Improving Nutrition Facts of Cassava and Soybean Residue through Solid-State Fermentation by Pleurotus ostreatus Mycelium: A Pathway to Safety Animal Feed Production

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Article

Improving Nutrition Facts of Cassava and Soybean Residue through Solid-State Fermentation by *Pleurotus ostreatus* Mycelium: A Pathway to Safety Animal Feed Production

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Abstract: The overwhelming amount of cassava residues and okara is a foremost challenge for the food processing industry environmental loading. The purpose of this article is to utilize *Pleurotus ostreatus* mycelium to ferment solid cassava and soybean residue, resulting in mycelial biomass with nutritional values and promising prebiotic activities from fermented waste-sources. By a blending ratio of 80% cassava and 20% soybean residues, the mycelium spread rapidly after 3 days of culture, from 1.73 mm on the first day to 13.32 mm on the third day, and completely covered the surface after 9 days of culture (120 mm). Using the SSF method allowed us to improve the content of substances isolated from mycelium biomass, where polysaccharide content rose by 2.1 times to 3.44 mg/g, and the protein content increased by 1.84 times over the initial substrate. The prebiotic activity of isolated-PS was greatest in *P. acidilactici* NBD8 (1.58); for *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1 strains, the indices were 0.11, 0.17, and 0.3, respectively. SSF process with *P. ostreatus* mycelium has the potential to be an effective method for improving the nutrition and digestibility of soybean and cassava residues for application in the production of nature-derived animal feed, as well as contributing to fully utilize agricultural residue, agriculture's circular economy, reducing environmental issues, and achieve net-zero carbon emissions target by 2050, as Vietnam government committed during the COP26 World Leaders' Summit in 2021.

Keywords: Oyster mushroom; *Pleurotus ostreatus*; polysaccharide; prebiotic activity; soybean residue; cassava residue; solid-state fermentation

1. Introduction

Solid state fermentation (SSF) refers to a fermentation process in which microorganisms grow on a solid substrate that lacks or limited free water, and complex materials are transformed into simpler ones [1–3]. It is considered one of the technologies for conversion of agricultural by-products into valuable secondary products for nutrition and human health [4,5], animal feed [6–8], biofuel [9–12], and bio-protection for grain crops [13–15]. SSF regularly employs agricultural and agro-industrial by-products as low-economic-value raw materials rather than discarding them as an environmental concern; these materials are used as substrates in the fermentation process [16,17]. Various agricultural substrates have been successfully used in SSF to produce ligninolytic enzymes, which decompose lignin, a major contributor to the total carbon of agro-industrial wastes, and produce polycyclic aromatic hydrocarbon compounds that can inhibit DNA synthesis and induce cancerous tumors in the liver, lung, larynx, and cervix in animals and humans [18,19]. *Pleurotus ostreatus* (*P. ostreatus*) is one of significant edible mushrooms, capable of decomposing lignocellulose without the need for chemical or biological preparation due to its enzymatic complex system, which contains phenol oxidases and peroxidases [1,20].

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In Vietnam, the cassava starch and tofu processing, and soybean milk production industries are large-scale sectors that generate significant amounts of residue. This source is often only partially used in its fresh form as feed for livestock or solid compost, with the majority not being utilized, leading to environmental pollution and the wastage of this recyclable resource. Soybean residue (also called okara) and cassava residues retain a substantial amount of fiber and nutrients, making them suitable substrates for the growth of edible and medicinal mushrooms. Wet okara contains about 6% fat, 8.08% protein, 12.01% carbohydrates, 5% dietary fiber, 1% ash, and 32.05% total solid, meanwhile, these compositions in dried okara were 12.06, 34.15, 48.9, 33, 2.05, and 95.04%, respectively [21–23]. In other studies, it was reported that, okara had an amount of 10% lipid, 25% protein, and 50% fiber [24–26]. As for cassava residue, Yimin (2015) announced that, crude protein and neutral detergent fiber content were 1.30 – 16.41% and 25.4 – 52.9% on a dry matter basis, respectively [27]. Parts of cassava plant contain different concentrations of lysine, ranged from 3.9 g/100g in leaves to 7.2 g/100g dry weight in roots, soluble and insoluble dietary fiber contents in cassava pulp by-products reached 2.29% and 15.07%, respectively [28,29]. In another study, it was reported that cassava residue had a high organic content, including approximately 2.19% crude protein, 60.37% starch, 0.52% crude lipid, total minerals at 2.01%, and 21.01% fiber [30].

As for recent achievements around the world, Zhu (2020) designed, synthesized, and characterized superabsorbent hydrogels that had high capacities of water absorbency, holding, and retention through graft copolymerization of soybean residue with acrylic acid and acrylamide [31]. Sabater (2020) made a minireview to introduce new ways of valorization of vegetable food waste and by-products through fermentation processes to improve nutritional value, or to produce biologically active compounds from those waste and by-products [32]. Oktaviani (2021) applied bioconversion of cassava peel residue into yeasts to produce cell wall mannoprotein as an antioxidant [33]. Suriyapha (2022) delved into how to make bioconversion of agro-industrial residues as a protein source supplementation for cows. In that study, authors tried to compare the effects of Holstein Thai crossbreed cows with citric waste fermented yeast waste on absorption, digestibility, fermentation activity in the stomach of ruminants, blood metabolites, cognation of purine, milk production, and economical efficiency of tropical lactating cows [34]. Verardi (2023) reviewed research on agricultural residue recovery through fermentation technology, analyzed the key steps in the agro-residue bioconversion process, and the most common microorganisms employed in this procedure [35]. Bala (2023) addressed pathways to convert agro-residues, into valuable bioproducts and bioactive compounds, as well as their applications [36]. Blasi (2023) reviewed the valorization methods employed for biotransformation of lignocellulosic agricultural waste into economically and environmentally valuable products, such as the production of biofuels, the synthesis of platform chemicals; the creation of materials that is composed entirely of matter taken from living microorganisms; the production of extracellular fungal enzymes, organic acids, and compounds that has a biological activity [37]. Cruz (2021) and Adnane (2024) provided a thorough examination or evaluation of anaerobic co-digestion technology as a biochemical recovery pathway of cassava residue and other agricultural residues for the production biogas that fulfill the global target of renewable energy [38,39]. In this study, we proposed a method for bioconversion of cassava and soybean residues to derive valuable bio-substances, such as polysaccharide (PS) and protein by the purple *P. ostreatus* mycelium through SSF, moreover the prebiotic activity of PS isolated from fermented substrate was also evaluated.

P. ostreatus is one of the most popular mushrooms cultivated in Vietnam due to eating culture and humid tropical monsoon conditions. It contains a variety of nutrients and bioactive substances such as proteins, fibers, lipids, carbohydrates, minerals, vitamins, and bioactive compounds like PS, phenols, and flavonoids [40]. The applications of PS obtained from mushroom mycelium mainly focus on antioxidant activities, anti-tumor effects, immune modulation, stimulation of macrophage functions, and lung cell protection [41]. The biologically active PS in purple oyster mushroom was reported to occur from the mycelial growth stage without the need for the fruiting body to fully develop [42]. Thus, SSF to harvest mycelial biomass may shorten the cultivation cycle, easily control

 doi:10.20944/preprints202503.1319.v1

the cultivation conditions on an industrial scale to obtain a large amount of mycelial biomass, opening prospects for the development of diverse substrate sources for producing mushroom fiber rich in polysaccharides, both of high medicinal value and nutritional value for use in the livestock industry, and reduce environmental issues caused by agricultural residue.

2. Materials and Methods

2.1. Cassava and Soybean Residue Sources

Fresh cassava residue was collected from a tapioca starch processing factory in Quang Nam province (Q86F+498, ĐT611, Que Cuong commune, Que Son district, Quang Nam, Vietnam). Fresh soybean residue was sampled from Hoa Khanh market (Dong Ke Street, Hoa Khanh Bac town, Lien Chieu district, Danang city, Vietnam). These residues then were sun-dried and ground into fine powder. The purple oyster mushroom (*P. ostreatus*) was provided by the Laboratory of Biology – Environment, University of Science and Education – The University of Danang (459 Ton Duc Thang Street, Lien Chieu district, Danang, Vietnam). Probiotic strains, *Lactiplantibacillus plantarum* WCFS1, *Lactiplantibacillus pentosus* NH1, *Lactiplantibacillus argentoraten* NH15, *Pediococcus acidilactici* NBD8, and the pathogenic strain *Escherichia coli* ATCC 85922 were used to assess the prebiotic activity of polysaccharides fermented from mushroom mycelia. Commercial prebiotics (FOS, inulin, and GOS) were distributed by Southeast Asia Pharmaceutical and Trading company (46 Lot 5, Den Lu 2 Urban Area, Hoang Van Thu Ward, Ha Noi, Ha Noi Vietnam).

2.2. Cultivation of Pleurotus ostreatus Mycelium in Liquid Medium

The PDB+ medium was prepared by adding 200 g of potatoes, 20 g of D-glucose, 2 g of peptone, and 2 g of yeast extract into 1 L of distilled water, sterilized twice. 100 mL of PDB+ medium was dispensed into 250 mL Erlenmeyer flasks and then sterilized at 121°C for 30 minutes. After cooling, a uniform piece of mycelium (approximately 1 cm^2) was inoculated into each flask from a first-generation culture tube to proceed with the shake culture at a speed of 150 rpm at a temperature range of 25 to 28°C. After 7 days, the mycelium was harvested.

2.3. Solid-State Fermentation of Cassava and Soybean Residue with Pleurotus ostreatus Mycelium

The experiment was conducted to evaluate the growth of the *Pleurotus ostreatus* mycelium on various experimental treatments containing a mixture of soybean and cassava residues as detailed in Table 1.

Table 1. Blending treatments for cassava and soybean residues.

Treatments [1]	Cassava residue (%)	Soybean residue (%)
CT1 (n = 15)	100	0
CT2 (n = 15)	90	10
CT3 (n = 15)	80	20
CT4 (n = 15)	70	30
CT5 (n = 15)	60	40

[1] *Each formula was prepared in 5 samples and repeated three times. Both cassava and soybean residues were in dry substrate.*

100 g of the substrate in each formula was placed into plastic boxes (with an upper-diameter of 12 cm and bottom-diameter of 10 cm) to sterilize at 121°C, 1 atm, for 20 minutes. After cooling down, 5 mL of the liquid mycelium solution was inoculated into each box. The cultivation phase of the mushroom mycelium was maintained at a temperature range of 25 – 28°C, and subsequently, the spread rate and morphological characteristics of the mycelium were evaluated after 15 days of cultivation.

 doi:10.20944/preprints202503.1319.v1

2.4. Extraction and Quantification of PS

Taking 25 g of dry, finely ground mycelial biomass obtained after the SSF and mixing it with 375 mL of distilled water, followed by incubation at 70°C for 3 hours. The mixture was then vigorously shaken and filtered through Whatman filter paper, and the filtrate was precipitated using 96% alcohol in a 1:4 ratio for 12 – 24 hours at 4°C. Subsequently, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the sediment was dissolved in 1M NaOH at 60°C for one hour. The quantification of PS was conducted using the phenol-sulfuric acid method [43].

2.5. Assessment of the Impact of PS on the Growth of Gut Probiotics

The experimental medium used to evaluate the impact of PS on the growth of beneficial gut bacteria were prepared as follows: GF: MRS medium with glucose removed; PS: GF medium supplemented with 1 g/L PS; Pre: GF medium supplemented with 1 g/L commercial prebiotic (used as a control); Glc: GF medium supplemented with 1 g/L glucose; GlPs: Glc medium supplemented with 1 g/L PS; and GlPre: Glc medium supplemented with 1 g/L commercial prebiotic.

The probiotic strains were inoculated into the above mediums and then cultivated at 37°C for 48 hours. Subsequently, the cell density of the experimental treatments was determined by measuring the optical density at a wavelength of 600 nm at time points of 0 hours, 24 hours, and 48 hours [44].

2.6. Calculation of Prebiotic Index

The prebiotic activity of PS extracted from the mycelium after the SSF of cassava and soybean residues was evaluated through the prebiotic index (PI). The beneficial bacterial strains were cultured in GF, PS, Pre, and Glc mediums. The strain *E. coli* was cultured in M9 medium with glucose eliminated, supplemented with 1 g/L PS, 1 g/L glucose, and 1g/L commercial prebiotic. The bacterial strains were incubated at 37°C for 48 hours. The optical density of the liquid samples at a wavelength of 600 nm (OD_{600}) was measured to determine the biomass accumulation capacity. The PI was calculated using the following formula [45].

$$PI = \left[\frac{\left(P_p^{24} - P_p^0\right) - \left(P_{GF}^{24} - P_{GF}^0\right)}{\left(P_G^{24} - P_G^0\right) - \left(P_{GF}^{24} - P_{GF}^0\right)}\right] - \left[\frac{\left(E_p^{24} - E_p^0\right) - \left(E_{GF}^{24} - E_{GF}^0\right)}{\left(E_G^{24} - E_G^0\right) - \left(E_{GF}^{24} - E_{GF}^0\right)}\right] \quad (1)$$

where, P_p^0, P_p^{24}, P_{GF}^0, P_{GF}^{24}, and P_G^0, P_G^{24} were OD_{600} values of beneficial gut bacteria cultured in Pre; GF; and Glc medium at 0 hours and 24 hours. E_p^0, E_p^{24}, E_G^0, E_G^{24} and E_{GF}^0, E_{GF}^{24} were OD_{600} values of *E. coli* cultured in Pre; Glc; and GF medium at 0 hours and 24 hours.

2.7. Assessment of Mycelial Biomass Quality

The protein content was determined according to ISO 5983-2: 2009 for livestock feed - this standard specifies the method for determining nitrogen content and calculating crude protein content using the Kjeldahl method [46]. Lipid content was analyzed in accordance with ISO 6492:1999 for livestock feed - this specifies the determination of fat content by hexane extraction [47]. The crude ash content was analyzed according to ISO 5984:2022 for livestock feed [48].

2.8. Data Analysis

Each experimental formula was replicated three times, with each replication consisting of five identical treatments. Descriptive statistics were used to determine mean values and standard deviations. The treatments were compared using one-way analysis of variance (ANOVA) and the Tukey HSD Test for a significance level of 95%.

 doi:10.20944/preprints202503.1319.v1

3. Results and Discussion

3.1. Impact of the Cultivation Substrate on the Growth of Pleurotus ostreatus Mycelium

Agricultural residue typically contains a variety of substances and numerous nutrients remaining after the processing phase. These provide a rich source of raw materials for SSF to produce mycelial biomass from various types of mushrooms. The nutrient content within the substrate significantly influences the growth and development of the mushroom mycelium during SSF. Changes in the diameter of the *Pleurotus ostreatus* mycelium on the surface of the substrate in the treatments during the SSF process are depicted in Table 2 and Figure 1.

Table 2. Changes in the diameter (mm) of *Pleurotus ostreatus* mycelium on various substrate during cultivation.

treatments	Culture time (days)					Characteristics of mycelium
	1st	3rd	5th	7th	9th	
CT1 (n = 15)	1.77 ± 0.15a	19.37 ± 1.32a	51.8 ± 3.93a	110.62 ± 3.45a	120 ± 0a	Fine, low density
CT2 (n = 15)	0.91 ± 0.05b	10.26 ± 1.42c	33.99 ± 2.04b	83.18 ± 3.59b	120 ± 0a	Fine, evenly white, low density
CT3 (n = 15)	1.73 ± 0.17a	13.32 ± 1.04b	34.56 ± 2.41b	82.51 ± 3.66b	120 ± 0a	Thick, evenly white, high density
CT4 (n = 15)	0.97 ± 0.03b	6.47 ± 0.81d	24.01 ± 1.68c	60.06 ± 2.99c	108.07 ± 3.85b	Thick, fluffy, evenly white, high density
CT5 (n = 15)	0.91 ± 0.03b	2.69 ± 0.53e	11.56 ± 1.29d	41.61 ± 2.35d	84.29 ± 3.52c	Thick, fluffy, high density

Note: *Values are mean ± standard error of the mean (n = 15). Means followed by the same letter within a column are not significantly different according to the Tukey HSD Test for a significance level of 95%.*

The findings indicated that the mixing ratio between cassava and soybean residues significantly affects the spread rate and morphology of the mushroom mycelium. Treatments with a higher ratio of cassava residue showed rapid mycelium spread and completed occupying of the whole substrate surface quicker than those with a higher ratio of soybean residue. In treatments CT1, CT2, and CT3, the mycelium spread rapidly and covered the surface of the medium after 9 days of cultivation (120 mm). In CT4 and CT5, the spread rate was slower, the mycelium covering approximately 90% and 70.24% of the substrate surface after 9 days (108.07 mm and 84.29 mm, respectively). CT1 (100% cassava residue) showed the fastest spread rate, with the mycelium covering 92% of the surface by the 7th day (110.62 mm), followed by CT2 and CT3 with about 70% (83.18 mm and 82.51 mm, respectively) (Table 2).

According to Nair (2012), the high fiber and carbohydrates content (14.88 g/100g and 63.85 g/100g dry weight), good thermal stability, and high crystallinity in cassava residue might make the substrate more porous, allowing the mycelium to develop and spread more quickly [49]. This can explain the early surface coverage of mycelium observed in CT1, CT2, and CT3, because soybean residue contains mostly crude fiber composed of cellulose, hemicellulose, and lignin, about 25% protein, 10-15% oil, but little starch or simple carbohydrates [50]. Hence, a high proportion of soybean residue in treatments will result in increasing compaction and reduce the porosity of the substrate, while the diversity and richness in nutrients remaining in the soybean residue often led to slower mycelium development due to a lack of oxygen for respiration and decomposition of complex compounds. This demonstrated the slower mycelium spread on the substrate in treatments CT4 and CT5 where soybean residue occupied 30% and 40%, respectively.

 doi:10.20944/preprints202503.1319.v1

Figure 1. The development of the *Pleurotus ostreatus* mycelium during SSF. Which, CT1: 100% cassava residue; CT2: 90% cassava + 10% soybean residue; CT3: 80% cassava + 20% soybean residue; CT4: 70% cassava + 30% soybean residue; CT5: 60% cassava + 40% soybean residue.

Sensory evaluation of the mycelium thickness on the substrate surface revealed distinct differences among the treatments. *Pleurotus ostreatus* mycelium in CT3, CT4, and CT5 had the highest density, was fluffy, evenly white, while CT1 and CT2 had a lower density. This might be due to the rich and higher nutrients content in soybean residue compared to those in cassava residue [28,50] with high levels of nitrogen and vitamins, facilitating the growth and biomass accumulation of the mycelium during fermentation. The productivity and quality of the mycelium depend on the nutritional status from the substrate source, such as the C/N ratio, vitamins, plant hormones, and trace and macro minerals [51].

After 30 days of cultivation, the mycelium had covered the entire substrate in all treatments (Figure 1). In CT4 and CT5, although the mycelium had covered the surface of the substrate, the bottom of the boxes still had sparse mycelium, and some boxes showed no further mycelium spread upon continued cultivation. In CT1 and CT2, the low nutrient content might have led to low mycelial biomass and the mycelium drying out and dying over time. In contrast, for CT3, the mycelium developed fully and appeared fluffy and white across the box when the SSF period was extended, showing signs of fruiting body development on the substrate surface. Therefore, the 80% cassava + 20% soybean residue mix in CT3 was selected for further experiments.

3.2. Impact of the Cultivation Substrate on the Growth of Pleurotus ostreatus Mycelium

As presented in Figure 2, the PS content in the substrate after SSF increased approximately 2.12 times compared to the unfermented raw substrate (from 1.62 mg/100g to 3.44 mg/100g). The increase in PS content could be attributed to the mycelium utilizing a rich carbon source, including compounds such as starch, cellulose, and lignocellulose, for growth and accumulating intracellular polysaccharides from the fungal fibers and unutilized crude polysaccharides following lignocellulose hydrolysis. The rise in PS content after SSF by the mushroom mycelium has also been demonstrated in the study by Lu (2023) when conducted on substrates of corn stalks and xyloma sawdust (a plant belonging to the Willow family, *Salicaceae*) using Shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*) mycelium [52].

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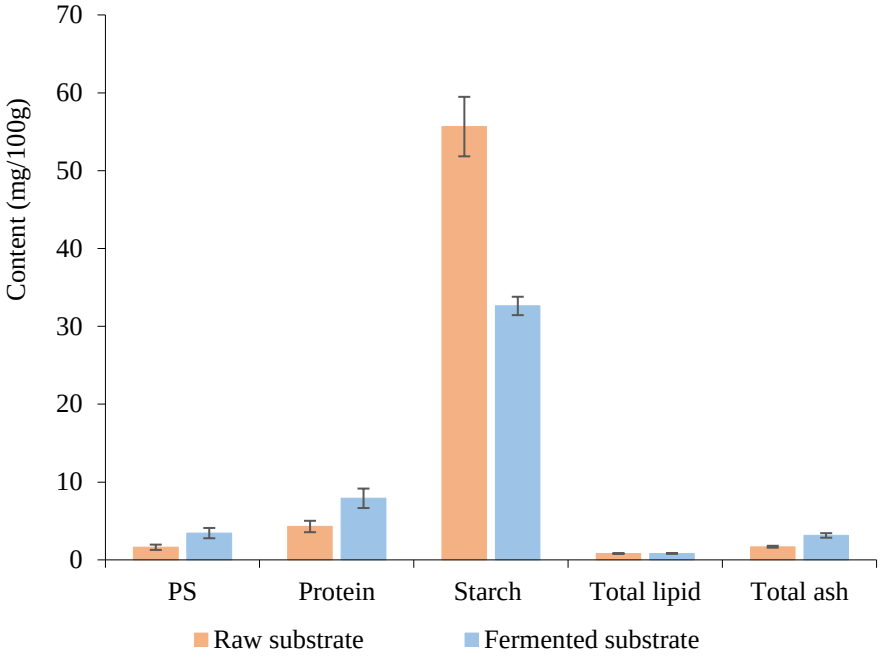

Figure 2. Content of PS, protein, starch, total lipid, and total ash (mg/100g) in the substrate before and after SSF.

Protein, lipid, carbohydrate, and total ash content are also important quality indicators of the product after SSF. Substrates from agricultural by-products typically contain relatively low nutrient levels. However, after fermentation with mushroom mycelium, the nutritional composition was significantly improved, especially in terms of protein content [53]. In this study, there was a significant increase in protein content in the substrate after SSF, about 1.84 times (from 4.29 mg/100g to 7.91 mg/100g). This increase might be due to the high protein content (28.85%) of the oyster mushroom [54,55], thus the increase in mycelium biomass during growth led to an increase in accumulated protein content. The total lipid content in the substrate before and after fermentation did not change significantly (0.82 – 0.83 mg/100g). Meanwhile, the starch content after SSF decreased by about 41.39% (from 55.66 mg/100g to 32.62 mg/100g). Starch usually constitutes a high proportion in the raw substrate composition. During fermentation, the mushroom mycelium used starch as a carbon source for growth and transformation into other compounds, leading to a decrease in starch content in the substrate after SSF [56]. The total ash content in the substrate after fermentation increased by 1.86 times (from 1.69 mg/100g to 3.14 mg/100g). The increase in total ash content could be due to the presence of minerals in the second-stage fermentation broth of the oyster mushroom when inoculating and some minerals from the water added to the substrate to maintain moisture.

3.3. Prebiotic Activity of PS after Extraction

Prebiotics, as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP), are non-digestible food components that selectively feed beneficial microorganisms [57]. Prebiotics added to the diet will not be digested and absorbed by poultry. However, they can play a crucial role in selectively promoting the growth of certain beneficial bacteria, improving the gut microbiome, and enhancing the absorption and utilization of nutrients [58]. In the present study, we evaluated the prebiotic activity of PS extracted from the substrate after SSF with purple oyster mushroom mycelium by assessing the ability of probiotic strains to utilize PS as a nutritional source and evaluating through the prebiotic activity index.

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3.3.1. The Impact of PS on the Growth of Beneficial Gut Probiotics

The prebiotic activity of PS is due to the selective metabolic capability of the probiotics (beneficial bacteria), thereby stimulating their growth [59]. The ability of probiotic strains to metabolize carbohydrates similarly to glucose is a prerequisite for assessing the prebiotic activity of a carbohydrate. In this study, four bacterial strains, *P. acidilactici* NBD8, *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1 were used to evaluate the ability to use PS extracted from the mixture of substrate and mycelium after SSF.

The three *Lactiplantibacillus* strains showed the highest growth rate in the medium with glucose as the carbon source (Figure 3). The control glucose medium (Glc) allowed mycelium development easily because glucose is a common metabolized sugar for living cells. However, *P. acidilactici* NBD8 grew better in the PS and Pre medium.

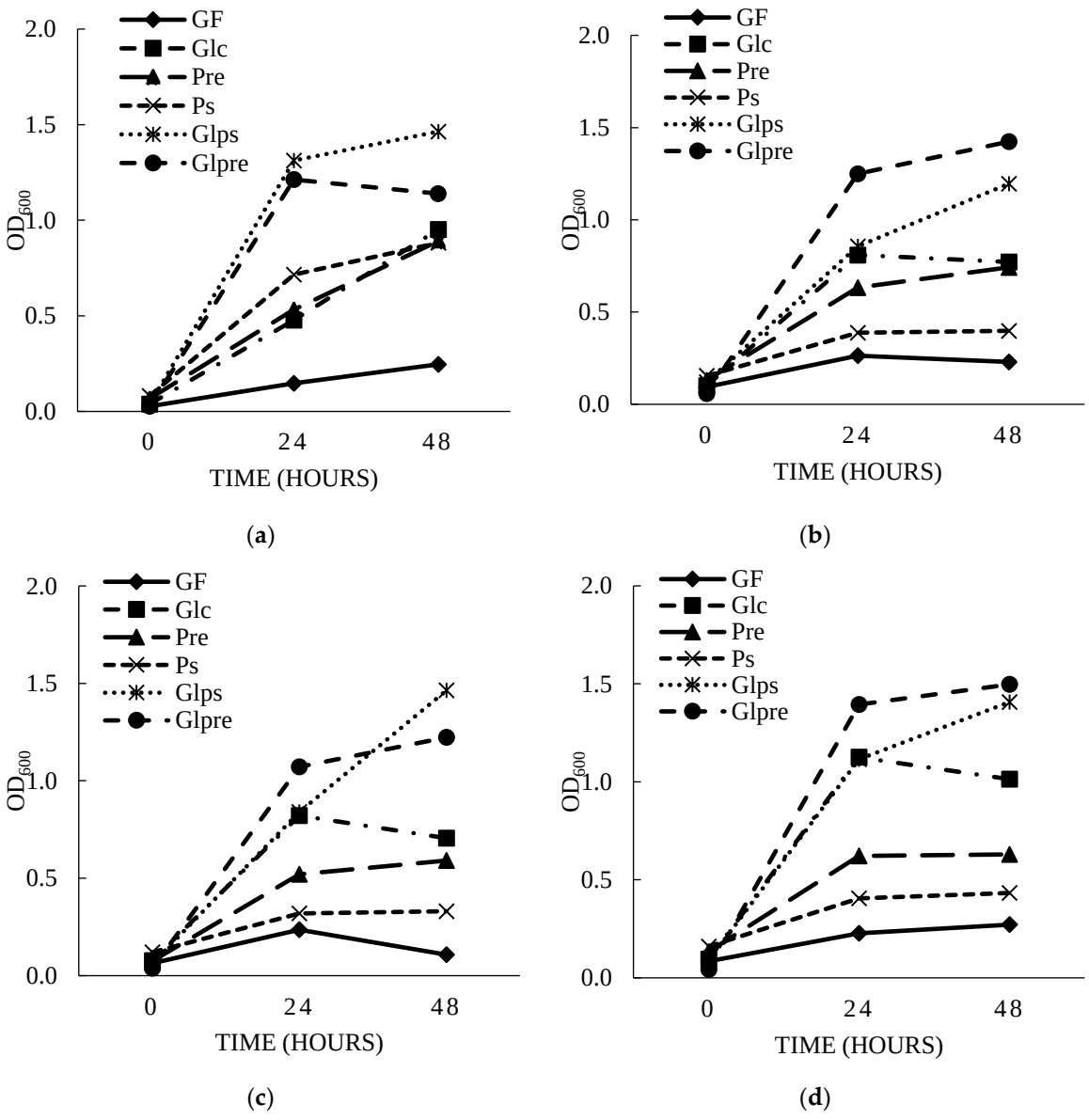

Figure 3. Impact of polysaccharides extracted from fermented substrate on probiotic growth. (**a**): *P. acidilactici* NBD8, (**b**): *L. pentosus* NH1, (**c**): *L. argentoraten* NH15, (**d**): *L. plantarum* WCFS1. Where: GF: MRS medium without glucose, Glc: GF medium supplemented with 1 g/L glucose, PS: GF medium supplemented with 1 g/L PS, Pre: GF medium supplemented with 1g/L commercial prebiotic, GlPs: Glc medium supplemented with 1g/L PS, and GlPre: Glc medium supplemented with 1g/L commercial prebiotic.

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In medium supplemented with PS, all four bacterial strains showed higher growth than in those without added carbon source. Thus, *P. acidilactici* NBD8, *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1 could use PS extracted from the substrate after SSF as a carbon source. However, in the PS medium, the accumulation of mycelial biomass was lower than in the Pre medium; this could be because the PS extracted from the substrate after SSF is crude PS not yet purified and contains some insoluble impurities, whereas commercial prebiotics are purified PS proven to selectively stimulate the growth of beneficial bacteria species. Yet, *P. acidilactici* NBD8 showed significant growth within 24 hours. This may be due to each probiotic strain producing different amounts of hydrolyzing enzymes and having varying rates of carbon source degradation for nutrition; hence, their growth rates differ. This finding aligns with the study conducted by Phirom-On (2021), where *L. plantarum* SKKL1, *L. plantarum* TISTR 2075, and *L. casei* TISTR 1463 could use cello-dextrin extracted from banana peels as a carbon source alternative to glucose for growth [44].

Both GlPs and GlPre mediums strongly stimulated the growth of probiotics. For *P. acidilactici* NBD8 cultured on GlPs medium, growth was better than on GlPre medium. After 48 hours of cultivation in GlPre medium, bacteria entered a degradation phase, cells were decomposed, so the OD_{600} value decreased, while in GlPs medium, the OD_{600} value continued to increase.

For the three beneficial probiotic strains *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1, after 24 hours of cultivation, the OD_{600} value in GlPs medium was lower than those in GlPre medium. And after 48 hours of cultivation, the OD_{600} value in both mediums continued to increase, especially *L. argentoraten* cultured in GlPs medium had a higher OD_{600} value than in GlPre medium. This may be because PS extracted from mycelium contains more complex structural components than commercial prebiotics, so the probiotic strains in the adaptation phase could not immediately use them for growth. They had to accumulate a certain biomass and produce enough extracellular enzymes to degrade and utilize these prebiotics, leading to a longer time required to achieve equivalent biomass levels. This result was similar to the findings of Nguyen Thi Bich Hang (2023), when supplemented 10 mg/mL PS extracted from *Cordyceps militaris* mycelium to evaluate the stimulatory effect on the growth of *L. plantarum* strain [60].

Aida (2009) also emphasized that the mycelium of many mushroom species contains PS with prebiotic activity (such as chitin, hemicelluloses, β and α–glucan, mannan, xylan, and galactan) [61]. α– (1–3)–glucan, one of the PS extracted from the cell walls of two species *P. ostreatus* and *P. eryngii*, has been shown to stimulate the growth of bifidobacterium and Lactobacillus [62].

3.3.2. Prebiotic Index (PI)

According to Huebner (2007), the activity of a prebiotic is defined as the ability of a specific substrate, typically a prebiotic substance, to support the growth of beneficial bacteria in the gut compared to other bacteria. To evaluate the activity of a prebiotic, it is often compared to the growth on a non-prebiotic substrate, usually glucose. Therefore, a carbohydrate exhibits a positive prebiotic activity index if it is metabolized like glucose by probiotic strains; and is selectively metabolized by probiotics rather than by other gut bacteria [63].

The OD_{600} value measured after 24 hours of culture clearly demonstrated that the culture medium influenced the growth of both beneficial and harmful bacteria. All strains grew slowly in MRS medium without carbon (GF) with OD_{600} values ranged from 0.06 – 0.26 but increased faster in medium supplemented with glucose and commercial prebiotics with OD_{600} values ranged from 0.29 to 1.13 for the Glc medium and 0.25 to 0.64 for the Pre medium. Notably, on medium supplemented with PS, growth stimulation was greater for probiotic strains, particularly *P. acidilactici* NBD8 (OD_{600} = 0.71) than for *E. coli* ATCC 85922 (OD_{600} = 0.09) (Figure 4).

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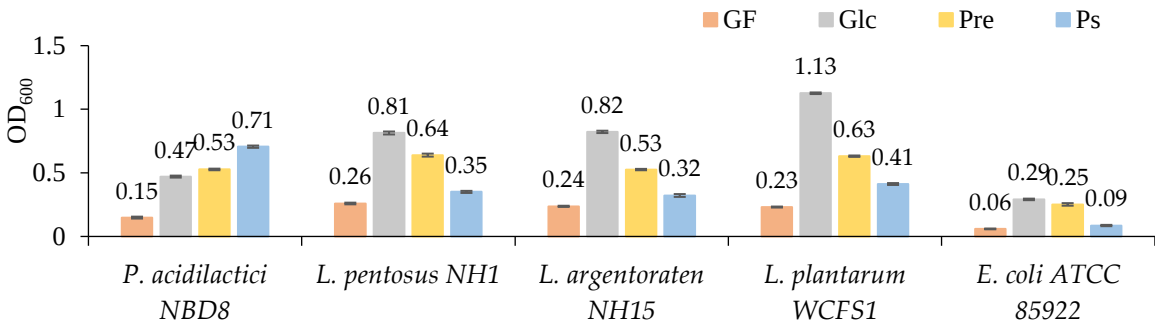

Figure 4. The optical density values of bacteria when cultivated in medium with different carbon sources at 37°C over 24 hours. Where GF: MRS medium without glucose, Glc: GF medium supplemented with 1 g/L glucose, Pre: GF medium supplemented with 1g/L commercial prebiotic; PS: GF medium supplemented with 1 g/L PS.

In treatments using PS as a carbon source, all probiotic strains showed positive prebiotic indices (ranging from 0.11 to 1.58). The highest prebiotic index was observed in the strain *P. acidilactici* NBD8 (1.58); the PI values of other the strains *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1 were 0.11, 0.17, and 0.30, respectively (Figure 5). The lower PI in the three *Lactiplantibacillus* strains could be due to their poorer growth in the medium supplemented with PS compared to the one supplemented with glucose. In the Pre medium, although the growth stimulation effect on the probiotic strains was higher than in the PS medium, the three *Lactiplantibacillus* strains showed negative prebiotic indices (–0.18; –0.32; and –0.41), and only the strain *P. acidilactici* NBD8 showed a positive PI (0.28). The reason for this negative value could be that the commercial prebiotic stimulated the growth of harmful microorganisms (*E. coli* ATCC 85922) as much as glucose did. According to Huebner (2007), strains *L. plantarum* 12006 and *L. acidophilus* 33200 showed negative PI when cultured on all prebiotics, except for purified GOS [63].

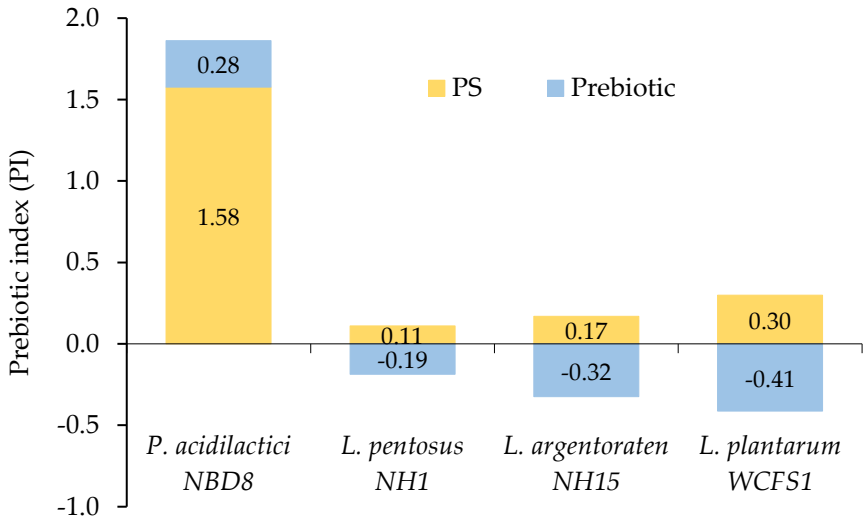

Figure 5. Selective prebiotic stimulation characteristics of bacterial strains through the prebiotic index.

This study also showed that the same type of PS can have different prebiotic indices in different bacterial strains. This can be explained by the fact that different microbial strains have different metabolic capabilities, leading to variations in prebiotic indices. Probiotic strains need specific hydrolytic enzyme systems to utilize prebiotics. Therefore, the genes encoding these metabolic systems may or may not be present in different strains, leading to different prebiotic activity indices. The hydrolytic enzyme systems play a crucial role in the ability of probiotic strains to use prebiotics.

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Each probiotic strain may have genetic variations and permutations in these systems, which can create diversity in their ability to utilize and metabolize prebiotics. This causes variations in prebiotic indices [64].

The positive prebiotic indices indicate that the level of PS extracted from the substrate after SSF with mycelium selectively promoted the growth of the strains *P. acidilactici* NBD8, *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1. Additionally, the strain *E. coli* ATCC 85922 was unable to use this PS as a carbon source for growth.

4. Conclusions

The blending ratio in the experimental formula containing 80% cassava and 20% soybean residues provided the most favorable conditions for the growth of the *P. ostreatus* mycelium, showing a rapid expansion after 3 days of cultivation (from 1.73 mm to 13.32 mm) and covering the entire plate surface after 9 days of cultivation (120 mm). The polysaccharide content extracted from the substrate after SSF reached 3.44 mg/g, increased by 2.1 times, with protein and mineral content increasing about 1.84 times compared to the initial substrate. The highest prebiotic index was observed in the strain *P. acidilactici* NBD8 (1.58); whereas, in the strains *L. pentosus* NH1, *L. argentoraten* NH15, and *L. plantarum* WCFS1, the indices were 0.11, 0.17, and 0.30, respectively. The SSF process using *P. ostreatus* mycelium holds potential as an effective method to enhance the nutritional and digestibility of soybean and cassava residues for application in the production of natural-origin animal feeds, as well as contributing to the circular economy in agriculture by fully utilizing by-products of the agricultural production industry.

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