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Upgrading the Nutritional Value of Rice Bran by Solid-State Fermentation with *Pleurotus sapidus*

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Abstract: Solid-state fermentation (SSF) of rice bran (RB) employing the edible fungus *Pleurotus sapidus* was investigated as a process strategy to improve the nutritional quality of this low-cost and abundant substrate. During fermentation, samples were withdrawn at different time intervals (4, 6, and 10 days) and further analyzed. Established methods were deployed to monitor the changes in nutritional composition (carbohydrates, proteins, ash, and lipids). Additionally, changes in fatty acid composition was studied as a function of culture progress. Results showed that the SSF of rice bran increased total carbohydrates from 36.6% to 50.2%, total proteins from 7.4% to 12.8%, and ash from 7.6% to 11.5%. However, the total lipid content was reduced from 48.5% to 27.8%. The fatty acid (FA) composition of RB included mainly oleic, linoleic, and palmitic acids. Upon fermentation with *P. sapidus*, small differences were found: linoleic acid and oleic acid content were increased by 0.4% and 1.1%, respectively, while palmitic acid content was reduced by 0.8%. This study demonstrated an improvement in the nutritional quality of RB after fermentation with *P. sapidus*, since protein, carbohydrates, minerals, and specific FA components were increased. As a whole, our results indicate that fermented rice bran could be used as a high-quality animal feed supplement.

Keywords: rice bran; solid-state fermentation; *Pleurotus sapidus*; fatty acids; nutritional composition; rice biorefinery; bioeconomy.

1. Introduction

Fermentation is one of the oldest processes used for biomass transformation into value-added products using microorganisms. Solid-state fermentation (SSF) processes have the benefits of dealing with substantial volumes of biomass, are simple to scale up, have a low environmental impact, and do not have a high water consumption [1,2]. Different types of agri-food industry side-streams have been used for SSF to produce enzymes, bioactive compounds, high-quality animal feed supplements, and flavors, among others. Besides, an increasing number of organisms have been used for SSF, like bacteria, yeast, ascomycetes, and basidiomycetes [2–4].

Rice represents one of the major crops produced in agricultural areas of Argentina (3.9 MT/year, according to FAO.org). Following grain processing, 5–8% remain as rice bran, which represents around 10% of the total weight of rough rice. Rice bran is an attractive raw material since it contains

minerals (Fe, P, and Mg), proteins, and fibers and it is an excellent source of lipids. Moreover, rice bran is a rich source of antioxidant compounds (e.g., polyphenols, vitamin E, tocotrienols) that prevent the oxidative damage of DNA and other body tissues [5,6]. The oil represents 90–96% of the lipid component of the rice bran, such as mono-, di- and triacylglycerols, free fatty acids, and waxes. Within the fatty acid (FA) fraction, palmitic acid (21–26%), linoleic acid (31–33%), and oleic acid (37–42%) are the predominant compounds. Due to the high content of mono- and polyunsaturated fatty acids (PUFAs), rice bran oil is considered a healthy food [7]. However, rice bran has a very short shelf life due to the high lipid percentage, and the lipase enzymes that degrade the oil make the bran rancid and transform it into a food that is not suitable for consumption [5]. Such as other low-cost agroindustry side-streams, it can be employed as a carbon source for microorganisms and fungus in SSF, allowing the production of natural bioactive compounds, enzymes, and other products such as feed additives for animals.

Several researchers have explored the effects of rice bran fermentation on its functional properties. After fermentation, an increase in nutrient availability, biosurfactant content, and mono- and polyunsaturated fatty acid content was observed. The same process also allowed the release and transformation of phenolic (e.g., ferulic acid) and volatile compounds—a fact that further enhances the potential benefits of the fermented substrates [2–4,6,8,9].

Pleurotus species are edible fungi that belong to the basidiomycota division; they can be grown in a wide variety of plant-based substrates [2,3,10]. This work focuses on the fermentation of rice bran by *P. sapidus*, which is a well-known edible fungus that is easy to cultivate in SSF, provides high nutritional quality food, and produces fascinating enzymes and metabolites [3,10–12].

More specifically, the present study aims to explore the capability of *P. sapidus* to upgrade the nutritional quality of the rice bran through SSF. In doing so, the changes in the nutritional composition and fatty acid profiles of fermented substrates were evaluated at different fermentation times. The fermented product may find applications as animal feed.

2. Materials and Methods

2.1. Rice Bran

Whole rice bran was supplied by the Rice Cooperative Villa Elisa Ltda., located in Villa Elisa, Entre Ríos, Argentina, and was kept at −20 °C until use. Rice bran preparation, as the substrate for the SSF processing, was standardized according to granulometry (0.35 mm to 0.70 mm particle size).

2.2. Microorganism and Inoculum Preparation

Fungus strain *Pleurotus sapidus* Dk3174 obtained by the mating of two compatible monokaryons, Mk31 and Mk74, was employed as a fermentative agent [12]. The fungus was maintained on Petri dishes containing potato/dextrose/agar medium (PDA), incubated at 25 °C for seven days, and then stored at 4 °C. The liquid culture was prepared using the same medium without agar (PD), and the incubation was performed on a shaker at 150 rpm for six days at 25 °C.

2.3. Culture Conditions for Solid-State Fermentation

P. sapidus was cultivated by SSF using rice bran (RB) as substrate and contained in 250 mL flasks as previously described [3,12]. The flasks were prepared by mixing RB (10 g) with water (12 mL). Flasks were sterilized at 121 °C for 20 min, cooled, and inoculated with 5 mL six-day-old pre-cultures previously grown on potato dextrose liquid medium; the inoculum was previously homogenized using an ultraturrax (Janke & Kunkel, Staufen, Germany). Flasks were incubated at 25 °C in the dark and the samples were taken on days 4, 6, and 10. A substrate control (RB) without fungus inoculation was prepared.

With the aim to have reference information related to the nutritional composition and fatty acid composition of *P. sapidus* DK3174 biomass (the strain used in this study) and to avoid the long incubation time to obtain the fruiting bodies, the fungus control sample was obtained by cultivating the mycelia in liquid media (PD), instead of rice bran. Cultivation was performed on a shaker at 150 rpm at

25 °C for six days, followed by centrifugation. The fungus biomass/pellets were then lyophilized and kept at −20 °C until used.

The fermented and unfermented rice bran samples were dried in an oven at 50 °C until constant weight and kept at −20 °C before use [13]. All samples were analyzed in triplicate.

2.4. Proximate Composition Analysis

The proximate composition of the control (fungus biomass and RB) and fermented samples was carried out in triplicate, based on official AOAC protocols [14]. The following fractions were obtained (Omarini et al. [13]): (i) moisture content (drying in an oven at 100 °C), (ii) ash content (by incineration at 550 °C), (iii) lipid content (determined in a Soxhlet apparatus for 8 h using hexane as solvent), and (iv) protein content (by the Kjeldahl method and the total protein was determined from the total nitrogen content using the correction factor 6.25 [10]). The non-nitrogen extractive fraction (% ENN), which included soluble carbohydrates, was calculated as dry matter not accounted in the sum of moisture, ash, lipid, and protein. Results were expressed as a percentage (%) on a dry matter basis. The energy value was calculated with total carbohydrates (including fiber) and expressed as Kcal/100 g [15].

2.5. Extraction and Esterification of Fatty Acids

Dried samples (*P. sapidus*, RB, and fermented RB) were used for the extraction and esterification of fatty acids following an established protocol with minor modifications [16]. First, oils were extracted separately with n-hexane in a Soxhlet apparatus for 8 h. The lipids were dried over anhydrous sodium, and the solvent was removed by vacuum distillation at 40 °C.

For fatty acid profile determination, the oil samples (1 g) were subjected to alkaline saponification with 2 M sodium hydroxide in methanol (10 mL), the tubes were sealed with Teflon tape and a screw cap, and they were placed in a microwave (2450 MHz and 750 W) for 20 s, twice. The unsaponifiable matter was extracted with n-hexane (5 mL), tubes were vortexed, and the upper phase was discarded (this step was repeated two times). The fatty acids (FAs) were converted to methyl esters (FAME) using 2 M H_2SO_4 in methanol (20 mL), and tubes were placed back into the microwave for 20 s, twice. Then, samples were cooled and n-hexane (10 mL) was added, vortexed, and the upper phase was collected (this step was repeated two times). The combined hexane extracts were evaporated to dryness and then brought to the appropriate volume with hexane before analysis.

2.5.1. Determination of the Fatty Acid Composition by Gas Chromatography

The FA methyl esters were analyzed by gas chromatography (GC) (Perkin-Elmer, Shelton, CT, USA) according to Maestri et al. [16]. Separations were made using a fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) CP Wax 52 CB (Varian, Walnut Creek, CA, USA); carrier gas N_2 at 1 mL/min; split ratio 100:1; column temperature programmed from 180 °C (5 min) to 220 °C, at 2 °C/min; injection and detector temperatures at 250 °C, FID. The identification of FAME was carried out by comparison of their retention times with those of reference compounds (Sigma-Aldrich, St. Lois, MO, USA). The concentration of each fatty acid was determined as a relative percentage of the total composition using the heptadecanoic acid methyl ester as the internal standard (Sigma-Aldrich, St. Lois, MO, USA).

2.6. Statistical Analysis

The data generated from this study were subjected to one-way analysis of variance (ANOVA) at 5% level of significance. Means were compared by the Tukey test. All determinations were carried out in triplicate.

3. Results and Discussion

3.1. Effects of the SSF on the Nutritional Composition of Rice Bran

The nutritional composition of the *P. sapidus* mycelium (as fungus reference information), the rice bran (RB), and the fermented rice bran (FRB) at different fermentation times are shown in Table 1.

Table 1. Nutritional composition of *P. sapidus*, rice bran (unfermented), and fermented rice bran at different cultivation times (4, 6, and 10 days).

Components *	Control Samples		Fermented Rice Bran Samples		
	P. sapidus	Rice bran	Day 4	Day 6	Day 10
moisture (%)	95.1 ± 1.3 [a]	20.7 ± 0.8 [b]	28.7 ± 6.2 [c]	35.2 ± 2.1 [c,d]	37.9 ± 8.1 [d]
Protein (%)	26.5 ± 0.1 [a]	7.4 ± 0.6 [b]	10.8 ± 1.3 [c]	9.7 ± 0.8 [c]	12.8 ± 0.6 [d]
Ash (%)	6.5 ± 0.05 [a]	7.6 ± 0.5 [b]	11.2 ± 1.4 [c]	10.3 ± 1.1 [c]	11.5 ± 0.05 [c]
Total carbohydrates	60.5 ± 0.6 [a]	36.6 ± 0.6 [b]	50.2 ± 2.1 [c]	43.2 ± 1.8 [d]	45.0 ± 0.8 [d]
Lipids (%)	6.6 ± 0.7 [a]	48.5 ± 3.9 [b]	27.8 ± 6.5 [c]	36.9 ± 0.2 [d]	30.8 ± 11.8 [c,d]
Energetic value	407.4	612.1	494.2	543.3	508.4
% RDD **	20.4	30.6	24.7	27.2	25.4

* Components were expressed on a dry basis; ** % RDD: percentage of the recommended daily dose based on a 2000 Kcal diet ; the results are presented as mean ± standard deviation (n = 3); a–d: in each line, a different letters indicates that the mean value of at least one sample is significantly different, $p < 0.05$ (significant differences are represented by different letters).

The fermentation of the rice bran performed by *P. sapidus* improved the nutritional composition of the substrate, enhancing the carbohydrate (from 18% to 37%), protein (from 32% to 74%), and ash content (from 35% to 51%), whereas the lipid percentage was reduced (from 24% to 43%) (Table 1). Similar performance was reported by several authors using rice bran as substrate but with different fungal species. They pointed out that during the solid-state fermentation, the fungus executes a repertory of extracellular enzymes allowing the fungus to obtain nutrients while simultaneously producing changes in the chemical composition of the substrate, in addition to the production of other metabolites [14,17,18].

Significant differences were found among the components between the unfermented and fermented samples (Table 1). The major component present in FRB was the total carbohydrates, followed by the lipids, whereas the protein and ash content were the minor components (Table 1). In contrast, the lipids were the main compound found in the unfermented RB followed by the total carbohydrates, whereas the protein and the ash contents were the minor constituents. The nutritional composition of the RB was in agreement with values already reported by other authors [5,8]. However, slight differences were noticed regarding the content of each component. This is due to the fact that rice bran is an industrial by-product and, therefore, its chemical composition depends on several factors associated with the variety, the agronomic aspects, or the industrial process and preservation conditions of the material.

In general, a significant change was observed in the nutritional composition of the rice bran at the beginning (4 days) and at the end of the SSF process (10 days). However, at day 6 of fermentation, the components showed a small decrease that can be associated with variations in the fungus metabolism during the substrate colonization. At day six of fermentation, the fungus was able to fully colonize the substrate (10 g) and this period is the process time of nutrient accumulation by mycelium metabolism (physiological maturation). At this moment the fungus could undergo metabolic and physiological modifications related to the fruiting body initiation process [19]. Another possible explanation is due to changes in the lignocellulolytic enzyme (e.g., laccase, peroxidase, xylanases, cellulases) activities during the time course of the fermentation, which are involved in the substrate degradation, bioconversion or regulation of the fruiting body initiation process [19,20].

On the other hand, the main components found in *P. sapidus* biomass were total carbohydrates and total protein, whereas lipids and ashes represented minor components. It is important to mention that the fungus nutritional composition used in this study was obtained from mycelia cultivated in liquid media and not from the fruiting bodies harvested from FRB. In spite of this, the carbohydrate content in the fungus biomass was 17% to 29% higher than the percentages achieved by FRB, and the protein content was 52% to 63% higher than in FRB. The ash and lipid values presented in the fungus

samples were 42% to 53% and 76% to 90%, respectively, and were inferior compared to FRB. As was mentioned before, the differences between the fungus and FRB could be associated with the culture conditions or the media composition used to cultivate the fungus. However, the nutritional benefits and energetic contributions of *P. sapidus* were similar to those found in the literature [13,21,22].

The results obtained in the present study demonstrate that the fermentation performed by *P. sapidus* enhanced the nutritional quality of the final product (FRB) and provided a good source of energy. In addition, the battery of extracellular enzymes produced during the fungus growth contributed to the breakdown of the lipids [10,22]. Abu et al. [22] suggested that *P. ostreatus* appeared to be lipolytic rather than lipogenic because the lipid content present in the sweet potatoes was increasingly depleted after ten days of SSF. A similar observation was reported by Oliveira et al. [18] and Kupski et al. [23] during the SSF of rice bran by *Rhizopus oryzae*.

From a nutritional point of view, FRB represents an attractive option to provide energy and nutrients using a sustainable process (SSF). Furthermore, the RB upgrading was achieved in four days, indicating that a short fermentation time was enough since the nutritional composition of RB did not vary substantially with longer fermentation times. This naturally fermented product can be included in the formulation of high-performance animal feed supplements to add value to a by-product of the rice industry, to innovate in the development of products and processes through the use of the edible fungus *P. sapidus*, and to improve regional economies, among other possibilities. Several authors reported the beneficial effects of the fermented rice bran using *Rhizopus oryza*e or *Lentinula edodes*, not only in the enhancement of the nutritional quality of the final product but also its bioactivity [2,4,24]. Oil removal after fermentation could avoid various drawbacks like lipids oxidation, generation of unpleasant flavors, and loss of nutritional value [5,6,25]. Moreover, the product could be stabilized, thus extending product shelf life (e.g., feed supplement). However, to make a commercial product, it will be necessary to study in more detail the nutritional quality during storage, the organoleptic characteristics, and the stability of the final product.

3.2. Effects of the SSF on the Fatty Acid Composition of Rice Bran

In the present study, the fatty acid (FA) composition of *P. sapidus* biomass (as fungus reference information), the rice bran and the fermented RB were analyzed (Table 2).

Table 2. Fatty acid composition of *P. sapidus*, rice bran (unfermented), and fermented rice bran at different cultivation times (4 and 6 days).

		Fatty Acid Profile (%)			
Fatty Acid	**Nº Carbons**	***P. sapidus***	**Rice Bran**	**FRB day 4**	**FRB day 6**
Undecanoic acid	**C11:0**	2.28 ± 0.00 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}
Lauric acid	**C12:0**	5.16 ± 0.28 ^{a}	0.02 ± 0.02 ^{b}	0.05 ± 0.02 ^{b}	0.06 ± 0.03 ^{b}
Myristic acid	**C14:0**	1.28 ± 1.11 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}
Pentadecanoic acid	**C15:0**	2.02 ± 1.67 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}
Palmitic acid	**C16:0**	16.03 ± 0.85 ^{a}	21.27 ± 0.14 ^{b}	22.09 ± 1.96 ^{b,c}	21.08 ± 0.62 ^{b}
Palmitoleic acid	**C16:1**	0.83 ± 0.26 ^{a}	0.22 ± 0.02 ^{b}	0.19 ± 0.01 ^{b}	0.17 ± 0.03 ^{b}
Margaric acid	**C17:0**	0.72 ± 0.00 ^{a}	nd ^{b}	nd	nd
Stearic acid	**C18:0**	8.50 ± 0.38 ^{a}	1.76 ± 0.53 ^{b}	2.04 ± 0.21 ^{b}	1.99 ± 0.10 ^{b}
Oleic acid	**C18:1**	19.62 ± 4.29 ^{a}	39.93 ± 0.35 ^{b}	40.26 ± 1.04 ^{b}	40.36 ± 0.28 ^{b}
Linoleic acid	**C18:2**	39.07 ± 4.52 ^{a}	33.94 ± 0.18 ^{b}	32.79 ± 0.87 ^{c}	34.07 ± 0.82 ^{b}
Linolenic acid	**C18:3**	0.42 ± 0.00 ^{a}	1.50 ± 0.01 ^{b}	1.35 ± 0.03 ^{c}	1.50 ± 0.24 ^{b,c}
Arachidic acid	**C20:0**	nd ^{a}	0.85 ± 0.01 ^{b}	0.83 ± 0.09 ^{b}	nd ^{a}
Eicosenoic acid	**C20.1**	0.67 ± 0.00 ^{a}	0.66 ± 0.18 ^{a,b}	0.69 ± 0.19 ^{a,b}	0.80 ± 0.06 ^{b}
Behenic acid	**C22:0**	1.05 ± 0.47 ^{a}	0.37 ± 0.00 ^{b}	0.05 ± 0.01 ^{c}	nd ^{d}
Lignoceric acid	**C24:0**	2.81 ± 0.95 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}

nd = not detected; FRB = fermented rice bran at day 4 and day 6; the results are presented as mean ± standard deviation (n = 3); a–d: in each line different letters indicates that the mean value of at least one sample is significantly different, $p < 0.05$ (significant differences are represented by different letters).

The FA content of the lipids produced during the SSF of rice bran by the *P. sapidus* is reported for the first time. The rice bran fermentation introduced a positive effect with some differences

observed in the FA composition. The unsaturated fatty acid levels were higher than the saturated ones. The main FAs identified in all samples (*P. sapidus* biomass, RB and FRB) were linoleic, oleic, and palmitic acids and these results are consistent with those mentioned in the literature [6–8,26]. In FRB, the linoleic and oleic FAs showed a small increase compared to the control substrate after six days of SSF, while the arachidic and behenic FAs disappeared after four days of fermentation, suggesting that the fungus could play a role in such (bio) transformations (Table 2).

Nevertheless, the predominant FAs (linoleic, oleic and palmitic) in FRB did not show great differences with unfermented RB. This fact could be associated with the high extraction temperature used during the lipid extraction by Soxhlet, which could favor FA configuration changes, impairing the distinction between the effect of fermentation and extraction method [8]. Despite this, Oliveira et al. [8] reported similar FA profiles for fermented rice bran using *Rhizopus oryzae*. The main compounds identified by the authors were linoleic (32%–36%), oleic (36%–39%), and palmitic (15%–20%) FAs and no significant differences were obtained after 120 h of SSF. However, Silveira et al. [27] observed a substantial increase in linoleic and palmitic content after 72 h of rice and wheat bran fermentation by *R. oryzae*, as well as a significant reduction of stearic and linolenic FAs when compared to control rice bran (unfermented). On the other hand, Massarolo et al. [4] reported that after 84 h of RB fermentation using *R. oryzae*, a decrease in oleic acid and an increase in linoleic acid was observed. The differences in the FA profiles or contents after SSF of rice bran described by several authors depend on the culture conditions (e.g., fermentation media, the addition of supplements, submerged or solid fermentation) or are due to the differences concerning the fungal species, their physiology, and/or the metabolic processes during the SSF, and, as was mentioned before, the lipid extraction conditions [3,8,13,22,28].

On the other hand, significant differences in the FA profiles and contents were identified between *P. sapidus* biomass used as reference fungus information and the fermented rice bran (FRB) or the unfermented RB (control substrate). According to the results shown in Table 2, *P. sapidus* biomass lipids were constituted mainly by 39.1% linoleic (C18:2), 19.6% oleic (C18:1), 16.0% palmitic (C16:0), 8.5% stearic (C18:0), and 5.2% lauric (C12:0) FAs. The results also showed that the fungus biomass attained a higher content in linoleic (up to 19.8%), stearic (up to 79.3%), and lauric (up to 99.6%) FAs compared to the FRB and a lower content in palmitic (up to 11%) and oleic (up to 51.4%) FAs than the FRB (Table 2). Additionally, FAs such as undecanoic acid, myristic acid, pentadecanoic acid, margaric acid, and lignoceric acid were only identified in the *P. sapidus* biomass. However, the differences in the FA composition between the fungus biomass and the fermented RB could be associated with the culture conditions or the media composition used to cultivate the fungus. Despite that, the FAs identified in the *P. sapidus* biomass were similar to those reported by different *Pleurotus* species where the main FAs were linoleic (30%–68%) followed by oleic (9%–40%) and palmitic (12 %–32%) FAs, while stearic acid was present in lower concentrations (<12%) [26,29].

This exploratory study demonstrated that SSF of rice bran, a low-cost industrial by-product, using *P. sapidus* can be applied for the production of an excellent nutritional quality product that can be used as an animal feed supplement. The high content of linoleic acid, an essential fatty acid, present in all samples and, mainly in the fungus biomass, is interesting from a nutritional and health point of view because humans and animals cannot synthesize it. However, further studies should be performed in order to obtain a commercial product, including the optimization of the fermentation conditions, the design of the bioreactor and the study of organoleptic properties, and the stability of the final product.

4. Conclusions

The present study revealed that solid-state fermentation of rice bran by *P. sapidus* is an attractive option for improving the nutritional quality of the substrate, adding value to this low-cost industrial by-product. After four days of fermentation, the final product composition enhanced the carbohydrates, proteins, and ashes and reduced the lipids, whereas the FA values remained almost constant. However, in-depth studies should be carried out to perform a complete bioactive metabolites profile in the fermented rice bran to highlight the added value contributed by *P. sapidus*.

Comprehensive knowledge about the vitamins, amino acids, antioxidants, and the mineral composition of the fermented rice bran using the edible fungus *P. sapidus* will give rise to the possibility of supplying natural products to the animal feed sector and providing them with a high-quality feed supplement.

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