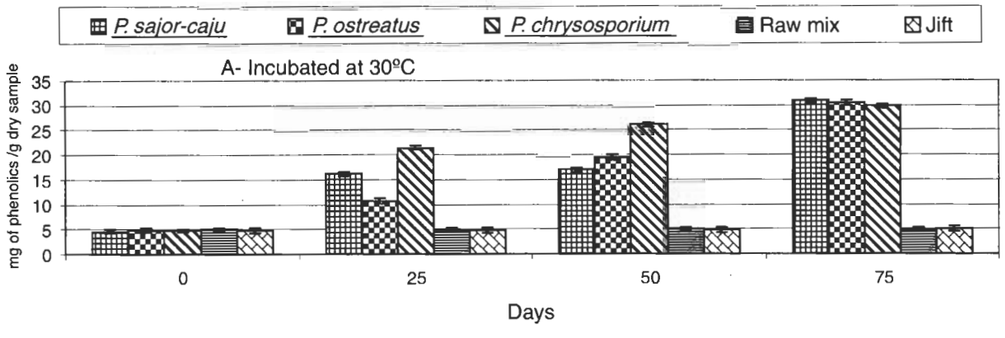


Figure 4. Total phenolics of Jift-mixes during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30 °C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation



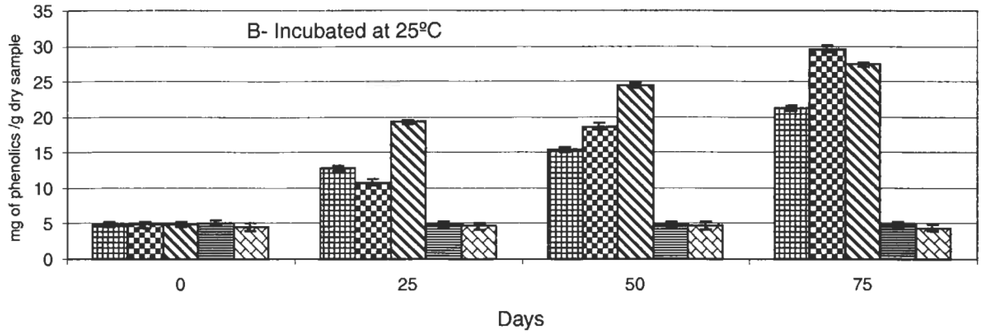


Figure 5. Total phenolics of Jift extract (liquid fraction) during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30 °C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation

3.1.4 pH Changes

All of fungi maintained the same trend in terms of the pH of the reaction medium during the 75-day solid-state fermentation period. There was a gradual change to acidic conditions from almost neutral pH after 25 days to an even more acidic pH after 50 and 75 days incubation. These changes were more pronounced in the case of *P. chrysosporium* compared with the two other fungi (Table 2).

Table 2. Changes in pH of Jift aqueous extract of the solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* after 25, 50 and 75 days incubation at 30°C (A) and 25°C (B)

Fungi	Change in pH		
	25 days	50 days	75 days
Incubated at 30°C			
P. sajor-caju	7.02	5.9	5.3
P. ostreatus	7.7	6.8	6.4
P. chrysosporium	6.49	5.4	5.08
Incubated at 25°C			
P. sajor-caju	7.53	6.3	6.12
P. ostreatus	7.4	7.01	6.1
P. chrysosporium	6.69	5.81	5.12

3.1.5 Ligninolytic enzymes

3.1.5.1 Laccase enzyme

Laccase activity was detected in Jift-straw mix colonized by each of the three fungi.(Figure 6A and 6B). By the end of 25 days of incubation at 30°C, *P. sajor-caju, P. chrysosporium* cultures exhibited 0.309 U/ml and 0.282 U/ml of laccase activity, respectively. The highest laccase activity was recorded for *P. ostreatus* (0.35 U/ml) after 50 days of incubation (Figure 6A). Laccase activity was lower in treatments maintained at 25°C, and after 75 days had dropped to almost zero.

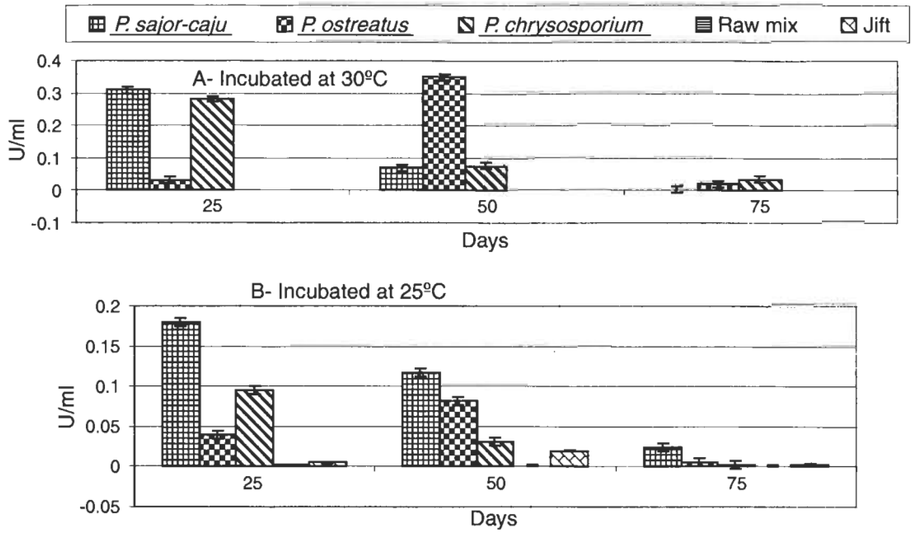


Figure 6. Laccase activity in the aqueous extract (liquid fraction) from bio-converted Jift during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30 °C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation

3.1.5.2 Lignin peroxidase (LiP)

Lignin peroxidase (LiP) was detected in Jift-straw mix colonized by each of the three fungi (Figure 7A and 7B), and enzyme activity remained high even at the end of the 75-day incubation period at 30°C. *P. chrysosporium* exhibited the highest value of 0.116 U/ml, followed by *P. sajor-caju* (0.101 U/ml) (Figure 7A). In the second treatment at 25°C, LiP activity began to decrease after 25 days incubation in the case of all three fungi (Figure 7B).

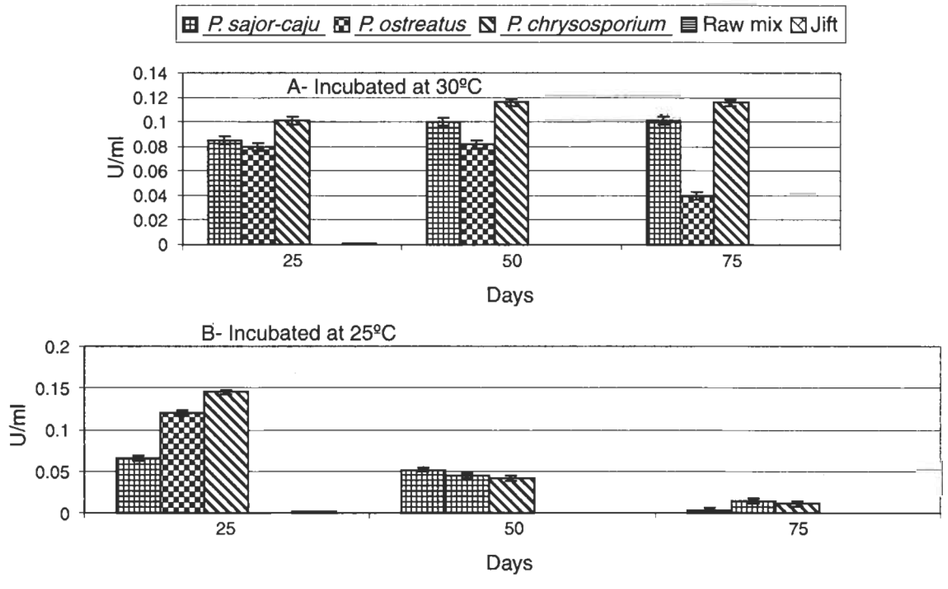


Figure 7. Lignin peroxidase activity in the aqueous extract (liquid fraction) from bio-converted Jift during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30 °C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation

3.1.5.3 Manganese peroxidase (MnP)

Manganese peroxidase (MnP) was detected only in *P. chrysosporium* and *P. sajor-caju*, cultures. In both cases, activity reached peak values of 0.312 U/ml and 0.256 U/ml, respectively after 50 days at 30°C (Figure 8A). In the second treatment at 25°C, MnP activity was lower for both fungi than at 30°C with values of 0.1638 U/ml and 0.173 U/ml detected after 50 days, respectively (Figure 8B).

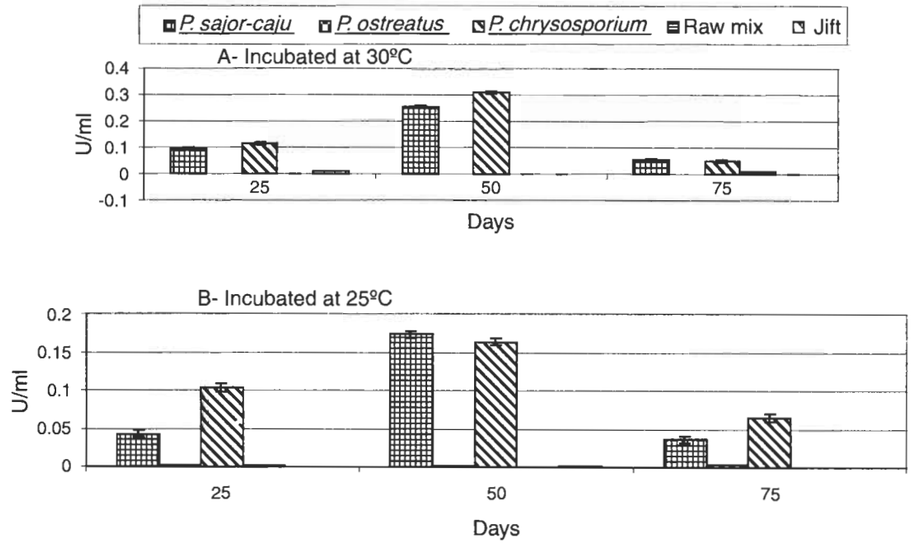


Figure 8. Manganese peroxidase activity in the aqueous extract (liquid fraction) from bio-converted Jift during solid state fermentation by *P. sajor-caju, P. ostreatus* and *P. chrysosporium* at 30 °C (A) and 25°C (B) after 0, 25, 50 and 75 days incubation

3.2 Biological bleaching

The dark brown color of the seed fragment in Jift was significantly bio-bleached after the biological treatment. *P. sajor-caju* resulting in a 16-fold increase in seed brightness. An 11-fold increase in brightness was achieved with *P. chrysosporium* (Table 3A), and *P. ostreatus* increased seed brightness 11.7-fold (Table 3B). The yellowness of the seed fragment was also assessed and the results were 22.7, 18 and 14-fold, respectively. The highest color change was caused by *P. sajor-caju* with a 26-degree change.

Table 3. Changes in biological bleaching of Jift during solid state fermentation by *P. sajor-caju, P. ostreatus, P. chrysosporium* at 30°C (A) and 25°C (B) after 25, 50 and 75 days incubation

Parameter	Degree in color change			Brightness index			Yellowness index		
	25	50	75	25	50	75	25	50	75
A- Incubated at 30°C									
P. sajor caju	6.2	22.01	26.12	10.6	15.47	16.02	21.1	22.1	22.7
P. ostreatus	2.1	6.50	10.51	4.1	6.8	7.4	10.2	10.4	11.4
P. chrysosporium	4.63	19.25	20.83	9.85	10.8	11.01	10.7	15.3	18.02
B- Incubated at 25°C									
P. sajor caju	5.8	21.37	27.06	9.90	12.81	14.1	16.8	20.14	20.4
P. ostreatus	3.5	8.70	15.09	5.8	9.6	11.7	12.06	13.8	14.6
P. chrysosporium	4.52	17.55	18.36	7.4	9.12	9.7	8.52	13.4	17.18

3.3 Feeding experiments

The results of utilizing the FP in animal feeding of rats and chicken indicated that there were no adverse effects upon the health of those animals (Tables 4 and 5).

Table 4. The effects of replacing the protein concentrates in rat feed by the float portion (FP) FP1 2.17%, FP2 4.33% and FP3 8.75% obtained from *P.sajor-caju* colonized Jift-mix on a protein-equivalent basis

Time (weeks)	Animal Body Weight per Each Treatment in Grams [1]								
	Contr.		FB1		FP2		FP3	Zero Port. Conc.	
	M	F	M	F	M	F	M	F	M
1st	343.25	238	345	287	314.5	260	422.8	248.3	372.3
2nd	346.87	244	347.3	284.6	313.8	265.3	427.1	243.3	375.5
3rd	350.72	247.6	348.3	277.3	318.3	269.6	431	225.7	383.1
4th	354.90	247.3	351.8	271.3	329.2	272.3	437.5	233	391.7
5th	365.30	249	363	271.3	337.7	278.3	450.5	239	402.8
6th	367.8	248.7	366.8	275.7	342.5	268.7	446.8	244	393.7
7th	365.3	281.7	369.5	268.3	340.3	285.3	431.5	229	395
8th	368.42	281.7	371.3	268.3	353.5	285.3	433.3	229	402.25

[1] All values are averages of three replicates; Control = regular feed prepared including protein concentrate; Fungal Protein fractions 1, 2, & 3 are the three concentrations of the float;
M & F, are male and female rats, respectively.

Table 5. The effects of replacing the corn component in chicken feed with the whole Jift-straw mix obtained from the *P. sajor-caju* colonized Jift-straw mix utilized as unwashed (UW) and washed (W) with tap water

Day	Control	UW 25%	UW 50%	W 25%	W 50%
1	50	50	50	50	50
7	125.51	108.33	104	108	108.695
14	205	125	130.434	160	133.333
21	351.041	321.944	218.181	255	251.136
28	831.25	611.111	450	562.5	440.909
35	921.875	875	657.5	821.615	684.545
Body gain	871.875	825	607.5	771.615	634.545

4 Discussion

Despite the fact that numerous biological and/or chemical solutions have been proposed for olive mill wastewater (OMWW) treatment,[16-18] very little attention was paid to the treatment of Jift as a solid by-product.[2] However, previous studies utilized composted Jift for various purposes.[19] Preliminary attempts made in this study proved Jift to be suitable for cultivating basidiomycetes fungi such as *P. sajor-caju, P. ostreatus* and *P. chrysosporium.* Such preliminary results suggested Jift to be a good candidate for further investigations for growing other types of fungi able to biodegrade or bioconvert the material and, at the same time, produce fungal secondary metabolites. Growth of those fungi on Jift-straw bed mix caused drastic changes in the physical and chemical properties of that substrate which may have improved its nutritional value. There was a significant reduction in the fiber content of the mix similar to that previously reported by Valmaseda et al.[20] on wheat straw, and Szebiotko et al.[21] on bean shells. Agosin et al,[22] suggested that any decrease in fiber content of fiber-rich substrates used as an ingredient in animal feed may improve and/or increase its digestibility. Therefore, cultivation of lignolytic fungi on Jift-straw mix renders this substrate to be a more suitable substitute for some components in animal rations. Moreover, the heavy colonization of Jift-straw mix by the white fungal mycelium mass enriched this substrate with protein to levels comparable to those previously observed in cases of oyster mushroom cultivation on coffee pulps.[23] The total protein content in the Jift bed mix treated with *P. sajor-caju* and *P. chrysosporium* reached 13% over the raw untreated control. The protein level, which was obtained on the basis of Kjeldhal total nitrogen value, may yet represent just a fraction of the real total protein content, i.e. the fungal biomass. Keeping in mind that the fungi utilized the nitrogen available in this substrate only, differences in treated Jift represent the outcome of various complementary bioconversions within this SSF. Although total nitrogen based analysis is still a valid measurement for protein content, certain specific direct or indirect measurements would have been more indicative of the real protein value. The presence of appreciably high amounts of phenolics and tannins in Jift may act as toxic and/or anti-nutritive factors.[24] Thus, reduction or removal of these ingredients by fungi render Jift more suitable as animal feed. This study revealed significant reductions in total phenolic compounds in treated Jift-straw bed mix by all the fungi tested, to reach 62.2, 77.3 and 90.1 mg/ml for *P. sajor-caju, P. ostreatus* and *P. chrysosporium*, respectively. Moreover, a significant reduction in condensed tannins was obtained. Those fungi reduced the tannin content of the Jift-straw mix to 0.68%, 0.88% and 0.5%, respectively. Similar results were reported by Lozano[23] and Wong & Wang[25] after growing *P. sajor-caju* on coffee grounds, where the potential nutritive value of this substrate as animal feed was greatly improved.

The three separate fractions obtained by washing the biological converted Jift bed mix, namely the floating portion (FP), the seed fragments (SF) and the liquid extract, reflected certain changes which occurred during SSF. Such changes are thought to be related hydrolytic processes involving lignin and other polymers as well as removal of other macromolecules, which took place during SSF. The decrease in the total weight of seed fragments from 17.28 mg to 10.65 mg, in the case of *P. sajor-caju*, indicated hydrolysis of its lignin and cellulose components. The partially hydrolyzed lignin in Jift renders it safer to the animal teeth when incorporated within its ration. Also, the possibility exists of channeling those seed fragments to cellulose conversion industries since they underwent a bio-bleaching process. The brightness of disc preparations of seed fragments from treated Jift-straw mix was increased 16-fold over controls. The liquid extract on the other hand could be used as a source of soluble lignin and enzymes like laccase, lignin peroxidase and manganese peroxidase. The floating portion which is mainly made up of fungus mycelium, could be used as protein concentrate.

All of the three ligninolytic enzymes, laccase, LiP and MnP, were detected at all intervals throughout the SSF processing of the Jift mix by the three fungi. However, lignin reduction was greater in case of *P. sajor-caju* and *P. chrysosporium*. These two fungi showed enzymatic activities for a longer duration throughout the SSF period. One of the contributions made by the present research work are the first reports of LiP and MnP production by *P. sajor-caju* and Lip production by *P. ostreatus*, based on their positive enzymatic activities seen with ABTS as a substrate for LiP, and DMP for MnP.[13] Bio-bleaching and bio-pulping of lignin-rich wood material is an

advancing technology,[15] in which ligninolytic enzymes are extensively utilized. The seed fraction of olive seed in Jift is very rich in lignin and it has dark brown appearance. All fungi tested were able to biologically bleach the seed fraction in treated Jift mix. Katherine and Frederick,[15] in Kraft pulp bleached by *Trametes versicolor*, obtained comparable finding. The bleaching observed in the present investigation was measured by the increase in brightness of the seed fraction up to 16-fold in the case of *P. sajor-caju* in comparison with the control. This bio-bleaching of the seed fragment in Jift offers new avenues for using this product, and this process in the pulping industries as an alternative to chlorine and caustic treatment.[26] The bio-bleached olive seed fragments could also be utilized instead of small gravels in the feed of egg laying hens.

The SSF of Jift by the basidiomycetous fungi *P. sajor-caju, P. ostreatus*, and *P. chrysosporium* caused a rapid drop in pH from 7.8 to 5.08 during a 75-days incubation period at 30°C, which was proportional with the ligninolytic activity of those fungi. White-rot fungi, including members of the genera used in the present investigation, caused similar decreases in pH during wheat straw fermentation.[27] Such increases in the acidity in the fermentation medium, which is rich in lignin and phenolic compounds, can be visualized as a consequence of increased phenolic acid due to the degradation of lignin side-chains.

One of the major positive bioconversions achieved in the present SSF of Jift was the increase in total soluble carbohydrate and reducing sugars in the aqueous extract which reached a maximum of 2.25 mg/ml and 2.06 mg/ml, respectively. This is a logical consequence of cellulose and hemicellulose degradation. This kind of conversion is in harmony with a previously reported cellulase and hemicellulase enzymes activity by these fungi.[20, 28]

Jift, as an ample and persistent organic by-product, has been considered as animal feed supplement either directly or mixed within the feedstuff. However, such kind of Jift utilization in animal feed of large animals is still at the experimental stage. The present study indicated that SSF treatment of Jift by fungi such as *P. sajor-caju* offers a genuine approach in the biological conversion of Jift into a promising valuable product, and a source of protein concentrate (FP). The results obtained from the feeding experiments are highly encouraging for further investigations toward using biologically converted Jift and/or the FP extracted from that product in animal food.

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