Progression Over Time of Nutritional Content and Antioxidant Activity of Grains Fermented with the Medicinal Mushrooms *Pleurotus ostreatus* and *Ganoderma sessile* (Agaricomycetes)

Antonella Mazzola,[a,b] Francisco Kuhar,[c,d,e] & Alina G. Greslebin[a,d,*]

[a]Laboratorio de Bioprospección en Investigación Aplicada en Plantas y Hongos (LaBIAPH), FCNyCS, Universidad Nacional de la Patagonia (UNPSJB), Esquel, Chubut, Argentina; [b]Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+i), Argentina; [c]Instituto Multidisciplinario de Biología Vegetal (INBIV, CONICET-UNC), X5016GCN Córdoba, Argentina; [d]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; [e]Innomy Biotech S.A. Astondo Bidea, 48160 Derio, Biscay, Basque Country, Spain

*Address all correspondence to: Alina G. Greslebin, Laboratorio de Bioprospección en Investigación Aplicada en Plantas y Hongos (LaBIAPH), FCNyCS, Universidad Nacional de la Patagonia (UNPSJB), Ruta 259 Km 16.4, Esquel, Chubut, Argentina; Tel.: +54 9 2945 687300, E-mail: agreslebin@unpata.edu.ar; agreslebin@gmail.com

ABSTRACT: Solid-state fermentation of cereals with edible fungi is a promising strategy for producing functional flours. Hypothetically, the nutritional and functional properties of these flours could be modulated by manipulating substrate composition, fungal species, and incubation conditions. This article reports the variation over time in nutritional, polyphenol, and triterpene contents, as well as the antioxidant activity of rice and wheat fermented with *Ganoderma sessile* and *Pleurotus ostreatus*. Solid-state fermentation significantly improved the antioxidant power of the substrates which seemed to be highly correlated with the increase of the phenolic compounds. This increase peaked in the second to third week and decreased after this point. Triterpene content also increased, especially in substrates fermented with *G. sessile.* Substrates fermented with *G. sessile* showed higher values than those fermented with *P. ostreatus* in all compounds, which could be a result of a higher growth rate. Fermented wheat showed higher values than fermented rice in all measured compounds except reducing sugars which can be related to a slower progress in the fermentation due to the more complex structure of the wheat grain. Our results reinforce the importance of substrate and strain selection for product modulation to meet the industry's growing needs.

KEY WORDS: *Ganoderma sessile*, *Pleurotus ostreatus*, antioxidant activity, bioactive compounds, cereals, medicinal mushrooms, mycelia, solid-state fermentation

ABBREVIATIONS: AA, ascorbic acid; **ANOVA**, analysis of variance; **BR**, brown rice; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl; **GA**, glucose asparagine medium; **LSD**, least significant difference; **MEA**, malt extract agar; **NCD**, non-communicable disease; **SSF**, solid state fermentation; **WW**, whole wheat

I. INTRODUCTION

Consumer demand from the food industry has changed continuously in recent decades, with a shift towards healthy foods to prevent nutrition-related diseases and improve physical and mental well-being. Accordingly, there is an increasing demand for functional foods, which have advantageous physiological effects, improve the general condition of the body, and contribute to the prevention of several health problems, such as non-communicable diseases (NCDs) cancer, diabetes, heart, and neurodegenerative diseases.[1]

Several NCDs are directly correlated with lifestyle (primarily diet and physical activity). Current literature supports the view that lifestyle-induced oxidative stress and inflammation are the primary drivers of the metabolic imbalances that cause NCDs.[2] Oxidative stress plays a role in degenerative

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senescence. Free radicals are involved in the pathogenesis of several processes, such as carcinogenesis, cardiovascular disease, ischemia, Alzheimer's disease, early aging, arteriosclerosis, liver injury, inflammation, diabetes mellitus, skin damage, and arthritis.[3] These aspects have sparked considerable interest in nutraceuticals and functional foods with antioxidant activity, which have become an essential focus of consumer demands.

Phenolic compounds have specific health effects on health associated with reduced risk of chronic and NCDs, possibly due to their ability to reduce oxidative agents and scavenge free radicals.[4] Free radical scavengers prevent cellular damage by reducing oxidative stress. Epidemiological studies have already revealed that a higher intake of antioxidants as food supplements reduces the risk of many diseases.[3] Also, phenolic extracts and pure polyphenols have been studied as potential alternatives to commercial medicines used to reduce the hydrolysis rate of starch because of their inhibitory activities against α-amylase and α-glucosidase.[5] In addition to inhibiting digestive enzymes, the effects of dietary polyphenols on starch digestion are also related to the direct interactions between polyphenols and starch because they alter the physical properties and micromolecular arrangements of starch.

Mushrooms are widely recognized as a healthy and nutritious food.[6] Even though their composition and nutritional value vary between species, mushrooms generally contribute to a healthy diet since they provide a significant number of complex polysaccharides, proteins, and micronutrients. In recent years, fungal protein has attracted more and more attention in the food industry due to its high potential as an alternative to animal protein.[7]

In addition to their nutritional value, mushrooms are also recognized as a source of nutraceuticals and functional food, modulating the human immune system, lowering proneness to disease, and acting as anti-inflammatory agents through their high content of antioxidants.[8,9] Among the different fungal bioactive compounds, polysaccharides and phenolic compounds are some of the main bioactive substances with reported potential for maintaining human health and reducing the risk of various diseases.[10] Numerous studies have shown that not only fruiting bodies, but also fungal mycelia represent an inexhaustible source of primary and secondary metabolites, including phenolic compounds and flavonoids, whose content is directly correlated with the significant antioxidant activity of mycelium extracts.[10]

Despite the demonstrated benefit of including fungal components and metabolites in the diet, the consumption of edible mushrooms may be limited due to their high cost, resulting from the time required for cultivation[11] and production yield and losses. Alternatively, mycelium can be produced faster, with fewer requirements and higher yields. Recent studies have demonstrated that mycelia, as well as the spent growth substrate or cultivation media, are a source of bioactive compounds, such as phenolic acids and ergosterol, both with antioxidant activity,[12] and lovastatin,[13] among others. In addition, when produced on edible substrates, mycelium biomass and substrate constitute a source of mycoprotein, such as the case of traditional Asian foods such as Kogi and tempeh or mycelia-based alternative meat (e.g. Quorn, Marlow Foods; Innomy Biotech; Ferment-IQ, MycoTechnology Inc.).

Solid-state fermentation (SSF) is the fermentation of solid substrates in the absence of free water. It consists of a three-phase system; the continuous gas phase, the liquid film, and the solid phase.[14] This technique, used for centuries in Asiatic cultures to preserve and enrich grain or cereal-based foods, has gained more attention in the Western world due to its potential for producing fungus-fermented products that may become novel functional food.[15] The nutritional and functional enrichment of several grains, cereals, and cereal by-products through SSF with mushrooms has been studied in the last years. In all cases, besides nutritional enrichment, the fermentation with mushrooms enhanced the total phenol content and antioxidant properties.[11,16,17]

Including fungal mycelia in the fermented substrate makes it possible to exploit the range of functional metabolites and components, namely those in the mycelia, those released into the substrate, as well as those in the fermented transformed substrate. Benson et al.[18] demonstrated that fungal SSF by

Trametes versicolor dramatically alters the biological effects of the fermented substrate and that the mushroom mycelium has distinctly different biological and immune-modulating properties from those of its fermented substrate. Fermentation also improves the digestibility and availability of proteins during digestion.[19]

Therefore, SSF of cereals constitutes a promising method for producing functional flours that can be used as an ingredient in a wide range of mass-consumption foods. Hypothetically, these flours, consisting of the dried and ground fermented grains and mycelia, could be modulated in their nutritional and functional properties by manipulating substrate composition, mushroom species, and incubation time and conditions. In this sense, this article reports the variation over time in nutritional, polyphenol, triterpene, and reducing sugar contents, as well as the antioxidant activity, of rice and wheat substrates fermented with *Ganoderma sessile* Murrill (Ganodermtaceae, Agaricomycetes) and *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (Pleurotaceae, Agaricomycetes). It also analyzes the relation between fungal biomass, polyphenol content, and antioxidant activity. Strains were selected because they correspond to widely studied species whose nutritional and functional value has been shown in different studies.[10,20,21] In particular, the strain E47 was widely researched in the past under the identity of *G. lucidum* before its placement within *G. sessile* was demonstrated using molecular tools.[20]

II. MATERIALS AND METHODS

A. Assay

1. Strains

G. sessile strains E47 (University of Guelph, Canada) and *P. ostreatus* (CERZOS, Bahía Blanca, Argentina) deposited in LaBIAPH strain collection were used. Colonies were grown and maintained in malt extract agar (MEA) medium. Agar explants 1 cm^2 in diameter were transferred to Petri dishes (90 mm in diameter) with MEA medium and incubated at 25°C in the dark for 7 d for *G. sessile* and 10 d for *P. ostreatus.*

2. Spawn

The spawn was prepared on a substrate consisting of cooked brown rice (BR) boiled for 15 min to reach 60% water content. Bags of 200 g final mass of substrate were sterilized in an autoclave for 45 min, and, after cooling, each bag was inoculated with one fully colonized Petri dish (prepared as described in "strains") and incubated at 25°C in the dark for 14 d.

3. Substrate Preparation

Two types of grains were used: BR (*Oryza sativa*) and whole wheat (WW) (*Triticum aestivum*). Both were bought in bulk directly from the producers. The grains were dried until constant weight. Dry grains were placed in 90 mm in diameter glass Petri dishes (50 g in each plate) and hydrated with distilled water (ratio 1:1) for 20 h. After the hydration period, plates were sterilized in an autoclave at 121°C (1.2 atm) for 90 min. The sterile plates were inoculated in a horizontal laminar flow hood (Biotraza, HB1500) with 5 g of spawn suspended in sterile water (ratio 1:2). Additionally, 6 uninoculated plates of each substrate were kept as controls. Plates were incubated at 25°C in the dark. Every week, six randomly selected Petri dishes (replicates) were extracted, dried at 60°C until constant mass, milled in a laboratory grinder to obtain a particle size smaller than 300 mesh, and kept in airtight plastic bags in the dark until processing.

4. Experimental Design

The effects of SSF with the two strains on the two substrates were evaluated through a factorial experiment with two factors: incubation time and substrate. Twenty-five Petri dishes of each substrate were inoculated with each strain, and six replicates were extracted in each incubation time (week). Controls were non-fermented substrates (indicated as time 0). Fungal biomass was estimated by registering the dry weight loss of the substrate, following the method described by Terebiznik and Pilosof.[22]

B. Chemical Analyses

1. Preparation of Extracts

One gram of each sample was mixed with 5 mL of distilled water or 5 mL of ethanol. Samples were stirred for 30 min for effective extraction and centrifuged at 2000 × *g* for 15 min. Supernatants were referred to as water extract and ethanolic extract respectively and stored at 4°C until the completion of the analysis.

Determination of the total phenol contents: The total phenol content of the water extracts was determined by the Folin-Cioncalteu colorimetric method[23] expressed in mg gallic acid (mgGA equivalents) per gram of sample, using a calibration curve of gallic acid (0.0007–0.005 mg).

2. Triterpenoid Content

This was determined by a colorimetric method described by Bidegain et al.[21] modified as follows: After transferring 50 aliquots of ethanolic extract to 10-mL test tubes, the solvent was evaporated at 60°C, and 0.5 mL of 70% perchloric acid was added to each tube. The mixtures were heated in a water bath at 60°C for 10 min, cooled at room temperature, and diluted with 2.5 mL of acetic acid and 0.15 mL of 5% vanillin (W310700; Sigma)–acetic acid solution. The absorbance was measured at 548 nm. The total triterpenoid content was expressed in mg of ursolic acid equivalents per g of dry matter using a calibration curve of ursolic acid (0.0006–0.005 mg).

3. Main Nutritional Component Assay

Water-soluble extracts were used to evaluate soluble protein and reduce sugar contents. The water-soluble protein contents were determined by the Lowry protein method.[24] The reducing sugar contents were quantified by the Somogyi-Nelson reaction.[25]

C. Analysis of Antioxidant Activity *In Vitro*

1. Potassium Ferricyanide Assay

The antioxidant activity was determined using the potassium ferricyanide assay described by Bibi Sadeer et al.[26] Briefly, the samples of water extract (0.5 mL) were mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1%). The resulting mixture was incubated at 50°C for 20 min. After the incubation period, 0.5 mL trichloroacetic acid (10%), 2.5 mL deionized water, and 0.5 mL ferric chloride (0.1%) were added to the mixture. The sample absorbance was read at 700 nm. Results are expressed as milligrams of ascorbic acid equivalent per gram of dry weight (mg AA/g) using a calibration curve of ascorbic acid (0.002–0.03 mg).

2. Scavenging Ability on DPPH Radical Assay

Each ethanolic extract was mixed with 1 mL of ethanolic solution containing DPPH radical, resulting in a final concentration of 0.1 mM DPPH. The mixture was shaken vigorously and allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm using an ultraviolet and visible spectrophotometer (Spectrum SP-2000 UV).[27] Results were expressed as mg ascorbic acid equivalent per g of dry weight (mg AA/g). For this, a calibration curve was performed with DPPH using ascorbic acid (0.0006–0.01 mg) as a radical scavenger.

D. Statistical Analysis

A two-way analysis of variance (ANOVA) was performed to verify differences in the response variables between incubation times and substrates. Fisher's least significant difference (LSD) was used as a *post hoc* test at $P < 0.05$ to determine which specific means were different. The two factors were incubation time, with five levels (0, 7, 14, 21, and 28 d), and substrate, with two levels (BR and WW). Response variables were total phenolic content, total triterpene content, antioxidant activity, soluble protein content, and reducing sugar content. When significant interactions between factors were found, the factors were analyzed separately in a one-way ANOVA using Fisher´s LSD at $P < 0.05$ as a *post hoc* test. Normality and homoscedasticity of data were checked through a Q-Q plot and Levene's test, respectively. When normality was rejected, the data set was analyzed through a Kruskal-Wallis non-parametric test. Differences between fungal strains were evaluated through a paired *t*-test where paired observation consisted of data from the same time and substrate. All statistical analyses were performed using InfoStat.[28]

III. RESULTS

All parameters were greater in fermented substrates than in controls (time 0), at least from time 14 d on (Figs. 1 and 2), except for the reducing sugar content of substrates fermented with *P. ostreatus* that was significantly greater from day 21 on in both substrates. The antioxidant profile and the content of soluble protein of the BR and WW substrates fermented by *G. sessile* and *P. ostreatus* strains varied according to the fermentation time. A significant increase in the antioxidant profile and the content of soluble proteins was observed, reaching a peak around days 14 to 21 and decreasing or slowing down afterward (Figs. 1 and 2C and 2D).

The evolution over time of the reducing-sugar content of WW fermented with *G. sessile* was markedly different from that of BR fermented with the same species. *Ganoderma sessile*-BR showed an abrupt and significant increase in reducing sugar up until day 14, remaining constant after that, whereas *G. sessile*-WW showed a progressive significant increment from the beginning, which seemed to slow down between days 21 and 28 (Fig. 2E). The substrates treated with *P. ostreatus* showed a much smaller increase in reducing sugar, which differs significantly from controls at 21 and 28 d of incubation and showed great variability (Fig. 2F).

All variables analyzed, i.e., dry weight loss, polyphenol content, triterpene content, reducing power, DPPH radical scavenging ability, soluble protein content, and reducing sugar content, were greater in substrates fermented with *G. sessile* than in those fermented with *P. ostreatus* strain on both substrates (Figs. 1 and 2 and Tables 1 and 2).

The effect of each strain on the two different substrates showed differences in some of the variables analyzed. Fermentation by *G. sessile* produced a significantly greater polyphenol content, reducing power, DPPH radical scavenging, and reducing sugar content in WW than in BR. Fermentation by *P. ostreatus* also produced significantly greater values in WW than in BR but only in polyphenol content and DPPH radical scavenging power (Figs. 1 and 2 and Table 3).

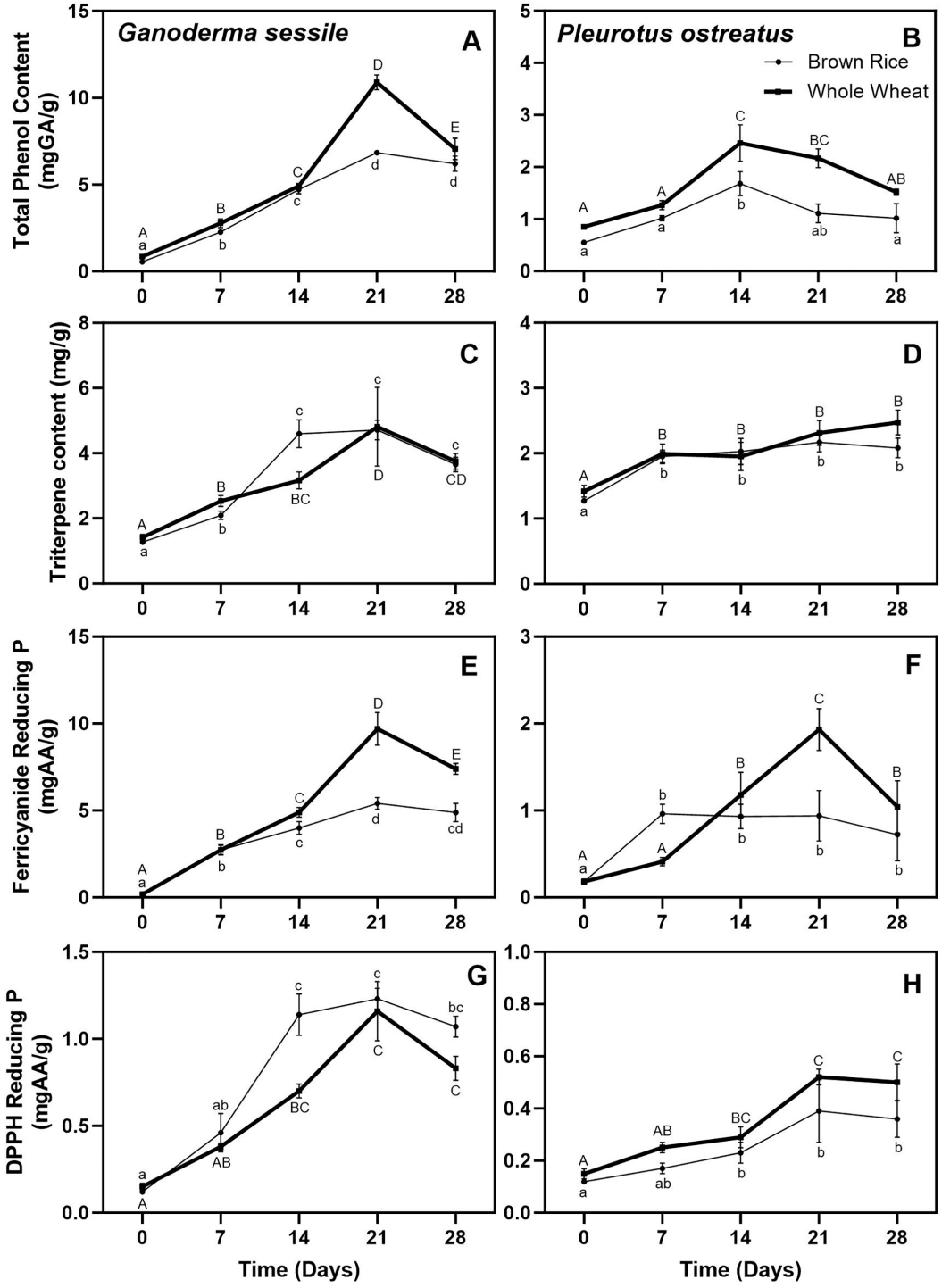

FIG. 1: Progression in time of: total polyphenol content (A and B), triterpene content (C and D), reducing power (E and F), and DPPH radical scavenging ability (G and H) of BR (thin line, circular points) and WW (thick line, quadrangular points) fermented with *Ganoderma australe* (left) and *P. ostreatus* (right). Different letters indicate significant differences ($P < 0.05$) between incubation times of WW (uppercase letters), and BR (lowercase letters). Time 0 corresponds to the controls (non-fermented substrates).

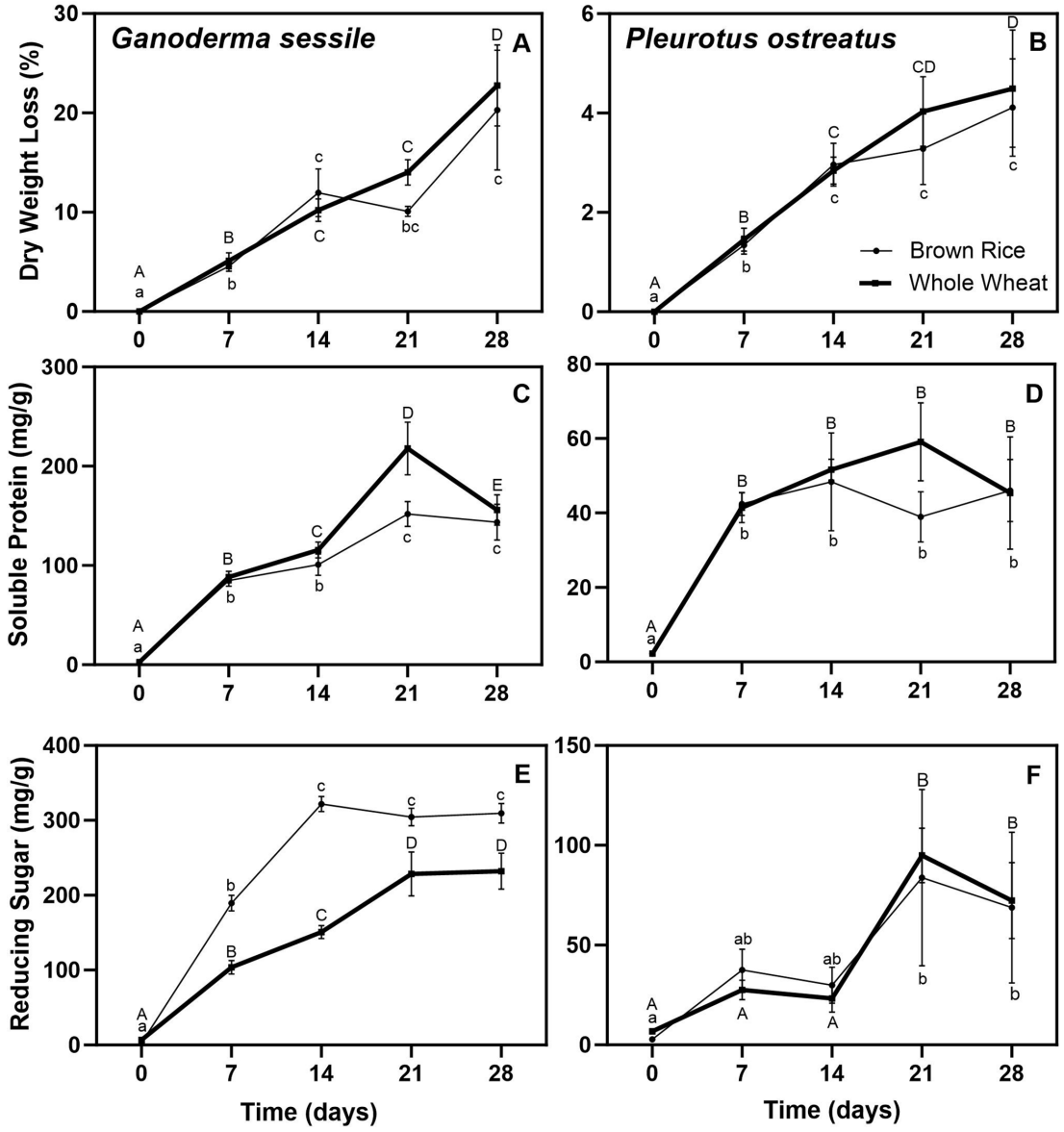

FIG. 2: Progression in time of dry weight loss (A and B), soluble protein content (C and D), and reducing sugar content (E and F) of brown rice (thin line, circular points) and whole wheat (thick line, quadrangular points) fermented with *G. sessile* (left) and *P. ostreatus* (right). Different letters indicate significant differences ($P < 0.05$) between incubation times of whole wheat (uppercase letters) and brown rice (lowercase letters). Time 0 corresponds to the controls (non-fermented substrates).

Nutritional and functional profiles correlated positively with weight loss of the substrate. Still, correlations were non-linear in most cases, as shown by the high and significant Spearman's coefficient (r_s) values of all variables and the low and non-significant Pearson's coefficient (r) values of most of them (Table 4 and the supplementary materials). These results are consistent with the pattern of progression over time observed for these variables, most of which peaked between days 14 and 21 and then decreased or slowed down the increment.

TABLE 1: Comparative paired *t*-test analysis of BR fermented by *G. sessile* (GS) versus that fermented by *P. ostreatus* (PO)

Variable	Median value of GS-BR	Median value of PO-BR	T value/(significance) GS–BR vs. PO-BR
Weight loss	10.6	2.64	4.87 (0.0001)
Polyphenol content	4.41	1.26	8.21 (< 0.0001)
Triterpene content	3.65	2.03	5.19 (0.0001)
Reducing power	3.89	0.91	10.52 (< 0.0001)
DPPH radical scavenging	0.90	0.26	7.13 (< 0.0001)
Soluble proteins	103.17	44.42	7.25 (< 0.0001)
Reducing sugars content	268.89	48.46	13.25 (< 0.0001)

N = 23 for all variables. GS–BR vs. PO-BR refers to the statistical comparison between the effect of the two strains on BR.

TABLE 2: Comparative paired *t*-test analysis of WW substrates fermented by *G. sessile* versus that fermented by *P. ostreatus*

Variable	Median value of GS-WW	Median value of PO-WW	T value/(significance) GS-WW vs. PO-WW
Weight loss	11.72	2.8	5.79 (0.0001)
Polyphenol content	5.22	1.84	6.38 (< 0.0001)
Triterpene content	3.31	2.09	4.56 (0.0002)
Reducing power	5.17	0.98	8.98 (< 0.0001)
DPPH radical scavenging	0.67	0.35	6.27 (< 0.0001)
Soluble proteins	124.44	47.97	7.63 (< 0.0001)
Reducing sugars content	165.04	43.43	10.86 (< 0.0001)

N = 23 for all variables. GS–WW vs. PO-WW refers to the statistical comparison between the effect of the two strains on WW.

TABLE 3: Comparative paired *t*-test analysis of the effect of each strain on the two different substrates (BR and WW) (*N* = 23). The mean value of each variable is presented in Tables 1 and 2

Variable	T value (significance) of GS-BR vs. GS-WW	T value/(significance) of PO-BR vs. PO-WW
Weight loss	–0.64 (0.527)	–0.50 (0.626)
Polyphenol content	–2.48 **(0.021)**	–3.66 **(0.001)**
Triterpene content	1.08 (0.292)	–1.64 (0.118)
Reducing power	–3.22 **(0.004)**	–0.50 (0.622)
DPPH radical scavenging	3.34 **(0.003)**	–2.20 **(0.040)**
Soluble proteins	–1.67 (0.108)	–0.31 (0.756)
Reducing sugars content	8.28 **(< 0.0001)**	0.42 (0.677)

GS–BR vs. GS-WW refers to the statistical comparison between the effect of *G. sessile* on the two substrates (BR and WW). PO-BR vs. PO-WW refers to the statistical comparison between the effect of *P. ostreatus* on the two substrates. Significant differences are shown in bold type.

The polyphenol content of both substrates (BR and WW) fermented by *G. sessile* showed a strong linear correlation with the antioxidant activity (reducing power). Similarly, the total polyphenol content of BR and WW fermented with *P. ostreatus* showed a strong linear correlation with the reducing power (Table 5).

TABLE 4: Analysis of the correlation among polyphenols, triterpene, soluble protein, and reducing sugar contents of fermented substrates and dry weight loss

Nutritional/functional	Linear correlation with weight loss Pearson *r* value (significance)	Spearman's rank correlation with weight loss Spearman *r* value, r_s (significance)
GS-BR polyphenol content	$r = 0.6$ ($P = 0.002$)	$r_s = 0.84$ ($P < 0.0001$)
GS-WW polyphenol content	$r = 0.46$ ($P = 0.02$)	$r_s = 0.87$ ($P < 0.0001$)
PO-BR polyphenol content	$r = 0.28$ (n.s.)	$r_s = 0.61$ ($P = 0.0004$)
PO-WW polyphenol content	$r = 0.30$ (n.s.)	$r_s = 0.75$ ($P < 0.0001$)
GS-BR triterpene content	$r = 0.30$ (n.s.)	$r_s = 0.77$ ($P < 0.0001$)
GS-WW triterpene content	$r = 0.45$ ($P = 0.03$)	$r_s = 0.76$ ($P < 0.0001$)
PO-BR triterpene content	$r = –0.16$ (n.s.)	$r_s = 0.49$ (0.011)
PO-WW triterpene content	$r = 0.31$ (n.s.)	$r_s = 0.43$ (0.02)
GS-BR soluble protein content	$r = 0.4$ (n.s.)	$r_s = 0.74$ ($P < 0.0001$)
GS-WW soluble protein content	$r = 0.47$ ($P = 0.019$)	$r_s = 0.83$ ($P < 0.0001$)
PO-BR soluble protein content	$r = 0.05$ (n.s.)	$r_s = 0.5$ (0.008)
PO-WW soluble protein content	$r = 0.55$ ($P = 0.006$)	$r_s = 0.77$ ($P < 0.0001$)
GS-BR reducing sugar content	$r = 0.48$ ($P = 0.002$)	$r_s = 0.75$ ($P < 0.0001$)
GS-WW reducing sugar content	$r = 0.70$ ($P = 0.0002$)	$r_s = 0.83$ ($P < 0.0001$)
PO-BR reducing sugar content	$r = 0.12$ (n.s.)	$r_s = 0.67$ (0.0003)
PO-WW reducing sugar content	$r = 0.63$ ($P = 0.001$)	$r_s = 0.69$ ($P < 0.0001$)

n.s., > 0.05.

TABLE 5: Analysis of the correlation between polyphenol contents and antioxidant activity (reducing power) of fermented substrates

Antioxidant content	Linear correlation with reducing power Pearson *r* value (significance)
GS-BR polyphenol content	$r = 0.70$ ($P = 0.0002$)
GS-WW polyphenol content	$r = 0.94$ ($P < 0.0001$)
PO-BR polyphenol content	$r = 0.70$ ($P = 0.0002$)
PO-WW polyphenol content	$r = 0.77$ ($P < 0.0001$)

IV. DISCUSSION

The results presented here show that SSF of cereals using strains of edible and nutraceutical species can significantly improve the antioxidant power of the substrates. This enrichment seems to be highly correlated with the increase in the soluble phenolic compounds measured in the extracts. Lignin (or insoluble polyphenol) degradation into smaller phenolic units (e.g., phloroglucinol, hydroquinone, and catechol) using laccases has been shown to result in improved antioxidant activities,[29] but hydrolytic enzymes such as α-amylase and β-glucosidase are also involved in the phenolic mobilization during solid-state growth.[30,31] Interestingly, this increase is not constant over time and shows a maximum around the third week and decreases after this point, even though dry-mass loss and, consequently, fungal mass growth remain linear.

The biological reasons for this dynamic are difficult to unveil with the results presented here. Growth and primary metabolism usually slow down due to the exhaustion of limiting nutrients[32,33] or to a decrease in oxygen concentration since an adequate oxygen supply stimulates enzyme activity.[34] Also, a low oxygen concentration can induce anaerobic respiration and the consequent accumulation of metabolites such as ethanol, acetaldehyde, and lactate, which have an inhibitory effect on mushroom mycelium growth.[35] Another reason might be the accumulation of relatively toxic metabolites such as phenols,[36] the concentration of which might have been increased by water loss during fermentation, or high concentration of carbon dioxide, which inhibits enzyme production.[37] Also, the decrease of free soluble-phenolic content could be due to the polymerization of the phenolics during the later course of growth in response to the stress induced on the fungus due to nutrient depletion, which activates the lignifying and tannin-forming peroxidase enzymes.[38]

In both strains, the water-soluble phenol content and the antioxidant power of fermented wheat were higher than those of rice, which may be due to the more complex structure of the grain covering of the former since a substrate richer in fiber provides a better source of phenolic compounds.[39] This coincides with the differences observed in the evolution of reducing sugar contents over time of substrates fermented with *G. sessile*: the substantial increase in fermented rice until day 7, remaining constant after that, compared with the progressive increment in fermented wheat suggests a slower progress in the degradation of the latter. Although the design of the assays performed does not allow us to explain this pattern, a possible cause might be the higher fiber content of WW[40] or the higher concentration of tannins[41] that might account for a more difficult degradation by filamentous fungi. These results suggest that a slightly longer fermentation might be needed in the case of wheat to fully exploit the potential of the fermentation.

Regarding the strain, the substrates fermented with *G. sessile* showed higher values of all measured compounds, which, besides being affected by the strain's particular properties, could result from a higher growth rate. A higher growth rate implies faster exhaustion of easily degradable nutrient sources and the consequent activation of secondary metabolism, which is known to involve the production of a great span of enzymes and diverse chemical compounds.[42] No conclusions at the species level can be made since the growth rate is highly strain dependent.

Total phenolic compound content correlates with antioxidant power in both strains, reinforcing the hypothesis that phenolic compounds are responsible for the antioxidant power. A higher content of phenol-derivatives is expected from *G. sessile* species than from *P. ostreatus*, judging from previous studies (e.g., Koutrotsios et al.[36]), in agreement with our results. Also, highly differentiated pigmented hyphal systems in substrates colonized by *Ganoderma* sp.[43] seem to reflect the incrustation with pigments such as terpenoids and phenol derivatives, which might contribute to the high concentrations shown by this species.

The dynamics observed in our experiments suggest that increments in fermentation times are not necessarily reflected in higher concentrations of the desired compounds and that the optimal times need to be determined for each combination of substrate, strain (or species), and incubation conditions. The lack of linear correlation of parameters such as growth and phenolic compound content is consistent with the fact that these substances are not constant constituents of fungal biomass, but intermediary metabolites involved in the exploitation of the substrates, differentiation of complex or pigmented hyphal systems, and stress response. Also, the production of enzymes responsible for the synthesis of these compounds is expected to increase more or less abruptly during the transition to secondary metabolism,[44] and not gradually following the mycelium proliferation.

This is a descriptive work aiming to determine the variation pattern of some components and metabolites throughout the SSF process. Future studies will test plausible explanations of the observed pattern hypothesized in this discussion. Our results support previous findings showing that white-rot fungi complex

oxidative metabolism can be used as a source of solutions for the food industry and many others, as reviewed by Kijpornyongpan et al.[45]

The main goal of this study consists in exploring possible products resulting from solid state fermentation. However, the complex behavior of the measured variables suggest that complex enzymatic dynamics might underly the release of phenolic compounds and other antioxidant molecules. The future assessment of the enzyme activities leading to the transformation of lignin, fiber and other structural constituents of the substrates would help understanding these interesting phenomena.

V. CONCLUSIONS

Our results show that the nutritional and functional profiles of fermented substrates vary according to the strain, the time of fermentation, and the substrate composition. The non-linear correlation between the fungal growth and the focal parameters indicates that an optimal incubation time can be derived from an adjusted function for each species. These functions can be constructed from repeated experiments of the kind presented here. Our results indicate that the optimal for *G. sessile* is around 14 d in BR and 21 d in WW. In the case of *P. ostreatus*, it could be around 14–21 d, but repetition of the experiment with longer incubation time is needed. Results agree with our hypothesis that these fermented flours' nutritional and functional properties could be modulated by manipulating substrate composition, incubation time, and mushroom species. The latter reinforces the importance of further prospection efforts in search of species and strains whose growth and metabolite production parameters meet the growing needs in a sustainable food production framework.

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