

THE UNIVERSITY OF DANANG
UNIVERSITY OF SCIENCE AND TECHNOLOGY

NGUYEN THI BICH HANG

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

**Major: Food Technology
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**SUMMARY OF DOCTORAL DISSERTATION IN
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SUPERVISORS

- 1. Assoc.Dr. DANG MINH NHAT**
- 2. Dr. NGUYEN HOANG DUNG**

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PREFACE

1. Introduction

Cordyceps militaris and *Trametes versicolor* are two highly valued medicinal mushrooms, known not only for their bioactive compounds with antioxidant, anticancer, and immunomodulatory properties but also for their potential to modulate gut microbiota through prebiotic effects. In addition to fruiting body cultivation, research on mycelial biomass production has demonstrated significant advantages in terms of both economic feasibility and medicinal value. Submerged fermentation for mycelial biomass or inoculum production offers several benefits, including shortened cultivation time and improved controllability for industrial-scale applications. Despite these promising prospects, the exploitation of polysaccharides (PS) derived from fungal mycelia still faces several challenges. Cultivation conditions exert a profound influence on fungal growth and PS biosynthesis; therefore, optimization of key factors such as carbon source, pH, temperature, agitation rate, and incubation time is essential to maximize both biomass yield and polysaccharide production.

Prebiotics are fermentable food components, such as non-digestible polysaccharides (e.g., β -glucans) and dietary fibers, that induce beneficial changes in the gut microbiota, thereby improving host nutrition and overall health. However, most commercially available prebiotics currently incorporated into functional foods to promote gut health are primarily derived from plant sources. In contrast, the prebiotic potential of extracts from medicinal mushrooms, particularly those obtained from fungal mycelia, remains insufficiently explored, especially with regard to their ability to stimulate the growth of beneficial probiotic bacteria such as *Lactobacillus* and *Bifidobacterium*. Elucidating this potential would not only enhance the value of medicinal mushrooms as functional food ingredients but also open new avenues for the development of natural products that support digestive health, strengthen immune function, and contribute to disease prevention. Therefore, we propose the study entitled: “Extraction and evaluation of the prebiotic activity of polysaccharides from mushroom mycelia (*Cordyceps militaris* and *Trametes versicolor*) and their biomass application in food supplement production”.

2. Objectives

2.1. General objectives

Establishing the scientific basis regarding the cultivation conditions, morphology, and bioactivities (antioxidant and prebiotic) of polysaccharide fractions from *C. militaris* and *T. versicolor* mycelia, and subsequently assessing the application of this biomass in producing bioactive-rich fermented functional beverages

2.2. Specific objectives

- To optimize the submerged fermentation conditions for mycelial growth,

aiming to maximize both the biomass yield and polysaccharide content from the two fungal strains, *Cordyceps militaris* and *Trametes versicolor*.

- To evaluate and compare the extraction yield, morphological characteristics, and bioactivities (prebiotic and antioxidant) of polysaccharide fractions obtained through sequential extraction using hot water (HWE), alkali (AE), and acid (AEc).

- To investigate the potential application of the mycelial biomass in developing a lactic acid-fermented oyster mushroom beverage that is rich in probiotics and possesses health-promoting bioactivities, including antioxidant and antibacterial properties

3. Materials and Scope

- Mushroom strains: The study utilized two fungal mycelium strains: *Cordyceps militaris* and *Trametes versicolor*.

- Study location: The research was conducted at the Biology laboratories, Faculty of Biology - Agriculture - Environment, University of Science and Education, and the Faculty of Chemistry, University of Science and Technology – The University of Da Nang.

- Study period: The study was carried out over a four-year period, from 2021 to 2025.

4. Study contents

Optimizing submerged fermentation conditions to harvest mycelial biomass and polysaccharides. Sequentially extracting and evaluating the morphological characteristics, antioxidant, and prebiotic activities of the polysaccharide extracts. Applying the fungal mycelia in the development of dietary supplements.

5. Scientific and practical significance

5.1. Scientific significance

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

The thesis delves into a comparative analysis of sequentially extracted polysaccharides (using water, alkali, and acid). The findings reveal and affirm the critical role of the acid-extracted fraction (A-PS) in prebiotic activity (exhibiting a high Prebiotic Index - PI and robust SCFA production). This offers a novel perspective on selecting appropriate extraction methods to tailor the functionality of target products, moving beyond the conventional reliance solely on traditional hot water extraction.

The research outcomes offer specific quantitative evidence on digestion resistance (against α -amylase), the Prebiotic Index (PI), and notably, the concentration of short-chain fatty acids (SCFAs - Acetate, Propionate, Butyrate) generated from the fermentation of fungal polysaccharides by

specific probiotic strains (*Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Bifidobacterium animalis*, *Lactiplantibacillus pentosus*, *Pediococcus acidilactici*). This significantly strengthens the theoretical foundation for utilizing medicinal mushroom mycelia as a viable prebiotic source.

Furthermore, the study establishes a robust scientific groundwork for further in-depth investigations into the molecular characteristics, chemical structures, and biological interactions of these polysaccharides within the gut ecosystem, as well as paving the way for the discovery of novel polysaccharide molecules from fungal mycelia.

5.2. Practical significance

- Establishing optimal conditions for the submerged fermentation process of *C. militaris* and *T. versicolor* to achieve high biomass yields and polysaccharide contents. This serves as a viable technological solution to generate an organic biomass source, proactively ensuring a stable raw material supply for the food and pharmaceutical industries. Furthermore, submerged fermentation acts as a rapid propagation method, enhancing the productivity and efficiency of mushroom cultivation, thereby modernizing current cultivation technologies.

- Diversifying health-promoting mushroom-based food products: The applied research has successfully developed a "Lactic acid-fermented oyster mushroom beverage supplemented with *Trametes versicolor* mycelia." This product exhibits excellent sensory qualities, ensures food hygiene and safety, and possesses verified biological activities (antibacterial and prebiotic). This represents a practical product model with significant commercialization potential, catering to the growing consumer trends towards green foods and gut health support.

6. Dissertation structure

The dissertation consists of 134 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 (3 chapters):
 - Chapter 1: literature review (38 pages): provides an overview of previous studies relevant to the research topic.
 - Chapter 2: materials and methods (22 pages): describes the materials, experimental design, and analytical methods employed in the study.
 - Chapter 3: results and discussion (63 pages): presents the findings of the research, accompanied by in-depth analysis and critical discussion.
- Conclusion and recommendations (3 pages).
- List of publications (1 pages): summarizes the scientific works

- published by the author related to the dissertation.
- References (23 pages): a total of 223 references in both English and Vietnamese, including peer-reviewed journal articles and scientific books.

The dissertation includes 24 tables and 30 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 polysaccharides 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 Polysaccharides (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. Studies 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 Polysaccharides and Extraction Methods

1.3.1. Polysaccharides in Mushrooms and Mycelia

1.3.2. Methods for Extracting Polysaccharides 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.

Chapter 2. Materials and Methods

2.1. Materials

C. militaris and *T. versicolor*. *Lactiplantibacillus plantarum*, *Lactiplantibacillus casei* 01, *Bifidobacterium animalis* YC381, *Lactiplantibacillus pentosus* NH1, *Pediococcus acidilactici* NBD8. *Escherichia coli* ATCC 85922 and *Staphylococcus aureus* ATCC 25023. 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 fermentation conditions to maximize mycelial biomass and polysaccharide yields

- a. Cultivation of mushroom mycelia
- b. Evaluating the effects of single factors on the biomass yield and polysaccharide (PS) content of *C. militaris*
- c. Evaluating the effects of single factors on the biomass yield and polysaccharide (PS) content of *T. versicolor*
- d. Optimization of cultivation conditions for the recovery of mycelial biomass and polysaccharides

- Methods for the recovery and determination of mycelial biomass yield
- Methods for the determination of mycelial PS content

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

a. Sequential extraction and quantification of mycelial polysaccharide fractions

b. Surface characterization of the polysaccharide fractions

c. Evaluation of antioxidant capacity using the ABTS assay

d. Evaluation of prebiotic activity

- Stimulation of beneficial microorganisms
- Prebiotic Index (PI)

- In vitro digestion resistance
- Inhibitory effect of *Lactiplantibacillus plantarum* culture broth supplemented with PS against harmful bacteria
- Quantification of short-chain fatty acids (SCFAs)

2.5.3. Content 3: Application of fungal mycelia in the development of dietary supplements

a. Safety evaluation of mycelial powder

b. Production process of fermented oyster mushroom beverage supplemented with *Trametes versicolor* mycelia

c. Methods for evaluating the quality of the fermented beverage

- Determination of total titratable acidity (TTA)
- Evaluation of the ABTS⁺ radical scavenging capacity of the oyster mushroom extract supplemented with *T. versicolor* mycelia
- Determination of *Lactiplantibacillus plantarum* cell density
- Evaluation of the growth inhibition against pathogenic microorganisms
- Sensory evaluation
- Evaluation of product safety and quality

2.6. Data collection and statistical analysis

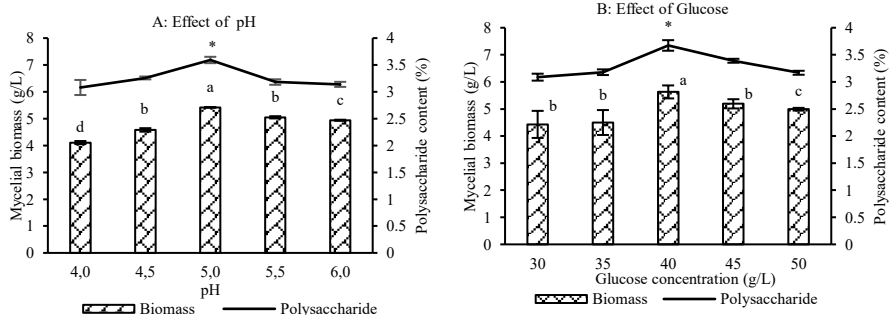
All experiments were conducted using a completely randomized design with three independent replications. Data comparison and statistical analysis were performed using Analysis of Variance (ANOVA) via Minitab 19.0 software. Differences were considered statistically significant at $p < 0.05$.

Chapter 3. Results and Discussion

3.1. Optimization of submerged cultivation conditions for the recovery of biomass and polysaccharides 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



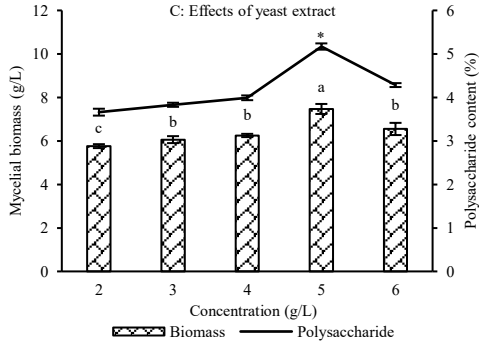


Figure 3.1. Effect of culture conditions (pH, glucose concentration, and yeast extract) in submerged medium on the mycelial biomass of *C. militaris*. A: Effect of pH; B: Effect of glucose; C: Effect of yeast extract.

3.1.1.2. Determination of optimal conditions for the recovery of biomass and polysaccharides 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:
 $Y_1 = -122,3 + 25,10 X_1 + 1,514 X_2 + 14,74 X_3 - 2,450 X_1^2 - 0,01910 X_2^2 - 1,467 X_3^2$

$$Y_2 = -44,73 + 10,93 X_1 + 0,6826 X_2 + 3,51 X_3 - 1,099 X_1^2 - 0,00847 X_2^2 - 0,339 X_3^2$$

Where: X_1 , X_2 , and X_3 represent pH, Glucose, and Yeast extract, respectively

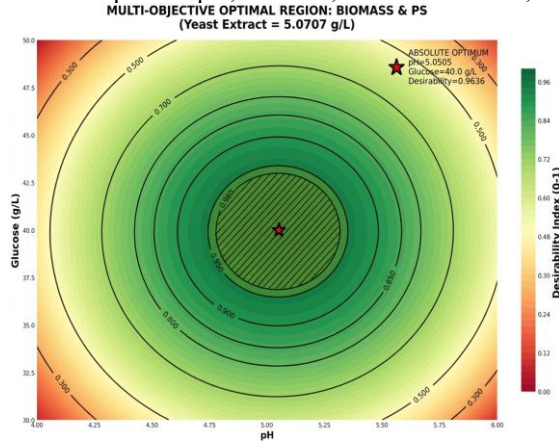


Figure 3.3. Multi-objective optimization analysis for *C. militaris*

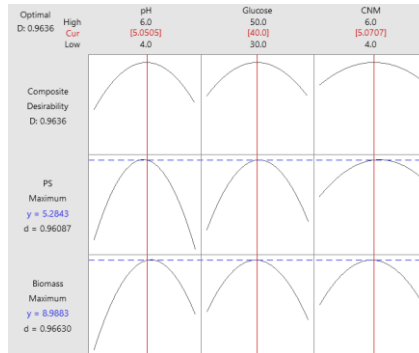


Figure 3.4. Predicted effects of factors on fungal biomass yield.

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

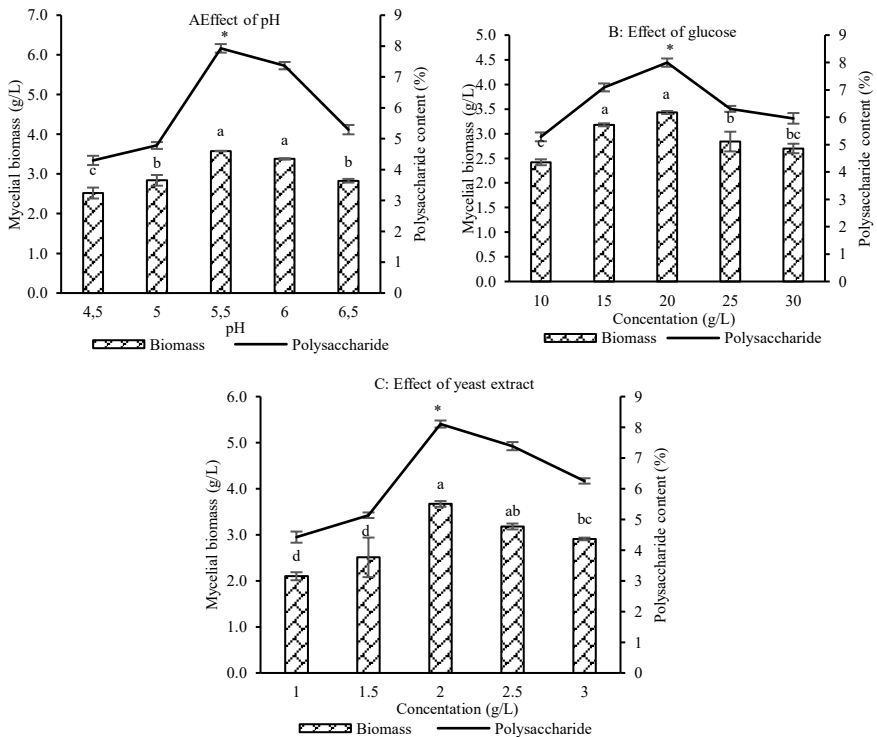


Figure 3.5. Independent effects of pH, glucose concentration, and yeast extract in

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: X_1 , X_2 , and X_3 represent pH, Glucose, and Yeast extract, respectively

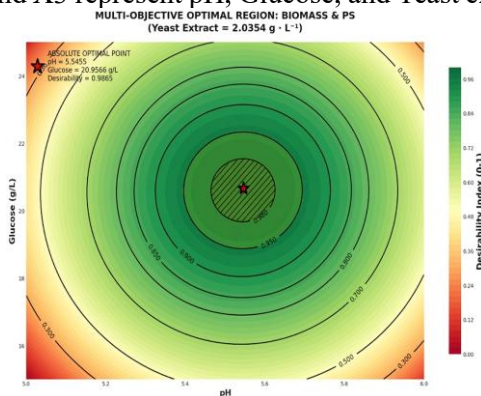


Figure 3.7. Multi-objective optimization analysis for *T. versicolor*

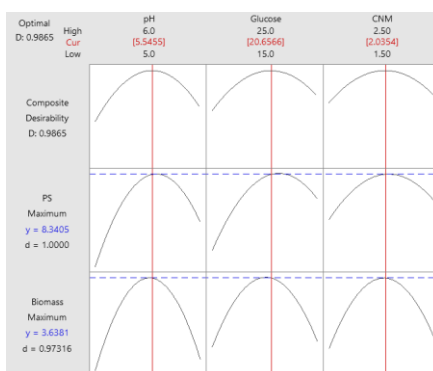


Figure 3.8. Multi-objective optimization plot of cultivation conditions for biomass and polysaccharide recovery

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

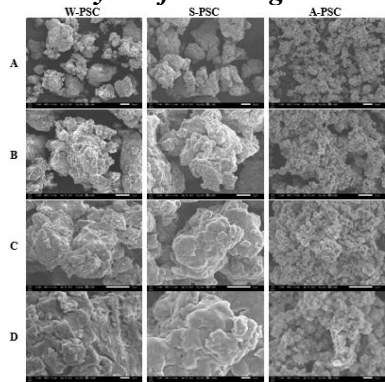


Figure 3.91. Scanning electron micrographs of *C. militaris* polysaccharide extracts at different magnifications: (A) 1000×; (B) 3000×; (C) 5000×; (D) 10,000×

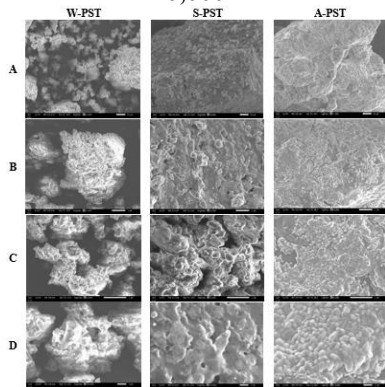


Figure 3.0. Scanning electron micrographs of *T. versicolor* polysaccharide extracts at different magnifications: (A) 1000×; (B) 3000×; (C) 5000×; (D) 10,000×

3.2.2. Content of sequentially extracted polysaccharides from fungal mycelia

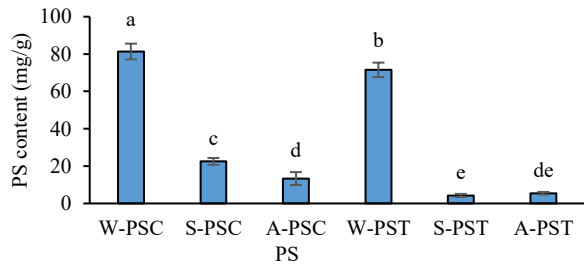


Figure 3.11. Content of PS extracted from *C. militaris* and *T. versicolor* mycelia



Figure 3.12. Fractionation of PS extracted from fungal mycelia.

3.2.3. Antioxidant capacity 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±0.31 ^a	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
IC ₅₀ (µg/mL)	2446.51±24.34 ^c	3273.33±18.5 ^a	2474.24±2.78 ^b

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
IC ₅₀ (µg/mL)	1142.5±21.72 ^b	1279.1±18.04 ^a	1070.6±18.25 ^c

In this assay, the IC₅₀ values of the three PS extracts (W-PSC, S-PSC, and A-PSC) from *C. militaris* were determined to be 2446.51 ± 24.34 µg/mL, 2474.24 ± 2.78 µg/mL, and 3273.33 ± 18.54 µg/mL, respectively. Meanwhile, the corresponding IC₅₀ 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 activity of mycelial PS extracts

3.2.4.1. Stimulatory effects on probiotic growth

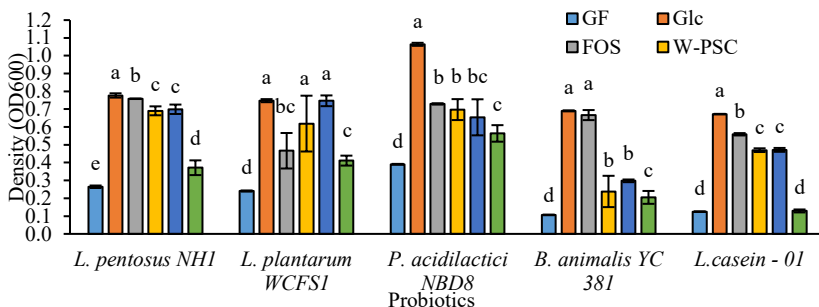


Figure 3.13. Growth of probiotic strains in media supplemented with PS from *C. militaris* mycelial extracts.

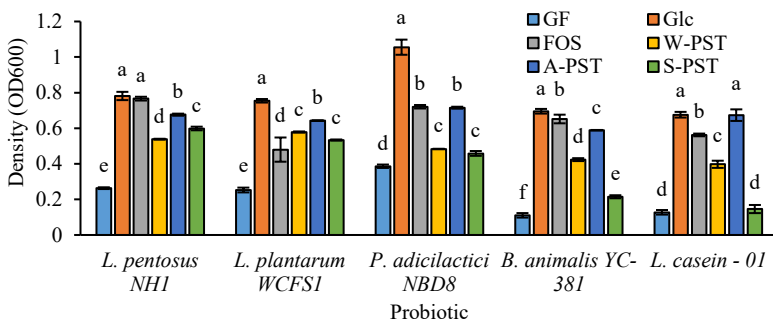
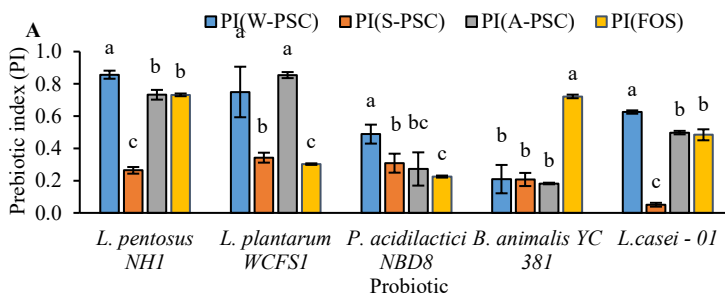


Figure 3.14. Growth of probiotic strains in media supplemented with PS from *T. versicolor* mycelial extracts

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



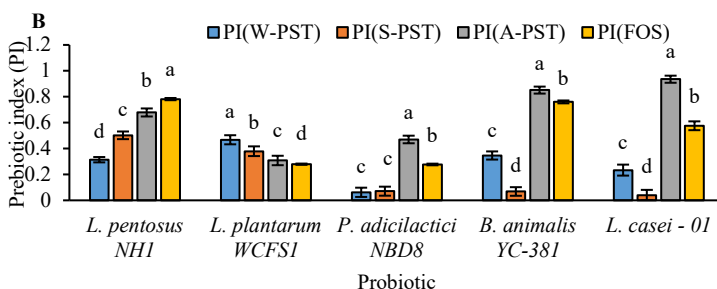
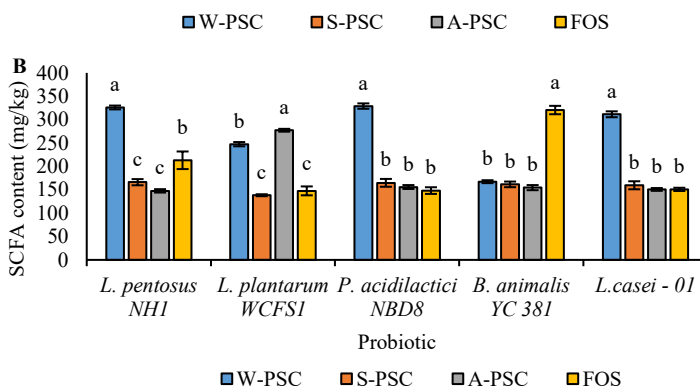
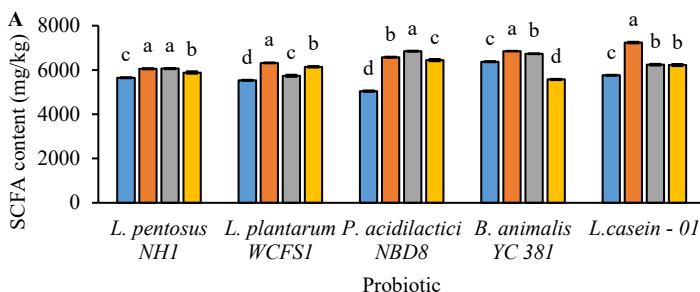


Figure 3.15. Prebiotic index (PI) of the polysaccharide fractions for the investigated probiotic strains. (A) Fractions from *C. militaris* mycelia; (B) Fractions from *T. versicolor* mycelia

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*



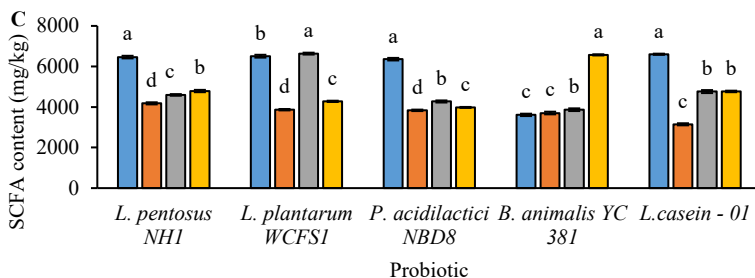
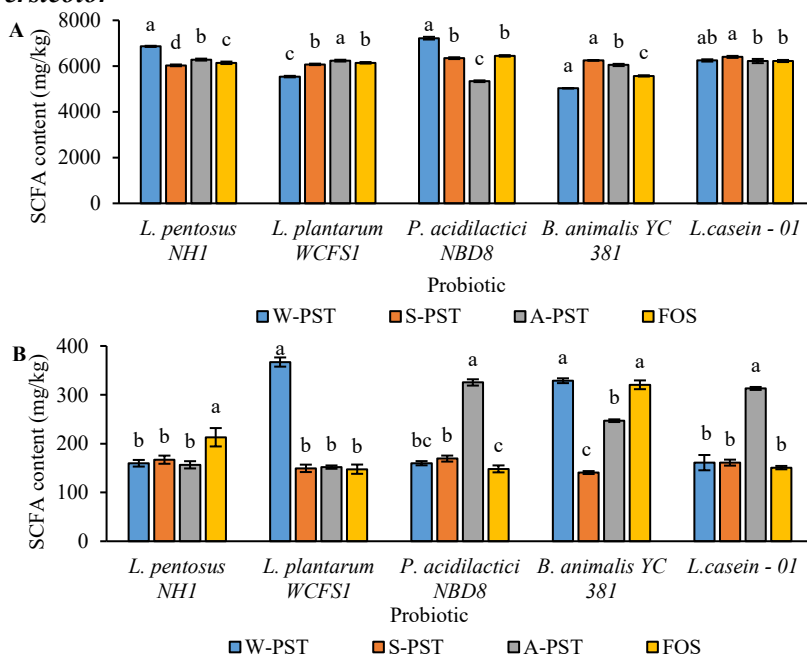


Figure 3.16. Effects of PS extracts on SCFA production: (A) butyric acid; (B) propionic acid; (C) acetic acid.

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*



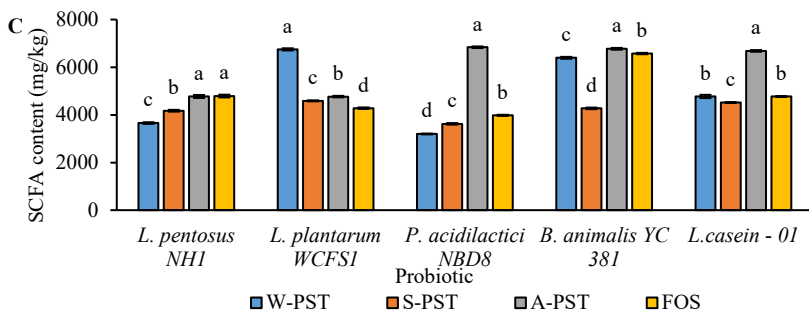


Figure 3.17. Effects of PS extracts on SCFA production: (A) acetic acid; (B) propionic acid; (C) butyric acid

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

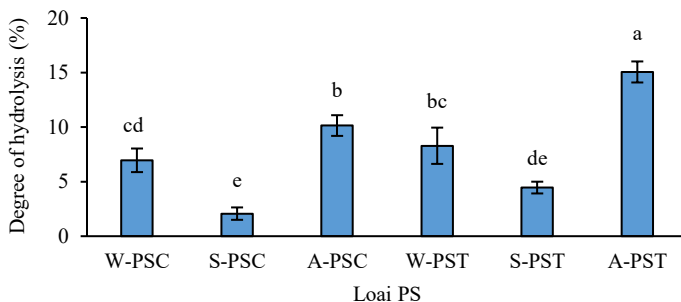


Figure 3.18. Percentage of PS hydrolyzed by α -amylase

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	<i>E. coli</i>		<i>S. aureus</i>	
	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
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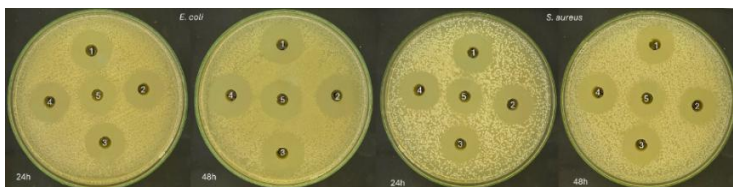


Figure 3.19. Antimicrobial activity of *L. plantarum* culture supernatants against *E. coli* ATCC 85922 and *S. aureus* ATCC 25023

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	<i>E. coli</i>		<i>S. aureus</i>	
	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	<i>Salmonella</i>	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^{•+} radical-scavenging activity (%) of oyster mushroom extracts supplemented with fermented *T. versicolor* mycelia

Treatment	Fermentation duration			
	Initial	12 h	Initial	48 giò
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 ^c	34.44 ± 0.86 ^c	33.48 ± 0.59 ^c	32.45 ± 0.71 ^c
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

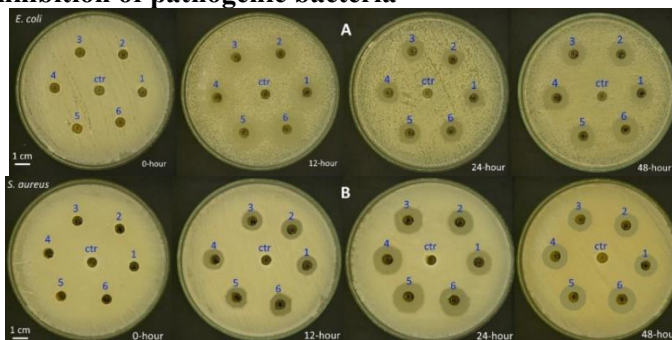


Figure 3.21. Antibacterial activity of fermented beverages against *E. coli* ATCC 85922 (A) and *S. aureus* ATCC 25023 (B).

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)		
<i>E. coli</i>			
CT1	6.53 ± 0.44 ^d	8.8 ± 0.64 ^c	6.95 ± 0.77 ^c
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
<i>S. aureus</i>			
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

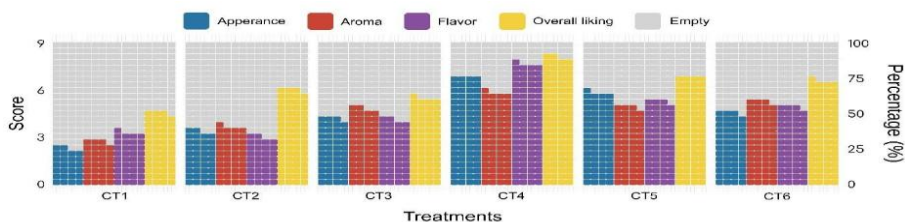


Figure 3.22. Waffle chart of sensory evaluation of fermented beverage after 24 h.

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	$3.33 \pm 0.02^b^c$	3.15 ± 0.03^b	3.07 ± 0.03^a
CT6	4.72 ± 0.09	$3.33 \pm 0.03^b^c$	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^c	4.244 ± 0.128^c	7.218 ± 0.100^c	9.158 ± 0.132^c
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	<i>Coliforms</i>	10 (CFU/ml)	ND (<1)	TCVN 6848:2007
4	<i>E. coli</i>	ND	ND (<1)	TCVN 7924-2:2008
5	Coagulase-positive staphylococci (<i>S. aureus</i> and other species)	ND	ND (<1)	ISO 6888-1:2021
6	<i>Cl. perfringens</i>	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

3.3.3. Development of the fermented beverage production process

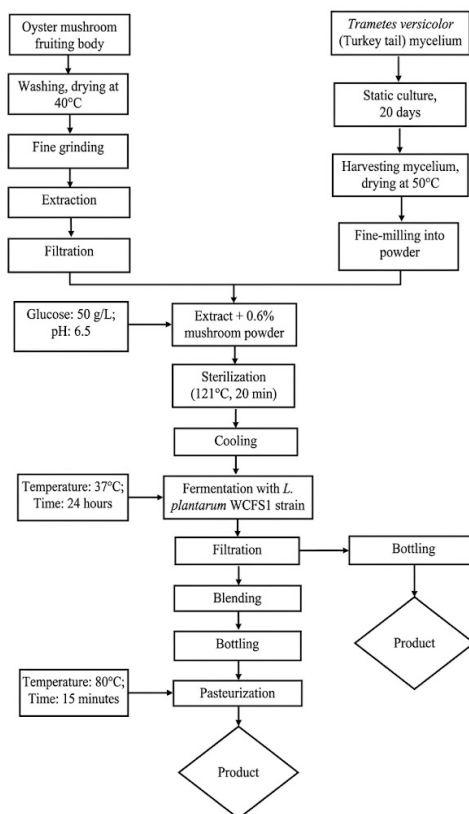


Figure 3.23. Flowchart of the production process for lactic acid fermented oyster mushroom beverage supplemented with *Trametes versicolor* mycelium

Conclusions and Recommendations

A. Conclusions

This thesis has successfully achieved its outlined objectives by focusing on the biological evaluation of polysaccharides (PS) derived from submerged mycelial cultures. The study specifically investigated their prebiotic properties, antioxidant capacities, and potential applications in the food industry. Based on the experimental findings, the following key conclusions are summarized as follows:

1. Determination of crucial optimal conditions for submerged cultivation to achieve high-yield and polysaccharide-rich biomass.

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

- For *Trametes versicolor*: The optimal conditions were a pH of 5.545, a glucose concentration of 20.65 g/L, and a yeast extract concentration of 2.025 g/L. Under these conditions, the dry biomass yield reached 3.59 ± 0.12 g/L, and the PS content was $8.23 \pm 0.27\%$.

2. Evaluation of the extraction efficiency and surface morphological characteristics of polysaccharides (PS)

The sequential extraction method using hot water, alkali, and acid, respectively, proved highly effective in fractionating PS with distinct characteristics.

- Regarding 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*). Subsequent sequential extraction successfully yielded alkali- and acid-extracted PS fractions with specific antioxidant and prebiotic activities.

- Regarding morphology: Extraction conditions significantly influenced the surface structure. While the hot water and alkali-extracted fractions generally exhibited an aggregated block-like appearance, the acid-extracted (A-PST) and alkali-extracted (S-PST) fractions of *T. versicolor* displayed distinct compact or finely granular structures.

3. Confirmation of the biological activities of the polysaccharide extracts

- Antioxidant activity: All PS fractions exhibited ABTS radical scavenging capacity. PS from *T. versicolor* showed stronger activity (IC_{50} ranging from 1070 to 1279 $\mu\text{g/mL}$) compared to *C. militaris* (IC_{50} ranging from 2446 to 3273 $\mu\text{g/mL}$).

- Prebiotic activity: The PS fractions demonstrated pronounced prebiotic potential through positive PI values and high resistance to digestion by α -amylase (hydrolysis degree ranging from only 2.07% to 15.05%). The most prominent were the W-PSC fraction (on the *Lactiplantibacillus pentosus* NH1

strain, PI = 0.857) and the A-PST fraction (on the *Lacticaseibacillus casei* 01 strain, PI = 0.935).

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

4. Practical application in fermented beverage products

- Successfully developed a formulation for a lactic acid-fermented oyster mushroom beverage supplemented with *T. versicolor* mycelial biomass:

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

- Product quality: The product achieved the highest sensory scores in color and flavor while possessing excellent biological activities. All food safety indicators (microbiology, heavy metals) were strictly within the permissible limits according to QCVN 20-1:2024/BYT.

B. NOVEL CONTRIBUTIONS OF THE DISSERTATION

The research findings of this dissertation have yielded the following novel academic and practical contributions:

***Academic contributions:**

- Systematization of the database on submerged cultivation technology: Established crucial optimal technological conditions for the submerged cultivation processes of *Cordyceps militaris* and *Trametes versicolor*, aimed at the biosynthesis of polysaccharide-rich biomass. This serves as a fundamental scientific basis for explaining the effects of environmental parameters (pH, carbon, and nitrogen sources) on the accumulation of biomass and polysaccharides within fungal cells.

- Elucidation of the efficacy of the sequential extraction method: Demonstrated the superiority of the sequential extraction approach in fractionating polysaccharide groups based on their structural characteristics and solubility. A significant novel contribution lies in the discovery that the acid-extracted polysaccharide fraction (A-PS) - which has received scarce attention in previous literature - exhibited superior prebiotic activity (highest PI index) compared to other fractions. This opens a novel avenue for exploiting tightly bound polysaccharides within the fungal cell wall.

- Confirmation of the prebiotic potential of mycelia: Provided reliable in vitro experimental evidence confirming that fungal mycelia act as an effective prebiotic, capable of selectively stimulating probiotic strains (*Lactobacillus*, *Bifidobacterium*, etc.) and inhibiting pathogenic bacteria (*E. coli*, *S. aureus*) through the mechanism of short-chain fatty acid (SCFA) production.

***Practical contributions:**

- The dissertation has demonstrated the feasibility of using fungal mycelium as a valuable organic raw material for the food and pharmaceutical industries. The research results provide an important foundation for developing

submerged cultivation of fungal mycelium at an industrial scale to obtain large quantities of biomass. This biomass can be utilized to produce biologically active and safe bio-products that support health and enhance immunity, meeting the increasingly growing demands of consumers. This is a vital premise for transitioning the production model of medicinal raw materials from conventional farming to industrial-scale manufacturing.

- Successful development of a probiotic-rich fermented beverage product line: Successfully established the technological process for producing a fermented oyster mushroom beverage supplemented with *Trametes versicolor* mycelial biomass. The product not only complies with national food safety standards but also exemplifies the successful application of the Synbiotic model (Prebiotic from mycelia + Probiotic from fermenting bacteria) to create a high-value-added functional food, highly ready for technology transfer and commercialization.

C. Recommendations and future research directions

- Continue investigating large-scale production processes for fungal mycelial biomass to generate organic material enriched in primary metabolites, particularly biologically active polysaccharides.

- Conduct molecular structural analysis and determine the molecular weight of each PS fraction to identify novel polysaccharides with solubility in different extraction solvents.

- Further investigate and elucidate additional biological activities of polysaccharides, including antidiabetic, anti-inflammatory, anticancer, and immunomodulatory effects.

- Explore the application of fungal mycelial biomass in the development of new products to diversify both product categories and functional properties, such as powdered milk, bird's nest drinks, biscuits, bread, cereal powders, and fermented fruit beverages.

- Utilize fungal mycelial biomass as a nutrient-rich powder with strong prebiotic activity.

- Employ purified polysaccharide powders obtained via hot-water extraction as pharmaceutical-grade ingredients for formulating health-enhancing products targeting gut health.

List of scientific research projects

1. Principal Investigator, City-level institutional project: Cultivation of *T. versicolor* on *Acacia mangium* substrate and development of health-protecting products from *T. versicolor* in Da Nang City – Completed in 2021.

2. Project secretary, University-level institutional project (Murata): Investigation of the prebiotic activity of polysaccharides extracted from the mycelia of *C. militaris* – Completed in 2023.

3. Main member, City-level project: Application of single-spore hybridization technology for the selection and breeding of high-yield, high-quality *C. militaris* strains – Completed in 2024.

4. Principal Investigator, University-level project: Evaluation of antioxidant and prebiotic activities of *T. versicolor* mycelia cultivated via submerged fermentation and application in the development of fermented mushroom beverages – Completed in 2026.

Publications

1. Nguyen Thi Bich Hang, Doan Chi Cuong, Truong Cong Phat, Bui Duc Thang, Do Phu Huy, Dang Minh Nhat (2024), Developing a health-supporting fermented lactic beverage from oyster mushrooms (*Pleurotus ostratus*). 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 *T. 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 (2025), 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 *C. militaris* mycelia, Vietnam Trade and Industry Review, 4, 413-420