Utilization of garlic and maize wastes supplemented with olive mill waste water for *Pleurotus ostreatus* cultivation

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ORIGINAL

Utilización de desechos de ajo y maíz suplementados con alpechín para el cultivo de *Pleurotus ostreatus*

Resumen. Los cultivos de ajo y de maíz están ubicados en la Argentina en áreas similares destinadas a la producción de oliva, lo cual es una ventaja para la eliminación de desechos producidos por la extracción de aceites de oliva por medio de la producción de hongos. El objetivo de este trabajo fue investigar el uso de diferentes porcentajes de alpechín (OMWW) mezclados con desechos de ajo y maíz para la producción de basidiomas de *Pleurotus ostreatus*. La cepa BAFC 2067 de *P. ostreatus* fue cultivada en desechos de ajo y maíz mezclados con 15, 30, 45 y 60% de alpechín. Bolsas con 0% (control) y 100% de alpechín fueron también inoculados. El tiempo de colonización y las cosechas obtenidas permitieron concluir que el mejor sustrato utilizado para la producción de basidiomas fue la mezcla compuesta de alpechín y desechos de maíz. El efecto negativo para el crecimiento de *Pleurotus ostreatus* en ajo humedecido con agua destilada fue revertido cuando los desechos de ajo contenían 15 y 30 % de alpechín. Un efecto deletéreo sobre las cosechas fue observado cuando 60 % de alpechín fue utilizado para humedecer tanto el desecho de ajo como de maíz.
Palabras clave: desechos agroindustriales, gírgolas, oliva, producción.

Abstract. Garlic and maize cultivations in Argentina are located in similar areas where olive is produced, an advantage for eliminating wastes produced for oil extraction by mushroom cultivation. The aim of this study was to investigate the use of different percentages of olive mill waste water (OMWW) mixed with garlic and maize wastes for the production of *Pleurotus ostreatus* basidiomes. Strain BAFC 2067 of *P. ostreatus* was cultivated in garlic and maize wastes mixed with 15, 30, 45 and 60% of OMWW. Bags with 0% OMWW (control) and 100% OMWW were also inoculated. The time of colonization and the yields obtained permitted us to conclude that the best substrate utilized for basidiomes production was a mixture composed of OMWW and maize wastes. The negative effect in the growth of *P. ostreatus* on garlic wetted with tap water was reverted when garlic wastes contained 15 and 30 % of OMWW. A deleterious effect on yield was observed when 60 % OMWW was utilized for wetting garlic and maize wastes.
Keywords: agro-industrial wastes, olive, oyster mushroom, production.

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Introduction

The olive oil extraction industry produces high amounts of olive mill waste water (OMWW) principally in the Mediterranean olive growing countries. Many other countries, such as Argentina, Australia and South Africa are considered emergent producers since they are promoting intensive olive tree cultivation. The extraction of olive oil generates huge quantities of wastes that may have a great impact on land and water environments because of their high phytotoxicity (Roig *et al.*, 2006). For every litre of oil produced, 2.5 L of wastewater is released by three phase olive mills (Fountoulakis *et al.*, 2002) and this waste has not only a high pollution potential but also its chemical oxygen demands (COD) could reach up to 200 g L^{-1} (Kalmis and Sargin, 2004). OMWWs contain polyphenols, sugars, tannins, polyalcohols, pectins and lipids (Hafidi *et al.*, 2005). The concentration of phenols reaches up to 10 g/L (Borja *et al.*, 1992), which contributes to a high toxicity and antibacterial activity. The origin and amount of the organic charge of OMWW permit the use of mushrooms as an aerobic biological method to be applied in bioremediation, and this process leaves a residue of lower toxicity and diminished phenol content. García García *et al.* (2000) reported that the species *Phanerochaete chrysosporium*, *Aspergillus niger* and *A. terreus* removed phenol compounds in olive mill waste-water aerobically. D'Annibale *et al.* (2004) showed that *Panus tigrinus* and *Lentinula edodes* removed toxic phenols from olive-mill wastewater. Several authors studied the use of *Pleurotus* strains for the production of biomass and basidiomes on OMWW, by achieving in parallel a significant decrease in the phenolic concentration (Sanjust *et al.*, 1991; Tsioulpas *et al.*, 2002).

Garlic and maize are cultivated in Argentina in similar areas where olive is produced, which is an advantage for the production and waste elimination by mushrooms. The cultivation of *Pleurotus* has increased generating a decrease in the production of traditional cultivations as *Agaricus bisporus* and *Lentinula edodes* (Chang, 1993). Eger (1965) developed the culture of *Pleurotus* on corn wastes and the industrial production of substrate for a later basidiome production was developed by several authors (Junková, 1971; Kalberer y Vogel, 1974; Stanér y Rysavá, 1971; Zadrazil, 1973; Zadrazil y Schneidereit, 1972). Several substrates for the oyster mushrooms cultivation were utilized, such as the utilization of paddy straw, maize stover, surgarcane bagasse, coir pith (Ragunathan *et al.*, 1996), leaves of hazelnut, leaves of tilia, leaves of aspen (Yildiz *et al.*, 2002) and coffee pulp (Salmones *et al.*, 2005).

The aim of this study was to examine the use of OMWW at different concentrations for moistening the garlic and maize wastes to produce basidiomes of *Pleurotus ostreatus* and to compare with substrate without OMWW.

Material and methods

The organism utilized was *Pleurotus ostreatus* (BAFC 2067, Italia, Commercial strain, 15- IX-1993). OMWW was obtained from a local olive oil producing company and used immediately for the preparation of the substrates. The percentage of water measured in OMWW was 79.37±1.46 and was used for the formulation of the different percentages with garlic and maize waste to adjust humidity in the substrate.

Wastes of garlic and maize were cut into pieces of 2-4 cm. Wastes of maize consisted on 50% leaves and stalks waste plus 50% corn cob waste. Each waste was mixed with 15, 30, 45 and 60% OMWW (dry weight). Six polypropylene bags were filled with 300 g (dry weight) of each formulation; humidity was adjusted in the substrate to 74 %. We inoculated bags without OMWW as control and bags with 100% OMWW. Bags were stopped with cotton plugs held by PVC

(polyvinyl chloride) cylinders before they were sterilized at 120 °C for 2 h. After cooling, the bags were inoculated with 5% (wt/wt) spawn and incubated in the dark at 25 °C. After bags were completely colonized by the mycelium they were moved to the basidiome production rooms. Cropping conditions to induce basidiome formation were 20 ºC, 9 h light/15 h dark photoperiod (20 W fluorescent light), 75 to 85 % humidity levels and watering by spray (fog type, pressure = 2 pounds / square inch) for 5 min every 3 h, which was automatically provided; the air in the cultivation room was renewed 6 times per hour. Eight cuts/bag were made to induce basidiomes formation. Basidiomes were harvested when mature and fresh weight was recorded during 70 days following induction. Biological efficiency (BE) was calculated as (fresh weight of harvested mushrooms / dry matter content of the substrate) × 100, and compared among treatments.

Six bags were used for each treatment; number of replications was confirmed by Rabinovich's test (Rabinovich, 1980). Tukey HSD (honest significant difference) test was used to determine the significant differences between groups in an ANOVA (analysis of variance). Normality and homogeneity assumptions were checked by KS and Bartlett tests respectively (P>0.05) for the validity of ANOVA method.

Results and discussion

For 100 % OMWW, a complete but weak growth was observed after 60 days of mycelial colonization and no basidiomes were produced during the cultivation.

The time required for complete colonization and primordium initiation on the different formulations of garlic and maize wastes supplemented with OMWW, 100% OMWW, and control are given in Tables 1 and 2. In garlic without OMWW colonization by mycelium was complete by day 34 and primordium formation started on day 7 after induction. On the garlic substrates containing 15 and 30 % OMWW complete colonization was delayed by 16 days. For the substrates to which 45 and 60% OMWW was added, colonization and primordium initiation were further delayed.

When maize waste was used (control), colonization of the substrate took 40 days, as on the maize substrate

Table 1. Mycelial colonization, primordium initiation after induction, weight of harvested basidiomes and BE of *P. ostreatus* BAFC 2067 on OMWW with garlic wastes

OMWW %	Mycelial colonization (days)	Primordium initation (days)	Weight of harvested basidiomes (g)			Total harvested (g)	BE (%)
			Flush 1	Flush 2	Flush 3		
0	34	7	157.0 ± 67.0 a	73.0 ± 42.6 a	33.3 ± 17.5 a	265.7 ± 49.2 a	88.6
15	50	7-8	154.0 ± 78.2 a	101.8 ± 40.6 a	90.3 ± 41.7 b	346.2 ± 46.1 a	115.4
30	50	18-20	170.2 ± 57.5 a	104.0 ± 29.7 a	66.6 ± 29.7 a	340.8 ± 48.5 a	113.6
45	42	28	142.3 ± 34.6 a	82.0 ± 50.7 a	33.0 ± 13.6 a	258.0 ± 52.0 a	86.0
60	38	47-50	85.0 ± 35.8 a	41.0 ± 29.6 a	20.3 ± 18.7 c[a]	146.3 ± 51.4 b	48.5
100	60	-	-	-	-	-	-

Means ± standard deviation in the same column followed by the same letter are not significantly different according to Tukey HSD test. Six replicates per substrate and treatment were performed.

Table 2. Mycelial colonization, primordium initiation, weight of harvested basidiomes and BE of *P. ostreatus* BAFC 2067 on OMWW with maize wastes

OMWW %	Mycelial colonization (days)	Primordium initation (days)	Weight of harvested basidiomes (g)			Total harvested (g)	BE (%)
			Flush 1	Flush 2	Flush 3		
0	40	6	258.0 ± 131.9 a	71.3 ± 57.1 a	24.3 ± 13.0 a	353.7 ± 53.4 a	117.9
15	40	13-14	212.0 ± 60.5 a	105.5 ± 39.0 a	71.0 ± 49.9 b[a]	388.5 ± 57.2 a	129.5
30	45	15-17	137.3 ± 43.1 a[b]	64.2 ± 30.0 a	17.7 ± 10.9 a	219.2 ± 64.2 b	73.1
45	49	12-13	135.7 ± 9.4 a[b]	36.7 ± 29.8 a	22.3 ± 11.1 a	194.7 ± 46.3 b	64.9
60	56	20-23	62.0± 30.2 b	41.0 ± 19.3 a	7.25 ± 5.9 c[a]	110.2 ± 47.7 b	36.7
100	60	-	-	-	-	-	-

Means ± standard deviation in the same column followed by the same letter are not significantly different according to Tukey HSD test. Six replicates per substrate and treatment were performed.

containing 15% of OMWW. The complete colonization was delayed by 5, 9 and 16 days when the percentages were 30, 45 and 60 % respectively. In the control, primordium initiation was observed 6 days after the substrate was completely colonized by mycelium, while on substrate wetted with 15, 30, 45 and 60 % it occurred 13-14, 15-17, 12-13 and 20-23 days after induction, respectively.

Results obtained in the both substrates showed that the sum of the days required for colonization and primordium initiation was always lower for maize than garlic wetted with OMWW. Only the time required for colonization and primordium initiation was lower for garlic wetted with tap water.

The highest yields were obtained with garlic waste wetted with 15% of OMWW (BE 115.4), but there were no significant differences ($p > 0.05$) with garlic wetted with tap water (control) and mixtures containing 30 and 45 % of OMWW; the mixture with 60 % OMWW (BE% 48.5) was significantly lower than the rest of the treatments. Three flushes of mushrooms were observed for each treatment (Table 1). Yield of *P. ostreatus* on garlic wetted with tap water and mixtures containing different percentages of OMWW were no statistically different ($p > 0.05$) among the first and the second flush yields; yields obtained with 15 % OMWW in the third flush was significantly higher than the rest of percentages, while 60 % OMWW was significantly ($p < 0.05$) lower. 44–59% of the total yield was harvested during the first flush in the control and all percentages, 27–31 % during the second flush and 12–26 % in the third. Securely, The high OMWW content increased the risk of contamination, and it could be observed on the high values of standard deviation (Tables 1 and 2).

When the substrate utilized was maize waste, best yield was obtained with 15 % OMWW (BE% 129.5) and was significantly ($p < 0.05$) higher than 30, 45 and 60 % OMWW; no significant differences ($p > 0.05$) were observed with the control (Table 2). Yields obtained in the first flush were higher than with garlic waste: in the control, 30 % and 45 % OMWW treatments, 62–73 % of the total produced was obtained from the first flush, 18–29 % from the second and 6–11 % from the third; while, in the 15 and 60 % OMWW treatment, 54–56 % of the total yield was obtained from the first flush, 27–37 from

the second and 6-18 from the third.

The present study aimed at examining to what extent and how efficiently could olive oil mill wastes mixed with two waste materials from agro-industry be transformed into a substrate for the production of basidiomes. Many of the potential products from fruit and vegetable residues have been developed using the SSF (Solid Substrate Fermentation) technique, and such products include mushrooms (Laufenberg *et al.*, 2003). The use of wastes for cultivation of edible mushrooms has two important utilities: to eliminate the wastes that could cause illnesses and to generate new products with nutritious values. We observed that the substrate discolored probably by the phenol discoloration. This could not be observed in bags with 90 % of OMWW.

The time of colonization and the yields obtained permitted us to conclude that the best substrate utilized for mixing with OMWW in this experiment was maize wastes. Cobs have important quantities of lignin and cellulose (Lu *et al.*, 1998; Okeke y Obi, 1994), which are important for the growth of the lignocellulolytic mushrooms. Times required in the present work for substrate colonization were 34-50 days for mixtures with garlic wastes and 40-56 days for mixtures with maize wastes. These times were higher than cited by other authors in similar experiences, as time required by *P. citrinopileatus* and *P. sajor-caju* on wheat straw with different percentages of OMWW, in which needed 19-32 days for the former and 21-36 days for the latter (Kalmis and Sargin, 2004).

Yields obtained with maize wetted with tap water were higher than garlic. The presence of substances, such as allicin, ajoene, thiosulfinates and a wide range of other organosulphurate compounds in garlic (Ledezma and Apitz-Castro, 2006), which probably are present in the wastes, have antifungal effects (Magro *et al.*, 2006) and could affect the growth of *P. ostreatus in this study*. However, this negative effect observed in garlic wetted with tap water was reverted when garlic wastes containing 15 and 30 % of OMWW. Cha *et al.* (2004) studied the production of *Pleurotus ferulae* on cotton seed supplemented with 5, 7 and 10% of garlic powder among other components. They obtained the highest yield with 7% level of garlic, and it was 83% higher than that of sawdust culture. According to Cha *et al.*, the reason why garlic did support growth was not clear but it is possible that garlic might contain effective components in the formation of basidiomes or that garlic contains compounds that stimulate ligninolytic enzyme production, leading to higher degradation and substrate utilization. Also, the presence of substances in garlic with antibacterial effects (Gomaa and Hashish, 2003) together with the substances with the same action found in OMWW (Capasso *et al.*, 1995; de la Rubia *et al.*, 2007; Obied *et al.*, 2005) could increase the growth of *P. ostreatus*. Thus, the growth of *P. ostreatus* on garlic and OMWW would be in a balance among the growth inhibition for antifungic compounds present in garlic and the benefit of the nutrients in the mixtures, together with compounds that could help for the elimination of organisms that could compete for the substrate. The statistically significant deleterious effects on yield when 60 % OMWW for wetting garlic and maize wastes are in line with the negative effects reported by Zervakis *et al.* (1996) and Kalmis and Sargin (2004).

According to yields obtained in first and second flush on garlic wetted with different percentages of OMWW, it could be possible utilizing higher percentages of OMWW without harvesting the third flush. Kalmis and Sargin (2004) obtained only 0-5.7% of basidiomes in the third flush of *Pleurotus cornucopiae* var. *citrinopileatus* in a cultivation on wheat straw and OMWW, and 6.5-9.2% of *Pleurotus sajor-caju*; we obtained 12–26 % for garlic and 6-18 % of the total yield for maize wastes. In general, better BEs were obtained in the present work with *P. ostreatus*, including the BE reached with the controls.

Argentina produces approximately 15,000,000 tons of maize per year, primarily in three provinces, Buenos Aires,

Santa Fe and Cordoba and 91,000 tons of garlic, principally in the provinces of Mendoza, San Juan and Buenos Aires. This production coincides, in great part of the region, with the production of olive. We conclude that it is possible to utilize garlic and maize wastes mixing with OMWW for cultivating *Pleurotus ostreatus*.

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References

Borja, R., A. Martin, R. Maestro, J. Alba, J. A. Fiestas, 1992. Enhancement of the anaerobic digestion of olive mill wastewater by the removal of phenolic inhibitors. Process Biochemistry 4: 231-237.

Capasso, R., A. Evidente, L. Schivo, G. Orru, M. A. Marcialis, G. Cristinzio, 1995. Antibacterial polyphenols from olive oil mill waste waters. Journal of Applied Bacteriology 4: 393-398.

Cha, W., D. Choi, S. Kang, 2004. Optimization of culture media for solid-state culture of *Pleurotus ferulae*. Biotechnology and Bioprocess Engineering 9: 369-373.

Chang, S. T., 1993. Mushroom biology: the impact on mushroom production and mushroom products. First International Conference on Mushroom Biology and Mushroom Products, Hong Kong.

D'Annibale, A., M. Ricci, D. Quaratino, F. Federici, M. Fenice, 2004. *Panus tigrinus* efficiently removes phenols, color and organic load from olive-mill wastewater. Research in Microbiology 7: 596-603.

de la Rubia, T., M. Lucas, J. Martinez, 2007. Controversial role of fungal laccases in decreasing the antibacterial effect of olive mill waste-waters. Bioresource Technology 99: 1018-1025.

Eger, G., 1965. Untersuchungen uber die Bildung und Regeneration von Fruchtkörpern bei Hutpilzen. I. *Pleurotus florida*. Archives of Microbiology 50: 343-356.

Fountoulakis, M.S., S.N. Dokianakis, M.E. Kornaros, G.G. Aggelis, G. Lyberatos, 2002. Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. Water Research 19: 4735-4744.

García García, I., P.R. Jimenez Pena, J.L. Bonilla Venceslada, A. Martn Martn, M.A. Martn Santos, E. Ramos Gomez, 2000. Removal of phenol compounds from olive mill wastewater using *Phanerochaete chrysosporium*, *Aspergillus niger*, *Aspergillus terreus* and *Geotrichum candidum*. Process Biochemistry 8: 751-758.

Gomaa, N.F., M.H. Hashish, 2003. The inhibitory effect of garlic (*Allium sativum*) on growth of some microorganisms. Journal of the Egyptian Public Health Association 78: 361-372.

Hafidi, M., S. Amir, J.C. Revel, 2005. Structural characterization of olive mill waster-water after aerobic digestion using elemental analysis, FTIR and 13C NMR. Process Biochemistry 8: 2615-2622.

Junková, A., 1971. Intensivni a extensivni zpusob pestovani hiivy ustricne. Mykologiky sbornik 8: 53-54.

Kalberer, P., E. Vogel, 1974. Untersuchungen zur Kultur von *Pleurotus*. Der Gemüseban 4: 37-44.

Kalmis, E., S. Sargin, 2004. Cultivation of two *Pleurotus* species on wheat straw substrates containing olive mill waste water. International Biodeterioration and Biodegradation 1: 43-47.

Laufenberg, G., B. Kunz, M. Nystroem, 2003. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. Bioresource Technology 87: 167-198.

Ledezma, E., R. Apitz-Castro, 2006. Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. Revista Iberoamericana de Micología 23: 75-80.

Lu, B., J. Yan, R. Wang, 1998. Integrated ecological engineering of corn utilization in Zhaodong County. Ecological Engineering 1-4: 139-146.

Magro, A., M. Carolino, M. Bastos, A. Mexia, 2006. Efficacy of plant extracts against stored-products fungi. Revista Iberoamericana de Micología 23: 176-178.

Obied, H.K., M.S. Allen, D.R. Bedgood, P.D. Prenzler, K. Robards, R. Stockmann, 2005. Bioactivity and analysis of biophenols recovered from olive mill waste. Journal of Agricultural and Food Chemistry 4: 823-837.

Okeke, B.C., S.K. C. Obi, 1994. Lignocellulose and sugar compositions of some agro-waste materials. Bioresource Technology 47: 283-284.

Rabinovich, J.E., 1980. Introducción a La Ecología de Poblaciones Animales. Compañía Editorial Continental, S. A. México, D.F.

Ragunathan, R., R. Gurusamy, M. Palaniswamy, K. Swaminathan, 1996. Cultivation of *Pleurotus* spp. on various agro-residues. Food Chemistry 2: 139-144.

Roig, A., M.L. Cayuela, M.A. Sanchez-Monedero, 2006. An overview on olive mill wastes and their valorisation methods. Waste Management 9: 960-969.

Salmones, D., G. Mata, K. N. Waliszewski, 2005. Comparative culturing of *Pleurotus* spp. on coffee pulp and wheat straw: biomass production and substrate biodegradation. Bioresource Technology 96: 537-544.

Sanjust, E., R. Pompei, A. Rescigno, A. Rinaldi, M. Ballero, 1991. Olive milling waste water as a medium for growth of four *Pleurotus* species. Applied Biochemistry and Biotechnology 31: 223-235.

Stanér, M., I. Rysavá, 1971. Application of thermophilic mnicroorganisms in the fermentation of the nutrient substrate for the cultivation of *Pleurotus ostreatus* (Jacq. Ex Fr.) Kummer. Mykologicky sbornik 8: 59-60.

Tsioulpas, A., D. Dimou, D. Iconomou, G. Aggelis, 2002. Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. Bioresource Technology 84: 251-257.

Yildiz, S., U.C. Yildiz, E.D. Gezer, A. Temiz, 2002. Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. Process Biochemistry 3: 301-306.

Zadrazil, F., 1973. Anbau. Etrag und Haltbarkeit von *Pleurotus florida* Fovose. Der Champignon 141: 17-24.

Zadrazil, F., M. Schneidereit, 1972. Die Grundlagen für die Inkulturnahme einer bisher nicht kultivierten *Pleurotus*-Art. Der Champignon 135: 25-32.

Zervakis, G., P. Yiatras, C. Balis, 1996. Edible Mushrooms from Olive Oil Mill Wastes. International Biodeterioration and Biodegradation 38: 237-243.