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EXTRACTION AND EVALUATION OF THE PREBIOTIC ACTIVITY OF POLYSACCHARIDE FROM MUSHROOM MYCELIA (*Cordyceps militaris*, *Trametes versicolor*) AND THEIR BIOMASS APPLICATION IN FOOD SUPPLEMENT PRODUCTION

Major: Food Technology
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SUMMARY OF DOCTORAL DISSERTATION IN ENGINEERING

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The dissertation can be accessed at:

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PREFACE

1. Introduction

The rapid increase in chronic diseases associated with metabolic syndrome has highlighted the essential role of the gut microbiota and prebiotic compounds. Prebiotics not only serve as a selective substrate for the growth of beneficial bacteria, but their fermentation process also produces short-chain fatty acids (SCFAs) that protect the colon and prevent gastrointestinal disorders. In the quest for novel prebiotic sources with high efficacy and safety, mushrooms have emerged as highly promising candidates due to their diverse polysaccharide (such as β-glucan, mannan, and xylan). Specifically, polysaccharide from Cordyceps militaris and Trametes versicolor possess a stable structure, resist gastric digestion, and can selectively stimulate the proliferation of beneficial bacterial strains such as Lactobacillus and Bifidobacterium.

However, the current exploitation of bioactive compounds from these two mushroom species primarily relies on traditional fruiting body cultivation on solid substrates—a time-consuming and costly process that is difficult to standardize on an industrial scale. To address this issue, submerged fermentation (SmF) technology for harvesting mycelial biomass serves as a superior alternative, offering a significantly shortened growth cycle, easy standardization of environmental parameters, and optimized accumulation of bioactive polysaccharide. Despite this, most current studies still focus on extracting compounds from fruiting bodies, leaving the prebiotic potential of SmF-derived mycelial biomass as a significant, underexplored research gap. Based on these practical and scientific rationales, the study "**Extraction and evaluation of the prebiotic activity of polysaccharide from mushroom mycelium (*Cordyceps militaris, Trametes versicolor*), and initial application of biomass in dietary supplement processing**" was conducted.

2. Objectives

2.1. General objectives

To establish the scientific basis regarding the cultivation conditions, morphological characteristics, and biological activities (antioxidant and prebiotic) of polysaccharide fractions derived from the mycelia of *Cordyceps militaris* and *Trametes versicolor*; thereby evaluating the potential application of this fungal biomass in the development of bioactive fermented beverage supplements.

2.2. Specific objectives

- To optimize submerged cultivation conditions to maximize biomass yield and polysaccharide content from two fungal strains, *Cordyceps militaris* and *Trametes versicolor*.

- To evaluate and compare the extraction yield, morphological characteristics, and biological activities (prebiotic and antioxidant) of

polysaccharide fractions sequentially extracted using hot water (HWE), alkali (AE), and acid (AEc).

- To assess the potential of utilizing mycelial biomass in the processing of a lactic acid-fermented abalone mushroom beverage, enriched with probiotics and possessing health-promoting biological activities such as antioxidant and antibacterial properties.

3. Objects and Scope of the Study

- Research Objects: Mycelia of two strains, *Cordyceps militaris* and *Trametes versicolor*.

- Spatial Scope: The research is conducted at the Biology Laboratories, Faculty of Biology - Agriculture - Environment, The University of Da Nang - University of Science and Education, and the Faculty of Chemistry, The University of Da Nang - University of Science and Technology.

- Timeframe: The study is conducted over a 4-year period, from 2021 to 2025.

4. Research methodology

4.1. Theoretical research methods

Searching, collecting, and systematically reviewing domestic and international literature, textbooks, and scientific publications related to: mycelial cultivation and condition optimization; sequential extraction of polysaccharide (PS) fractions from plant and mycelial sources; evaluation of antioxidant and prebiotic activities of PS and other prebiotics; and the processing of fermented products.

4.2. Experimental research methods

- Optimization of culture conditions for polysaccharide-rich mycelial biomass production

+ One-Factor-At-a-Time (OFAT) method.

+ Response Surface Methodology (RSM).

- Evaluating the morphology, antioxidant, and prebiotic activities of the extracted PS fractions.

- Processing dietary supplement products.

- Data collection and statistical analysis.

5. Significance of the Study

5.1. Scientific Significance

- The thesis provides a comprehensive and systematic scientific dataset on the effects of nutritional (Carbon, Nitrogen) and environmental (pH) factors on the biomass and polysaccharide accumulation of *Cordyceps militaris* and *Trametes versicolor* mycelia under submerged cultivation.

- The study provides an in-depth analysis and comparison of sequentially extracted polysaccharide fractions (aqueous, alkaline, and acidic).

- The findings provide specific quantitative evidence regarding digestion resistance (anti-α-amylase activity), the prebiotic index (PI), and notably, the production of short-chain fatty acids (SCFAs - Acetate, Propionate, Butyrate) generated from fungal PS fermented by specific probiotic strains (*L. plantarum*, *L. casei*, *B. animalis*, *L. pentosus*, *P. acidilactici*). This strengthens the theoretical foundation for utilizing medicinal mushroom mycelia as a prebiotic source.

- The research establishes a scientific foundation for further in-depth studies on the molecular characteristics, chemical structures, and biological interactions of PS with the gut microbiome, as well as the discovery of novel PS molecules from mycelia.

5.2. Practical Significance

- The study establishes the optimal conditions for the submerged cultivation of *C. militaris* and *T. versicolor* to achieve high biomass yield and polysaccharide content. This provides a viable solution for generating organic biomass, ensuring a stable raw material supply for the food and pharmaceutical industries. Furthermore, submerged cultivation acts as a rapid propagation method, enhancing mushroom cultivation efficiency and modernizing cultivation technology.

- The applied research has successfully developed a Lactic acid-fermented abalone mushroom beverage supplemented with *Trametes versicolor* mycelia. This product exhibits excellent organoleptic properties, ensures food safety, and possesses verified biological activities (antibacterial and prebiotic). It serves as a practical product model with initial application potential in the dietary supplement sector, catering to the growing consumer trend towards green foods and gut health support.

6. Dissertation structure

The dissertation consists of 136 pages (excluding appendices and the reference list) and is organized as follows:

- Introduction (5 pages): presents the research rationale, objectives, scope and methodology, as well as the scientific and practical significance of the dissertation.
- Main content (4 chapters):

  Chapter 1: literature review (39 pages): provides an overview of previous studies relevant to the research topic.

  Chapter 2: materials and methods (23 pages): describes the materials, experimental design, and analytical methods employed in the study.

  Chapter 3: results and discussion (61 pages): presents the findings of the research, accompanied by in-depth analysis and critical

discussion.

- Conclusion and recommendations (4 pages).
- List of publications (2 pages): summarizes the scientific works published by the author related to the dissertation.
- References (22 pages): a total of 221 references in both English and Vietnamese, including peer-reviewed journal articles and scientific books.

The dissertation includes 24 tables and 28 figures/graphs to illustrate the results and analyses.

Chapter 1. Literature Review

1.1. Introduction to *Cordyceps militaris* and *Trametes versicolor* mushrooms

1.1.1. Taxonomical characteristics and traditional value

1.1.2. Major bioactive compounds of *Cordyceps militaris*

1.1.3. Major bioactive compounds of *Trametes versicolor*

1.2. Overview of polysaccharide in mushrooms

1.2.1. History and Definition of Prebiotics

The concept of "prebiotics" was first introduced by Gibson and Roberfroid in 1995 and was later refined in 1999. In 2017, an expert panel of the International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the official definition to incorporate new scientific insights and broaden its scope. Prebiotics are now defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit on the host".

1.2.2. Classification criteria and mechanisms of prebiotic

Three essential criteria underpin this definition. First, prebiotics must resist digestion and absorption in the small intestine. Second, they should be selectively fermented by specific beneficial microorganisms within the gut microbiota. Third, and most importantly, prebiotics must exert measurable physiological health benefits on the host.

1.2.3. Prebiotic Potential of Cordyceps militaris and Trametes versicolor

C. militaris and *T. versicolor* stand out as two representative species due to their structural diversity of Polysaccharide (PS), their ability to biosynthesize valuable bioactive compounds, and their wide application potential in functional foods and modern pharmaceuticals.

1.2.4. Current State of Prebiotic Research

1.2.4.1. Studise in Vietnam

Research focuses on cultivation and evaluating the quality and activity of fruiting bodies, with limited studies on fungal mycelia. Commercial prebiotic products in Vietnam are mainly plant-derived (e.g., FOS, GOS). Prebiotics remain a relatively new field in the country, requiring more extensive research.

1.2.4.2. *Global studies*

Numerous studies exist on production methods and the growth-stimulating effects on probiotics. However, most research focuses on plant-based sources. Currently, fungi are gaining attention as a new, safe, and effective source for prebiotic production.

1.3. Overview of Fungal Polysaccharide and Extraction Methods

1.3.1. Polysaccharide in Mushrooms and Mycelia

1.3.2. Methods for Extracting Polysaccharide from Fungi

1.3.3. Scientific Basis for Using HWE, AE, and AEc in Polysaccharide Extraction

a. Classicality, reliability, and solid scientific foundation.
b. Wide-spectrum recovery of various polysaccharide types.
c. Technical and economic feasibility in the Vietnamese context.
d. Stability and reduced risk of structural modification.
e. Economic aspects and research resources.

1.4. Submerged Fermentation (SmF)

1.4.1. Introduction to Submerged Fermentation

Submerged fermentation (SmF), also known as liquid fermentation, is a method of cultivating microorganisms in a nutrient-rich liquid medium. This process involves constant agitation and aeration to optimize cellular growth and/or the biosynthesis of metabolic compounds.

1.4.2. Advantages of submerged fermentation

Precise control of environmental conditions such as pH, temperature, dissolved oxygen concentration, agitation speed, and aeration; providing higher biomass yield in a shorter period of time; easier capacity for biomass recovery and active compound extraction; ease of scaling up through bioreactor systems with volumes ranging from several liters to thousands of liters, while maintaining the repeatability and stability of the product.

1.4.3. Influencing Factors in Submerged Fermentation

1.4.3.1. Culture Medium Composition

a. Carbon sources

b. Nitrogen sources

c. Micronutrients and minerals

1.4.3.2. Physical Conditions

a. Medium pH

b. Temperature

c. Dissolved oxygen and aeration

1.4.3.3. Mycelial Morphology and Metabolic Capacity

1.4.3.4. Biological Factors and Inoculation Conditions

a. Fungal strains

b. Inoculum age and inoculation ratio

1.5. Overview of Antioxidant Capacity

1.5.1. Concept and Mechanism

Antioxidant capacity is a term used to describe the ability of a substance or a biological system to neutralize free radicals and Reactive Oxygen Species (ROS), thereby protecting cells from oxidative damage (oxidative stress).

1.5.2. Antioxidant Capacity of Compounds in *Cordyceps militaris* and *Trametes versicolor*

Medicinal mushrooms have long been utilized in traditional medicine and are increasingly recognized as rich sources of natural antioxidants. Among them, *C. militaris* and *T. versicolor* are prominent due to their diverse bioactive compounds that combat oxidative stress through multiple synergistic mechanisms.

1.6. Rationale for the Selection of Abalone Mushroom and the Role of *Lactiplantibacillus plantarum* in Lactic Acid Fermentation

1.6.1. Rationale for Selecting Abalone Mushroom as a Fermentation Substrate

1.6.2. Lactic Acid Fermentation of Abalone Mushroom and Changes During the Fermentation Process

1.6.3. The Role of *Lactiplantibacillus plantarum* in Fermentation

Chapter 2. Materials and Methods

2.1. Materials

C. militaris and *T. versicolor*. *Lactiplantibacillus plantarum, Lactiplantibacillus casein* 01, *Bifidobacterria animalis* YC381, *Lactiplantibacillus pentosus* NH1, *Pediococci acidilactici* NBD8. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Commercial prebiotics FOS. *Pleurotus ostreatus*.

2.3.1. Chemicals and reagent

2.3.2. Equipment and tools

2.3. Research scope

2.4. Research diagram

2.5. Research methods for study contents

2.5.1. Content 1: Optimization of submerged cultivation conditions for biomass and polysaccharide production from mycelia

2.5.1.1. Mycelial cultivation methods

2.5.1.2. Experimental design

a. One-Factor-At-a-Time (OFAT) method

b. Response Surface Methodology (RSM)

2.5.1.3. Methods for harvesting and determining mycelial biomass yield

2.5.1.4. Methods for extraction and determination of mycelial polysaccharide (PS) content

2.5.2. Content 2: Sequential extraction and evaluation of the morphology, antioxidant, and prebiotic activities of PS extracts

2.5.2.1. Sequential extraction and quantification of mycelial polysaccharide fractions

2.5.2.2. Surface morphological analysis of the polysaccharide fractions

2.5.2.3. Evaluation of antioxidant capacity using the ABTS assay

2.5.2.4. Evaluation of the prebiotic potential of polysaccharide

2.5.3. Content 3: Application of mycelia in processing dietary supplement products

2.5.3.1. Safety evaluation of the mycelial powder

2.5.3.2. Production process of the fermented abalone mushroom beverage supplemented with *Trametes versicolor* mycelia

2.5.3.3. Methods for quality assessment of the fermented beverage product

- Determination of total acidity
- Evaluation of the $ABTS^+$ radical scavenging capacity of the abalone mushroom extract supplemented with *T. versicolor* mycelia
- Determination of *Lactobacillus plantarum* cell density
- Evaluation of growth inhibition against harmful microorganisms

Sensory evaluation

- Product safety and quality assessment

2.6. Data collection and statistical analysis

All experiments were conducted using a completely randomized design with three independent replications. Data comparison and statistical analysis were performed using Analysis of Variance (ANOVA) via Minitab 19.0 software. Differences were considered statistically significant at $p < 0.05$.

Chapter 3. Results and Discussion

3.1. Optimization of submerged cultivation conditions for the recovery of biomass and polysaccharide from fungal mycelia

3.1.1. Optimization of cultivation conditions for C. militaris mycelia

3.1.1.1. Effect of culture conditions on biomass and polysaccharide (PS) accumulation of C. militaris mycelium

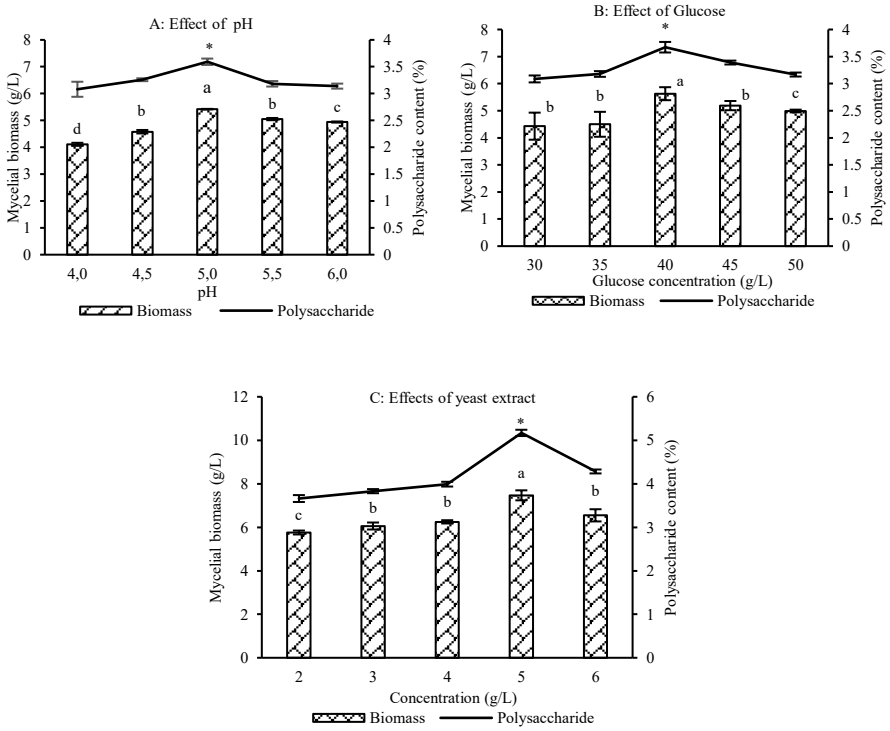


Figure 3.1. Effect of culture conditions (pH, glucose concentration, and yeast extract) in submerged medium on the mycelial biomass of *C. militaris*. A: Effect of pH; B: Effect of glucose; C: Effect of yeast extract.

3.1.1.2. Determination of optimal conditions for the recovery of biomass and polysaccharide from *C. militaris* mycelia

The mathematical models describing the relationship between biomass yield (Y_1) and PS content (Y_2) with the influencing factors are expressed as follows:

$Y1 = – 122,3 + 25,10\ X_1 + 1.514\ X_2 + 14,74\ X_3 – 2,450\ X_1^2 – 0,01910\ X_2^2 – 1,467\ X_3^2$

$Y2 = – 44,73 + 10,93\ X_1 + 0,6826\ X_2 + 3,51\ X_3 – 1,099\ X_1^2 – 0,00847\ X_2^2 – 0,339\ X_3^2$

Where: X1, X2, and X3 represent pH, Glucose, and Yeast extract, respectively

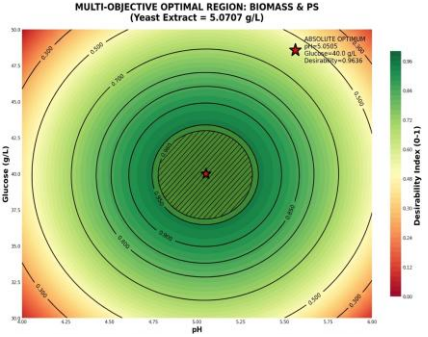


Figure 3.3. Multi-objective optimization analysis for *C. militaris*

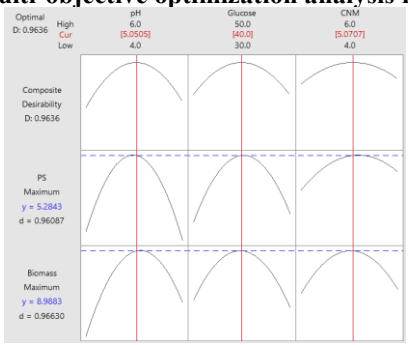
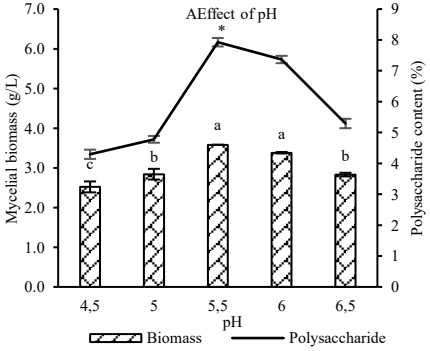


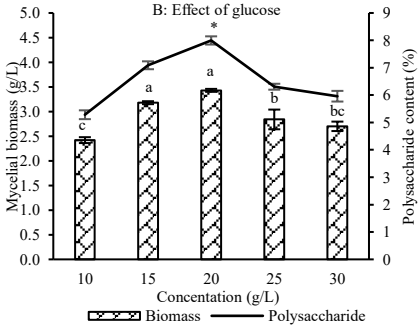
Figure 3.4. Predicted effects of factors on fungal biomass yield.

3.1.1.3. Experimental validation of the optimal conditions for *C. militaris* mycelium

3.1.2. Optimization of cultivation conditions for *T. versicolor* mycelia

3.1.2.1. Effects of single factors on the biomass yield and PS content of *T. versicolor* mycelia





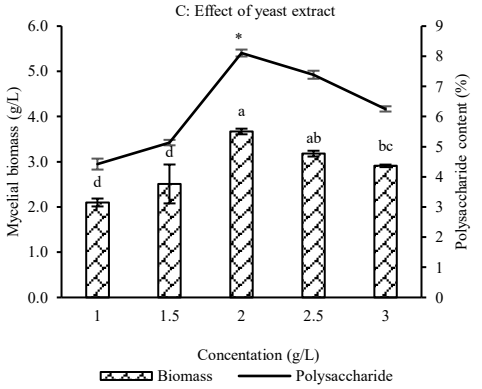


Figure 3.5. Independent effects of pH, glucose concentration, and yeast extract in submerged medium on T. versicolor mycelial biomass. A: Effect of pH; B: Effect of glucose; C: Effect of yeast extract.

3.1.2.2. Determination of optimal conditions for T. versicolor mycelial biomass and PS production

The mathematical models describing the relationship between biomass yield (Y_1) and PS content (Y_2) with the influencing factors are expressed as follows:

Y_1 (g/L) = –109,69 + 34,18 X_1 + 0,956 X_2 + 9,36 X_3 – 3,098 X_1^2 – 0,02378 X_2^2 – 2,318 X_3^2

Y_2 (%) = –232,2 + 73,52 X_1 + 1,962 X_2 + 14,02 X_3 – 6,587 X_1^2 – 0.04597 X_2^2 – 3,397 X_3^2

Where: X1, X2, and X3 represent pH, Glucose, and Yeast extract, respectively

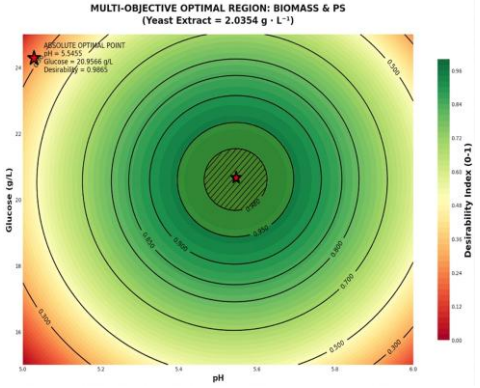


Figure 3.7. Multi-objective optimization analysis for *T. versicolor*

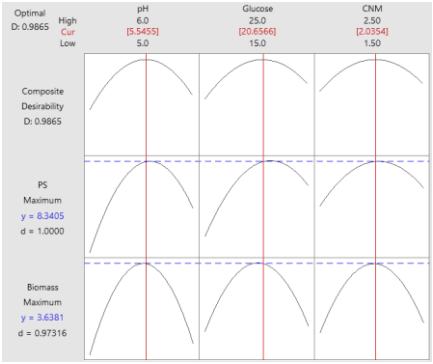


Figure 3.8. Multi-objective optimization plot of cultivation conditions for biomass and polysaccharide recovery

3.1.2.3. Experimental validation of optimal conditions for *T. versicolor* mycelium.

3.2. Sequential extraction and evaluation of the morphology, antioxidant, and prebiotic activities of PS extracts

3.2.1. Surface structural analysis of PS using scanning electron microscopy

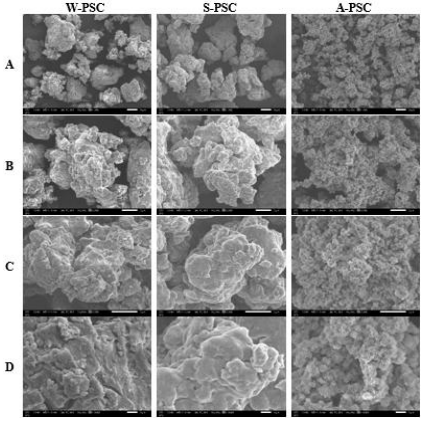


Figure 3.91. Scanning electron micrographs of *C. militaris* polysaccharide extracts at different magnifications: (A) 1000×; (B) 3000×; (C) 5000×; (D) 10,000×

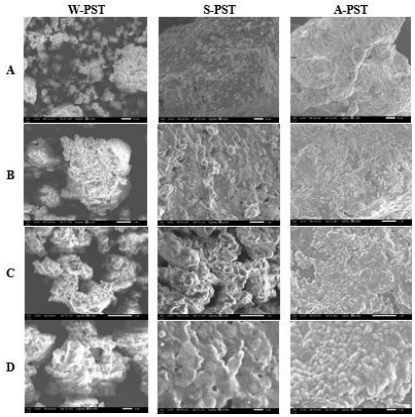


Figure 3.0. Scanning electron micrographs of *T. versicolor* polysaccharide extracts at different magnifications: (A) 1000×; (B) 3000×; (C) 5000×; (D) 10,000×.

3.2.2. Content of sequentially extracted polysaccharide from fungal mycelia

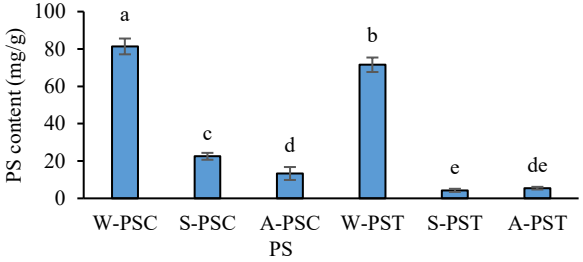


Figure 3.11. Content of PS extracted from *C. militaris* and *T. versicolor* mycelia

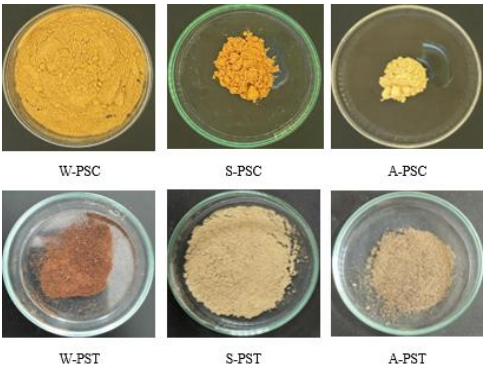


Figure 3.12. Fractionation of PS extracted from fungal mycelia.

3.2.3. ABTS radical-scavenging activity *of PS extracts from fungal mycelia*

Table 3.7. ABTS radical-scavenging activity of PS extracted from *C. militaris*.

PS extracts conc. (µg/mL)	ABTS radical-scavenging activity (%)		
	W-PSC	S-PSC	A-PSC
100	11.84±0.34^{a}	7.49±0.74^{b}	13.88±0.13^{a}
200	18.58±0.81^{a}	8.83±0.44^{b}	16.99±0.13^{a}
400	21.58±0.54^{a}	13.73±0.44^{b}	22.88±0.47^{b}
600	22.25±0.47^{b}	17.82±0.65^{c}	26.94±0.59^{a}
800	26.14±0.29^{b}	21.19±0.67^{c}	30.36±0.42^{a}
1000	31.37±0.91^{a}	24.54±0.34^{b}	32.59±0.37^{a}
2000	44.88±0.31a	36.70±0.18^{b}	46.91±0.27^{a}
5000	86.15±0.79^{a}	69.64±0.51^{b}	81.51±0.56^{a}
IC50 (µg/mL)	2474.24±2.78^{b}	3273.33±18.5^{a}	2446.51±24.34^{c}

Table 3.8. ABTS radical-scavenging activity of PS extracted from *T. versicolor*

PS extracts conc. (µg/mL)	ABTS radical-scavenging activity (%)		
	W-PST	S-PST	A-PST
100	6.86±1.3^{b}	10.14±1.7^{ab}	11.45±1.1^{a}
200	9.61±0.3^{c}	14.27±1.3^{b}	17.93±1.0^{a}
400	20.38±1.9^{b}	21.36±3.3^{b}	29.09±2.2^{a}
600	27.13±2.2^{b}	28.73±1.7^{b}	36.72±1.4^{a}
800	37.83±2.9^{b}	34.81±0.5^{b}	43.43±0.3^{a}
1000	44.21±1.7^{b}	43.86±0.5^{b}	49.63±0.4^{a}
2000	84.64±3.1^{a}	71.78±1.6^{c}	79.08±0.5^{b}
IC50 (µg/mL)	1142.5±21.72^{b}	1279.1±18.04^{a}	1070.6±18.25^{c}

In this assay, the IC_{50} values of the three PS extracts (W-PSC, S-PSC, and A-PSC) from *C. militaris* were determined to be 2474.24 ± 2.78 µg/mL, 2446.51 ± 24.34 µg/mL, and 3273.33 ± 18.54 µg/mL, respectively. Meanwhile, the corresponding IC_{50} values for *T. versicolor* were 1142.50 ± 21.72 µg/mL, 1279.10 ± 18.00 µg/mL, and 1070.60 ± 18.30 µg/mL.

3.2.4. Prebiotic potential of polysaccharide extracted from mycelia

3.2.4.1. Stimulatory effects on probiotic growth

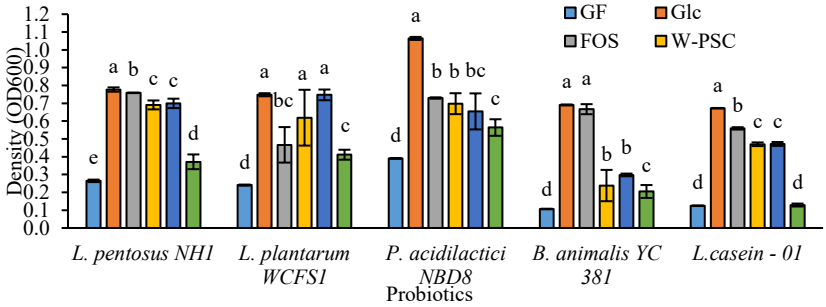


Figure 3.13. Growth of probiotic strains in media supplemented with PS from *C. militaris* mycelial extracts.

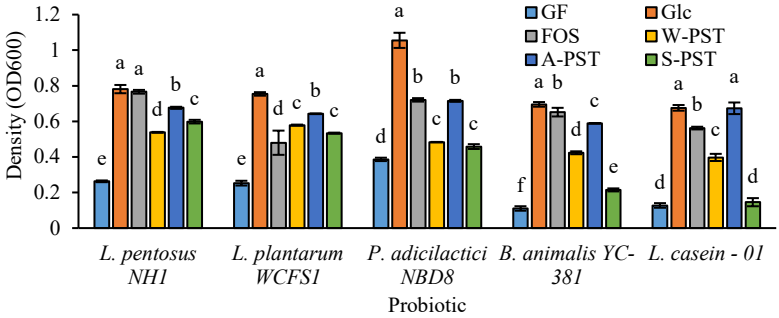


Figure 3.14. Growth of probiotic strains in media supplemented with PS from *T. versicolor* mycelial extracts

The results indicated that all probiotic strains tested were able to grow in the media supplemented with PS extracts..

3.2.4.2. Prebiotic index

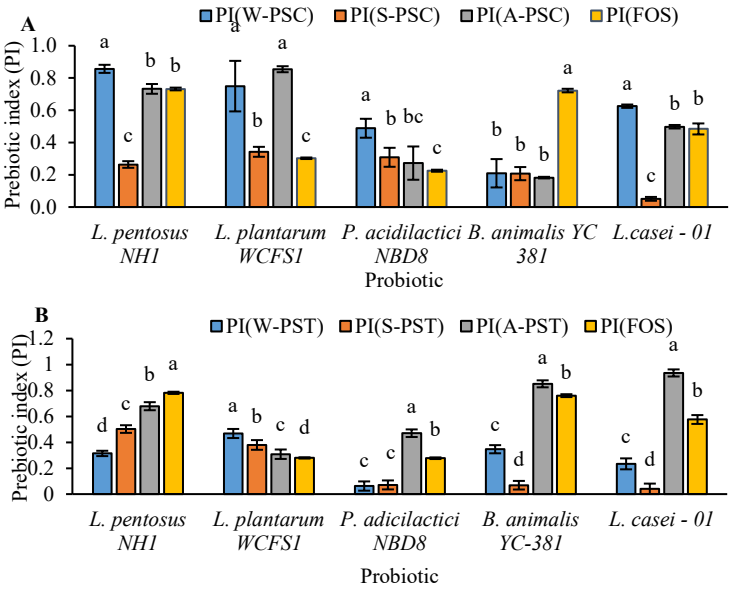


Figure 3.15. Prebiotic index (PI) of the polysaccharide fractions for the investigated probiotic strains. (A) Fractions from *C. militaris* mycelia; (B) Fractions from *T. versicolor* mycelia

The PI values of all PS extracts were greater than zero, confirming their ability to support the growth of beneficial microorganisms. However, notable variations were observed between different PS extracts and among probiotic strains, suggesting strain-specific and substrate-dependent effects.

3.2.4.3. Effects of PS extracts on SCFA production

a. SCFA concentrations in media supplemented with PS extracted from *C. militaris*

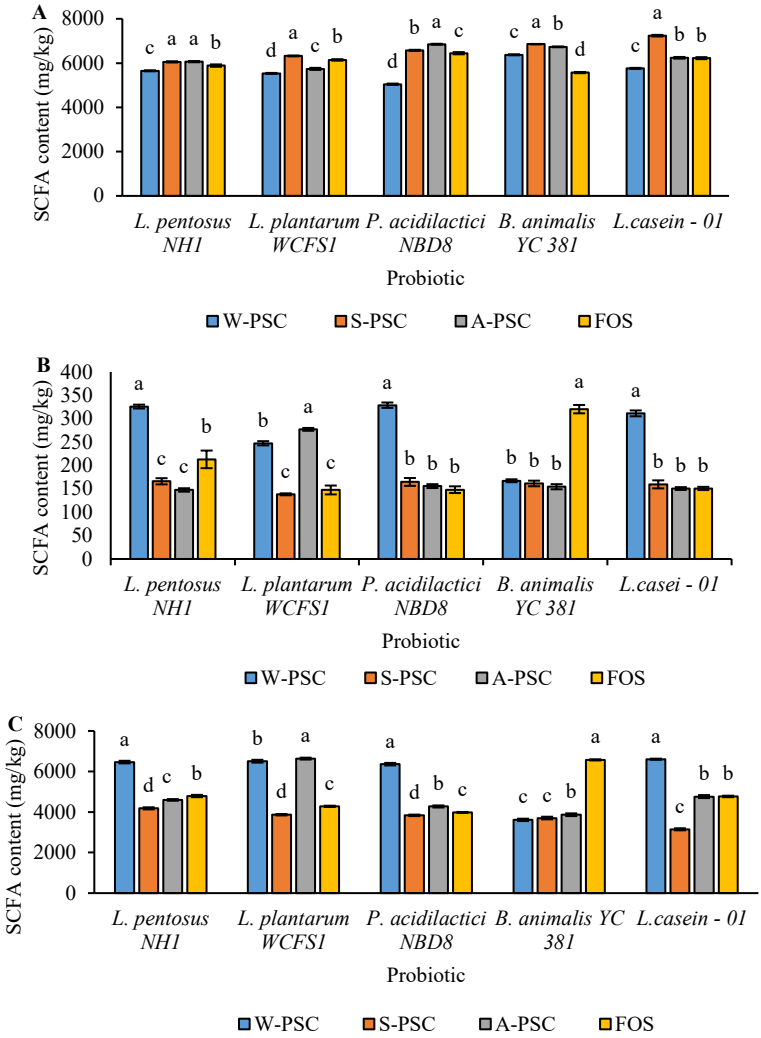


Figure 3.16. Effects of PS extracts on SCFA production: (A) butyric acid; (B) propionic acid; (C) acetic acid.

Acetic acid was the most abundant SCFA, ranging from 5044.35 to 7237.35 mg/kg, followed by butyric acid (3147.95–6634.99 mg/kg), while propionic acid was detected at the lowest levels (138.31–328.97 mg/kg).

b. SCFA concentrations in media supplemented with PS extracted from *T. versicolor*

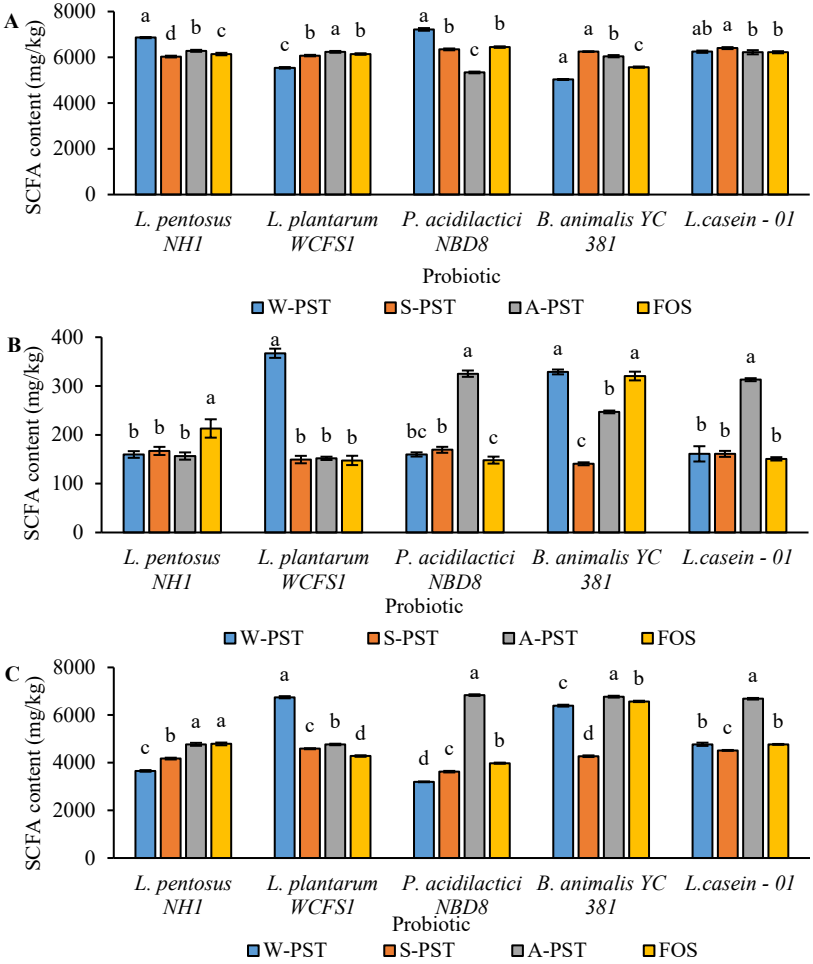


Figure 3.17. Effects of PS extracts on SCFA production: (A) acetic acid; (B) propionic acid; (C) butyric acid

The total and individual SCFA concentrations varied significantly among treatments with different probiotic strains supplemented with PS extracts. Overall, acetic acid was the most abundant SCFA, ranging from 5800 to 7200 mg/kg, followed by butyric acid (3200–7000 mg/kg). Propionic acid consistently showed the lowest concentrations, between 100 and 360 mg/kg.

3.2.4.4. Inhibitory activity against α-amylase

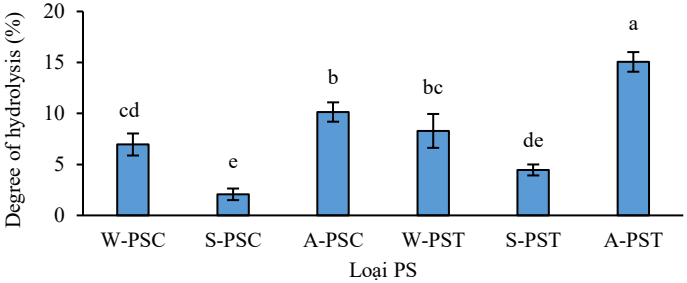


Figure 3.18. Percentage of PS hydrolyzed by α-amylase

The research results indicated that the degradation of PS extracts by α-amylase was relatively low, ranging from 2.07% to 15.05%. In both fungal strains, the S-PS fraction was less digested compared to the other two extracts.

3.2.4.5. Antimicrobial activity against pathogenic bacteria

a. Antibacterial activity of probiotic culture broth supplemented with the PS fraction extracted from C. militaris mycelia

Table 3.9. Inhibition zone diameters against *E. coli* and *S. aureus* (mm)

Treatment	*E. coli*		*S. aureus*	
	24h	48h	24h	48h
CT1	27.06 ± 1.21^{a}	27.68 ± 0.62^{ab}	26.93 ± 0.50^{a}	25.01 ± 0.35^{b}
CT2	25.67 ± 0.67^{a}	27.21 ± 0.40^{ab}	26.47 ± 0.30^{a}	23.80 ± 0.47^{c}
CT3	27.00 ± 0.38^{a}	27.11 ± 0.39^{b}	24.88 ± 0.40^{b}	24.63 ± 0.42^{bc}
CT4	26.49 ± 0.92^{a}	28.66 ± 0.82^{a}	27.22 ± 0.66^{a}	26.27 ± 0.38^{a}
CT5	22.47 ± 0.54^{b}	25.56 ± 0.48^{c}	23.42 ± 0.38^{c}	22.35 ± 0.49^{d}

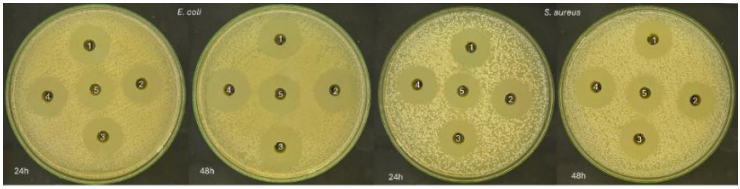


Figure 3.19. Antimicrobial activity of *L. plantarum* culture supernatants against *E. coli* ATCC 85922 and *S. aureus* ATCC 25023

b. Antibacterial activity of probiotic culture broth supplemented with the PS fraction extracted from *T. versicolor* mycelia

Table 3.10. Inhibition zone diameters against *E. coli* and *S. aureus* (mm).

Treatment	*E. coli*		*S. aureus*	
	24h	48h	24h	48h
1	28.28 ± 0.30^{a}	27.88 ± 0.76^{a}	28.47 ± 0.81^{a}	27.04 ± 0.25^{b}
2	28.47 ± 0.85^{a}	26.91 ± 0.39^{a}	27.35 ± 0.68^{ab}	26.05 ± 0.35^{c}
3	28.48 ± 0.62^{a}	27.74 ± 0.29^{a}	26.60 ± 0.56^{b}	27.49 ± 0.50^{ab}
4	28.86 ± 0.34^{a}	27.65 ± 0.92^{a}	27.53 ± 0.25^{ab}	28.19 ± 0.15^{a}
5	23.86 ± 0.74^{b}	24.33 ± 0.34^{b}	23.57 ± 0.50^{c}	24.21 ± 0.20^{d}

At both 24 h and 48 h, all formulations supplemented with PS extracts and FOS exhibited significantly stronger antimicrobial activity compared to the non-supplemented control ($p < 0.05$).

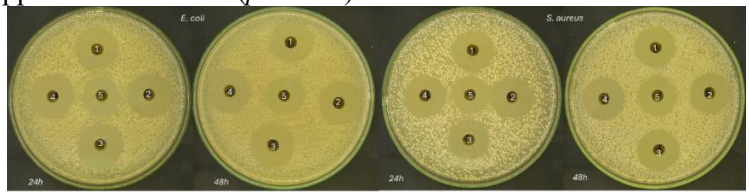


Figure 3.20. Antimicrobial activity of *L. plantarum* culture supernatants against *E. coli* ATCC 85922 and *S. aureus* ATCC 25023.

3.3. Application of fungal mycelia in the development of dietary supplements

3.3.1. Safety evaluation of mycelial powder

Table 3.111. Results of biosafety assays for fungal mycelial powder

No.	Parameters	Unit	Test methods	Results	Allowable threshold
1	Pb	mg/kg	AOAC 999.11	0,13	5
2	Cd	mg/kg	AOAC 999.11	0,11	3
3	Hg	mg/kg	AOAC971.21	ND (MDL=0,01)	0,1
4	As	mg/kg	AOAC986.15	ND (MDL=0,01)	2
5	Total Aflatoxin (B1, B2, G1, G2)	µg/kg	AOAC991.31	ND (MDL=1,0)	10
6	Total aerobic microorganisms	CFU/g	TCVN4884-1:2015	ND (<10)	10
7	Colifoms	CFU/g	TCVN 6848:2007	ND (<10)	10
8	Total yeast mold spores	CFU/g	TCVN8275-2:2020	ND (<10)	10
9	*Salmonella*	CFU/g	TCVN 10780-1:2017	negative/25g	Not detect

3.3.2. Effects of mycelial powder supplementation on the quality of fermented oyster mushroom beverage

3.3.2.1. Effects of mycelial biomass supplementation ratio on the antioxidant activity of the fermented beverage

Table 3.12. $ABTS^{\bullet+}$ radical-scavenging activity (%) of oyster mushroom extracts supplemented with fermented *T. versicolor* mycelia

Treatment	Fermentation duration			
	Initial	12 h	Initial	48 giờ
CT1	25.98 ± 0.51^{f}	26.05 ± 0.31^{f}	25.63 ± 0.53^{f}	25.46 ± 0.51^{f}
CT2	35.52 ± 0.88^{e}	34.44 ± 0.86^{e}	33.48 ± 0.59^{e}	32.45 ± 0.71^{e}
CT3	40.34 ± 0.89^{d}	39.39 ± 0.89^{d}	37.83 ± 0.46^{d}	37.66 ± 0.47^{d}
CT4	46.49 ± 0.81^{c}	48.6 ± 0.86^{c}	44.43 ± 1.25^{c}	43.27 ± 0.42^{c}

CT5	54.43 ± 1.38[b]	54.83 ± 1.07[b]	47.07 ± 0.56[b]	46.90 ± 0.38[b]
CT6	59.69 ± 0.38[a]	58.13 ± 2.42[a]	49.63 ± 1.01[a]	48.44 ± 0.21[a]

The results showed that, across all experimental treatments, antioxidant activity increased proportionally with the amount of *T. versicolor* mycelia supplemented into oyster mushroom extracts. Radical-scavenging capacity increased from 25.84% in CT1 to 59.79% in CT6 immediately after supplementation; from 25.92% to 57.79% after 12 h of fermentation; from 25.85% to 49.57% after 24 h; and from 25.51% to 48.35% after 48 h.

3.3.2.2. Effects of mycelial biomass supplementation ratio on the growth of probiotics

Table 3.13. Cell density of *L. plantarum* in fermented oyster mushroom beverages (log CFU/mL).

Treatment	Fermentation duration			
	Initial	12 h	24 h	48 h
CT1	8.38 ± 0.04[a]	10.9 ± 0.04[d]	10.99 ± 0.12[b]	10.97 ± 0.09[c]
CT2	8.38 ± 0.06[a]	11.19 ± 0.13[c]	11.4 ± 0.15[a]	11.21 ± 0.24[b]
CT3	8.39 ± 0.01[a]	11.21 ± 0.12[c]	11.51 ± 0.18[a]	11.5 ± 0.12[a]
CT4	8.38 ± 0.03[a]	11.50 ± 0.16[a]	11.55 ± 0.17[a]	11.51 ± 0.16[a]
CT5	8.36 ± 0.02[a]	11.41 ± 0.23[ab]	11.52 ± 0.19[a]	11.38 ± 0.15[a]
CT6	8.36 ± 0.05[a]	11.28 ± 0.26[bc]	11.56 ± 0.12[a]	11.46 ± 0.17[a]

After 12 h of fermentation, the density of *L. plantarum* increased markedly in all treatments compared to the initial level. The highest density was recorded in CT4, reaching 11.49 log CFU/mL.

3.3.2.3. Inhibition of pathogenic bacteria

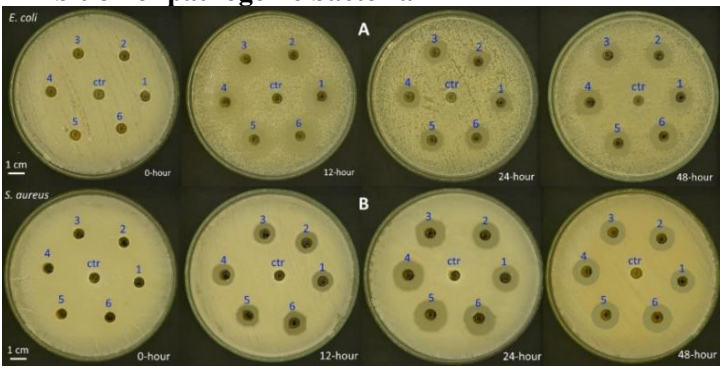


Figure 3.21. Antibacterial activity of fermented beverages against *E. coli* ATCC 85922 (A) and *S. aureus* ATCC 25023 (B).

Table 3.14. Inhibition zone diameters (mm) of fermented beverages against *E. coli* and *S. aureus* at different fermentation times

Treatments	12 h	24 h	48 h
	Inhibition zone diameters (mm)		
E. coli			
CT1	6.53 ± 0.44^{d}	8.8 ± 0.64^{c}	6.95 ± 0.77^{e}
CT2	7.95 ± 0.45^{c}	8.98 ± 0.29^{c}	9.18 ± 0.61^{d}
CT3	7.78 ± 0.37^{c}	10.54 ± 0.64^{a}	11.07 ± 0.37^{c}
CT4	8.07 ± 0.35^{c}	9.58 ± 0.38^{b}	12.26 ± 0.36^{b}
CT5	10.39 ± 0.70^{b}	10.45 ± 0.28^{a}	11.88 ± 0.52^{b}
CT6	11.71 ± 0.40^{a}	10.97 ± 0.56^{a}	13.42 ± 0.47^{a}
S. aureus			
CT1	7.55 ± 0.40^{d}	11.77 ± 0.72^{d}	9.74 ± 0.53^{d}
CT2	8.31 ± 0.94^{bc}	11.76 ± 0.62^{d}	11.08 ± 1.26^{c}
CT3	7.81 ± 0.44^{cd}	13.97 ± 0.86^{c}	11.35 ± 0.50^{c}
CT4	8.02 ± 0.52^{bcd}	15.31 ± 0.88^{b}	12.38 ± 0.59^{b}
CT5	8.62 ± 0.50^{b}	16.13 ± 1.21^{ab}	12.55 ± 0.70^{b}
CT6	9.41 ± 0.67^{a}	16.65 ± 0.74^{a}	13.58 ± 0.91^{a}

3.3.2.4. Sensory quality of the fermented product

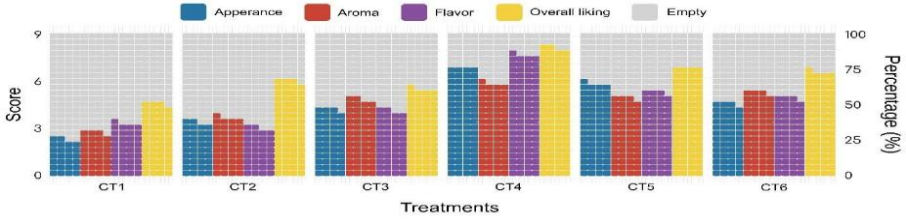


Figure 3.22. Waffle chart of sensory evaluation of fermented beverage after 24 h.

The sensory evaluation results indicated that the formulation supplemented with 0.6% T. versicolor mycelia and fermented for 24 h (CT4-24) achieved the highest overall acceptability score (7.0 ± 0.63).

3.3.2.5. Changes in pH and total titratable acidity (TTA)

Table 3.15. Changes in pH of oyster mushroom extracts supplemented with *T. versicolor* mycelia during fermentation

Treatment	Fermentation duration			
	Initial	12 h	Initial	48 giờ
CT1	5.62 ± 0.17	3.40 ± 0.14^{a}	3.19 ± 0.03^{a}	3.06 ± 0.02^{b}
CT2	5.28 ± 0.27	3.35 ± 0.05^{b}	3.15 ± 0.06^{b}	3.05 ± 0.01^{c}
CT3	5.10 ± 0.12	3.34 ± 0.02^{b}	3.14 ± 0.03^{c}	3.04 ± 0.05^{c}
CT4	4.89 ± 0.13	3.32 ± 0.02^{c}	3.14 ± 0.05^{c}	3.05 ± 0.04^{b}
CT5	4.90 ± 0.07	3.33 ± 0.02b^{c}	3.15 ± 0.03^{b}	3.07 ± 0.03^{a}
CT6	4.72 ± 0.09	3.33 ± 0.03^{bc}	3.16 ± 0.05^{b}	3.07 ± 0.02^{a}

Table 3.16. Changes in total titratable acidity (calculated as lactic acid) of oyster mushroom extracts supplemented with *T. versicolor* mycelia during fermentation

Treatment	Fermentation duration			
	Initial	12 h	Initial	48 giờ
CT1	1.362 ± 0.074^{f}	3.702 ± 0.118^{f}	6.611 ± 0.101^{f}	8.026 ± 0.059^{f}
CT2	1.437 ± 0.026^{e}	4.244 ± 0.128^{e}	7.218 ± 0.100^{e}	9.158 ± 0.132^{e}
CT3	1.606 ± 0.030^{d}	5.466 ± 0.049^{d}	7.435 ± 0.041^{d}	9.442 ± 0.099^{d}
CT4	1.744 ± 0.028^{c}	5.782 ± 0.034^{c}	7.825 ± 0.071^{c}	9.769 ± 0.045^{c}
CT5	1.875 ± 0.043^{b}	6.017 ± 0.089^{b}	8.16 ± 0.059^{b}	10.362 ± 0.106^{b}
CT6	2.021 ± 0.036^{a}	6.617 ± 0.027^{a}	8.643 ± 0.033^{a}	10.678 ± 0.027^{a}

3.3.2.6. Safety evaluation of the fermented mushroom beverage supplemented with mycelia

Table 3.17. Results of microbiological and chemical safety assessment of fermented beverages

No.	Parameters	Allowable threshold (TCVN 13368:2021)	Results	Test methods
1	Total aerobic microorganisms	100 (CFU/ml)	ND (<1)	TCVN 4884-1:2015
2	Total yeast and mold spores	10 (CFU/ml)	ND (<1)	TCVN 8275-1:2010
3	*Coliforms*	10 (CFU/ml)	ND (<1)	TCVN 6848:2007
4	*E. coli*	ND	ND (<1)	TCVN 7924-2:2008
5	Coagulase-positive staphylococci (*S. aureus* and other species)	ND	ND (<1)	ISO 6888-1:2021
6	*Cl. perfringens*	ND	ND (<1)	TCVN 4991:2005
7	Cadmi (Cd)	1 (mg/kg)	<0,05	AOAC 999.11
8	Chì (Pb)	0,05 (mg/kg)	<0,05	AOAC 999.11
9	Thủy Ngân (Hg)	0.05 (mg/kg)	<0,05	AOAC 971.21

Conclusions and Recommendations

A. CONCLUSIONS

The thesis has successfully accomplished its proposed research objectives, focusing on evaluating the in vitro biological activities of polysaccharide (PS) obtained from mycelial biomass cultivated in submerged conditions, including prebiotic activity, antioxidant capacity, and their application orientation in food products. From the achieved results, the following main conclusions can be drawn:

- Identified several crucial optimal conditions for submerged cultivation to harvest high-yield, polysaccharide-rich biomass.

• For Cordyceps militaris: The optimal conditions are pH 5.05, glucose concentration of 40 g/L, and yeast extract of 5.07 g/L. Under these conditions, the dry biomass yield reached 8.90 ± 0.25 g/L, and the PS content reached 5.28 ± 0.16%.

• For Trametes versicolor: The optimal conditions are pH 5.545, glucose concentration of 20.65 g/L, and yeast extract of 2.0354 g/L. Under these conditions, the dry biomass yield reached 3.59 ± 0.12 g/L, and the PS content reached 8.23 ± 0.27%.

- Evaluation of extraction efficiency, surface structural characteristics, and biological activities of Polysaccharide extracts:

• Extraction yield: The aqueous solvent demonstrated a high polysaccharide recovery yield in both strains (81.35 mg/g for C. militaris and 71.53 mg/g for T. versicolor). Sequential extraction further recovered PS in alkaline and acidic environments with distinct antioxidant and prebiotic activities.

• Surface morphology: Extraction conditions significantly influenced the surface structure. While the aqueous and alkaline-extracted fractions exhibited a clumped block-like structure, the acidic (A-PST) and alkaline (S-PST) extracted fractions of T. versicolor displayed specific compact or fine-grained structures.

• ABTS radical scavenging capacity: All PS fractions exhibited ABTS radical scavenging capacity. PS from T. versicolor showed stronger activity (IC_{50} ranging from 1070 – 1279 µg/mL) compared to C. militaris (IC_{50} ranging from 2446 – 3273 µg/mL).

• Prebiotic potential: The PS fractions demonstrated distinct prebiotic potential through positive PI indices and high resistance to alpha-amylase hydrolysis (hydrolysis degree ranging only from 2.07 – 15.05%). The most prominent were the W-PSC fraction (on the L. pentosus NH1 strain, PI = 0.857) and A-PST fraction (on the L. casei 01 strain, PI = 0.935).

• Mechanism of action: The supplementation of PS not only promoted the growth of beneficial bacteria but also enhanced the biosynthesis of short-chain fatty acids (SCFAs) and improved the inhibitory efficiency against pathogenic bacteria (E. coli, S. aureus).

- Practical application in fermented beverage products:

Successfully developed a formula for processing lactic acid-fermented abalone mushroom beverage supplemented with *T. versicolor* mycelial biomass:

• Optimal formula: Supplementation of 0.6% mycelial biomass, fermentation time of 24 hours.

• Product quality: The product achieved high sensory values in color and flavor while possessing good biological activities. Food safety indicators (microbiology, heavy metals) were all within the permissible limits according to QCVN 20-1:2024/BYT.

B. NOVEL CONTRIBUTIONS OF THE THESIS

The research findings of the thesis have been identified as making the following novel academic and practical contributions:

* *Academic contributions:*

- Systematizing the database on submerged cultivation technology: Established several optimal technological conditions for the submerged cultivation process of two mushroom strains, Cordyceps militaris and Trametes versicolor, directed towards the biosynthesis of polysaccharide-rich biomass. This provides a crucial scientific basis for explaining the effects of environmental conditions (pH, C, N sources) on biomass and polysaccharide accumulation in fungal cells.

- Elucidating the efficiency of the sequential extraction method: Demonstrated the efficacy of the sequential extraction method in separating polysaccharide groups based on structural characteristics and solubility. A novel contribution lies in the discovery that the acid-extracted polysaccharide fraction (A-PS) — which has received little attention in previous studies — exhibited potential prebiotic activity (highest PI index with certain probiotics) compared to other fractions. This opens a new approach to exploiting tightly bound polysaccharide in the fungal cell wall.

- The thesis's findings serve as a foundation for further purification, extraction, and discovery of novel polysaccharide from mycelial biomass with specific antioxidant and prebiotic activities.

- Providing data on the prebiotic potential of mycelia: Provided reliable experimental evidence proving the in vitro prebiotic activity of mycelial PS: indigestibility, ability to selectively stimulate beneficial bacterial strains (Lactobacillus, Bifidobacterium, etc.) and inhibit harmful bacteria (E. coli, S. aureus) through the mechanism of short-chain fatty acid (acetic, propionic, butyric acids) production.

* *Practical contributions:*

- Proposing technological solutions for a proactive raw material source: The thesis proved the feasibility of submerged cultivation for harvesting mycelial biomass. This is a highly potential method to generate valuable organic raw materials for the food and pharmaceutical industries due to its

stability, high yield, short duration, scalability for industrial production, and ease of cultivation condition control and automation. This is an important prerequisite for diversifying raw material production models alongside traditional fruiting body cultivation.

- Diversifying prebiotic raw material sources in the market: Currently, prebiotics on the market are primarily produced from plants (FOS, inulin) and seaweed (fucoidan, carrageenan). The research results indicate that mycelial biomass is also a potential prebiotic source whose cultivation conditions can be proactively controlled to obtain products on an industrial scale.

- Successfully developing a probiotic fermented beverage product line rich in antioxidant activity: Successfully established a technological process for producing fermented beverages from abalone mushroom supplemented with Trametes versicolor mycelial biomass. The product met microbiological and chemical safety standards, demonstrating the successful application of a model combining Prebiotics (mycelia) and Probiotics (fermenting bacteria) to create high-value-added dietary supplements.

C. RECOMMENDATIONS AND FUTURE RESEARCH DIRECTIONS

- Analyze the molecular structure using FTIR/NMR methods, molecular weight, and monosaccharide composition of each PS fraction to discover novel PS soluble in different solvents.

- Investigate and further elucidate the biological activities of PS: anti-diabetic, anti-inflammatory, anti-cancer, and immune-enhancing properties.

- Conduct in vivo studies to evaluate the prebiotic activity of mycelial PS to clarify the mechanism of action and confirm the prebiotic efficacy of mycelia on the gut microbiome and host health.

- Evaluate product stability and research the potential for expanding the production scale.

LIST OF SCIENTIFIC PUBLICATIONS

Scientific Research Projects:

1. Principal Investigator of City-level grassroots project: Research on cultivating Trametes versicolor mushroom on Acacia auriculiformis wood substrate and processing some health supplement products from Trametes versicolor mushroom in Da Nang city – Accepted in 2021.

2. Secretary Member of Murata University-level grassroots project: Investigation of prebiotic activity of polysaccharide extracted from Cordyceps militaris mycelia – Accepted in 2023.

3. Key Member of City-level project: Application of single-spore hybridization technology to select and breed Cordyceps militaris strains for high yield and quality – Accepted in 2024.

4. Principal Investigator of University-level project: Evaluation of antioxidant and prebiotic activities of Trametes versicolor mycelia under submerged cultivation and application in processing fermented mushroom beverage – Accepted in 2026.

Domestic and International Journal Articles:

1. Nguyen Thi Bich Hang, Doan Chi Cuong, Truong Cong Phat, Bui Duc Thang, Do Phu Huy, Dang Minh Nhat (2024), Developing a health-suppoting fermented lactic beverage from oyster mushrooms (Pleurotus ostreatus). The New Diverse Facets of Sensory Evaluation (SPISE), 127-138.

2. Nguyen Thi Bich Hang, Dang Minh Nhat, Bui Duc Thang, Vo Van Minh, Nguyen Sy Toan, Munehiro Tanaka, Doan Chi Cuong (2025), Optimization of Lactic Fermented Beverages: Integrating Trametes versicolor Mycelium and Pleurotus ostreatus Extract for Enhanced Functional Properties, Mycobiology, Volume 53, Issue 4, 1-14.

3. Hang Thi-bich Nguyen, Cuong Chi Doan, Thang Duc Bui, Nhat Minh Dang (2026), Prebiotic Properties of Polysaccharide Extracts from Cordyceps militaris Mycelium: Potentials for Functional Food and Drink Applications, Journal of Applied Biology & Biotechnology. Vol. 14(1), pp. 46-55, January-February, 2026.

4. Nguyen Thi Bich Hang, Dang Minh Nhat, Doan Chi Cuong, Bui Duc Thang (2023), Prebiotic properties of polysaccharide isolated from Cordyceps militaris mycelia, Vietnam Trade and Industry Review, 4, 413-420.

Monographs (Chapters 1, 3, 5)

Monograph: Mycelial Polysaccharide: Biosynthesis, Characteristics, and Applications; Chief Editor: Doan Chi Cuong, Co-authors: Nguyen Thi Bich Hang, Dang Minh Nhat, Vo Chau Tuan, Bui Duc Thang. Hue University Publishing House, 2026, ISBN: 978-632-627-353-3.