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# Optimizing the Antioxidant Activity of Solid State Fermentation Systems with *Pleurotus floridanus* and *Paecilomyces variotii* on Rice Straw

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SOLID state fermentation system (SSFS) efficiency depends on the proper selection of the microorganisms, substrates and environmental conditions. In this study, we investigated and optimized the antioxidant activity of the SSFS constructed using *Pleurotus floridanus* or *Paecilomyces variotii* on rice straw. The genotypic effect on antioxidant activity surpassed that of the environmental conditions. Peroxidase (POX) activity was expressed only in *Pleurotus floridanus*, but catalase (CAT) activity was expressed in both fungi, in favor of *Paecilomyces variotii.* The activities of both enzymes were maximized at low pH, low temperature and with ammonium chloride as the N source, but high moisture was optimal for POX and low moisture was optimal for CAT. Phenolic and flavonoid contents were higher in *Paecilomyces variotii* than *Pleurotus floridanus*, with preference for high moisture, low pH and urea in *Paecilomyces variotii*, and low moisture, low pH, and sodium nitrate in *Pleurotus floridanus*. The reducing power of *Paecilomyces variotii* was higher than that of *Pleurotus floridanus*, with positive moisture-dependence, low temperature, and gelatin preferences in *Paecilomyces variotii*, and negative moisture- and temperature-dependence and urea-preference in *Pleurotus floridanus*. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was higher in *Paecilomyces variotii* than *Pleurotus floridanus*, with negative dependence on moisture and temperature and limited nitrogen source effects in both fungi. Regarding the incubation period, maximal antioxidant activity was observed with a shorter incubation in the *Paecilomyces variotii* SSFS and a longer incubation in the *Pleurotus floridanus* SSFS.

**Keywords:** Catalase, Flavonoids, Peroxidase, Phenolics, Reducing Power, Solid state fermentation.

## Introduction

Solid state fermentation enabled the controlled, efficient, and economical production of bioactive products such as enzymes, metabolites, and antibiotics from solid plant wastes (Inácio et al., 2015). The principle of SSF is creating contact between the microorganism and the solid substrate with just adequate moisture requirements to dominate the associating microflora and reduce the risk of contamination (Šelo et al., 2021). During fermentation, microorganisms produce various extracellular enzymes that aid in antioxidant release from the substrate, and antioxidant production via microbial metabolic pathways (Dey et al., 2016). Microorganisms are valuable sources of medicinal and pharmaceutical products and can participate in the production of antioxidants such as phenolics, flavonoids, tocopherols, ascorbic acid and carotenoids (Mwangi et al., 2022). However, the disadvantages of SSF include the discrimination against organisms with high water demands; the difficulties monitoring pH, temperature and oxygen tension, and the generation of heat, which can slow down microbial growth (Šelo et al., 2021). Thus selecting the ideal microorganism and substrate, and optimizing the growth conditions are critical for constructing efficient SSFS.

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Various agro-industrial wastes have been used as SSFS substrates with their efficiencies depending on the particle size, porosity, and nutrient content. Rice straw is a valuable agro-residue with appreciable nutrient and phytochemical contents; containing approximately 39% cellulose, 27% hemicellulose, 12% lignin, and 11% ash. However, in Egypt, rice straw is wastefully eliminated via open incineration in the field, which has serious environmental impacts (El-Dewany et al., 2018). Rice straw has several beneficial uses, e.g. organic fertilizer and soil conditioner for vegetable crops, substrate for mushroom cultivation and, fodder for livestock (upon enrichment with urea and ammonia) (El-Fallal et al., 2009; Utami & Susilawati, 2017). Recently, rice straw has been used in SSF to produce bioethanol (Singh et al., 2021), enzymes (Isaac & Abu-Tahon, 2016; Maftukhah & Abdullah, 2018), and single cell protein (Novita et al., 2019).

In addition to the choice of substrate, several physicochemical conditions control SSFS efficiency, including moisture content, pH, temperature, and incubation period (Szymczak et al., 2021). These factors can affect the microbial growth and the release of metabolites, and hence the efficiency of the process. Although the term SSF implies fermentation with low water content, the optimal water content varies according to the type of substrate and fungal species (Thomas et al., 2013). The SSFS reaction (pH) is an important factor that must be adjusted within an optimal narrow range. Most fungi prefer moderately acidic media, whereas bacteria prefer neutral to slightly alkaline substrates (Thomas et al., 2013). Moreover, microbes necessitate a balanced nutrient supply of carbon, nitrogen, phosphorous and minerals for optimal growth; thus the C/N ratio of agro-residues has a pivotal role in the reaction efficiency. Generally, a high C/N ratio will slow down the biodegradation rate, whereas a low C/N ratio will lead to excess ammonia, which may inhibit microbial growth after the initial active growth period (Zhou & Wen, 2019).

Optimizing the fermentation conditions is necessary to increase the SSFS yield, save time and effort, and consequently reduce costs (Ismail & Hassan, 2020). In the present work, we investigated and optimized the production of enzymatic and non-enzymatic antioxidants from SSFS using rice straw and the basidiomycete *Pleurotus floridanus* or the ascomycete *Paecilomyces variotii*. Basidiomycete and ascomycete fungi are important sources of biologically active compounds with different efficacies and environmental requirements (Blackwell, 2011). Therefore we aimed to determine whether the optimal antioxidant activity conditions of SSFS vary between the basidiomycete *Pleurotus floridanus* and the ascomycete *Paecilomyces variotii*, as well as between the enzymatic and non-enzymatic antioxidants.

## Material and Methods

*Fungal species, substrate and chemicals*

Two fungal species, the basidiomycete *Pleurotus floridanus* Sing (38539) and the ascomycete *Paecilomyces variotii* (Biourge & Bainier) were selected for SSFS. The collection, identification and maintenance of the test fungi are described in El-Katony et al. (2019). Rice straw, obtained from local farms, was washed with distilled water, air-dried, ground into a fine powder, and passed through a 1 mm sieve. To initiate SSF, the powdered rice straw was inoculated with two discs (2-cm diameter) from the two fresh fungi cultures. The water holding capacity (WHC) of rice straw was calculated according to Piper (1974). The stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, USA, and 2,4-dichlorophenol (2,4-DCP), 4-aminoantipyrine, Folin-Ciocalteu phenol reagent, gallic acid, quercetin and trichloroacetic acid were obtained from Fisher Scientific, UK. All other reagents and solvents were of analytical grade.

*SSFS condition optimization*

Five environmental factors were investigated, viz. moisture content, incubation period, pH, temperature, and nitrogen source. The moisture content was adjusted by moistening the powdered rice straw with distilled water to 10%, 20%, 30%, 50%, 70%, or 100% of the rice straw WHC, the pH was adjusted to 5, and the SSFS was incubated at 25°C for 14 days. Thereafter, the moisture content was set at 10% and 30% of the rice straw WHC for *Paecilomyces variotti* and *Pleurotus floridanus,* respectively. The effect of incubation period was investigated by monitoring the antioxidant activity of the SSFS (with rice straw adjusted to pH 5 and incubated at 25°C) at 3-day intervals for up to 15 days. The effect of pH was investigated by adjusting the substrate pH to 4, 5, 6, 7, 8, or 9 using either 1N HCl or 1N NaOH at an incubation temperature of 25°C for 14 days. The effect of temperature was

investigated by incubating the SSFS with pH 4, at 20, 25, 30, 35 and 40°C for 14 days. The effect of nitrogen source was investigated by supplying rice straw with sodium nitrate, ammonium chloride, gelatin, peptone, or urea at a dose of 0.5 g N equivalent (at pH 4, and 25°C for 14 days).

*Enzyme and antioxidant extraction*

For enzyme extraction, the whole culture was thoroughly mixed with 50 mL of phosphate buffer (0.1M, pH 7.0), and incubated in a rotary shaker at 180rpm and 30°C for 2h. The mixture was centrifuged at 10,000 × *g* for 15min at 4°C and the supernatant was collected for enzyme assay. For non-enzymatic antioxidants, 50mL of 95% ethanol were added to the culture, and the mixture was incubated for 2 hours at room temperature in a rotary shaker at 250rpm. The mixture was centrifuged at 2,800 × *g* for 10min at room temperature.

*Protein and antioxidant enzyme activity assay*

Peroxidase (EC 1.11.1.x) activity was assayed using 2,4-DCP as the substrate (Ramachandra et al., 1988). The reaction mixture (3mL) contained 0.6mL of potassium phosphate buffer (0.1M, pH 7.0), 0.6 ml of 4-aminoantipyrine (16mM), 0.6mL of 2,4- DCP (25mM), and 0.6mL of the culture supernatant. The reaction was initiated by adding 0.6mL of $H_2O_2$ (50mM), and the mixture was incubated at 37°C for 1min. The increase in absorbance, resulting from 4-aminoantipyrine oxidation was measured at 510nm. One unit of enzyme activity was defined as the amount of enzyme required to increase absorbance by one unit/minute.

Catalase (EC 1.11.1.6) activity was assayed by adding 0.1mL of the culture supernatant to 1mL of 2mM $H_2O_2$ and 1.9mL of phosphate buffer (0.1M, pH 7.5). The mixture was incubated at 30°C for 5min, then 4mL of titanium reagent were added to stop the reaction. The mixture was centrifuged at 3000 × *g* for 10min and absorbance was measured at 410nm (Teranishi et al., 1974). The residual $H_2O_2$ of the sample was calculated by reference to a standard curve in the range of 0–200µmole $H_2O_2$. Enzyme activity was expressed as µmole $H_2O_2$/mg protein/minute.

The protein content was determined by adding 0.1mL of the supernatant to 5mL of Coomassie brilliant blue reagent, and reading absorbance at 595nm after 2min (Bradford, 1976). The protein content was estimated with reference to a standard curve of bovine serum albumin in the range of 0–100µg/mL.

*Non-enzymatic antioxidants assay*

Total phenolics were assayed by adding 0.4mL of the ethanolic extract to a mixture of 2mL Folin-Ciocalteu phenol reagent (0.25N) and 1.6mL of 7.5% $Na_2CO_3$ (Luque-Rodríguez et al., 2007). The mixture was heated in a water bath at 50°C for 5min, cooled to room temperature in darkness and the absorbance was read at 760nm. The total phenolic content was estimated with reference to a standard curve of gallic acid in the range of 0–100µg/mL, and the concentration was expressed as µg gallic acid equivalent (µg GAE)/mL culture.

Total flavonoids were assayed by adding 0.25 ml of the ethanolic extract to 1.25mL distilled water and 75µL of 5% $NaNO_2$ (Yang et al., 2009). After 6min, 150µL of 10% $AlCl_3$ were added. After another 5min, 0.5 mL of 1 M NaOH and 775µL distilled water were added, and the absorbance was read at 510nm. The total flavonoid content was estimated with reference to a standard curve of quercetin in the range of 0–500µg/mL, and concentrations were expressed as µg quercetin equivalent (µg QE)/mL culture.

The reduction potential of the culture was estimated by mixing 1 mL of the ethanolic extract with 2.5mL of phosphate buffer (0.2 M, pH 6.6) and 2.5mL of 1% $K_3Fe(CN)_6$ (Oyaizu, 1986). The mixture was incubated at 50°C for 20min, and 2.5mL of 10% trichloroacetic acid were added, followed by centrifugation at 1000 × *g* for 10min. An aliquot of 2.5mL of the supernatant was mixed with 2.5mL distilled water and 0.5mL of 0.1% $FeCl_3$, and absorbance was measured at 700nm. A higher absorbance indicates a greater reduction potential.

The DPPH-scavenging activity of the SSF was monitored by mixing 0.3mL of the ethanolic extract with 2.7mL of DPPH solution ($6 \times 10^{-5}$mol/L) (Moraes-de-Souza et al., 2008). The mixture was shaken vigorously and left to stand for 60min in the dark. The decrease in DPPH radical concentration was measured by monitoring the absorbance at 517nm. The DPPH-scavenging activity was calculated as follow:

DPPH scavenging activity = (Ad-Ac)/Ad × 100

where $A_c$ is the absorbance of the sample and $A_d$ is

the absorbance of the DPPH solution.

*Statistical analysis*

Data were subjected to two-way ANOVA, using SPSS version 22, to reveal the effect of the main factors (fungal species and environmental conditions) and their interaction on the antioxidant activity of the SSF. Phenolics and flavonoids data were log-transformed and, DPPH-scavenging activity data were arcsine transformed to ensure homogeneity of variance before the ANOVA. The ANOVA was followed by mean separation using Duncan's multiple range test at $P< 0.05$. Because the N source is a categorized factor, the relative effect of the N source on the antioxidant activity of each fungus was expressed as the coefficient of variation (CV):

$$CV = \frac{S \times 100}{\bar{Y}}$$

where $S$ and $\bar{Y}$ are the standard deviation and the general mean of the different N sources, respectively. Principal component analysis was performed to summarize the relationships between different measures of antioxidant activity under the different environmental conditions.

## Results

The effects of the main factors, the fungal species and the environmental conditions (substrate water content, incubation period, substrate pH, incubation temperature and nitrogen source) and their interaction on the enzymatic and non-enzymatic antioxidant activity of the SSFS ranged from significant ($P< 0.05$) to highly significant ($P< 0.01$), with relatively moderate effects of pH and nitrogen source (Table 1).

Peroxidase (POX) activity was not detected in the ascomycete *Paecilomyces variotii*; but was expressed in the basidiomycete *Pleurotus floridanus* with a minimum water requirement of 30% of the rice straw WHC, beyond which POX activity exhibited a progressive 19-fold increase with increasing water content up to 100% of the rice straw WHC. In contrast, Catalase (CAT) activity was higher in *Paecilomyces variotii* but was expressed in both fungi with different moisture-dependence. The CAT activity of *Pleurotus floridanus* was reduced with increasing substrate moisture, but that of *Paecilomyces variotii* peaked at 30% WHC (Table 2). The overall phenolic content of the *Paecilomyces variotii* SSFS was about four times that of the *Pleurotus floridanus* SSFS, and a contrasting effect of substrate moisture on phenolic content was observed between the two fungi. Increasing the rice straw water content from 10% to 100% WHC doubled the phenolic content of *Paecilomyces variotii*, but halved that of *Pleurotus floridanus* (Table 2). Similarly, the overall flavonoid content of the SSFS was 40% higher in *Paecilomyces variotii* than *Pleurotus floridanus*, also with contrasting responses to water content. Only a mild 19% increase in flavonoid content was observed in *Paecilomyces variotii* compared with a marked 62% decrease in *Pleurotus floridanus* across the whole water content range (Table 2). The overall reducing power of the *Pleurotus floridanus* SSFS was slightly higher than the *Paecilomyces variotii* SSFS, and the effect of water content on reducing power was contrasting between the two fungi. Increasing the water content of rice straw from 10% to 100% WHC increased the reducing power of *Paecilomyces variotii* by about four folds, and reduced that of *Pleurotus floridanus* by 28%. The high DPPH-scavenging activity of *Paecilomyces variotii* was not significantly affected by moisture content, whereas the low DPPH-scavenging activity of *Pleurotus floridanus* was halved by increasing the water content from 10% to 100% WHC (Table 2).

*Pleurotus floridanus* POX activity peaked on day 6 of incubation. The high CAT activity of *Paecilomyces variotii* was not significantly dependent on incubation time, whereas the low CAT activity of *Pleurotus floridanus* was reduced by approximately 30% from day 3 to day 15 (Table 3). The high phenolic content of the *Paecilomyces variotii* SSFS exhibited a 75% increase from day 3 to day 15, and the low phenolic content of *Pleurotus floridanus* showed a 60% reduction in the same period (Table 3). The significant genotypic difference in flavonoid content of the SSFS systems, in favor of *Paecilomyces variotii*, was associated with a non-significant effect of the incubation period in the two fungi (Table 3). Where the overall high reducing power of *Paecilomyces variotii* was increased by about 50% across the incubation period, no time dependence was observed in the *Pleurotus floridanus* SSFS (Table 3). The high-DPPH scavenging activity of the *Paecilomyces variotii* SSFS was time-independent, but the *Paecilomyces variotii* SSFS exhibited a 31% DPPH-scavenging activity across the incubation period (Table 3).

**TABLE 1. Two-way ANOVA showing the effect of the main factors and their interaction on the antioxidant activity of SSFS [The main factors were 1) fungal species in interaction with 2) one of the environmental factors (moisture content, incubation period, pH, temperature and form of nitrogen)]**

| Variable and source of variation | df | F | P | F | P | F | P |
|---|---|---|---|---|---|---|---|
| | | **Moisture** | | | | | |
| | | **Peroxidase** | | **Catalase** | | **Phenolics** | |
| Fungus | 1 | 60.14 | 0.000 | 4950 | 0.000 | 98.11 | 0.000 |
| Moisture | 5 | 1423 | 0.000 | 6.802 | 0.000 | 60.14 | 0.000 |
| Fungus × Moisture | 5 | 1423 | 0.000 | 6.498 | 0.000 | 160.9 | 0.000 |
| | | **Flavonoids** | | **Reducing power** | | **DPPH scavenging** | |
| Fungus | 1 | 325.3 | 0.000 | 975.9 | 0.000 | 869.9 | 0.000 |
| Moisture | 5 | 6.418 | 0.000 | 26.25 | 0.000 | 37.24 | 0.000 |
| Fungus × Moisture | 5 | 15.44 | 0.000 | 109.9 | 0.000 | 12.48 | 0.000 |
| | | **Incubation period** | | | | | |
| | | **Peroxidase** | | **Catalase** | | **Phenolics** | |
| Fungus | 1 | 190858 | 0.000 | 55.55 | 0.000 | 4189 | 0.000 |
| Period | 4 | 11886 | 0.000 | 5.417 | 0.004 | 33.80 | 0.000 |
| Fungus × Period | 4 | 11886 | 0.000 | 3.035 | 0.042 | 233.9 | 0.000 |
| | | **Flavonoids** | | **Reducing power** | | **DPPH scavenging** | |
| Fungus | 1 | 193.2 | 0.000 | 1993 | 0.000 | 55.55 | 0.000 |
| Period | 4 | 1.259 | 0.319 | 51.70 | 0.000 | 5.423 | 0.004 |
| Fungus × Period | 4 | 2.623 | 0.065 | 66.40 | 0.000 | 3.027 | 0.042 |
| | | **pH** | | | | | |
| | | **Peroxidase** | | **Catalase** | | **Phenolics** | |
| Fungus | 1 | 1121 | 0.000 | 143.9 | 0.000 | 613.4 | 0.000 |
| pH | 5 | 6.356 | 0.001 | 13.10 | 0.000 | 1.494 | 0.229 |
| Fungus × pH | 5 | 6.356 | 0.001 | 1.087 | 0.393 | 0.880 | 0.510 |
| | | **Flavonoids** | | **Reducing power** | | **DPPH scavenging** | |
| Fungus | 1 | 66.16 | 0.000 | 445.6 | 0.000 | 12.06 | 0.002 |
| pH | 5 | 88.55 | 0.000 | 0.776 | 0.576 | 0.833 | 0.539 |
| Fungus × pH | 5 | 65.65 | 0.000 | 0.983 | 0.449 | 0.187 | 0.965 |
| | | **Temperature** | | | | | |
| | | **Peroxidase** | | **Catalase** | | **Phenolics** | |
| Fungus | 1 | 1593 | 0.000 | 1380 | 0.000 | 3763 | 0.000 |
| Temp. | 3 | 184.4 | 0.000 | 121.7 | 0.000 | 748.2 | 0.000 |
| Fungus × Temp. | 3 | 184.4 | 0.000 | 38.58 | 0.000 | 534.1 | 0.000 |
| | | **Flavonoids** | | **Reducing power** | | **DPPH scavenging** | |
| Fungus | 1 | 9.731 | 0.007 | 48.09 | 0.000 | 306.1 | 0.000 |
| Temp. | 3 | 31.06 | 0.000 | 4.725 | 0.015 | 22.38 | 0.000 |
| Fungus × Temp. | 3 | 26.71 | 0.000 | 2.507 | 0.096 | 14.74 | 0.000 |
| | | **Nitrogen source** | | | | | |
| | | **Peroxidase** | | **Catalase** | | **Phenolics** | |
| Fungus | 1 | 99.79 | 0.000 | 173.3 | 0.000 | 2921 | 0.000 |
| N source | 4 | 7.273 | 0.001 | 4.040 | 0.015 | 39.66 | 0.000 |
| Fungus × N source | 4 | 7.273 | 0.001 | 2.403 | 0.084 | 38.66 | 0.000 |
| | | **Flavonoids** | | **Reducing power** | | **DPPH scavenging** | |
| Fungus | 1 | 2922 | 0.000 | 432.1 | 0.000 | 778.5 | 0.000 |
| N source | 4 | 39.68 | 0.000 | 8.227 | 0.000 | 1.716 | 0.186 |
| Fungus × N source | 4 | 38.67 | 0.000 | 9.384 | 0.000 | 1.023 | 0.420 |

**TABLE 2. The enzymatic (POX and CAT) and non-enzymatic (phenolics, flavonoids, reducing power and the DPPH scavenging) antioxidant activity of an SSFS employing the ascomycete *Paecilomyces variotii* or the basidiomycete *Pleurotus floridanus* on rice straw at different substrate water contents**

| Fungus and water content (% WHC) | Peroxidase (U/min) | Catalase (µmole $H_2O_2$/mg protein/min) | Phenolics (µg gallic acid equivalent/mL) | Flavonoids (µg quercetin equivalent/mL) | Reducing power ($A_{700}$) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|---|
| *Paecilomyces variotii* | | | | | | |
| 10 | 0.000 ± 0.000$^{a}$ | 12.5 ± 0.32$^{bc}$ | 127 ± 5.8$^{e}$ | 807 ± 56$^{ef}$ | 0.045 ± 0.003$^{a}$ | 86.0 ± 2.50$^{f}$ |
| 20 | 0.000 ± 0.000$^{a}$ | 14.2 ± 2.31$^{cd}$ | 151 ± 5.8$^{f}$ | 831 ± 63$^{ef}$ | 0.065 ± 0.006$^{b}$ | 85.8 ± 0.32$^{f}$ |
| 30 | 0.000 ± 0.000$^{a}$ | 15.9 ± 0.04$^{d}$ | 213 ± 7.2$^{g}$ | 856 ± 64$^{ef}$ | 0.074 ± 0.000$^{b}$ | 87.2 ± 0.77$^{f}$ |
| 50 | 0.000 ± 0.000$^{a}$ | 13.3 ± 1.19$^{bc}$ | 233 ± 4.4$^{gh}$ | 882 ± 52$^{ef}$ | 0.084 ± 0.002$^{c}$ | 86.7 ± 2.60$^{f}$ |
| 70 | 0.000 ± 0.000$^{a}$ | 13.8 ± 0.00$^{bc}$ | 250 ± 5.8$^{hi}$ | 908 ± 53$^{ef}$ | 0.093 ± 0.004$^{cd}$ | 76.8 ± 0.83$^{e}$ |
| 100 | 0.000 ± 0.000$^{a}$ | 11.6 ± 0.16$^{b}$ | 269 ± 7.3$^{i}$ | 933 ± 65$^{f}$ | 0.129 ± 0.004$^{h}$ | 73.1 ± 2.80$^{fe}$ |
| *Pleurotus floridanus* | | | | | | |
| 10 | 0.000 ± 0.000$^{a}$ | 1.25 ± 0.02$^{a}$ | 89.6 ± 2.4$^{d}$ | 882 ± 10$^{ef}$ | 0.121 ± 0.001$^{g}$ | 62.4 ± 0.85$^{d}$ |
| 20 | 0.000 ± 0.000$^{a}$ | 1.13 ± 0.06$^{a}$ | 82.8 ± 1.0$^{d}$ | 783 ± 2.2$^{de}$ | 0.106 ± 0.003$^{f}$ | 56.4 ± 2.82$^{cd}$ |
| 30 | 0.014 ± 0.002$^{a}$ | 1.07 ± 0.04$^{a}$ | 72.3 ± 1.5$^{c}$ | 681 ± 3.2$^{d}$ | 0.106 ± 0.001$^{e}$ | 53.5 ± 2.02$^{c}$ |
| 50 | 0.074 ± 0.004$^{b}$ | 0.76 ± 0.03$^{a}$ | 49.7 ± 3.2$^{b}$ | 554 ± 0.7$^{c}$ | 0.094 ± 0.001$^{d}$ | 38.2 ± 1.67$^{b}$ |
| 70 | 0.166 ± 0.004$^{c}$ | 0.65 ± 0.06$^{a}$ | 46.5 ± 3.8$^{b}$ | 458 ± 11$^{b}$ | 0.093 ± 0.001$^{cd}$ | 32.7 ± 3.14$^{b}$ |
| 100 | 0.276 ± 0.005$^{d}$ | 0.47 ± 0.03$^{a}$ | 36.0 ± 0.5$^{a}$ | 255 ± 4.3$^{a}$ | 0.087 ± 0.003$^{cd}$ | 23.5 ± 1.45$^{a}$ |

Each value is the mean of three replicates ± SE.

Means with common letters are non-significantly different at P<0.05

**TABLE 3. The enzymatic (POX and CAT) and non-enzymatic (phenolics, flavonoids, reducing power and the DPPH scavenging) antioxidant activity of an SSFS employing the ascomycete *Paecilomyces variotii* or the basidiomycete *Pleurotus floridanus* on rice straw at different time periods**

| Fungus and time period (day) | Peroxidase (U/min) | Catalase (µmole $H_2O_2$/mg protein/min) | Phenolics (µg gallic acid equivalent/mL) | Flavonoids (µg quercetin equivalent/ mL) | Reducing power ($A_{700}$) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|---|
| *Paecilomyces variotii* | | | | | | |
| 3 | 0.000 ± 0.000 | 1.397 ± 0.008$^{a}$ | 170.7 ± 2.3$^{e}$ | 2066 ± 20$^{d}$ | 0.165 ± 0.001$^{c}$ | 69.85 ± 0.4$^{a}$ |
| 6 | 0.000 ± 0.000 | 1.426 ± 0.116$^{a}$ | 203.5 ± 2.0$^{f}$ | 2149 ± 28$^{f}$ | 0.179 ± 0.001$^{d}$ | 71.29 ± 5.8$^{a}$ |
| 9 | 0.000 ± 0.000 | 1.355 ± 0.005$^{ab}$ | 247.5 ± 4.3$^{g}$ | 2181 ± 38$^{ef}$ | 0.189 ± 0.003$^{d}$ | 67.73 ± 0.3$^{ab}$ |
| 12 | 0.000 ± 0.000 | 1.366 ± 0.008$^{ab}$ | 284.3 ± 3.5$^{h}$ | 2215 ± 50$^{def}$ | 0.243 ± 0.006$^{e}$ | 68.30 ± 0.4$^{ab}$ |
| 15 | 0.000 ± 0.000 | 1.340 ± 0.081$^{ab}$ | 297.9 ± 1.6$^{i}$ | 2114 ± 37$^{de}$ | 0.250 ± 0.006$^{e}$ | 67.00 ± 4.1$^{ab}$ |
| *Pleurotus floridanus* | | | | | | |
| 3 | 0.045 ± 0.000$^{a}$ | 1.285 ± 0.005$^{abc}$ | 133.9 ± 5.0$^{d}$ | 1775 ± 43$^{ab}$ | 0.110 ± 0.002$^{b}$ | 64.26 ± 0.28$^{abc}$ |
| 6 | 0.183 ± 0.000$^{e}$ | 1.210 ± 0.011$^{bc}$ | 86.10 ± 0.5$^{bc}$ | 1743 ± 26$^{ab}$ | 0.105 ± 0.002$^{ab}$ | 60.50 ± 0.56$^{bc}$ |
| 9 | 0.173 ± 0.000$^{d}$ | 1.124 ± 0.116$^{c}$ | 88.90 ± 1.6$^{c}$ | 1821 ± 46$^{bc}$ | 0.101 ± 0.003$^{ab}$ | 56.28 ± 5.78$^{c}$ |
| 12 | 0.102 ± 0.001$^{c}$ | 0.917 ± 0.005$^{d}$ | 77.40 ± 1.0$^{b}$ | 1725 ± 72$^{ab}$ | 0.097 ± 0.003$^{a}$ | 45.86 ± 0.22$^{d}$ |
| 15 | 0.062 ± 0.001$^{b}$ | 0.886 ± 0.070$^{d}$ | 53.43 ± 8.3$^{a}$ | 1862 ± 22$^{c}$ | 0.106 ± 0.003$^{ab}$ | 44.30 ± 3.48$^{d}$ |

Each value is the mean of three replicates ± SE.

Means with common letters are non-significantly different at P<0.05

The POX activity of *Pleurotus floridanus* showed a progressive 42% reduction with the increase in substrate pH from 4 to 9. With an increase in pH from 4 to 5, the high CAT activity of *Paecilomyces variotii* was reduced by 57%, and that of *Pleurotus floridanus* was reduced by 30%. The CAT activity of both fungi was low at higher pH (Table 4). The high phenolic content of *Paecilomyces variotii* and the low phenolic content of *Pleurotus floridanus* exhibited 10% and 27% reductions, respectively with an increase in pH from 4 to 9. The flavonoid contents of the two fungal SSFS were comparable in the two fungal species across the pH range of 4 to 7 but were substantially higher in *Paecilomyces variotii* than *Pleurotus floridanus* at higher pH. In the *Paecilomyces variotii* SSFS, increasing the substrate pH from 4 to 7 reduced the flavonoid content by 28%, and the content remained steady at higher pH, whereas in the *Pleurotus floridanus* SSFS, the flavonoid content was reduced by 62% as the pH increased from 7 to 9. The reducing power and DPPH scavenging activity of the two fungi were not significantly affected by the substrate pH (Table 4).

In the *Pleurotus floridanus SSFS*, the activities of POX and CAT were progressively reduced with increasing incubation temperatures, reaching complete cessation at 40°C. The CAT activity of *Paecilomyces variotii* peaked at 30°C. The high phenolic content of *Paecilomyces variotii* and the low content of *Pleurotus floridanus* exhibited marked reductions with increases in temperature; but the greatest reduction occurred within the low range (25°C –30°C) in *Pleurotus floridanus* and the high range (30°C –40°C) in *Paecilomyces variotii* (Table 5). The comparable flavonoid contents of the two fungi exhibited a peak at 30°C for *Paecilomyces variotii* and 35°C for *Pleurotus floridanus*. The high reducing power of *Paecilomyces variotii* and the low reducing power of *Pleurotus floridanus* were mildly reduced with the increase in temperature from 30°C to 40°C. The high DPPH scavenging activity of *Paecilomyces variotii* and the low DPPH-scavenging activity of *Pleurotus floridanus* were reduced by an average of 27% as temperature increased from 25°C to 40°C, but the inhibition occurred within the higher range (35°C –40°C) in *Paecilomyces variotii* and the lower range (25°C–30°C) in *Pleurotus floridanus* (Table 5).

**TABLE 4. The enzymatic (POX and CAT) and non-enzymatic (phenolics, flavonoids, reducing power and the DPPH scavenging) antioxidant activity of an SSFS employing the ascomycete *Paecilomyces variotii* or the basidiomycete *Pleurotus floridanus* on rice straw at different pH values**

| Fungus and pH | Peroxidase (U/min) | Catalase (µmole $H_2O_2$/mg protein/min) | Phenolics (µg gallic acid equivalent/mL) | Flavonoids (µg quercetin equivalent/mL) | Reducing power ($A_{700}$) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|---|
| *Paecilomyces variotii* | | | | | | |
| 4 | 0.000 ± 0.000 | 5.46 ± 0.68$^{a}$ | 430 ± 40.4$^{a}$ | 1319 ± 62$^{a}$ | 0.217 ± 0.019$^{b}$ | 65.7 ± 2.9$^{ab}$ |
| 5 | 0.000 ± 0.000 | 3.81 ± 0.72$^{b}$ | 409 ± 19.4$^{ab}$ | 945 ± 31$^{e}$ | 0.233 ± 0.019$^{b}$ | 63.6 ± 2.2$^{abc}$ |
| 6 | 0.000 ± 0.000 | 3.29 ± 0.08$^{b}$ | 411 ± 35.2$^{a}$ | 1052 ± 31$^{cd}$ | 0.241 ± 0.011$^{a}$ | 63.8 ± 2.3$^{abc}$ |
| 7 | 0.000 ± 0.000 | 3.99 ± 0.18$^{b}$ | 351 ± 28.0$^{b}$ | 980 ± 31$^{de}$ | 0.216 ± 0.005$^{b}$ | 61.3 ± 5.8$^{abc}$ |
| 8 | 0.000 ± 0.000 | 3.81 ± 0.02$^{b}$ | 379 ± 12.4$^{ab}$ | 963 ± 41$^{de}$ | 0.212 ± 0.008$^{b}$ | 65.4 ± 2.9$^{ab}$ |
| 9 | 0.000 ± 0.000 | 4.06 ± 0.39$^{b}$ | 390 ± 23.1$^{ab}$ | 1034 ± 41$^{cde}$ | 0.224 ± 0.009$^{b}$ | 68.2 ± 1.2$^{a}$ |
| *Pleurotus floridanus* | | | | | | |
| 4 | 0.163 ± 0.005$^{a}$ | 3.76 ± 0.06$^{b}$ | 130 ± 5.8$^{c}$ | 1101 ± 9$^{bc}$ | 0.107 ± 0.001$^{c}$ | 62.5 ± 2.3$^{abc}$ |
| 5 | 0.152 ± 0.021$^{ab}$ | 1.62 ± 0.15$^{c}$ | 102 ± 5.0$^{c}$ | 1181 ± 14$^{b}$ | 0.112 ± 0.001$^{c}$ | 56.0 ± 2.3$^{c}$ |
| 6 | 0.133 ± 0.028$^{bc}$ | 1.60 ± 0.03$^{c}$ | 104 ±6.7$^{c}$ | 1094 ± 8$^{bc}$ | 0.111 ± 0.000$^{c}$ | 58.4 ± 2.0$^{bc}$ |
| 7 | 0.140 ± 0.007$^{b}$ | 1.68 ± 0.08$^{c}$ | 116 ± 5.8$^{c}$ | 1145 ± 1$^{b}$ | 0.122 ± 0.002$^{c}$ | 56.5 ± 3.2$^{c}$ |
| 8 | 0.119 ± 0.007$^{c}$ | 1.42 ± 0.08$^{c}$ | 99.4 ± 4.2$^{c}$ | 481 ± 11$^{f}$ | 0.110 ± 0.001$^{c}$ | 58.3 ± 3.8$^{bc}$ |
| 9 | 0.094 ± 0.004$^{d}$ | 1.13 ± 0.05$^{c}$ | 95.2 ± 4.1$^{c}$ | 441 ± 19$^{f}$ | 0.109 ± 0.003$^{c}$ | 60.0 ± 2.9$^{abc}$ |

Each value is the mean of three replicates ± SE.

Means with common letters are non-significantly different at P<0.05.

**TABLE 5. The enzymatic (POX and CAT) and non-enzymatic (phenolics, flavonoids, reducing power and the DPPH scavenging) antioxidant activity of an SSFS employing the ascomycete *Paecilomyces variotii* or the basidiomycete *Pleurotus floridanus* on rice straw at different temperatures**

| Fungus and temperature (°C) | Peroxidase (U/min) | Catalase (µmole $H_2O_2$/mg protein/min) | Phenolics (µg gallic acid equivalent/mL) | Flavonoids (µg quercetin equivalent/mL) | Reducing power ($A_{700}$) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|---|
| *Paecilomyces variotii* | | | | | | |
| 25 | 0.000 ± 0.000 | 3.05 ± 0.07$^{c}$ | 275.4 ± 2.9$^{a}$ | 808 ± 2.4$^{cd}$ | 0.190 ± 0.016$^{a}$ | 82.5 ± 0.5$^{a}$ |
| 30 | 0.000 ± 0.000 | 4.74 ± 0.00$^{a}$ | 291.1 ± 2.4$^{a}$ | 1097 ± 1.4$^{e}$ | 0.185 ± 0.018$^{a}$ | 82.2 ± 3.8$^{a}$ |
| 35 | 0.000 ± 0.000 | 3.92 ± 0.25$^{b}$ | 160.5 ± 6.0$^{b}$ | 759 ± 57.5$^{bc}$ | 0.181 ± 0.006$^{a}$ | 82.2 ± 1.2$^{a}$ |
| 40 | 0.000 ± 0.000 | 2.10 ± 0.11$^{d}$ | 48.13 ± 2.4$^{d}$ | 584 ± 3.2$^{a}$ | 0.136 ± 0.009$^{b}$ | 58.7 ± 0.2$^{b}$ |
| *Pleurotus floridanus* | | | | | | |
| 25 | 0.077 ± 0.005$^{a}$ | 1.31 ± 0.05$^{e}$ | 93.3 ± 5.0$^{c}$ | 687 ± 0.8$^{b}$ | 0.134 ± 0.006$^{b}$ | 59.0 ± 1.8$^{b}$ |
| 30 | 0.066 ± 0.001$^{b}$ | 1.24 ± 0.04$^{e}$ | 33.6 ± 0.2$^{f}$ | 730 ± 17.3$^{bc}$ | 0.122 ± 0.001$^{b}$ | 43.7 ± 0.9$^{c}$ |
| 35 | 0.060 ± 0.001$^{c}$ | 0.51 ± 0.01$^{f}$ | 42.1 ± 1.2$^{e}$ | 891 ± 0.8$^{d}$ | 0.128 ± 0.000$^{b}$ | 45.1 ± 2.9$^{c}$ |
| 40 | 0.000 ± 0.000$^{d}$ | 0.00 ± 0.00$^{g}$ | 41.8 ± 1.5$^{e}$ | 666 ± 64.1$^{ab}$ | 0.121 ± 0.000$^{b}$ | 46.1 ± 3.5$^{c}$ |

Each value is the mean of three replicates ± SE.

Means with common letters are non-significantly different at P<0.05.

The POX activity of the *Pleurotus floridanus* SSFS was highest with ammonium chloride as the nitrogen source, intermediate with urea, gelatin, and peptone and lowest with sodium nitrate. The high CAT activity of *Paecilomyces variotii* was mildly affected by the N source, whereas *Pleurotus floridanus* showed a substantial response. In both fungi, the highest CAT activity was observed with ammonium chloride; but the N source with the lowest CAT activity was urea for *Paecilomyces variotii* and gelatin for *Pleurotus floridanus* (Table 6). The genotypic differences in phenolic and flavonoid contents, which were higher in the *Paecilomyces variotii* SSFS, were lowest with ammonium chloride, which also generated the lowest contents of phenolics and flavonoids in both fungi. The maximal phenolic and flavonoid contents were found with urea and peptone in *Paecilomyces variotii*, but with sodium nitrate in *Pleurotus floridanus* (Table 6). The genotypic difference in the reducing power, which was higher in *Paecilomyces variotii*, was particularly evident with the polymeric organic N sources (gelatin and peptone). In the *Paecilomyces variotii*, SSFS, the reducing power was relatively high with gelatin and low with urea, and the reverse was true in the *Pleurotus floridanus* SSFS (Table 6). The high DPPH scavenging activity of *Paecilomyces variotii* and the low DPPH-scavenging activity of *Pleurotus floridanus* were not significantly affected by the N source (Table 6).

## Discussion

The SSFS of rice straw enhances the breakdown of cell wall networks, leading to the release or de novo biosynthesis of various bioactive compounds with the aid of fungal metabolic machinery. Moreover, fermentation can enrich rice straw with glucose and microbial proteins improving its palatability and digestibility for animals (Singh & Arya, 2021). Although plant products represent the primary source of natural antioxidants, particularly phenolics and flavonoids, phenolic compounds are also produced as secondary metabolites by basidiomycetes (Umeo et al., 2015) and ascomycetes (Sharma & Gautam, 2017).

In addition to the main components of the SSFS (fungus and substrate), SSFS antioxidant activity is influenced by several environmental conditions such as moisture content, pH, temperature, nitrogen source and incubation period. The substrate moisture content is the most important factor affecting the SSFS efficiency (Thomas et al., 2013), and the optimal moisture content may vary according to the fungus species and taxonomic group. The present work reveals a positive effect of increasing rice straw moisture content on the antioxidant activity of the ascomycete *Paecilomyces variotii* and a negative effect on that of the basidiomycete *Pleurotus floridanus*. However, the extent of this effect varied according to the antioxidant activity indicator. Šelo et al. (2021) postulated that increasing the moisture content of the substrate,

beyond a certain limit could lower the substrate porosity and reduce oxygen availability, ultimately restricting fungal growth. In contrast, lowering the moisture content below a certain threshold reduces nutrient availability, and leading to poor microbial growth. According to Bellettini et al. (2019), the satisfactory growth of *Pleurotus* spp. necessitates substrate moisture in the range of 50% –75% of substrate dry weight.

Another important environmental factor affecting SSFS performance is the substrate pH, which modulates fungal growth, enzyme activity and protein conformation (Job et al., 2010). In addition to affecting the enzymatic antioxidant activity, substrate pH is also expected to affect the non-enzymatic antioxidant activities because the later is the output of enzymatic action. The present work reveals a relatively moderate effect of pH on the antioxidant activity of the SSFS relative to the other environmental factors, with a preference for low pH observed with both fungi. pH had a stronger effect on the enzymatic activity (POX and CAT) than on the non-enzymatic antioxidants, among which the effect on the DPPH scavenging activity was marginal. In general, the response to substrate pH was more evident in *Paecilomyces variotii* than *Pleurotus floridanus*. In accordance with the present findings, the optimum pH for tannase activity in *Paecilomyces variotii* SSFS was reported to be 5–7 (Raaman et al., 2010) or 4.5–6.5 (Battestin & Macedo, 2007). For *Pleurotus ostreatus,* the maximum laccase activity occurred at pH 5.0 (Elsayed et al., 2012), and the optimum CAT activity occurred at pH 7.5 (Susmitha et al., 2014).

The two investigated fungi preferred moderate temperature ranges (~ 25°C). Similar to pH, the effect of incubation temperature was stronger on the enzymatic antioxidant activity (POX and CAT) than on the non-enzymatic antioxidants and more apparent in *Paecilomyces variotii* than *Pleurotus floridanus*. Consistent with our findings, Wang et al. (2017) reported a moderate temperature of 28°C as optimal for CAT production by *Pleurotus ostreatus* and enzyme activity declined as temperature increased beyond 40°C. This is interesting because greater production of $H_2O_2$-manipulating enzymes is expected under heat stress. The optimum temperature might vary widely among enzymes; for example, it was lower for tannase (29°C –34°C) (Raaman et al., 2010; Battestin & Macedo, 2007) than for invertase (< 60°C) of *Paecilomyces variotii* (Giraldo et al., 2012).

**TABLE 6. The enzymatic (POX and CAT) and non-enzymatic (phenolics, flavonoids, reducing power and the DPPH scavenging) antioxidant activity of an SSFS employing the ascomycete *Paecilomyces variotii* or the basidiomycete *Pleurotus floridanus* on rice straw with different nitrogen sources**

| Fungus and nitrogen source | Peroxidase (U/min) | Catalase (µmole $H_2O_2$/mg protein/min) | Phenolics (µg gallic acid equivalent/mL) | Flavonoids (µg quercetin equivalent/mL) | Reducing power ($A_{700}$) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|---|
| *Paecilomyces variotii* | | | | | | |
| Ammonium chloride | 0.000 ± 0.000 | 5.44 ± 0.63$^{d}$ | 594 ± 37$^{c}$ | 2374 ± 146$^{c}$ | 0.150 ± 0.02$^{c}$ | 92.5 ± 0.4$^{c}$ |
| Gelatin | 0.000 ± 0.000 | 4.66 ± 0.60$^{d}$ | 1141 ± 8$^{de}$ | 4562 ± 34$^{de}$ | 0.180 ± 0.017$^{d}$ | 90.8 ± 0.5$^{c}$ |
| Sodium nitrate | 0.000 ± 0.000 | 4.25 ± 0.93$^{d}$ | 983 ± 68$^{d}$ | 3932 ± 272$^{d}$ | 0.098 ± 0.005$^{b}$ | 93.3 ± 0.2$^{c}$ |
| Peptone | 0.000 ± 0.000 | 4.83 ± 0.01$^{d}$ | 1180 ± 47$^{de}$ | 4721 ± 188$^{de}$ | 0.137 ± 0.008$^{c}$ | 90.2 ± 0.7$^{c}$ |
| Urea | 0.000 ± 0.000 | 2.82 ± 0.42$^{c}$ | 1212 ± 2.6$^{e}$ | 4847 ± 10.5$^{e}$ | 0.092 ± 0.004$^{b}$ | 91.2 ± 0.3$^{c}$ |
| *Pleurotus floridanus* | | | | | | |
| Ammonium chloride | 0.100 ± 0.014$^{c}$ | 1.50 ± 0.23$^{a}$ | 32.1 ± 0.8$^{a}$ | 128.7 ± 3.1$^{a}$ | 0.018 ± 0.000$^{a}$ | 35.9 ± 11.2$^{b}$ |
| Gelatin | 0.048 ± 0.002$^{b}$ | 0.15 ± 0.02$^{b}$ | 33.5 ± 1.6$^{a}$ | 134.1 ± 6.3$^{a}$ | 0.015 ± 0.001$^{a}$ | 20.8 ± 1.0$^{a}$ |
| Sodium nitrate | 0.013 ± 0.004$^{a}$ | 0.99 ± 0.07$^{ab}$ | 41.7 ± 6.3$^{b}$ | 166.7 ± 25.1$^{b}$ | 0.016 ± 0.002$^{a}$ | 25.7 ± 3.3$^{ab}$ |
| Peptone | 0.042 ± 0.015$^{b}$ | 0.53 ± 0.10$^{ab}$ | 38.1 ± 1.6$^{ab}$ | 152.2 ± 6.3$^{ab}$ | 0.008 ± 0.001$^{a}$ | 21.6 ± 0.9$^{a}$ |
| Urea | 0.060 ± 0.016$^{b}$ | 0.67 ± 0.17$^{ab}$ | 37.1 ± 0.5$^{ab}$ | 148.6 ± 2.1$^{ab}$ | 0.019 ± 0.001$^{b}$ | 24.4 ± 1.1$^{a}$ |

Each value is the mean of three replicates ± SE.

Means with common letters are non-significantly different at P<0.05.

The nitrogen source is an important nutritional factor that affects fungal growth and metabolism, constricting preferences were observed between *Paecilomyces variotii* and *Pleurotus floridanus*. The current study reveals that the activities of POX in *Pleurotus floridanus* and of CAT in both fungal species were favored with the reduced inorganic N (i.e., ammonium). In accordance with these findings, ammonium was the best nitrogen source for laccase activity in *Pleurotus eryngii*, *Pleurotus ostreatus* (Stajić et al., 2006) and *Paecilomyces variotii* (Battestin & Macedo, 2007; Elsayed et al., 2012). The best nitrogen sources for POX activity in *Pleurotus ostreatus* and laccase activity of *Pleurotus* species were peptone and amino acids (Stajić et al., 2006; Giraldo et al. 2012). In contrast, the non-enzymatic antioxidant activity–except DPPH scavenging activity– of the two test fungi were relatively high with the organic nitrogen sources, particularly urea.

The appropriate incubation period for maximal antioxidant activity might vary according to the fungal species, incubation conditions, and the antioxidant activity assayed. The present study reveals the advantage of a brief incubation period (6 days) for increasing POX activity versus a late period (12–15 days) for increasing CAT activity of *Pleurotus floridanus*; however, no effect of incubation period on CAT activity was observed in *Paecilomyces variotii*. Job et al. (2010) reported a 4-day incubation period was ideal for maximal β-glucosidase production by *Paecilomyces variotii***,** whereas the optimal laccase production by *Pleurotus* species was obtained on day 10 (Edae & Alemu, 2017). The optimal incubation period for non-enzymatic antioxidant activity (phenolics, flavonoids, and reducing power) was contrasting between the two fungal species, with a progressive increase in the *Paecilomyces variotii* SSFS and a progressive decrease in the *Pleurotus floridanus* SSFS with increasing incubation time.

The different measures of antioxidant activity assayed in the present work showed different degrees of association (Fig. 1). The POX activity was distinct from the other activities by virtue of its radical differences between the fungal species, POX activity was detected only in the basidiomycete *Pleurotus floridanus* SSFS, and was absent from the ascomycete *Paecilomyces variotii* SSFS. POX activity was also distinctly negatively correlated with the other antioxidant measures. A positive correlation was evident between phenolics, flavonoids, and DPPH-scavenging activity but was weakly expressed between CAT and the other non-enzymatic activities.

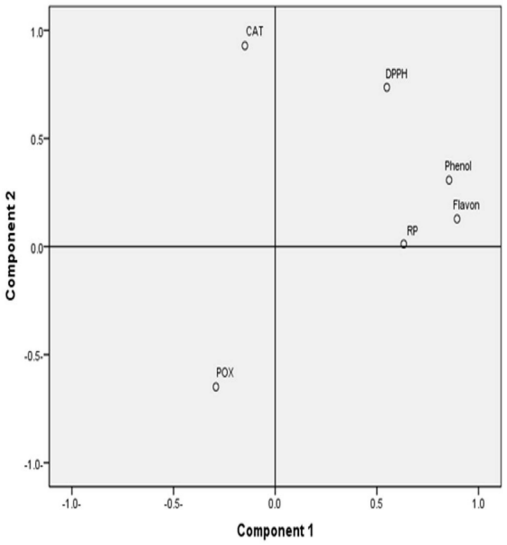


**Fig. 1. Principle component analysis (PCA) to summarize the relationships between the different antioxidant activity measures of the SSFS consisting of *Pleurotus floridanus* or *Paecilomyces variotii* grown on rice straw under different conditions of moisture, pH, temperature, nitrogen source and incubation period**

## Conclusions

The optimum conditions for SSFS antioxidant activity vary between fungal species and enzymatic and non-enzymatic activities. Low moisture content and a brief incubation period were preferable in the basidiomycete *Pleurotus floridanus* SSFS, whereas high moisture content and a long incubation period were ideal for the ascomycete *Paecilomyces variotii* SSFS. Low pH and temperature were optimal for both fungi. However, the effects of pH and temperature were more evident on *Paecilomyces variotii* SSFS than on *Pleurotus floridanus* SSFS, and on POX and CAT activities than on the non-enzymatic antioxidants. Whereas the appropriate N source for POX and CAT was ammonium, the non-enzymatic antioxidants preferred urea. The appropriate incubation period for POX was shorter than that of CAT in *Pleurotus floridanus* SSFS, with no effect of time on CAT activity of *Paecilomyces variotii* SSF. Whereas the non-enzymatic antioxidant activity was increased with

incubation time in *Paecilomyces variotii* SSF, the reverse was evident in *Pleurotus floridanus* SSF. Overall, the antioxidant activity was affected by the fungal species to a greater extent than by the environmental conditions. The antioxidant activities of the ascomycete *Paecilomyces variotii* were substantially higher than those of the basidiomycete *Pleurotus floridanus*, however, POX was observed only in *Pleurotus floridanus*. CAT activity was observed in both fungi but was higher in *Paecilomyces variotii*. POX activity was distinctly segregated from and negatively correlated with the non-enzymatic antioxidant activity; whereas CAT activity and the non-enzymatic antioxidants exhibited a weak positive correlation.

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## تحسين نشاط مضادات الأكسدة لنظام تخمر الحالة الصلبة باستخدام الفطر البازيدي *Pleurotus floridanus* والفطر الزقي *Paecilomyces variotii* على قش الأرز

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تتحدد كفاءة نظام التخمير الصلب (SSFS) باختيار الكائن الدقيق والركيزة والظروف البيئية. تم اختبار تحسين النشاط المضاد للأكسدة لـ SSFS من فطر *Pleurotus floridanus* أو *Paecilomyces variotii* على قش الأرز. تجاوز تأثير الفطر على النشاط المضاد للأكسدة تأثير الظروف البيئية. ظهر نشاط البيروكسيديز (POX) فقط في *Pleurotus floridanus* بينما ظهر نشاط الكتاليز (CAT) في الفطرين وبأفضلية لـ *Paecilomyces variotii*. اشترك كلا الإنزيمين في تفضيل الأس الهيدروجيني ودرجة الحرارة المنخفضتين و $NH_4Cl$ كمصدر نيتروجيني ولكن الرطوبة العالية كانت أفضل لـ POX مقابل الرطوبة المنخفضة لـ CAT . كانت محتويات الفينولات والفلافونويد أعلى في *Pleurotus variotii* من *Pleurotus floridanus* ، مع تفضيل الرطوبة العالية والأس الهيدروجين المنخفض واليوريا في *Paecilomyces variotii* مقابل الرطوبة المنخفضة والأس الهيدروجين المنخفض والنترات في *Pleurotus floridanus* . كانت القدره الاختزالية أعلى في *Paecilomyces variotii* من *Pleurotus floridanus* ، مع ارتباط إيجابي مع الرطوبة ودرجة الحرارة المنخفضة وتفضيل الجيلاتين في *Paecilomyces variotii* مقابل ارتباط سلبى مع الرطوبة ودرجة الحرارة وتفضيل اليوريا في *Pleurotus floridanus* ،كان نشاط كسح DPPH أعلى في *Paecilomyces variotii* من *Pleurotus floridanus* مع ارتباط سلبي بالرطوبة ودرجة الحرارة وتأثير محدود لمصدر النيتروجين في الفطرين. بخصوص فترة التحضين كان أقصى نشاط مضاد للأكسدة لـ *Paecilomyces variotii* في فترات قصيرة مقابل فترات متأخرة لـ *Pleurotus floridanus* .