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Artur Fernando Poffo Costa

UTILIZAÇÃO DA CASCA DE ARROZ *IN NATURA* E CARBONIZADA NA COMPOSIÇÃO DE SUBSTRATOS PARA O CULTIVO DE *Pleurotus ostreatus*

Santa Maria, RS
2022

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência do Solo, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de Mestre em Ciência do Solo.

Orientador: Prof. Dr. Rodrigo Josemar Seminoti Jacques
Co-orientadora: Dr. ª Gerusa Pauli Kist Steffen

Santa Maria, RS
2022

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Artur Fernando Poffo Costa

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Aprovado em 25 de fevereiro de 2022.

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Rodrigo Josemar Seminoti Jacques, Prof. Dr. (UFSM)
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Santa Maria, RS
2022

À minha mãe, Cintia Raquel Poffo.

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AULA DE VOO

O conhecimento
caminha lento feito lagarta.
Primeiro não sabe que sabe
e voraz contenta-se com cotidiano orvalho
deixado nas folhas vividas das manhãs.

Depois pensa que sabe
e se fecha em si mesmo:
faz muralhas,
cava trincheiras,
ergue barricadas.

Defendendo o que pensa saber
levanta certeza na forma de muro,
orgulha-se de seu casulo.

Até que maduro
explode em voos
rindo do tempo que imagina saber
ou guardava preso o que sabia.

Voa alto sua ousadia
reconhecendo o suor dos séculos
no orvalho de cada dia.

Mas o voo mais belo
descobre um dia não ser eterno.
É tempo de acasalar:
voltar à terra com seus ovos
à espera de novas e prosaicas lagartas.

O conhecimento é assim:
ri de si mesmo
E de suas certezas.
É meta de forma
metamorfose
movimento
fluir do tempo
que tanto cria como arrasa

a nos mostrar que para o voo
é preciso tanto o casulo
como a asa.

Mauro Iasi

RESUMO

TÍTULO: UTILIZAÇÃO DA CASCA DE ARROZ *IN NATURA* E CARBONIZADA NA COMPOSIÇÃO DE SUBSTRATOS PARA O CULTIVO DE *Pleurotus ostreatus*

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A casca de arroz é um resíduo gerado em grande quantidade em diversos países, porém seu reaproveitamento é limitado devido à baixa qualidade nutricional e ao elevado teor de sílica. O objetivo do estudo foi avaliar a utilização da casca de arroz *in natura* ou carbonizada na substituição parcial da serragem de eucalipto nos substratos para a produção do cogumelo comestível *Pleurotus ostreatus*. Os substratos foram formulados com proporções de 0 a 100% de serragem de eucalipto e casca de arroz *in natura* ou carbonizada. O *P. ostreatus* foi cultivado por 60 dias nestes substratos para avaliação do crescimento do fungo, produção da massa fresca, eficiência biológica, teores de proteína e gordura. A qualidade organoléptica dos cogumelos produzidos foi avaliada através de análise sensorial. A umidade, o pH, os teores de C e N dos substratos foram avaliados antes e após o cultivo. Os substratos com 25 e 50% da casca de arroz *in natura* ou carbonizada em mistura com a serragem disponibilizaram nitrogênio e água de forma mais adequada ao fungo, o que resultou em maior produção de massa fresca, teor de proteína dos cogumelos e eficiência biológica (75 a 83%) em relação ao substrato de referência (100% serragem de eucalipto), sem haver alteração da qualidade sensorial. Devido aos problemas ambientais resultantes da carbonização, recomenda-se o uso da casca de arroz *in natura* em mistura de até 50% com a serragem de eucalipto para a produção do *P. ostreatus.*

Palavras-chave: Resíduo. Cogumelo. Pleurotaceae. Eficiência Biológica. Análise Sensorial.

ABSTRACT

TITLE: THE USE OF RICE HUSK IN THE SUBSTRATE COMPOSITION INCREASES *Pleurotus ostreatus* MUSHROOM PRODUCTION AND QUALITY

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Rice husk is a waste generated in large quantities in various countries, although its reuse is limited due to low nutritional quality and high silica content. Therefore, this study sought to evaluate using raw or carbonized rice husks to partially substitute eucalyptus sawdust in substrates to produce the edible mushroom *Pleurotus ostreatus*. The substrates were formulated with proportions of 0 to 100% of eucalyptus sawdust and raw or carbonized rice husks. *P. ostreatus* was grown for 90 days on the substrates to evaluate mycelial growth, fresh mass production, biological efficiency, and protein and fat content. The organoleptic quality of the mushrooms produced was evaluated by sensory analysis. The substrates' moisture, pH, and C and N contents were evaluated before and after cultivation. The substrates with 25 and 50% of raw or carbonized rice husk in a mixture with sawdust provided nitrogen and water more adequately to the fungus, improving the fresh mass production, the protein content of mushrooms, and biological efficiency (75–83%) compared to the reference substrate (100% eucalyptus sawdust), without altering the sensory quality. Given the environmental issues caused by carbonization, using raw rice husk in a mixture of up to 50% eucalyptus sawdust is recommended for *P. ostreatus* production.

Keywords: By-product. Bioconversion. Edible mushroom. Biological efficiency. Sensory quality.

SUMÁRIO

1 INTRODUÇÃO GERAL

1.1 COGUMELOS COMESTÍVEIS

A relação humana com os cogumelos é tão antiga quanto da agricultura e, quem sabe por isso, igualmente fascinante. Ao longo da história, os cogumelos foram utilizados com as mais distintas finalidades, por diferentes civilizações e em várias regiões do planeta (CHANG e MILES, 1989). Atualmente, um grande número de espécies de cogumelos, silvestres e cultivadas, são consideradas ingredientes da gastronomia em todo o mundo, especialmente devido ao fato de possuir naturalmente o sabor umami (CHANG e MILES, 2004). Em revisão realizada por Li et al (2021), os autores constataram que existem 2.189 espécies de cogumelos comestíveis relatadas na literatura, no entanto, apenas cerca de 35 espécies são cultivadas comercialmente (AIDA et al., 2009).

Os cogumelos possuem grande valor nutricional por serem alimentos ricos em proteínas, pobres em gorduras e energia, com importante teor de aminoácidos essenciais, minerais e fibras. Além disso, os cogumelos comestíveis fornecem um conteúdo significativo de vitaminas (B1, B2, B12, C, D e E) (HELENO et al., 2010) e são reconhecidos pelas suas propriedades medicinais, entre elas, por apresentarem compostos bioativos, tais como polissacarídeos com atividades antivirais, antifúngicas, antitumorais e antidiabéticas (ABREU et al., 2015). Por essa razão, Valverde et al. (2015) se referem aos cogumelos como um dos suplementos dietéticos mais importantes devido aos seus vários benefícios para a nutrição e saúde humana.

Os cogumelos pertencem ao Reino dos Fungos, são seres heterotróficos que dependem de outros organismos para a sua alimentação, absorvendo nutrientes do substrato orgânico vivo ou morto que habitam (OEI e VAN NIEUWENHUIJZEN, 2006). A nutrição ocorre pela liberação de inúmeras enzimas sobre o substrato, seguido da absorção de substâncias parcialmente degradadas; ou parasitando tecidos vivos de outros organismos; ou integrando relações simbióticas, onde o organismo associado fornece os recursos nutritivos necessários para a vida do fungo e em troca recebe outros benefícios (TIMM, 2018). A produção de uma grande diversidade de enzimas, permite aos fungos, especialmente do grupo dos Basidiomycota, a possibilidade de degradar componentes poliméricos da matéria vegetal, como celulose, hemicelulose e lignina (MANDEEL et al., 2005). Este é um serviço ambiental de grande importância, pois está ligado à ciclagem dos nutrientes, processo que sustenta os ecossistemas naturais (VIRIATO et al., 2021).

Por essa razão, os cogumelos possuem a capacidade de degradar inúmeros substratos lignocelulósicos (SÁNCHEZ, 2009). Estes fungos podem ter um papel significativo no gerenciamento de resíduos agrícolas, que representam matérias-primas potencialmente sustentáveis para a produção de cogumelos comestíveis (CHANG e MILES, 2004; MANDEEL et al., 2005; KURT e BUYUKALACA, 2010). A fungicultura tem se mostrado uma área da micologia com grande potencial de expansão e para aumentar a renda dos produtores, principalmente em pequenas propriedades.

Existem cerca de 200 tipos de resíduos nos quais as espécies de cogumelos podem ser produzidas (POPPE, 2000). São, em sua maioria, resíduos agroindustriais gerados em grandes quantidades, causando problemas de disposição, por conseguinte, podendo resultar em poluição ambiental (GARG e GUPTA, 2009). Nesse sentido, o cultivo de cogumelos comestíveis em substratos de resíduos agroindustriais pode ser uma alternativa para a reciclagem de resíduos orgânicos lignocelulósicos. De acordo com Beetz e Kustudia (2004), esse é um dos únicos processos que combina a produção de alimentos ricos em proteínas com a redução da poluição ambiental e é considerada a segunda tecnologia microbiana comercial mais importante depois da levedura (PATHAK et al., 2009).

1.2 O FUNGO *Pleurotus ostreatus*

O cogumelo *Pleurotus ostreatus* (Jacq.) Kumm. (1871), popularmente conhecido como cogumelo ostra, shimeji ou hiratake, é um dos cogumelos comestíveis mais cultivados em todo o mundo, especialmente na Ásia, América e Europa (HOA et al., 2015; ROYSE et al., 2017). De acordo com Fernandes et al. (2015), este gênero está entre os mais apreciados no mundo, tanto que alcançaram a terceira posição na produção de cogumelos comestíveis, atrás das espécies dos gêneros *Agaricus* e *Lentinula*. O gênero *Pleurotus* inclui aproximadamente 40 espécies, que têm sido usadas pelos humanos há muitos anos, em todo o mundo (VALVERDE et al., 2015). As espécies são facilmente encontradas em seus habitats naturais: as florestas tropicais e subtropicais, e podem ser cultivadas artificialmente (BONATTI, 2004).

Assim como outros cogumelos comestíveis, o *P. ostreatus* é um alimento rico em proteínas, minerais (P, Ca, Fe, K e Na) e vitaminas (tiamina, ácido fólico riboflavina e niacina), além do baixo teor de gorduras (SZABOVÁ et al., 2013). Além de ser um alimento excelente do ponto de vista nutricional, é uma espécie utilizada como cogumelo medicinal há muito tempo, pois produz moléculas bioativas importantes, tais como compostos fenólicos, terpenos, esteroides e polissacarídeos com ação antifúngica e antioxidante, por exemplo (YANG et al., 2013; ZHANG et al., 2016). Jedinak e Silva (2008) relatam ainda que o *P. ostratus* possui potenciais efeitos terapêuticos/preventivos no câncer de mama e cólon.

Em comparação com outros cogumelos comestíveis, o *P. ostreatus* requer menor tempo de crescimento. Além disso, este cogumelo exige poucos controles ambientais para o cultivo e seus corpos frutíferos não são frequentemente atacados por doenças e pragas, podendo ser cultivado de forma simples e com baixo custo. Outra vantagem do cultivo desta espécie é que uma alta porcentagem do substrato é convertida em corpos de frutificação, aumentando a lucratividade em comparação com outros cogumelos, tornando o *P. ostreatus* uma excelente escolha para o cultivo (SÁNCHEZ, 2010; TESFAW et al., 2015; BAYSAL et al., 2003). Por essa razão, e também devido às características nutricionais e funcionais, o *P. ostreatus* é considerado cada vez mais popular do ponto de vista comercial (FERNANDES et al., 2015).

As pesquisas sobre cogumelos comestíveis têm se voltado para o desenvolvimento de tecnologias capazes de reduzir os custos de produção, permitindo preços mais baixos para o consumidor e assim estimulando o consumo de cogumelos (CARDOSO et al., 2013). Por isto, muitos resíduos orgânicos têm sido estudados para a produção de *P. ostreatus*, como uma alternativa sustentável para redução de custos e reciclagem dos resíduos agrícolas (RIZKI e TAMAI, 2011; ASHRAFI et al., 2014; VIEIRA e ANDRADE, 2016; VIEIRA et al., 2019; ECONOMOU et al., 2020; VIRIATO et al., 2021). Normalmente, o cultivo do *P. ostreatus* engloba o uso de serragem de *Eucalyptus* sp. ou palha de gramíneas como substrato (BADU et al., 2011; HOA et al., 2015). Mas existes muitas outras possibilidades, devido à elevada quantidade e diversidade de resíduos agroindustriais produzidos globalmente. Mais pesquisas devem ser realizadas buscando avaliar outros resíduos, especialmente considerando aqueles produzidos localmente, que apresentam alta disponibilidade e baixo custo de aquisição.

1.3 CASCA DE ARROZ

De acordo com os dados mais recentes da FAOSTAT (2020), no ano de 2018 o Brasil foi o nono maior produtor de arroz do mundo, tendo colhido na safra de 2017-2018 cerca de 11,7 milhões de toneladas de arroz em casca. O estado do Rio Grande do Sul (RS) é o maior produtor nacional, concentrando 71% desta produção (IBGE, 2018). Dentre os subprodutos gerados no beneficiamento do arroz, a casca é o mais expressivo, de modo que seu volume representa cerca de 20% da massa do arroz em casca (LORENZETT et al., 2012). Com isto, o RS produz aproximadamente 1,6 milhões de toneladas de casca de arroz por ano (IBGE, 2018). Esta é uma matéria-prima de baixo valor agregado, de difícil reaproveitamento, baixas propriedades nutritivas e elevado teor de sílica (LORENZETT et al., 2012). Como é produzida em alta quantidade, esse resíduo se caracteriza como um problema para a indústria arrozeira, já que as possibilidades de destinação correta são limitadas.

A composição da casca de arroz varia de acordo com a variedade plantada, o clima e as condições do solo, além da localização geográfica (POUEY, 2006). Os principais componentes orgânicos da casca são a celulose, a hemicelulose e a lignina, nas proporções de aproximadamente 40%, 30% e 20%, respectivamente (TOUHAMI et al., 2017). A composição da casca de arroz ainda apresenta teor de cinzas de 11,4%, sendo que essas geralmente contêm 80-90% de SiO_2, 5% de K_2O, 4% de P_2O_5 e 1-2% de CaO, além de pequenas quantidades de Mg, Fe e Na (FERREIRA, 2005).

A baixa densidade da casca de arroz, aproximadamente 130 kg m^{-3}, resulta em grande volume desse resíduo, necessitando de um local igualmente grande para sua disposição (MAYER et al., 2006). Porém, segundo este autor, a maior parte da casca é depositada no solo, em terrenos a céu aberto, causando sérios impactos ao ambiente. O maior problema verificado nessa situação é que a casca possui lenta decomposição e, quando decomposta pode liberar metano (CH_4), um gás de efeito estufa 28 vezes mais prejudicial ao ambiente que o dióxido de carbono (CO_2) (LORENZETT et al., 2012; GUIMARÃES et al., 2014). Além disso, Walter e Rossato (2010), descrevem que outra prática comum no estado do RS é o descarte da casca de arroz em margens de rios ou beiras de estrada. Há ainda quem realize a queima descontrolada deste material, outro destino inadequado, uma vez que a combustão resulta em emissão de poluentes particulados para atmosfera, além de monóxido e dióxido de carbono (MAYER et al., 2006). Todas as práticas descritas até então são nocivas ao ambiente, apesar disso são frequentemente utilizadas devido à dificuldade de proporcionar ao resíduo uma destinação correta e viável.

De acordo com Vale et al. (2014), uma alternativa sustentável para o uso da casca de arroz é a geração de energia a partir da queima dessa biomassa. No entanto, em pesquisa realizada por Walter e Rossato (2010), apenas 30% das unidades beneficiadoras de arroz pesquisadas, pertencentes à Microrregião de Restinga Seca – RS, utilizavam a casca de arroz como fonte energética a partir da queima desse resíduo. Isso demonstra que há casos de reaproveitamento da casca pela própria indústria beneficiadora, porém uma grande parcela desse resíduo segue tendo como destino final práticas que são contrárias à preservação do ambiente.

Recentemente, uma destinação ambientalmente correta tem sido disponibilizada às indústrias arrozeiras do estado do RS. São nove usinas termelétricas que produzem energia a partir da combustão da casca de arroz (NOGUEIRA, 2019). Mas esta destinação ainda consome somente uma pequena porcentagem do total de casca gerada neste estado, pois é alto o seu custo de transporte a partir de locais distantes das termelétricas. Por essa razão, a alternativa de incorporar esse resíduo lignocelulítico no substrato para o cultivo de cogumelos comestíveis é uma solução sustentável que pode reduzir o custo de produção e contribuir para dar um destino ambientalmente correto a uma parcela da casca de arroz (SÖZBIR et al., 2015; MYRONYCHEVA et al., 2017).

1.4 HIPÓTESE

É possível melhorar a eficiência biológica e a produtividade de *P. ostreatus* pela substituição parcial da serragem de eucalipto pela casca de arroz na composição do substrato, sendo que a casca de arroz carbonizada tem eficiência biológica superior à casca de arroz *in natura*.

1.5 OBJETIVO GERAL

Avaliar a utilização da casca de arroz *in natura* ou carbonizada na substituição parcial da serragem de eucalipto nos substratos para a produção do cogumelo comestível *Pleurotus ostreatus*.

1.5.1 Objetivo específicos:

1) Avaliar substratos de cultivo à base de serragem de eucalipto e casca de arroz *in natura* ou carbonizada nos parâmetros produtivos e nutricionais do cogumelo *Pleurotus ostreatus*;
2) Verificar se o uso da casca de arroz *in natura* ou carbonizada nos substratos altera as propriedades sensoriais dos cogumelos;
3) Caracterizar as alterações físico-químicas dos substratos resultantes da substituição parcial da serragem de eucalipto pela casca de arroz *in natura* ou carbonizada.

2 ARTICLE: THE USE OF RICE HUSK IN THE SUBSTRATE COMPOSITION INCREASES P*leurotus ostreatus* MUSHROOM PRODUCTION AND QUALITY

2.1 ABSTRACT

Rice husk is a waste generated in large quantities in various countries, although its reuse is limited due to low nutritional quality and high silica content. Therefore, this study sought to evaluate using raw or carbonized rice husks to partially substitute eucalyptus sawdust in substrates to produce the edible mushroom *Pleurotus ostreatus*. The substrates were formulated with proportions of 0 to 100% of eucalyptus sawdust and raw or carbonized rice husks. *P. ostreatus* was grown for 90 days on the substrates to evaluate mycelial growth, fresh mass production, biological efficiency, and protein and fat content. The organoleptic quality of the mushrooms produced was evaluated by sensory analysis. The substrates' moisture, pH, and C and N contents were evaluated before and after cultivation. The substrates with 25 and 50% of raw or carbonized rice husk in a mixture with sawdust provided nitrogen and water more adequately to the fungus, improving the fresh mass production, the protein content of mushrooms, and biological efficiency (75–83%) compared to the reference substrate

(100% eucalyptus sawdust), without altering the sensory quality. Given the environmental issues caused by carbonization, using raw rice husk in a mixture of up to 50% eucalyptus sawdust is recommended for *P. ostreatus* production.

Keywords: by-product, bioconversion, edible mushroom, biological efficiency, sensory quality.

2.2 INTRODUCTION

Rice is produced by numerous countries in the world and is among the most produced cereals (Okigbo et al., 2021). In the processing of grains, the byproduct generated in greater quantity is the husk, representing 20% of the total mass of the grain (Lorenzett et al., 2012). Rice husk has about 20% lignin and 15% ash, which comprise ~90% silica (Faustino et al., 2019). Due to these characteristics, economically viable disposal of this waste in Brazil is challenging, as it is the largest rice producer outside the Asian continent (FAOSTAT, 2023). Recently, thermal power plants burning exclusively rice husk have been installed in southern Brazil, albeit in a handful of locations, making it unfeasible to dispose of the husk generated over longer distances. For this reason, a significant percentage of the husk is disposed of inappropriately in large piles near the rice processing plants. This generates environmental problems, including contaminating watercourses when the husk is transported by rainfall, releasing methane by microbial degradation, attracting rodents, and reducing land use caused by large deposits that remain for many years and degrade slowly (Guimarães et al., 2014; Gómez-Pozuelo et al., 2021; Shukla et al., 2022).

Growing edible mushrooms on substrates based on lignocellulosic organic waste can be an alternative for correctly disposing of rice husk; this process combines the production of protein-rich food and reduces pollution (Pathak et al., 2009; Jeznabadi et al., 2016). In addition, mushroom consumption has been proliferating around the world due to its gastronomic potential and the search for healthier alternatives (Bellettini et al., 2019; Cirlincione et al., 2022). The availability of new substrates for mushroom cultivation can reduce production costs and the final price of mushrooms, allowing more consumers access to this nutritionally-rich food (Szabová et al., 2013). *P. ostreatus* cultivation in southern Brazil is commonly performed in substrates of eucalyptus sawdust. However, given that it has other uses, sawdust is scarce in some regions, justifying the search for other more easily accessible substrates (Franco et al., 2021; Viriato et al., 2022).

P. ostreatus is an edible mushroom known as Shimeji or Hiratake and has rapid growth, including in lignocellulosic substrates of difficult decomposition such as rice husk (Thongklang; Luangharn, 2016). Obodai et al. (2003) and Frimpong-Manso et al. (2011) evaluated this alternative and found that using 100% raw rice husk resulted in rapid substrate colonization by *P. ostreatus*. Nevertheless, the mycelia could not utilize this substrate, and satisfactory yields were not obtained. According to these authors, the high porosity of rice husk and its low moisture retention capacity allows its use only as a complement of substrates for mushroom production. In another study, Baysal et al. (2003) utilized rice husk in low proportions mixed with waste paper (1:9 and 2:8, respectively) and achieved higher mushroom yields than a pure paper substrate.

Few studies have employed this residue because of the low efficiency of raw rice husks in mushroom production, although other possibilities may make its use feasible. Carbonizing rice husk modifies the physicochemical properties of the material. There is an increase in total porosity, specific surface area, and surface reactivity, consequently increasing water and nutrient adsorption capacity (Hodgson, 2016). Furthermore, microorganisms are reduced or eliminated during carbonization in the rice husk, making the substrate less contaminated. If feasible, using rice husk, raw or carbonized, in *P. ostreatus*, would be a low-cost alternative to transform potentially harmful waste generated in large quantities into healthy food with added value. Given the above, this study hypothesizes that rice husk mixed with eucalyptus sawdust increases the biological efficiency and productivity of *P. ostreatus*, which is higher when carbonized rice husk is used. Therefore, this study sought to evaluate the use of raw or carbonized rice husks to partially replace eucalyptus sawdust in substrates to produce the edible mushroom *Pleurotus ostreatus*.

2.3 MATERIALS AND METHODS

2.3.1 Inoculum and substrate materials

The inoculum of *Pleurotus ostreatus* (Jacq.) P. Kumm (SB strain) in the form of mycelium inoculated in wheat grains was purchased from the company Funghi & Flora (Valinhos, SP, Brazil) and stored for 15 days at 5 °C until substrate inoculation. The sawdust of *Eucalyptus* sp. obtained in a wood processing industry was sun-dried, sieved in 2-mm mesh, and packed in plastic bags until use. The raw rice husk and rice bran were obtained by processing organically produced grains. Part of the husk was sieved with a 2-mm mesh and stored in plastic bags, and another part was carbonized according to a method adapted from Haefele et al. (2011). For this, 30 L of raw rice husk was piled on a low fire resulting from wood burning and maintained for 20 h so that the carbonization occurred from the inside to the outside of the pile in a process in which there is a low amount of oxygen and gasification occurs at low levels. Afterward, the pile of rice husk was completely carbonized, then spread, cooled with a small amount of water, dried in the sun, sieved with a 2-mm mesh, and packed in plastic containers until its use.

2.3.2 Experimental design and substrate preparation

The experimental design was entirely randomized, with nine treatments and eight repetitions, totaling 72 experimental units. The treatments consisted of substrates formed by different percentages in volume of eucalyptus sawdust (S) and raw rice husk (RRH) or carbonized (CRH), as follows: 100S, 75S+25RRH, 50S+50RRH, 25S+75RRH, 100RRH, 75S+25CRH, 50S+50CRH, 25S+75CRH, and 100CRH. The 100S treatment was considered the reference because it is frequently used by growers in cultivating *P. ostreatus*. All treatments were supplemented with 5% (v/v) rice bran as a source of N and 2% (v/v) hydrated lime to increase the substrate’s pH. The ingredients of each treatment and enough water to reach a gravimetric moisture content of 70% (m/m) were added to a mechanical mixer (140 L capacity). Each experimental unit was composed of a heavy-duty polypropylene bag (H x W x D = 22 x 20 x 12 cm), produced explicitly for mushroom growing, equipped with an air filter for gas exchange. Each bag received 4 L of the substrate and was sterilized in an autoclave at 121 °C for 90 min. After cooling, the substrates were inoculated on the surface with 70 g (5% w/w) of *P. ostreatus* inoculum in a laminar flow chamber.

2.3.3 Mushroom cultivation

Mushroom cultivation was conducted in a climate-controlled environment for approximately 90 days. The inoculated bags were incubated in a climate-controlled room at 25 ± 2 °C, with a relative moisture content of 70–75%, under the complete absence of light during the mycelial growth stage (24–34 days according to the substrate composition). After complete colonization of the substrates, the culture bags were transferred to a room (3 x 4 m) with a temperature varying from 15 to 25 °C, relative moisture content of 75–95 %, and natural light from a window (1 x 2 m). The day length was ~10 h. Four X-shaped incisions were made with a scalpel on the sides of each bag to allow the fruiting bodies to grow. The mushrooms were harvested manually over 60 days when they reached the point of harvest for commercialization before the edges were in the same plane as the pileus surface.

2.3.4 Mushroom analysis

The following mushroom production components were determined: i) earliness, defined as the sum of the elapsed time from mycelial growth and bag opening to the first harvest; ii) productivity, expressed as the total fresh mass of harvested mushrooms; and iii) biological efficiency (BE), calculated as the percentage between the total fresh mass of mushrooms and the initial dry mass of the substrate (Melanouri et al., 2022b).

The dry mass of the fresh mushrooms was determined by weighing them after drying them at 60 °C for 72 h. Crude protein was determined by grinding the dry samples, digestion in sulfuric acid, and determining the total nitrogen content by the macro-Kjeldahl method (Ezeibekwe et al., 2009). In calculating the crude protein, the conversion factor 4.38 was used instead of the 6.25 commonly used because mushrooms contain significant amounts of nitrogenous organic compounds that are not rich in protein, such as chitin (Barros et al., 2008). The Bligh-dye method quantified the fat content after extraction with chloroform-methanol-water (Bligh; Dyer, 1959). Briefly, 2-g samples of ground mushrooms in triplicate were packed in falcon tubes and received 8 mL of chloroform, 16 mL of methanol, and 5 mL of water. The mixture was then centrifuged for 30 min at 1.030 g. Another 8 mL of chloroform was added, and the mixture was centrifuged at 1.030 g for 5 min. Afterward, the liquid phase was collected and filtered with anhydrous sulfate powder and filter paper in a test tube. A 5-mL aliquot of the filtrate was kept in an oven at 70 °C until drying to determine the fat mass.

2.3.5 Substrate evaluation

Before and after mushroom production, the substrate samples were dried at 60 °C and ground in a ball mill. A dry combustion elemental analyzer determined total organic C and total N contents (FlashEA 1112, Thermo Finnigan, Italy). Moisture was determined by drying the samples at 105 °C until they reached constant mass, according to AOAC (1990).

2.3.6 Mushroom sensory analysis

In the sensory analysis laboratory, a trained team of 7 judges acquainted with mushroom preparation and consumption performed the sensory analysis according to the NBR ISO 11136 standard (ABNT, 2016). The mushrooms produced on the substrates that obtained the best biological efficiencies were selected: 50S + 50CRH, 75S + 25RRH, 75S + 25CRH, 50S + 50RRH, plus the reference treatment 100S. After harvesting, the mushrooms were dehydrated in a forced air circulation oven at 40 ºC until constant mass. The following protocol was established for sample preparation: 1) hydration: 100 g of dehydrated mushroom were added to 2 L of water at room temperature (25 ºC) for 30 min; 2) removal of excess water: the samples were drained on a sieve (60 mesh) for 10 min; 3) preparation: samples were cooked in boiling water (4 L) for 15 min, 1% (w/w) salt added; 4) removal of excess water: drained on a sieve (60 mesh) for 3 min. Afterward, the samples were presented in 30 g portions to the judges, in a balanced block design in monadic presentation (each sample was evaluated individually), in white plastic dishes, coded with three random digits. Salt crackers and water were provided to clean the palate between samples, a napkin, and an evaluation form. The verbally structured hedonic scale was applied, ranging from “disliked a lot” (1) to “liked a lot” (7), according to NBR ISO 11136 (ABNT, 2016). The attributes evaluated were: color, odor, flavor, texture, and overall appearance. The preference-order test was employed to evaluate preference, in which the judges ordered the samples, served simultaneously, in descending order of their preference, according to NBR ISO 8587 (ABNT, 2015). For each judge, the sample placed first was recognized as “the most preferred,” receiving a value of 1, the second a value of 2, and so on, until the “least preferred” sample (value 5). The study was approved by the Ethics Committee on Human Research (CEP) of the Universidade Federal de Santa Maria under registration number 87072418.6.0000.5346.6.

2.3.7 Statistics

Data were subjected to a normality test (Shapiro-Wilk with $p < 0.05$) and analysis of variance (ANOVA). Statistically significant mean values were compared by Tukey’s test with $p < 0.05$. Data regarding the variable “Bag opening to the first harvest” were transformed to log (x) to follow normality assumptions. For the data obtained from the sort-preference test, the sum of all the judges for each sample was calculated and analyzed by Friedman’s test using the Newell and Mac Farlane table (Naes et al., 2018), which defines the value of critical differences between the sort-preference totals at the 5% level. The acceptability index (AI%) was calculated by the formula: mean acceptance score x 100/maximum acceptance score. Statistical analyses were performed in the R statistical software and SASM (version 4) (R Core Team, 2020; Althaus et al., 2001; Canteri et al., 2001).

2.4 RESULTS

P. ostreatus cultivation in the reference substrate (100S) resulted in the fastest mycelial growth, although it was statistically indifferent from the other five substrates (Figure 1A). The only substrates with lower performance than 100S were those with a higher proportion of rice husk: 100CRH, 100RRH, and 25S+75RRH. The period from the opening of the bags to the first harvest showed no statistical difference among the substrates, except for 25S+75RRH, which, on average, was two days slower than the others (Figure 1B).

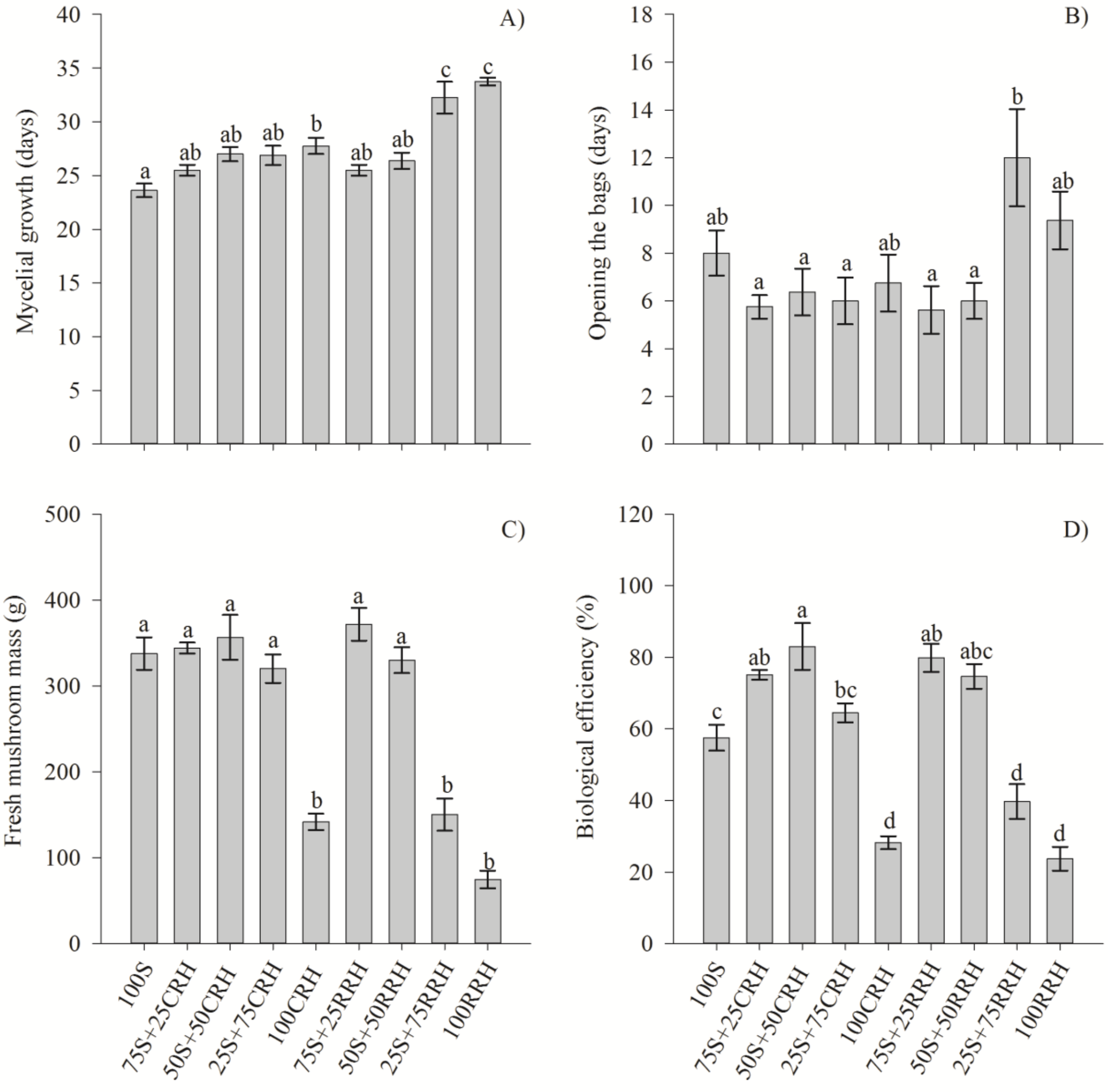

Figure 1. Production components of the edible mushroom *P. ostreatus* grown on substrates with different proportions of eucalyptus sawdust (S) and raw (RRH) or carbonized (CRH) rice husk. All substrates were supplemented with 5% (v/v) rice bran and 2% (v/v) hydrated lime. Means followed by the same letters in the columns do not differ by Tukey’s test (p ≤ 0.05).

When quantifying the fresh mass of mushrooms, again, the treatments 100CRH, 100RRH, and 25S+75RRH showed the worst performance, with mushroom production lower than half of the other substrates, which showed no statistical differences among themselves (Figure 1C). The BE showed the greatest variation among the substrates, from 23.71% (100CRH) to 82.98% (50S+50CRH) (Figure 1D).

The substrates that produced the highest amounts of fresh mushroom mass were the same ones that presented the highest BE values. The highest BE was observed in substrate 50S+50CRH, without statistically differing from 50S+50RRH, 75S+25CRH, and 75S+25RRH. Once again, substrates 100CRH, 100RRH, and 25S+75RRH showed the lowest BE values, less than 40%. The reference substrate (100S) showed an intermediate performance, with a BE of 57.51%.

In the evaluation of the protein content of the mushrooms, the highest values were quantified in the samples grown on the substrates that showed the worst yields and biological efficiency (100CRH, 100RRH, and 25S+75RRH) (Figure 2A). The substrates that showed the best performances in these variables (50S+50CRH, 50S+50RRH, 75S+25CRH, and 75S+25RRH) had intermediate protein content, an mean of 17.32%. Nevertheless, the reference substrate produced mushrooms with the lowest percentage of protein. The fat content was also higher in the two treatments with the lowest yields and biological efficiencies (25S+75RRH and 100RRH) (Figure 2B). The other treatments did not differ statistically and had an mean fat content of 3.10%.

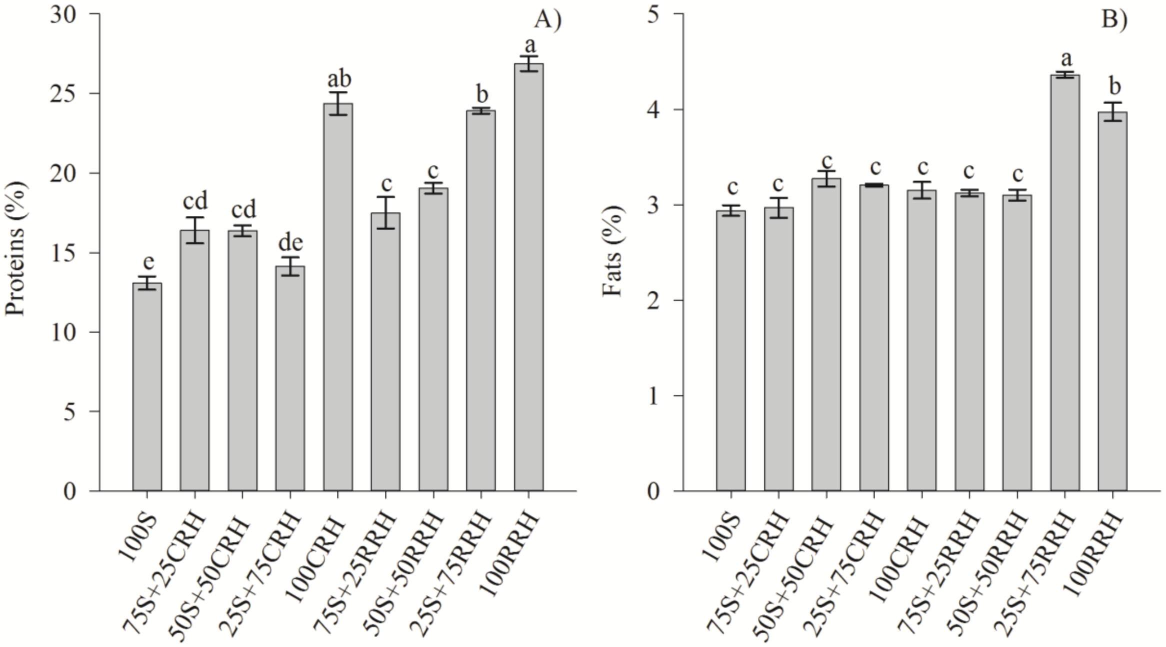

Figure 2. Protein and fat content of the edible mushroom *P. ostreatus* cultivated in substrates with different proportions of eucalyptus sawdust (S) and raw

(RRH) and carbonized (CRH) rice husk. Means followed by the same letters in the columns do not differ by Tukey's test ($p \leq 0.05$).

In the physicochemical evaluation of the substrates, the lowest initial moisture contents were quantified in those with the lowest BE values (100CRH, 100RRH, and 25S+75RRH) (Table 1). However, the higher the rice husk content in the substrate, the lower the moisture losses between the beginning and the end of the cultivation. Carbonizing the rice husk resulted in higher moisture retention capacity by the substrate, a mean of 3.35% points higher, although it reached 6.37% points in treatment 25S+75CRH. The pH of all substrates was acidified during mushroom growth, on average, by 2 to 3 units. The initial total C content was the highest in the 100S treatment and the lowest in the substrates with 100% rice husk. In the other treatments, the values were proportional to the percentages used of these materials. Carbonizing the rice husk reduced the C content by around 17%, and during cultivation, the carbon content decreased by an average of 20% in the substrates. The initial total N content was approximately double in the substrates with 100% rice husk than in the substrate with 100% sawdust. Intermediate levels proportional to the mixtures were observed in the other substrates. The reference substrate showed a ~70% increase in N content, while this increase in the substrates with rice husk averaged 16%. The lower the C/N ratio, the higher the proportion of rice husk in the substrate.

Table 1. Physicochemical characteristics of the substrates before and after cultivation of the edible mushroom *P. ostreatus* produced in substrates with different proportions of eucalyptus sawdust (S) and raw (RRH) and carbonized (CRH) rice husk.

Substrates	Moisture content (%)		pH		Total C (%)		Total N (%)		C/N ratio	
	Before	After	Before	After	Before	After	Before	After	Before	After
100S	67.10	38.92	8.90	5.90	41.70	33.74	0.26	0.44	158.08	76.13
75S+25CRH	69.24	55.34	8.87	5.86	37.07	28.78	0.29	0.37	127.17	78.83
50S+50CRH	71.62	63.96	7.95	6.26	32.68	23.75	0.35	0.40	92.40	59.59
25S+75CRH	68.15	65.88	8.40	6.04	29.09	20.22	0.41	0.54	70.13	37.31
100CRH	63.92	62.71	8.73	5.88	23.63	18.29	0.52	0.54	45.58	33.99
75S+25RRH	68.85	53.77	8.21	6.06	39.82	32.04	0.37	0.38	108.35	85.28
50S+50RRH	66.55	61.13	8.43	5.91	37.98	30.92	0.41	0.41	93.58	75.11
25S+75RRH	61.78	54.81	8.00	6.58	35.63	32.33	0.51	0.45	70.52	71.99
100RRH	62.35	53.29	8.53	7.02	33.81	31.79	0.48	0.64	70.92	49.57

The four substrates with the highest biological efficiency and the reference substrate were selected for the sensory evaluation test to determine whether using raw or carbonized rice husk modified the organoleptic quality of the mushrooms (Table 2). All the judged criteria (color, odor, flavor, texture, and general appearance) showed similar scores and no statistical differences. Additionally, all samples presented a high acceptance index (mean of 80.9) by the judges. In the sorting test, most judges preferred the sample produced on substrate 75S+25RRH, differing statistically from the samples produced on substrates 100S and 50S+50CRH (Table 4).

Table 2. Mean scores of the acceptance test, acceptability index (%), and values of the sorting test of the samples of the edible mushroom *P. ostreatus* produced in substrates with different proportions of eucalyptus sawdust (S) and raw (RRH) or carbonized (CRH) rice husk.

Substrates	Acceptance Test						Sorting test
	Color	Odor	Flavor	Texture	General appearance	Index of acceptability	
100S	5.71 ± 0.95^{ns}	5.86 ± 0.90^{ns}	5.57 ± 0.79^{ns}	5.57 ± 0.98^{ns}	5.57 ± 0.79^{ns}	80.82^{ns}	29^{b}
75S+25CRH	6.00 ± 0.80	5.71 ± 0.95	5.43 ± 0.79	5.86 ± 0.69	5.86 ± 0.69	82.45	16^{ab}
50S+50CRH	5.57 ± 1.27	5.86 ± 0.90	4.86 ± 1.35	5.86 ± 1.07	5.71 ± 0.76	79.59	27^{b}
75S+25RRH	5.71 ± 1.11	5.86 ± 0.90	5.71 ± 1.11	5.86 ± 0.90	6.14 ± 0.90	83.67	10^{a}
50S+50RRH	5.14 ± 1.07	5.42 ± 0.79	5.43 ± 0.98	5.86 ± 0.69	5.43 ± 0.79	77.96	21^{ab}

NS: not significant. Means followed by the same letters in the column do not differ by Tukey's Test ($p \leq 0.05$) for acceptance and by Friedman's Test ($p \leq 0.05$) for ordering; n = 7; acceptance test scale: 1 = disliked very much, 7 = liked very much.

2.5 DISCUSSION

Our findings prove the hypothesis that rice husk mixed with eucalyptus sawdust increases the biological efficiency and productivity of *P. ostreatus*, increasing when carbonized rice husk is used. If the raw rice husk is chosen, it should not exceed 50% of the substrate volume; this proportion can reach 75% for carbonized rice husk. The use of larger amounts than these results in lower productivity and biological efficiency due to the low nutritional quality of the substrate, as demonstrated in other studies (Obodai et al., 2003; Frimpong-Manso et al., 2011; Okigbo et al., 2021) To our knowledge, these results are unprecedented in demonstrating that *P. ostreatus* can obtain high yields when grown in a substrate consisting of a mixture of high percentages of rice husk with eucalyptus sawdust. These results also demonstrate the feasibility of producing food of excellent nutritional qualities from a residue generated in large quantities of difficult destinations and potential environmental impacts, such as rice husk.

The moisture retention capacity of the substrates was identified as a determining factor for *P. ostreatus* production in substrates consisting of a mixture of rice husk and eucalyptus sawdust. The substrates that presented the lowest mushroom yields comprised the highest percentages of rice husk (100CRH, 100RRH, and 25S+75RRH). These substrates presented lower moisture retention capacity at the beginning of the cultivation due to the higher porosity observed visually. The lower moisture content reduced fungal growth, substrate colonization, and productivity (Melanouri et al., 2022a). Nevertheless, rice husk is more efficient than eucalyptus sawdust in maintaining substrate moisture during cultivation because the higher the rice husk content in the substrate, the lower the moisture losses between the beginning and end of cultivation.

Carbonizing the rice husk increased the moisture-holding capacity of the substrate. This explains why the 25S+75CRH treatment was statistically superior to 25S+75RRH in yield and biological efficiency. While 25S+75CRH had 68.15% moisture content at the beginning of the experiment, 25S+75RRH had only 61.78%. Prakongkep et al. (2013) stated that carbonizing rice husks increases water-holding capacity due to increased total porosity.

The use of rice husk in the substrate composition for *P. ostreatus* cultivation increased N content, promoted greater moisture retention in the substrate, and resulted in a greater amount of protein in the mushrooms, increasing consumer acceptance. Thus, the use of rice husk, in addition to being a form of reuse of a residue of a difficult destination, represents an alternative for use in the composition of the substrate in *P. ostreatus* cultivation.

The reference substrate (sawdust) showed low water retention capacity, as justified by the higher moisture content loss since the beginning of mushroom production. Additionally, the small particles of sawdust resulted in a more compact substrate at the end of the production period, impairing the oxygenation necessary for *P. ostreatus* growth (Bellettini et al., 2015). Another factor that explains the lower productivity in the substrate with 100% sawdust is the lower total N content among the evaluated treatments. Nitrogen is fundamental for fungal growth in various aspects, with a significant effect in stimulating the production of lignocellulose enzymes, which favors the decomposition of the substrate and obtaining more nutrients by the fungus (Bagewadi et al., 2016; Melanouri et al., 2022b).

The presence of rice husk in proportions of 50 or 25% contributed to a better balance in the C:N ratio of the substrate, which increased *P. ostreatus*. The C:N ratio values observed in all treatments corroborate the values indicated for mushroom production (Bellettini, 2019). The C:N ratio of the culture substrate is a critical factor for mycelial growth and, consequently, mushroom production (Figueiró; Gracioolli, 2011). Rice husk has lower C and N content than eucalyptus sawdust. This resulted in higher N values in the substrates with a higher percentage of rice husk, consequently, higher N contents available to the fungus, higher N uptake, and higher protein content in the mushrooms. Because of this, the protein content was higher in the substrates with a higher rice husk percentage. The most productive substrates with higher BE values had intermediate protein values because they consisted of a mixture of 25 or 50% rice husk and sawdust.

The highest nitrogen availability in the substrate results in the highest synthesis of proteins, nucleic acids, purines, pyrimidines, and polysaccharides constituting the cell wall of the fungi (Drozdowski et al., 2010; Abdullah et al., 2015). The mean protein value observed in the mushrooms produced on these substrates (17.3%) corroborates the values commonly reported in the literature (Valverde et al., 2015). Similarly, the percentages of fat found in the present study ranged from 2.94 to 4.36% and are within the range of 0.5 to 5% reported by Khan and Tania (2012).

According to Kim et al. (2011), mushrooms are rich in fiber and protein and low in fat, which can help reduce unhealthy cholesterol levels.

The substrates 50S+50CRH, 75S+25RRH, 75S+25CRH, and 50S+50RRH obtained the highest BE percentages (82.98–74.64%), much higher than the 7.6 and 14% achieved with wheat straw and corn cob, respectively, by Bhatti et al. (2007) and Adjapong et al. (2015). Owaid et al. (2015) used a substrate containing only cardboard and found a BE value of 68.1%. In contrast, the authors who used legume residues (rich in N) obtained higher BE values. Ivarsson et al. (2021) used the bark of vetch (*Vicia faba*) and obtained a BE of 109%. Patil et al. (2010) obtained a BE of 85.16% in mushroom cultivation when grown on soybean straw.

The mushrooms produced on the different substrates showed the same sensory quality (color, odor, taste, texture, and overall appearance). Before the test, there was a concern that mushrooms produced on the substrate with CRH might show organoleptic differences due to the characteristic smell of charred biomass. This is because, according to Oyetayo and Ariyo (2013), the composition of substrates can affect mushrooms' chemical, functional, and sensory characteristics. However, this did not prove to be the case, and all samples showed high acceptance ratings, with the mushrooms produced on the 75S+25RRH substrate standing out.

The acceptability index informs about a given product's acceptance in the consumer market, considering its sensory properties, and should be at least 70% (Saint-Denis, 2018). The five samples evaluated presented an acceptability index above 77%, indicating that the composition of the substrates used did not interfere with the acceptance of the mushrooms. It is also highlighted that the number of studies that perform the sensory evaluation of mushrooms, especially fresh ones, is limited, so it is recommended that this evaluation be used more by researchers, considering that this is one of the main ways to commercialize mushrooms and that the products should obtain good acceptance by consumers. The substrates with the best performance comprised 25 or 50% rice husk regardless of whether raw or carbonized, likely by combining greater availability of N and water to mushrooms. Thus, the carbonization of rice husk is not justified, as it is a costly process of labor, time, and wood to burn and releases smoke into the atmosphere.

Furthermore, if done frequently, it should be licensed by environmental agencies. According to Mayer et al. (2006), carbonizing rice husks should be avoided because it emits atmospheric pollutants, especially carbon monoxide and carbon dioxide. However, raw rice husk is produced in large quantities in the region and has no acquisition costs (Walter; Rossato, 2010).

2.6 CONCLUSIONS

Using 25 or 50% of raw or carbonized rice husk in partial replacement of eucalyptus sawdust in substrates for *Pleurotus ostreatus* cultivation increases water and nitrogen availability, increasing the productivity, biological efficiency, and protein content of mushrooms. There is no change in the sensory quality of mushrooms produced in substrates using 25 or 50% raw or carbonized rice husk in their composition compared to those produced in substrates with 100% eucalyptus sawdust. Due to the problems resulting from carbonized rice husk, using raw rice husk is recommended in substrates for *P. ostreatus* cultivation.

2.7 ACKNOWLEDGMENTS

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