Australian Journal of Basic and Applied Sciences, 3(4): 3352-3360, 2009
ISSN 1991-8178

Nutritional Analysis and Enzyme Activities of *Pleurotus Ostreatus* Cultivated on *Citrus Limonium* and *Carica Papaya* Wastes

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Abstract: Utilization of three food processing wastes (lemon pulp, papaya fruit waste and rice straw) for the production of fruit bodies mushroom with edible fungi *Pleurotus ostreatus* NRRL-0366 was done using solid state fermentation technique. Rice straw supplemented with different concentrations of the other two wastes forming eight substrates was used in this study. The highest mushroom harvested was with a substrate no. 2 (equal concentration of lemon pulp and rice straw) giving 957.80 g/Kg fresh fruit bodies with biological efficiency 26.98% followed by substrate no. 7 (double amounts of papaya fruit waste supplemented with one amount of rice straw) giving 431.1g/Kg fresh fruit bodies with biological efficiency 13.50%. The results revealed that the fruit bodies containing 26.0-31.5% digestible protein, 20.9 -33.0% total soluble carbohydrates and 2.0-5.9% fat (on dry basis). Calorific values for the eight fruit bodies ranged189-253 Kcal per 100 g of dry mushrooms. GLC analysis of the eight fatty acids of the fruit bodies has revealed that the unsaturated fatty acids were at higher concentration (68.2-75.2%) than the saturated one. Studies were carried out on the activities of amylase, cellulase, invertase, polygalacturonase and pectinlyase enzymes of *Pleurotus ostreatus* fruit bodies cultivated in the previous eight substrates. The substrates affect the enzyme activities of *Pleurotus ostreatus* fruit bodies. A higher activity of amylase was observed in the unsupplemented lemon pulp and papaya waste. No significant change was obtained in cellulase and invertase activities within the eight substrates except substrate no. 7 using papaya waste. This fungus is able to produce high levels of pectinlyase in fruit bodies during solid state fermentation on lemon pulp.

Key words: Food processing by-products, *Pleurotus ostreatus*, enzymes, mushroom, nutritional evaluation.

INTRODUCTION

Mushrooms are saprophytic, growing on dead organic matter of vegetative origin. Therefore they can utilize almost all agricultural wastes as substrates (Miles and Chang, 1997).

Saprophytic species are cultivated for mushroom production on lignin and cellulose containing substrates such as wood logs, saw dust, straw and cotton waste, besides many other agricultural and food industrial wastes, which can easily be transformed into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and regenerating the environment (Bano and Rajarathnam, 1982). The proficiency of fungi in converting substrate to protein is far superior to that of several plants and even animals (Yang, 1986).

Mushrooms serve as delicacies for human consumption and as neutriceuticals "food that also cures". Mushrooms, the fruiting bodies of basidomycetous fungi, contain substances of various kinds that are highly valued as medicines, flavoring and perfumes (Chang and Buswell, 1996; Fan *et al*., 2008). It considered healthy food as it is low in calories, fat and cholesterol, while rich in protein, carbohydrate, fibers, vitamins and minerals (Racz *et al*., 1996).

Pleurotus ostreatus is an edible and are among the easiest mushroom to cultivate (Kues and Liu, 2000). It is a white rot basidiomycete which belongs to the subclass of ligninolytic microorganisms that produce laccases, manganese peroxidases, amylase, cellulase, pectinase and protease (Rashad and Abdou 2001; Palmieri *et al*., 2001; Abdou, 2003; Fan *et al*., 2008 and Rashad *et al*., 2009).

During the growth of mushroom mycelia and the development to mature fruitbodies (or sporophores), biochemical changes are known to occur, as a result of which enzymes are secreted extracellularly to degrade the insoluble materials in the substrates into simple and soluble molecules which are subsequently utilized by intracellular enzymes within the mushroom. Consequently, enzymes play significant role in mushroom

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development; in addition, they also affect the food nutrient, flavour and shelf life of these fungi (Baardseth, 1979; Paranjpe and Chen, 1979; Wang, 1989; Zadrazil *et al*., 2004; Kuforiji and Fasidi, 2008; Kapoor *et al*., 2009). Accumulation of lignocellulosic materials in large quantities in places where agricultural residues present a disposal problem result in deterioration of the environment. In Egypt, rice straw used extensively as a substrate in cultivating *Pleurotus ostreatus* and *Agaricus* species for production of fruit bodies mushroom.

Unprocessed citrus pulp wastes (orange, lemon,……etc.) could be alternatively utilized as carbon sources to grow microorganisms (LoCurto *et al*., 1992; Rashad *et al*., 2009). Many workers used papaya (*Carica papaya*) peel and latex for preparation of papain, proteolytic enzymes, ethanol and other products (Poulter and Caygill, 1985; Arimura, 1989; Espin and Islam, 1998; Chaiwut *et al*., 2007; Akin-Osanaiye *et al*., 2008).

To our knowledge, papaya fruit waste and lemon pulp have never been tested for growing of *P. ostreatus*. So, the aim of this work was undertaken to study the production of edible mushroom by solid state fermentation using different concentrations of either lemon pulp waste or papaya fruit waste supplemented with rice straw. Also the chemical composition of the produced fruiting bodies was investigated. Moreover, the study was aimed to evaluate the effect of substrates type on the enzyme activities in *Pleurotus ostreatus* fruit bodies.

MATERIALS AND METHODS

Organism:

Pleurotus ostreatus NRRL-0366, obtained from Agriculture Research Service (Peoria, II). The fungi were established on potato dextrose agar (PDA) at 28 °C for 7 days (Jodon and Royse, 1979).

Substrates:

Lemon (*Citrus limonium*) pulp is collected from a citrus factory which processes mainly orange and lemon fruits for essential oil and juice extraction. Papaya (*Carica papaya*) fruit and rice straw were collected from Egyptian local markets. Samples (1 Kg) of fresh lemon pulp and papaya fruit waste (peels and central seeds) cut into small pieces. Rice straw was soaked in water for 3 h, dried and cut into small pieces.

Media and Cultivation:

Eight different substrates were used in this study. Lemon pulps unsupplemented with rice straw (substrate no.1) and a mixture of lemon pulps with small pieces of rice straw at different ratios (substrate no. 2- 4).

The other four substrates (5 - 8) consist of different mixture of papaya fruit waste supplemented with rice straw except no.5 was unsupplemented one. Each substrate was mixed with 1% $CaCO_3$ (w/w) suspension in water, pressed to expel excess water and sterilized at 121°C for 1 h, then cooled. Each treatment was replicated 3 times.

The mushroom seeds were prepared using sorghum grain as spawn base material (Sivaprakasam, 1980). The cultured-spawn mixture was placed in polyethylene bags containing freshly sterilized media. The bags were closed and incubated for 15 days at 20-25°C in darkness. The bags were opened, perforated along the upper sides, and humidified daily by spraying with water to initiate the development of fruit bodies. The time taken for the appearance of pin-heads and quantity of mushroom produced in each flush were recorded. The yields on the basis of the total quantity of substrates were compared and the biological efficiencies calculated as described by Gujral *et al*. (1987).

Analytical Methods:

The moisture content was determined according to A.O.A.C. (1980). Lipid content was determined after extraction the dried fruit bodies with cold 1:1 mixture of methanol and chloroform (Pederson, 1962) and purified according to Folch *et al*. (1957). The prepared fatty acid methyl esters (Christie, 1973) were analyzed by a Hewlett Packard GC (HP 6980 series). The method described by Dubois *et al*. (1956) was used to estimate the total carbohydrates of fruit body hydrolysate (Rashad and Abdou, 2002). Crude protein content was measured by Kjeldahl method as N x 6.25 (Harris, 1974). Energy values (Kcal per 100 g dry wt. = 2.62 x % protein + 8.37 x % fat + 4.2 x % carbohydrates) were calculated as mentioned by Crisan and Sands (1978).

Preparation of Samples for* Enzyme *Assay:

To obtain crude enzyme extract, 7.0 g of fresh fruit bodies of *P. ostreatus* was ground with 100 ml distilled water in a rotor at 45 rev/min for 30 min. The suspension was centrifuged twice at 8500 xg. over 15 min at 4 °C then filtered through Whatman no. 1 filter paper. The supernatant obtained assayed for enzyme activities.

Enzyme Assays:

Amylase activity was assayed by incubating 1 ml of crude enzyme with 1% soluble starch in 0.05 M sodium acetate buffer (pH 4.8) at 37 °C for 10 min, the increase of the reducing power was determined by Somogyi (1952) and Nelson (1944) method.

Cellulase activity was measured as filter paper activity units. Whatman no. 1 filter paper strip (1.0 cm x 6.0 cm) in 1 ml of 0.1 M sodium citrate buffer, pH 5.0 was used as a substrate and incubated with 1 ml of enzyme extract for 1 h at 50 °C. The reaction was terminated by boiling the reaction mixture. Finally, the reducing sugar released as a result of enzymatic action was estimated spectrophotometrically (Somogyi, 1952; Nelson, 1944).

Invertase activity was determined from the amount of reducing sugars produced from sucrose. The reaction mixture contained 1.5 ml of 0.1 M acetate buffer (pH 5.0), 1 ml of 2% sucrose and 1 ml enzyme solution, and incubated for 1h at 40°C (DelRosario and Santisopasri, 1977). The increase of the reducing sugar was determined by (Somogyi 1952; Nelson, 1944).

Polygalacturonase (PGase) activity was assayed by determining the liberated reducing end products by Somogyi (1952) and Nelson (1944) method using polygalacturonic acid as a standard. The reaction mixture containing 0.8 ml citrus pectin (1% in 0.2 M acetate buffer, pH 5.0) and 0.2 ml of crude enzyme incubated at 55°C for 10 min.

Pectinlyase (PL) activity was determined by measuring the increase in absorbance at 235 nm of substrate solution (0.8 ml 1% citrus pectin in 0.2 M Tris-HCl buffer, pH 8.5) hydrolyzed by 0.2 ml enzyme solution, at 55°C. One unit of PL was defined as the amount of enzyme which releases 1 µmol of unsaturated uronide per minute, based on the molar extinction coefficient (5500) of the unsaturated products (Albersheim, 1966). International (IU) unit of the enzyme was expressed as 1 µmol of reducing sugar released per gram of fruit body in min under the standard assay conditions.

RESULTS AND DISCUSSION

Studies were done for producing mushroom fruit bodies through growing the edible fungi *Pleurotus ostreatus* on different food processing wastes (lemon pulp, papaya fruit waste and rice straw) as substrates.

P. ostreatus grew well on each substrate with different degrees. The first flush appeared in all the substrates after 36 days of inoculation. The second flush appeared within 7 days after the first flush. In general, the first flush of fruit bodies gave much higher yields than that of the second flush in all the conditions used (Fig. 1 a and b).

Fig. (2) shows the effect of different types of the substrates on the productivity of the mushroom fruit bodies. The data showed that supplementation of the two wastes with rice straw plays a role in increasing the productivity of the mushroom. The maximum yield was obtained (957.80 g/kg wet substrate) by using equal concentration of lemon pulp and rice straw (substrate 2) with 26.98% biological efficiency. This was followed by using a mixture of lemon pulp and rice straw at ratio (3:1) (409.3 g/kg wet substrate) with biological efficiency 13.64%.

Also, it can be seen from these data (Fig. 2) that the productivity with the substrate of unsupplemented papaya fruit waste (substrate 5) was very limited (25.20 g/kg substrate) with biological efficiency (0.53%) when compared to those supplemented with rice straw at equal ratio (345.80 g/kg substrate), biological efficiency (8.14 %) or double amount of papaya fruit waste (431.10 g/kg substrate) with biological efficiency (13.50 %). Variable ranges of biological efficiency (6.4-19.6%) have been reported when different lignocellulosic wastes (rice straw, mango waste, date waste and cabbage wastes) were used as substrates for production of *P. ostreatus* NRRL-0366 mushroom (Jwanny *et al*., 1995, Rashad and Abdou 2002; Rashad *et al*., 2003) which are in agreement with our results.

While using a substrate of lemon pulp supplemented with the same concentration of rice straw gave fruitful results with higher biological efficiency (26.98 %) more than any substrate used by above authors.

In recent years, the development of commercial culture methods for mushroom of *Pleurotus* species production has led to an increasing interest in proximate composition of these fruit bodies. Due to the limited information available on the true nutritive value of the mushroom especially when lemon pulp or papaya were used for their production. Studies have been carried out to find proximate composition of these cultivated fruit bodies.

The data in Table 1 illustrate the chemical analysis of the eight *P. ostreatus* fruit bodies. The protein contents in the fruit bodies of the mushroom cultivated on lemon pulp supplemented with rice straw at different ratios (1:1, 2:1, 3:1) were 27.0, 29.2 and 31.5% respectively.

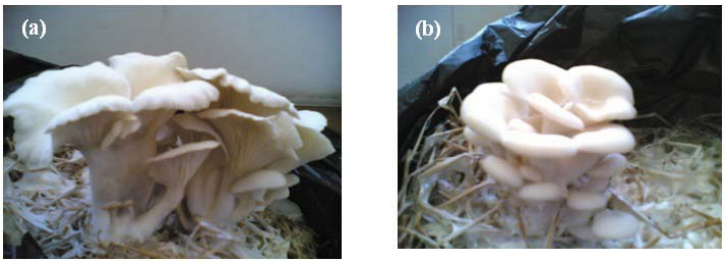

Fig. 1: Fruit bodies of *P. ostreatus* on mixture of lemon pulp + rice straw at ratio 1:1 (a) and mixture of papaya fruit waste + rice straw at ratio 2:1 (b).

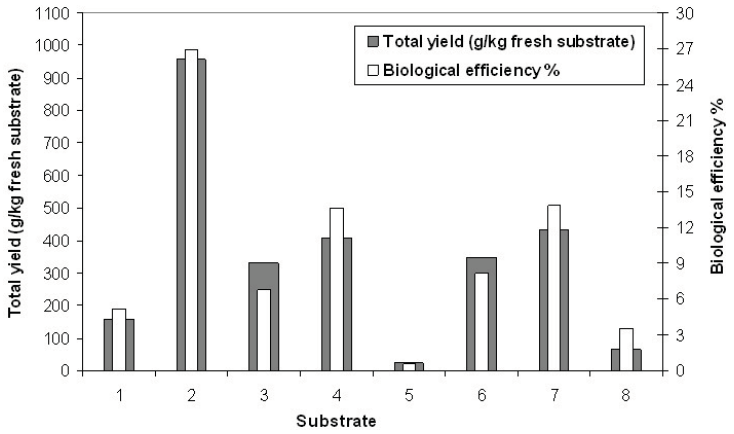

Fig. 2: The average production of fruit bodies and their biological efficiency of mushroom *P. ostreatus* cultivated on different substrates. 1- Lemon pulp alone, 2- Mixture of lemon pulp + rice straw at ratio 1:1, 3- Ditto → 2:1, 4- Ditto → 3:1, 5- papaya waste alone, 6- papaya waste + rice straw at ratio 1:1, 7- Ditto → 2:1, 8- Ditto → 3:1.
Biological Efficiency (%) = g dry fruit bodies per 100 g dry substrate.

Table 1: Chemical composition of the fruit bodies of *P. ostreatus* cultivated on different substrates.

Substrates	Protein %	Total carbohydrate %	Crude fat %	Energy value K. cal./100 g
1	26.30	24.08	5.92	219.60
2	27.00	25.92	4.00	213.10
3	29.20	29.72	2.00	217.60
4	31.50	33.00	3.00	253.20
5	26.00	20.88	3.99	189.20
6	27.20	24.07	4.20	207.00
7	28.50	26.68	4.00	220.20
8	28.80	27.04	3.89	221.60

Table 2: Fatty acid composition of the fruit bodies *of P. ostreatus* cultivated on different substrates.

Fatty acids	Fruit bodies of *P. ostreatus*							
	1	2	3	4	5	6	7	8
C14:0	-	-	2.80	3.0	-	-	-	2.9
C16:0	14.0	13.5	13.0	13.2	15.2	14.0	14.5	12.9
C16:1	12.0	11.5	11.7	10.2	13.9	14.8	14.5	14.0
C18:0	7.2	12.0	11.5	12.2	11.2	10.8	9.8	9.0
C18:1	10.2	10.0	9.5	8.6	13.2	11.3	11.7	17.2
C18:2	50.0	49.2	48.6	46.4	43.7	43.6	45.5	44.0
C20:0	6.6	3.8	2.9	3.4	2.8	3.0	4.0	-
C20:4	-	-	-	3.0	-	2.9	-	-
Saturated	27.8	29.3	30.2	31.8	29.2	27.8	28.3	24.8
Unsaturated	72.2	70.7	69.8	68.2	70.8	72.6	71.7	75.2
Unsat:Sat ratio	2.6	2.4	2.3	2.2	2.4	2.5	2.5	3.0

The protein contents in fruit bodies cultivated on papaya waste and rice straw at different ratios (1:1, 2:1, 3:1) were 27.2, 28.5 and 28.8% respectively while the lowest one (26%) recorded when using unsupplemented papaya waste (substrate 5).

Rashad and Abdou (2002) observed a significant variation in the protein (20.59- 26.84%) when *P. ostreatus* NRRL – 0366 was cultivated on a mixture of cabbage waste and rice straw at different ratios as a substrate. While, Thomas *et al*. (1998) reported that the protein content of *P. sajor-caju* cultivated on coconut palm as a substrate was 17.2 - 25.7 %.

Differences were observed in the amount of total carbohydrates (20.88 - 33.00%) as shown in Table 1. The highest value (33%) was obtained from a substrate contained triple amount of lemon pulp mixed with rice straw. These variations were similar to Rai *et al*. (1988) who found that the carbohydrate content in seven different *Pleurotus* species was 20-32%. While they are lower than the results reported by Jwanny *et al*. (1995); Rashad and Abdou (2002) using *P. ostreatus* (21.88 - 66.00 %).

There were great similarities in the content of fat of the eight fruit bodies formed in the eight substrates (2-6%). These results were in the same range of those reported with Rashad and Abdou (2002) of *P. ostreatus* NRRL – 0366 fruit bodies formed on different substrates (cabbage waste, rice straw, date waste,…etc.). In general, several investigators stated that, the crude fat content in different species of *Pleurotus* could comprise from about 1% to as high as 9.4 % on the dry weight basis being on an average of 2.85% (Crisan and Sands, 1978; Bano and Rajarathnam, 1982; Jwanny *et al*., 1995; Thomas *et al*., 1998; Rashad *et al*., 2001, 2003).

Mushrooms are healthy foods, poor in calories. Calorific values for eight fruit bodies ranged 189-253 Kcal/100 g of dry mushrooms. These results are in agreement with the results reported by Rashad *et al*. (2003) using water hyacinth as a substrate (212-321 Kcal %), while they are lower than that of *P. ostreatus* NRRL-0366 mushroom cultivated on a mixture of cabbage waste and rice straw recorded (431-550 Kcal%) (Rashad and Abdou, 2002).

The fatty acid composition of the purified and esterified lipids in *P. ostreatus* fruit bodies, cultivated on different substrates, are shown in Table 2. Fatty acids with even and odd numbered carbon atoms between C14 and C18 could be observed. GLC analysis of the fatty acids has revealed that the main fatty acid was C18:2 (Linoleic) followed by C16:0 (Palmitic) and C18:1 (Oleic). C18:2 was found to be the main unsaturated component which represents about 43.6-50.0 % of the total fatty acids in the eight fruit bodies cultivated in different substrates. These results were similar to some extent to those of some investigators (Breene, 1990; Stancher *et al*., 1992; Jwanny *et al*., 1995; Yilmaz *et al*., 2006; Rashad *et al*., 2006) revealing that the major fraction of fatty acids of *P. ostreatus* mushrooms consisted of 18:0, 18:1 and 18:2. Also, Hanijir and Taniguchi (2006) stated that the major fatty acids in papaya fruit lipid were C16, C18:1, C18:2, C18:3. It is well known that the unsaturated fatty acids especially Oleic and Linoleic acids are important essential dietary constituents and has effect as reducing agents in chronic heart diseases.

It can be concluded that fruit bodies of *P. ostreatus* cultivated on different substrates have relatively high concentration of nutritionally valuable unsaturated fatty acids (68.2-75.2%), which are interesting from the nutritional stand point.

The activities of the enzymes of *P. ostreatus* are dependent on the nature of the substrate (Kuforiji and Fasidi, 2008). From another point of view, Baardseth (1979); Kadiri (1990); Kuforiji and Fasidi (2008) reported that levels of food nutrients, flavour and discoloration in fruit bodies are controlled by amylase, cellulase, peroxidase and respiratory enzymes. The higher their activities in the fruit bodies, the greater their depreciation. So, studies were done to estimate the activity of some enzymes formed in the fruit bodies to evaluate their effects on food nutrients. Fig. 3 shows the levels of five enzymes (amylase, cellulase, invertase, polygalacturonase and pectinlyase) in the crude extract of the eight fruit bodies of edible mushroom *P. ostreatus* NRRL-0366 cultivated on eight substrates (mentioned before).

The maximum activities of *P. ostreatus* amylase enzyme (32.45 and 31.88 U/g fresh fruit bodies) were achieved with unsupplemented lemon pulp and papaya fruit waste respectively. El-Zalaki and Hamza (1979) detected different levels of amylase in *Lentinus edodes* propagated on different food wastes. Kuforiji *et al*. (2005) also found that the total amylase activities of *Volvariella volvacea* are related to the carbohydrate contents in the mushroom, thus affecting the nutritional composition of the fungus in each substrate. Kuforiji and Fasidi (2008) stated that higher total amylase activity was observed in by *Pleurotus tuber-regium* sporophores.

Cellulase activities of the fruit bodies produced by growing *P. ostreatus* on different substrates (substrate 1-6, 8) showed no significant difference (4.2 - 7.0 U/g fresh fruit bodies). While using double amount of papaya waste mixed with rice straw (substrate 7) gave 14.2 U/g fresh fruit bodies. Kuforiji *et al*. (2005) also reported that cellulase activities of 0.8, 0.6, 0.6 and 0.5 mg/h/mg protein in *V. volvacea* grown on cotton

wastes, rice straw, sorghum chaff and sawdust of *M. altissima*, respectively. Kuforiji and Fasidi (2008) reported that cellulase activities of *Pleurotus tuber-regium* fruit bodies grown on cotton waste, rice straw and cocoyam peels were 2.4. 1.8 and 0.9 mg/h/mg protein, while those on sawdusts of *M. altissima*, *Khaya ivoresis* and *B. angustifolia* had 1.5, 2.1 and 1.0 mg/h/mg protein, respectively.

The invertase activity increased 2-2.5 times than the control (unsupplemented) with increasing the amount of lemon pulp. While no change in the activities were observed in the different papaya substrates (Fig. 3c). Polygalacturonase (PGase) in fruit bodies extract using unsupplemented lemon pulp (9.8 U/g fresh fruit bodies) was higher than the other substrates (Fig. 3d). While fruitful results of pectinlyase enzyme achieved with lemon pulp substrates. The highest activity obtained (373.5 U/g fresh fruit bodies) when double amount of lemon pulp was mixed with rice straw (Fig. 3e). On the other hand, the pectinlyase enzyme was not detected by using papaya waste either supplemented or unsupplemented.

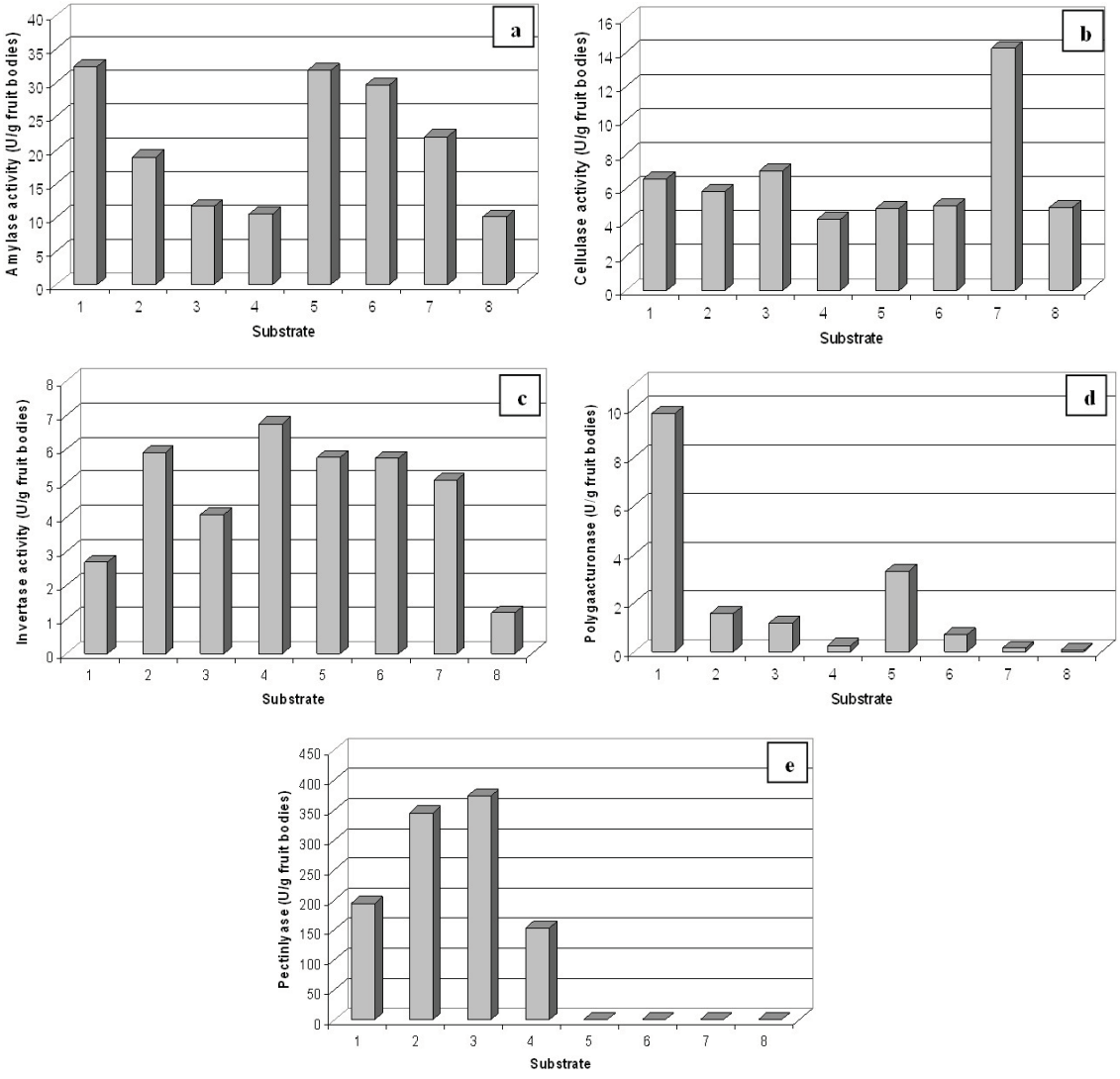

Fig. 3: Effect of different substrate types on the enzyme activities in the fruit bodies of P. ostreatus NRRL-0366. (a) amylase, (b) cellulase, (c) invertase, (d) polygalacturonase, (e) pectinlyase.

To our knowledge, the production of pectinases and invertase enzymes in mushroom fruit bodies has never been tested. While intensive studies were done on extracellular mushroom enzymes (Zheng and Shetty, 2000; Kusuda *et al*., 2004; Freixo *et al*., 2008; Rashad *et al*., 2009).

Conclusion:

The present study indicates that it is feasible to use fruit processing wastes (lemon pulp and papaya fruit waste as such or supplemented with rice straw) as raw materials for the production of fruit bodies and some enzymes by a food grade fungus *Pleurotus ostreatus* NRRL-0366. Also the different substrates affect the enzyme activities of *Pleurotus ostreatus* fruit bodies. This fungus is able to produce high levels of pectinlyase in fruit bodies during solid state fermentation on lemon pulp. Moreover, utilization of these agro wastes for the cultivation of this mushroom is an effective method for the production of nutritional food and offers a holistic approach to waste management. As it is possible to use the fermented viticulture waste to feed ruminants.

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