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Innovation and Creative Technology Association & Rajamangala University of technology

RMUTCON 2025

PROCEEDING BOOK

THE 13TH RAJAMANGALA UNIVERSITY OF TECHNOLOGY INTERNATIONAL CONFERENCE

9 RMUT: DRIVING SOCIAL INNOVATION TOWARDS SUSTAINABLE DEVELOPMENT
JULY 22 - 24, 2025

RMUTCON 2025
14TH RMUTNC & 13TH RMUTIC / 6TH RMUT INNOVATION AWARDS 2025

Proceeding Book
The 13th Rajamangala University of Technology International Conference

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The 13th Rajamangala University of Technology International Conference
9 RMUT: Driving Social Innovation Towards Sustainable Development

July 22 - 24, 2025
Asawin Grand Convention Hotel, Bangkok

Organized by

- Creative Innovation and Technology Association
- Rajamangala University of Technology Phra Nakhon
- Rajamangala University of Technology Krungthep
- Rajamangala University of Technology Rattanakosin
- Rajamangala University of Technology Thanyaburi
- Rajamangala University of Technology Tawan-ok
- Rajamangala University of Technology Isan
- Rajamangala University of Technology Lanna
- Rajamangala University of Technology Suvarnabhumi
- Rajamangala University of Technology Srivijaya

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Abstract Book:
The 13th Rajamangala University of Technology International Conference
9 RMUT: Driving Social Innovation Towards Sustainable Development

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Preface

The Association of Innovation and Creative Technology, together with Rajamangala University of Technology Phra Nakhon, is honored to host the 14th Rajamangala University of Technology National Conference (14th RMUTNC), the 13th Rajamangala University of Technology International Conference (13th RMUTIC), and the 6th RMUT Innovation Awards 2025. These events will take place from July 22–24, 2025, at Asawin Grand Convention Hotel, Laksi District, Bangkok.

This conference provides a platform to share research, inventions, and innovations from faculty, researchers, students, and staff across all nine Rajamangala Universities of Technology. It also welcomes participants from other institutions and organizations in Thailand and abroad. The goal is to promote the exchange of knowledge, experiences, and academic ideas among researchers, scholars, and stakeholders, leading to the development of practical innovations for the country's sustainable economic and social growth.

Rajamangala University of Technology Phra Nakhon, as the host, would like to sincerely thank all administrators, organizing committees, distinguished experts, and participants for their valuable support and contributions to this event.

We hope this conference will inspire new ideas, expand knowledge, and encourage meaningful collaborations that will benefit the nation in the future.

Rajamangala University of Technology Phra Nakhon
July 2025

Message from Ms. Sudawan Wangsuphakijkosol
Minister of Higher Education Science Research and Innovation

The Ministry of Higher Education, Science, Research and Innovation (MHESI) is dedicated to advancing higher education to meet the demands of a rapidly changing world. By fostering research and innovation within universities and promoting collaboration across all sectors—both nationally and internationally—the Ministry underscores its commitment to driving sustainable development that aligns with the nation's strategic goals.

Recognizing the crucial role of research and innovation in responding to the needs of communities and society, MHESI has placed these elements at the heart of its mission. The 14 th National RMUT Conference, the 13 th International RMUT Conference, and the 6th RMUT Invention and Innovation Contest, held under the theme "Innovation for Society: 9 RMUTs Driving Development towards Sustainability", stand as significant milestones. These events showcase the outcomes of rigorous academic inquiry and creative innovation from researchers, academics, and students, while fostering knowledge exchange and collaboration at national and international levels.

On behalf of the Ministry, I extend my heartfelt congratulations to the Association of Innovation and Technology, the nine Rajamangala Universities of Technology, and all supporting organizations for their collective efforts in organizing these important events. This collaboration reflects the pivotal role of higher education institutions in developing innovations that are deeply connected to and beneficial for communities and society.

I am confident that RMUTCON 2025 will achieve outstanding success and lead to meaningful outcomes—transforming academic research into valuable innovations that contribute to the well-being of communities, strengthen society, and advance national development.

สุดาวรรณ

Ms. Sudawan Wangsuphakijkosol
Minister of Higher Education Science Research and Innovation

Message from Professor.Dr. Supachai Pathumnakul Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation

The Ministry of Higher Education, Science, Research and Innovation (MHESI) is steadfast in its commitment to advancing the development of the nation's human capital. Our goal is to equip individuals with the knowledge, skills, and competencies necessary to thrive in the 21st century, enabling them to contribute effectively to national progress. Central to this endeavor is the application of science, research, and innovation as catalysts for sustainable development, ensuring that progress aligns with both societal needs and environmental stewardship.

In alignment with this vision, the Ministry emphasizes the importance of data-driven decision-making and the strategic utilization of knowledge within universities of technology. These institutions are expected to take a leading role in driving technological advancement and fostering innovation, which are critical components for improving quality of life, safeguarding the environment, and achieving the United Nations Sustainable Development Goals (SDGs). Of particular relevance is SDG 9—"Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation"—which underscores the essential role of higher education in supporting resilient and sustainable national development.

The Ministry is pleased to acknowledge the collaborative efforts of the nine Rajamangala Universities of Technology and their dedicated partners in organizing three significant events: the 14th International Conference on Technology and Innovation for Sustainable Development, the 13th National Conference on Technology and Innovation, and the 6th Rajamangala University of Technology National Conference (RMUTCON 2025). These gatherings provide a vital platform for scholars, researchers, and practitioners to share knowledge, exchange ideas, and present groundbreaking research findings that offer tangible benefits to society.

It is my sincere hope that RMUTCON 2025 will serve as a catalyst for fostering innovation and academic excellence, inspiring participants to create impactful knowledge and solutions that address the pressing challenges of our time. May these collective efforts continue to drive forward innovation-led development and promote sustainable progress for the nation and beyond.

Prof. Dr. Supachai Pathumnakul
Permanent Secretary Ministry of Higher Education,
Science, Research and Innovation

Message from the Chairman of the Rajamangala University of Technology Presidents Council

By Assoc.Prof.Dr. Udomvit Chaisakulkiet

President of Rajamangala University of Technology Rattanakosin

Chairman of the Rajamangala University of Technology Presidents Council

On behalf of the Rajamangala University of Technology Presidents Council, I would like to express my heartfelt appreciation for the organization of the 14 th Rajamangala University of Technology National Conference, the 13 th International Conference, and the 6 th RMUT Innovation and Invention Contest under the theme “Innovation for Society: 9 Rajamangala Universities Driving Sustainable Development.” This reflects the significant role of the nine Rajamangala University of Technology institutions in collaboratively advancing research, technology, and innovation to strengthen the nation’s capacity based on knowledge and sustainability.

Rajamangala University of Technology Phra Nakhon has been honored as the main host, in collaboration with the eight other Rajamangala University of Technology institutions and the Association of Creative Innovation and Technology. The event features a variety of key activities, including special lectures by invited speakers, national and international academic conferences, innovation and invention contests, RMUT Startup presentations, the RMUT IP Fair exhibition, business matching sessions, and academic showcases from educational institutions and organizations both within and outside the network. This conference serves as an important mechanism to foster academic collaboration and provides a platform for the exchange of knowledge among researchers, scholars, students, as well as representatives from government, private, and civil society sectors, both domestically and internationally. Such integration of knowledge will further the development of technologies, innovations, and research that can be applied practically and sustainably.

I extend my sincere thanks to all parties involved in organizing this event. I reaffirm the Rajamangala University of Technology network’s commitment to continuously driving knowledge and innovation for the benefit of society. It is my earnest hope that this conference will serve as a vital force in enhancing the quality of the nation’s research and innovation, contributing sustainably to society, the nation, and the global community.

Assoc.Prof.Dr. Udomvit Chaisakulkiet
Chairman of the Rajamangala University of Technology Presidents Council

Message from the Chairperson of the Rajamangala University of Technology Research and Development Institute Network

By Asst. Prof.Dr. Bowonkitti NekomanurakDirector
Research and Development Institute, Rajamangala University of Technology Rattanakosin Chairperson,
Research and Development Institute Network, Rajamangala University of Technology

The Research and Development Institute Network of the nine Rajamangala Universities of Technology has continuously collaborated to promote, support, and develop the management of research to enhance the quality of research outputs, inventions, and innovations. These efforts aim to produce tangible benefits for communities and the nation, both in the fields of social sciences and industry. This is achieved through multi-sectoral cooperation, integrating knowledge to deliver concrete outcomes.

This conference serves as a vital platform for exchanging academic knowledge and showcasing the research and innovation potential of all nine Rajamangala Universities of Technology. It also creates an opportunity for networking and brainstorming among faculty members, researchers, and students at both national and international levels. Such interactions will inspire the development of new research, inventions, and innovations that can truly address the country's sustainable development goals.

On behalf of the Chairperson of the Rajamangala University of Technology Research and Development Institute Network, I extend my heartfelt thanks to Rajamangala University of Technology Phra Nakhon for hosting this event, as well as to all stakeholders involved in its strong organization. I firmly believe that this conference will be another powerful driver in advancing research, inventions, and innovations toward wider recognition and contributing positively and sustainably to society and the nation.

Asst. Prof. Dr. Bowonkitti Nekomanurak
Chairperson, Research and Development Institute Network,
Rajamangala University of Technology

Message from Dr. Natworapol Rachsiriwatcharabul
President of Rajamangala University of Technology Phra Nakhon (RMUTP)

The network of nine Rajamangala Universities of Technology has continuously collaborated in organizing academic conferences. This year, Rajamangala University of Technology Phra Nakhon is honored to serve as the main host, in partnership with the Association of Creative Innovation and Technology, for the 14th National Rajamangala University of Technology Academic Conference, the 13th International Rajamangala University of Technology Academic Conference, and the 6 th Rajamangala Innovation and Invention Contest (RMUTCON 2025). The theme of the event, "Innovation for Society: 9 Rajamangala Universities Driving Development Towards Sustainability," reflects the role of higher education institutions in the contemporary era, not limited to teaching and learning but extending to applying knowledge for research and innovation to solve societal problems, meet community needs, and serve as a crucial force in driving the nation toward stable and sustainable development.

This academic conference not only serves as a platform for faculty members, researchers, and students to present their research but also provides an excellent opportunity to exchange knowledge and experiences with academics from both domestic and international institutions, thereby fostering global academic collaboration networks.

On behalf of Rajamangala University of Technology Phra Nakhon, I would like to express my sincere gratitude to all sectors involved in making this conference a success. May this event be a significant step forward in academic cooperation and serve as an inspiration for all participants to continue creating meaningful and impactful work for society.

Dr. Natworapol Rachsiriwatcharabul
President, Rajamangala University of Technology Phra Nakhon

The 13th Rajamangala University of Technology International Conference 9 RMUT: Driving Social Innovation Towards Sustainable Development

July 22 - 24, 2025
Asawin Grand Convention Hotel, Bangkok

Rationale

Rajamangala University of Technology Phra Nakhon is an institution of higher learning committed to cultivating practice-oriented graduates equipped with advanced knowledge and expertise in science and technology. The University has established a comprehensive vision, mission, strategic priorities, and clearly defined objectives that align with Group 2 of the strategic higher education institutions, which focus on technological advancement and the promotion of innovation. Emphasis is placed on enhancing teaching and learning, research, and innovation development as mechanisms to propel the nation's targeted industries. These endeavors are further reinforced by efficient organizational management, research capacity-building, and the fostering of innovation ecosystems.

The academic conference is organized to serve as a platform for the dissemination of research findings, inventions, and innovations produced by university personnel, as well as a medium for showcasing the institution's scholarly contributions to the broader public. Additionally, the event provides opportunities for faculty members and researchers to engage in academic exchange, strengthen collaborative networks in research and academic services, and cultivate a dynamic research environment. These efforts are aimed at generating impactful research, fostering technological advancement, and producing knowledge that contributes to societal and national development. As part of its core mission, the University's Office of Research and Development is tasked with organizing the Rajamangala University of Technology National Conference in collaboration with the network of nine RMUT institutions. Past conferences have been hosted as follows:

1. **10th RMUT National Conference**
   Theme: "Rajamangala Driving Innovation Forward Towards Thailand 4.0"
   Date: 1–3 August 2018
   Venue: Rua Rasada Hotel, Trang Province
   Host: Rajamangala University of Technology Srivijaya
2. **11th RMUT National Conference**
   Theme: "Rajamangala Innovation Pathways for Economic and Social Advancement"
   Date: 24–26 July 2019
   Venue: Chiang Mai International Exhibition and Convention Centre
   Host: Rajamangala University of Technology Lanna
3. **12th RMUT National Conference**
   Theme: "Nine RMUTs Driving Innovation, Leading Economic Development, and Fostering Green Technology for Sustainable Growth"
   Date: 18–20 May 2022
   Venue: Royal Cliff Grand Hotel, Chonburi Province
   Host: Rajamangala University of Technology Thanyaburi
4. **13th RMUT National Conference**
   Theme: "Nine RMUTs Empowering Sustainable Innovation and Shaping Future Lifestyles through the BCG Model"
   Date: 30 August – 1 September 2023
   Venue: Nongnooch Tradition Center Hall, Nongnooch Tropical Garden, Chonburi Province
   Host: Rajamangala University of Technology Tawan-ok

In 2025, Rajamangala University of Technology Phra Nakhon, in collaboration with eight other RMUTs and the Association of Innovation and Creative Technology, will assume the role of host for the following events:

- The 14th Rajamangala University of Technology National Conference
- The 13th Rajamangala University of Technology International Conference
- The 6th RMUT Innovation Awards

These will be held under the overarching theme:

"Innovation for Society: 9 RMUTs Driving Development Towards Sustainability"

Key Activities

- Special lectures by distinguished guest and keynote speakers
- National and international academic paper presentations
- Invention and innovation competitions
- RMUT Startup project showcases and competitions
- RMUT IP Fair and business matching activities
- Academic exhibitions featuring research and innovation outputs from all nine RMUTs and partner institutions

This academic gathering will serve as a significant venue for the exchange of scholarly knowledge and research experiences among academics, researchers, and professionals from the public and private sectors. Furthermore, it aims to promote the dissemination and application of research-based knowledge and innovation in practical settings, thereby fostering the development of high-impact, quality research.

Objectives

1. To provide a platform for the presentation and dissemination of research, inventions, and creative innovations in the fields of science, technology, humanities, and social sciences at both national and international levels by faculty members, researchers, students, and academic personnel from within and beyond the RMUT network.
2. To strengthen academic cooperation and promote the exchange of knowledge and experiences among researchers from the nine RMUTs and external academic institutions.

Conference Activities

1. National and international academic presentations
   - Oral presentation sessions
   - Poster presentation sessions
2. Invention and innovation competitions
3. RMUT Startup competitions
4. RMUT IP Fair and business matching sessions
5. Academic exhibitions by RMUTs and partnering organizations
   Session 1 Humanities and Social Sciences
   Session 2 Agricultural and Food Innovation
   Session 3 Science and Engineering Technology
   Session 4 Architecture and Creative Works
   Session 5 Business and Economics

Fields Open for Submission Topics for Oral Presentation and Poster Presentation

National Level — Divided into 7 sessions as follows:

- Session 1: Humanities and Social Sciences
- Session 2: Agricultural Technology and Food Innovation
- Session 3: Engineering
- Session 4: Science and Information Technology
- Session 5: Architecture, Arts, and Creative Works
- Session 6: Business Administration and Economics
- Session 7: Routine to research

International Level — Divided into 6 sessions as follows:

- Session 1: Humanities and Social Sciences
- Session 2: Agricultural and Food Innovation
- Session 3: Science and Engineering Technology
- Session 4: Architecture and Creative Works
- Session 5: Business and Economics
- Session 6: Resource Management

Topics for the Invention, Innovation, and RMUT Startup Competition

Eligible participants: faculty members, students, and the general public.

- Group A: Food Technology and Food Innovation
- Group B: Engineering and Technology
- Group C: Science and Information Technology
- Group D: Architecture, Arts, and Design
- Group E: Quality of Life Development and Creative Economy

Awards for Poster and Oral Presentation Competitions

The following distinctions were conferred in recognition of outstanding performance in the Poster and Oral Presentation categories:

1. **Gold Award**: Recipients were awarded a gold medal and a certificate of achievement.
    - National Level: 7 awards
    - International Level: 5 awards
2. **Silver Award**: Recipients were awarded a silver medal and a certificate of achievement.
    - National Level: 7 awards
    - International Level: 5 awards
3. **Bronze Award**: Recipients were awarded a bronze medal and a certificate of achievement.
    - National Level: 7 awards
    - International Level: 5 awards

Awards for the Invention and Innovation Competition

Category: Faculty Members and the General Public

1. **Gold Award**: Recipients received a commemorative plaque, a gold medal, and a monetary prize of 8,000 THB (5 awards).
2. **Silver Award**: Recipients received a silver medal and a monetary prize of 4,000 THB (5 awards).
3. **Bronze Award**: Recipients received a bronze medal and a monetary prize of 2,000 THB (5 awards).

Category: Students

1. **Gold Award**: Recipients received a commemorative plaque, a gold medal, and a monetary prize of 5,000 THB (5 awards).
2. **Silver Award**: Recipients received a silver medal and a monetary prize of 2,500 THB (5 awards).
3. **Bronze Award**: Recipients received a bronze medal and a monetary prize of 1,500 THB (5 awards).

RMUTP Startup Competition Awards

Category: Students

1. **Gold Award**: The recipient was awarded a commemorative plaque, a gold medal, and a monetary prize of 5,000 THB (1 award).
2. **Silver Award**: The recipient was awarded a silver medal and a monetary prize of 2,500 THB (1 award).
3. **Bronze Award**: The recipient was awarded a bronze medal and a monetary prize of 1,500 THB (1 award).

Note: All awardees will receive a certificate of achievement.

Tentative Schedule for the National and International Academic Conference (14th Rajamangala University of Technology National Conference – RMUTNC, and 13th Rajamangala University of Technology International Conference – RMUTIC)

Period	Activity
15 June 2025	Notification of review results
25 June 2025	Deadline for submission of final revised papers
25 June - 20 July 2025	Early bird registration for paper presenters
10 - 20 July 2025	Regular registration for paper presenters
1–15 July 2025	Submission of oral presentation files
22–24 July 2025	RMUTCON 2025 Academic Conference

Tentative Schedule for the 6th Rajamangala Innovation Awards and RMUT Startup Award

Period	Activity
15 June 2025	Announcement of shortlisted entries (preliminary selection results)
25 June - 10 July 2025	Early bird registration fee payment
10 - 20 July 2025	Regular registration fee payment
21 July 2025	Installation of exhibition booths for innovations, inventions, and startups
22–24 July 2025	RMUTCON 2025 Academic Conference and Innovation Exhibition

Venue
Asawin Grand Convention Hotel
88 Vibhavadi Rangsit Road (Kamphaeng Phet 6), Talat Bang Khen Subdistrict, Lak Si District, Bangkok, Thailand.

Conference Website: https://rmutcon2025.rmutp.ac.th/

Schedule

The 14th Rajamangala University of Technology National Conference
The 13th Rajamangala University of Technology International Conference
The 6th Innovation and Invention Contest (RMUTCON 2025)
22 – 24 July 2025
At Asawin Grand Convention Hotel, Grand Conference Room, 4th Floor, Bangkok

Tuesday, 22 July 2025		
Opening Ceremony		
Time	**Details**	**Venue**
08.30 – 09.00 a.m.	Registration for invited guests/media/speakers	Grand Conference Room A B C, 4th Fl.
09.00 – 09.10 a.m.	Opening Performance	
09.10 – 09.30 a.m.	**Opening Ceremony** - Dr. Natworapol Rachsiriwatcharabul, President of Rajamangala University of Technology Phra Nakhon, delivers the report. - Lt. Gen. Chainaronk Kijrungrojjarean, Acting President of the University Council, presides over and officially opens the conference.	
09.30 – 10.15 a.m.	**Special Lecture: "Developing Technological Human Resources through Teaching, Research, and Social Needs"** by Prof. Dr. Yuttana Kumsuwan, Faculty of Engineering, Chiang Mai University	
10.15 – 11.00 a.m.	**Special Lecture: "The Language of Change: How Communication Powers Sustainable Social Innovation"** by Assoc. Prof. Dr. Jinlong Zhang, Director of Media Practice Teaching Center, Yangtze Normal University, People's Republic of China (PRC).	
11.00 – 12.00 a.m.	**Exhibition and Poster Presentations of Research, Innovation, and RMUT Startups** by the Chairperson and Executive Teams	
12.00 – 01.30 p.m.	Lunch	3rd Fl.
Project and Contest Presentations		
Time	**Details**	**Venue**
09.00 a.m. – 05.00 p.m.	RMUT Startup Contest Presentations	Meeting Room, 2nd Fl.
01.00 – 05.00 p.m.	Oral Presentations: Technology and Innovation (national and international levels)	Meeting Room, 2nd Fl.
01.00 – 05.00 p.m.	- Poster Presentations: Technology and Innovation (national and international levels) - Innovation and Invention Contest Presentations - Academic Exhibition	Grand Conference Room A, 4th Fl.

Wednesday, 23 July 2025		
Project and Contest Presentations		
Time	**Details**	**Venue**
08.30 – 12.00 a.m.	Oral Presentations: Technology and Innovation (national and international levels)	Meeting Room, 2nd Fl.
09.00 – 12.00 a.m.	- Poster Presentations: Technology and Innovation (national and international levels) - Innovation and Invention Contest Presentations - RMUT Startup Presentations - Academic Exhibition	Conference Room A, 4th Fl.
09.00 – 12.00 a.m.	E-Sports Competition: Arena of Valor (ROV)	Grand Hall B C, 4th Fl.
12.00 – 01.30 p.m.	Lunch	3rd Floor
01.00 – 05.00 p.m.	Oral Presentations: Technology and Innovation (national and international levels)	Meeting Room, 2nd Fl.
	- Poster Presentations: Technology and Innovation (national and international levels) - Innovation and Invention Contest Presentations - RMUT Startup Presentations - Academic Exhibition	Conference Room A, 4th Fl.
06.00 – 10.00 p.m.	Welcome Party	Grand Conference Room B C, 4th Fl.
Thursday, 24 July 2025		
Closing Ceremony		
Time	**Details**	**Venue**
08.30 – 09.00 a.m.	Registration for invited guests/award recipients	Grand Conference Room B C, 4th Fl.
09.00 a.m. – 04.00 p.m.	**Closing Ceremony** - Launching Ceremony: "Innovation Partnership" by Association and related organizations - Award Presentations for Oral and Poster Presentations - Award Presentations for Innovation and Invention Contest - RMUT Startup Awards - Closing Remarks by Dr. Natworapol Rachsiriwatcharabul, President of Rajamangala University of Technology Phra Nakhon - Handover of the RMUTCON 2026 host flag	Grand Conference Room B C, 4th Fl.

Note : Coffee breaks : 10.30 – 10.45 a.m. and 02.30 – 02.45 p.m.
Lunch : 12.00 – 01.30 p.m.

List of International Articles – Full paper

Session 1
Humanities and Social Sciences

Learning with PRIDE: A Decade of English Camp Innovation and Student Empowerment

Dhunyawat Treenate[a], Thanya Pakpoom2[b]

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Abstract

Over ten years of managing English camps at Rajamangala University of Technology Krungthep (RMUTK) led to the creation of the PRIDE model, which includes planning, role preparation, idea exchange, doing, and evaluating. The model began as a support system for rural students to learn English, as well as a means for undergraduates to gain practical experience, and has evolved into a recognized method for promoting experiential learning and developing 21st-century competencies. The PRIDE Model combines theoretical classroom instruction with practical application by applying Kolb's experiential learning theory (1984) alongside Dewey's philosophy of experiential learning (1938). This paper defines the theoretical background of the model and details its practical application while demonstrating its usefulness as a structured framework for student development within higher education, especially for language learning and soft skills development.

Keywords: The PRIDE Model, Experiential Learning, 21st-Century Education

1. Introduction

The English Camp organized by RMUTK's English for International Communication Department since 2009 transformed into a powerful experiential learning platform. The camp delivers English language education to students who live in remote and underserved regions throughout multiple Thai provinces, including Tak, Nong Bua Lamphu, Phetchabun, Buriram, Phrae, Prachuap Khiri Khan, Kanchanaburi, and the latest addition being Phetchaburi. For 13 years, the camp operated as a demonstration of RMUTK's dedication to community outreach initiatives alongside student-focused academic advancement.

The RMUTK English Camp started with two main goals. The camp aims to improve English language skills among rural school students who have restricted access to quality English education and genuine communication opportunities. The camp provides undergraduate English majors with real-world experiential learning experiences. Undergraduates majoring in English take charge of planning and organizing camp activities while executing them to develop practical skills in communication and leadership.

Through repeated reflection and refinement over many years, this process eventually led to the development of the PRIDE Model—a structured framework that captures the five key stages of student-led experiential learning: The PRIDE Model identifies five essential stages for student-led experiential learning, which include planning, role preparation, idea exchange, doing, and evaluation. The PRIDE Model functions beyond management capabilities by providing an educational structure that nurtures language acquisition together with 21st-century skills development and follows principles of experiential learning as proposed by Kolb (1984) and Dewey (1938).

The primary goal of this paper is to investigate how the PRIDE Model developed its current form and structure and to discuss its educational benefits for language teaching and student empowerment through community-based experiential learning in higher education settings.

1.1 Objectives

This article explains how the PRIDE Model serves as an impactful framework for student learning through experiential methods while providing transferable skills for real-world situations. The article proposes that university instructors can adapt the PRIDE Model to function as a pedagogical tool which will improve teaching and learning in higher education settings.

The development and implementation of the PRIDE Model is grounded in two major theoretical underpinnings: The PRIDE Model draws from two foundational theories: experiential learning theory and the 21st-century skills framework. The design and results of the English camp project at Rajamangala University of Technology Krungthep (RMUTK) reflect its foundation in these theories, especially in promoting student-driven action-based learning and skill development, which reaches beyond classroom boundaries.

1.2 Theoretical Framework

The development and implementation of the PRIDE Model is grounded in two major theoretical underpinnings: The PRIDE Model draws from two foundational theories: experiential learning theory and the 21st-century skills framework. The design and results of the English camp project at Rajamangala University of Technology Krungthep (RMUTK) reflect its foundation in these theories, especially in promoting student-driven action-based learning and skill development, which reaches beyond classroom boundaries.

1) Experiential Learning Theory

According to Kolb (1984), experiential learning involves creating knowledge through transforming experiences. Kolb's model emphasizes a four-stage learning cycle: The four stages of Kolb's learning cycle consist of concrete experience followed by reflective observation, which leads to abstract conceptualization and concludes with active experimentation. Through engagement with real-life tasks, learners build understanding, which develops through reflection and conceptual integration followed by the application of insights in fresh contexts.

The educational philosophy of John Dewey (1938) serves as a basis for this approach by asserting that learning should be experience-based and most effective when students work on real-world problems within authentic settings. Dewey believed that learning becomes continuous and transferable through the practice of reflecting on experiences.

The PRIDE Model represents experiential learning principles through multiple student opportunities to plan their English camp activities and afterwards reflect on their experiences. The phases of the model—planning, role preparation, idea exchange, doing, and evaluation—correspond directly with Kolb's learning cycle. The camp preparation and implementation stage provide students with direct experiences while post-camp evaluations serve as a platform for reflective observation, which leads to the creation of abstract organizational concepts followed by active experimentation in subsequent camps. The method converts students from knowledge receivers into proactive learners who practice their skills.

Multiple research studies demonstrate that experiential learning approaches effectively support profound learning experiences and skill acquisition. Yardley, Teunissen, and Dornan (2012) demonstrated that professional competencies improve substantially when experiential learning takes place in real-world settings, particularly within health and education domains. Healey and Jenkins's 2000 research demonstrated how experiential learning combined with inquiry-based methods increases student engagement and comprehension among undergraduate learners.

2) 21st-Century Skills Framework

The 21st-century skills framework works in tandem with experiential learning theory to emphasize the essential competencies needed for success in our modern world. The researchers Trilling and Fadel proposed in 2009 that critical thinking together with communication skills, collaboration abilities, and creativity form essential core skills for success in modern personal and professional life. These competencies are frequently grouped into three domains: The three main skill categories include learning and innovation capabilities along with digital literacy abilities and life and career competencies.

Binkley et al. (2012) broadened the original framework to include components of self-regulation, problem-solving abilities, and global awareness. Their educational framework highlights the need for student-focused learning spaces with problem-solving elements that support social interaction and match the educational design and principles of the PRIDE Model.

The RMUTK English Camp requires student organizers to engage in activities that demand simultaneous application of several 21st-century skills. Students develop time management abilities together with task delegation and problem-solving skills during the planning and coordination phases. Students leading activities need to overcome cultural and language differences through effective communication. Through critical thinking and team reflection combined with constructive feedback during evaluation activities, students develop metacognitive abilities and learn to improve continuously.

Contemporary educational research shows that higher education programs need to develop courses that include 21st-century skills. Voogt and Roblin (2012) argued for educational programs that support creative thinking and group work among students. Silva (2009) stated that traditional educational methods need to develop by incorporating soft skills instruction to equip students for work in diverse international settings. The results validate the PRIDE Model's effectiveness as a tool to promote academic advancement while supporting lifelong learning and professional preparedness.

Experiential learning methods connect strongly with contemporary skills, and they play an essential role in teaching languages, which is particularly useful for English as a Foreign Language (EFL) instruction. Language education in our globalized and interconnected world requires teaching beyond vocabulary and grammar to develop learner skills in intercultural communication and adaptability while enhancing their critical thinking and collaboration abilities (Trilling & Fadel, 2009). Experiential learning supports these requirements by combining practical activity with reflective thinking and direct application to real-world situations (Kolb, 1984). Students can achieve meaningful engagement and improved language retention by participating in real-world communication contexts. Integrating experiential approaches into EFL education enables learners to practice English across various practical scenarios while enhancing their language abilities and developing essential modern skills through authentic interactions.

The connection between experiential learning methods and essential 21st-century skills holds significant importance for language education, specifically within English as a Foreign Language settings. Task-based and experiential learning methods place importance on authentic communication practices and collaborative cultural exchanges (Ellis, 2003), while traditional classrooms focus on grammar rules and memorization. Through the PRIDE Model, students gain linguistic proficiency while simultaneously building social and cognitive abilities during genuine communication exercises.

Studies by Beckett & Slater (2005) demonstrate that the inclusion of project-based learning (PBL) within language education frameworks enhances student motivation along with engagement levels and improves their ability to acquire new skills. The RMUTK English Camp demonstrates how the PRIDE Model creates a PBL learning atmosphere where language serves as both the learning tool and the educational result. Functional English usage enables students to organize activities, deliver instructions, support peers, and reflect on processes that establish practical language contexts while developing leadership abilities and collaboration skills.

In summary, the PRIDE Model stands at the intersection of two powerful educational paradigms: experiential learning and 21st-century skill development. Applying it to language education transforms teaching approaches from passive instruction into active learner-centered participation, which helps students develop English language proficiency alongside leadership and adaptive skills for global success.

2. PRIDE Model Overview

The PRIDE Model originated from more than ten years of accumulated practical experience in directing English camps at Rajamangala University of Technology Krungthep (RMUTK).

2.1 Origin and Development of the PRIDE Model

The PRIDE Model emerged from more than ten years of ongoing learning and practical experience in organizing English camps at Rajamangala University of Technology Krungthep (RMUTK). The English for International Communication Department started annual English camps across Thailand's provinces in 2009 to help under-resourced schools and communities. The community outreach program transformed into a learning ecosystem led by undergraduate English majors who organized and managed the camps with faculty supervision.

Faculty members and student leaders engaged in reflection about the learning process due to ongoing patterns of successful practices and persistent challenges. A structured framework emerged through repeated cycles of preparation followed by implementation and reflection, which led to improvements. The PRIDE model emerged as a result, which stands for Planning, Role Preparation, Idea Exchange, Doing, and Evaluation in an acronym format. The PRIDE Model captures experiential learning cycles within camp management while serving as both a management instrument and educational framework to foster soft skills, practical language use, and learner independence. By studying the chronological development of the RMUTK English Camp, one can better comprehend how the PRIDE Model evolved during twelve years of active educational practice. The following timeline presents important developmental stages such as the expansion of camp activities and the integration of experiential learning theories, which eventually led to the formation of the PRIDE framework.

2009	2011	2013	2015	2017	2019	2021	2023
First camp started	English Camp	Structure roles assignment introduces	Integration of Experiential Learning Principles	Initial Outline of PRIDE Stages Emerges	Student-led Evaluations Shape Camp Design	No English Camp (COVID 19)	Model Refined
Expansion to more provinces of Thailand	English Camp	English Camp	English Camp	English Camp	No English Camp (COVID 19)	PRIDE Acronym Formalized and Piloted	Comprehensive PRIDE Framework Established
2010	2012	2014	2016	2018	2020	2022	2024

Figure 1: Timeline of the Evolution of the RMUTK English Camp Leading to the PRIDE Model

The RMUTK English Camp evolved from 2009 to 2025, which coincided with the development of the PRIDE Model. The RMUTK English Camp reached its first milestone in 2009 when it launched its first camp, followed by important developments such as role structure formalization in 2013, experiential learning theory integration in 2015, PRIDE stage emergence in 2017, and full model establishment in 2023. The English Camps operated on an annual basis but stopped in 2020 due to COVID-19 before restarting in 2022 with the formalized PRIDE acronym introduced and tested. The model evolved to become an all-encompassing experiential learning structure that supported student development.

2.2 Stages of the PRIDE Model

The PRIDE Model's stages perform unique educational and structural functions. The model's structured stages create deep learning experiences by combining active involvement with reflection exercises and repetitive practice.

P—Planning

During the first stage, students team up to establish the camp's main structure. This includes:
Establishing which audience to target and understanding their specific learning requirements

- Defining the learning objectives
- Drafting the activity schedule and lesson plans
- Allocating budget, materials, and logistical responsibilities

Students develop skills in establishing objectives and managing resources while practicing strategic planning during this stage. The stage replicates professional project start-up phases, which helps students learn educational concepts and organizational abilities.

R – Role Preparation

During this stage, team members receive defined roles and necessary training to perform their tasks efficiently. Teams typically include:

- Academic Team: Designs educational content and language games.
- Hospitality Team: Manages meals and participant comfort.
- Recreation Team: Organizes ice-breaking and bonding activities.
- General Affairs: Handles logistics, venues, and equipment.
- Participant Support: Mentors and assists student participants.

Students participate in team-building activities followed by rehearsal simulations and training based on their assigned roles. The stage fosters teamwork along with leadership and accountability clarity throughout the team.

I – Idea Exchange

Students participate in collaborative group discussions to brainstorm and improve ideas before moving forward with their plans. Brainstorming sessions allow for:
Students contributed original ideas for camp activities alongside themes and language content elements.

- Anticipation of potential challenges and solutions
- Cross-functional feedback between teams

This stage encourages students to develop analytical skills while fostering creative expression and transparent communication. The process shows that high-quality programming develops from collective discussions and joint responsibility.

D – Doing (Implementation)

During this execution phase, the English camp gets conducted following the established plan. Students:

- Deliver language games and lessons
- Facilitate group activities and manage transitions.
- Respond to real-time issues and participant needs.
- Monitor engagement and adapt as necessary.

During this stage, students demonstrate their language abilities alongside interpersonal and leadership skills in practical settings. Students face unexpected situations that help them develop resilience and learn to adapt while making decisions under stress.

E – Evaluation

Students critically evaluate their experiences by analyzing both the accomplishments and limitations after camp activities end. Evaluation includes:

- Collecting feedback from participants and team members
- Reviewing individual and team performance
- Documenting lessons learned and best practices
- Proposing improvements for the next iteration

The final phase develops both metacognitive awareness and self-assessment practices while establishing a continuous improvement mindset, which exemplifies experiential learning as described by Kolb in 1984.

The PRIDE Model operates through a repeating cycle instead of a straight line. Every iteration results in enhancements that enhance future results. Evaluation results directly shape the planning phase of the subsequent cycle while promoting continuous learning and development. Below is the PRIDE loop through circular arrows that connect P to R to I to D to E and back to P.

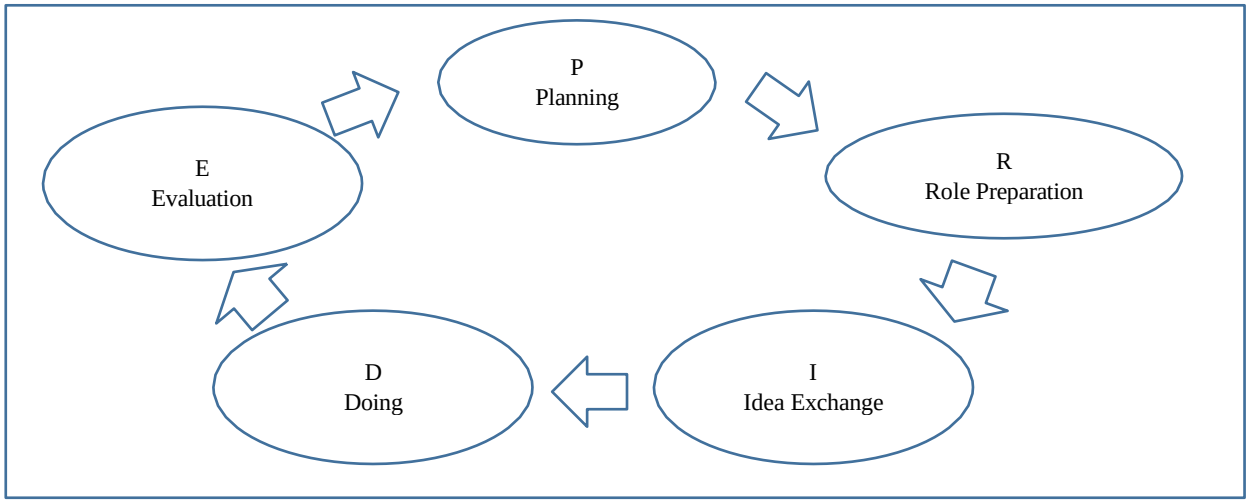

Figure 2: PRIDE Model for Experiential Learning

3. PRIDE in Action: Case from RMUTK English Camp

The RMUTK English Camp exemplifies real-world application of the PRIDE Model within an educational framework. English major students assume full responsibility for organizing the camp each year as faculty advisors provide guidance. The camp functions as both an outreach initiative for rural educational institutions and a practical setting where students develop soft skills while applying their academic knowledge.

3.1 Team Structure and Roles

Students are grouped into five main teams that mirror professional organizational structures through their functional responsibilities. The Academic Team focuses on creating language games and educational materials, while the Hospitality Team takes care of food provision and participant comfort logistics; additionally, the General Affairs Team is in charge of setting up equipment and preparing venues. The Entertainment Team creates fun and interactive activities that keep participants entertained during the entire training program. The Participant Services Team delivers comprehensive support to trainees so they can effectively participate in all program activities. The variety of organizational roles enables students to develop key abilities in leadership while learning responsibility and teamwork skills.

3.2 Skills Developed Across PRIDE Stages

The PRIDE Model intentionally designs each stage to develop particular soft skills that students need to succeed academically and professionally. Students build their strategic planning capabilities together with organizational skills during the planning phase as they establish objectives and timelines. During role preparation, students strengthen their teamwork abilities and learn to be accountable through designated role assignments and practice sessions. The Idea Exchange stage helps students develop creative solutions and problem-solving skills by working together to improve camp activities. Students must utilize adaptability and communication to execute their plans while adjusting to unforeseen issues during the Doing phase. Evaluation promotes reflective thinking and ongoing development by providing feedback and analyzing activities after camp completion. These stages create an integrated framework for experiential learning, which facilitates ongoing personal and professional development.

3.3 Qualitative Outcomes

Students demonstrate their development in leadership abilities and teamwork skills through their reflective statements, which also show increased personal confidence. Participants commonly state that their experience strengthened their work readiness and expanded their knowledge of teamwork dynamics alongside personal awareness. The camp evolves from a simple academic exercise to a powerful journey of personal transformation.

4. Educational Impact

Through its application, the PRIDE Model has shown a major educational influence by connecting academic learning with practical real-world scenarios. The systematic involvement in the model's stages enables RMUTK students to strengthen their scholastic understanding while developing key 21st-century competencies such as leadership and critical thinking, which are essential for the evolving demands of the global workforce (Binkley et al., 2012; Trilling & Fadel, 2009).

The English Camp setting delivers genuine immersive learning experiences where students practice English in teaching roles and team interactions. The learning approach requires students to modify their language use during real-life interactions, which boosts their fluency and confidence. The approach matches both task-based language teaching and communicative methods because they emphasize contextual use as essential for linguistic development (Ellis, 2003; Richards & Rodgers, 2014).

The camp experience helps young learners develop personal growth by teaching responsibility along with resilience and reflection. Participants consistently observe enhancements in their ability to manage emotions alongside better time organization and cross-cultural understanding. During the final evaluation stage, learners analyze their roles and receive peer feedback, which helps them to pinpoint areas for improvement, thus fostering continuous learning and self-development (Kolb, 1984; Dewey, 1938).

The framework of the PRIDE Model allows teachers to transform their role from traditional instructors to learning facilitators while building educational spaces where students manage their own growth. The PRIDE approach presents a replicable instructional model that promotes student independence alongside profound involvement and practical skills necessary for achievements across educational and career environments (Silva, 2009; Voogt & Roblin, 2012).

5. Implications

The PRIDE Model's creation and application hold essential consequences for educational practice and higher education curriculum design. When students receive structured guidance and reflective opportunities, they can successfully lead meaningful real-world learning experiences. The approach opposes conventional teacher-led models while promoting environments that empower students through project-focused learning.

The PRIDE framework extends beyond language education and camp settings. This scalable framework is adaptable for practicum-based courses and service-learning initiatives as well as community outreach programs and interdisciplinary projects. The model promotes enduring skill development by employing learning cycles that connect active participation with reflective practice according to experiential education theory (Kolb, 1984).

PRIDE provides educators with a structured approach to help students develop outside the classroom environment while transforming instructors into coaching figures instead of lecturers. This model enables institutions to create transformative educational experiences that equip students for challenges they will face in both their local communities and the wider world. Upcoming studies should investigate how well the model applies to different academic fields and its effects on student success and motivation alongside their preparedness for graduate life.

6. Conclusions

The PRIDE Model, which RMUTK refined during more than ten years of English camp administration, delivers a flexible framework for experiential learning. The model facilitates student development through planning, role preparation, idea exchange, doing, and evaluation stages, which enables learners to transfer academic knowledge to real-world applications while building crucial 21st-century skills like leadership and collaboration.

The model promotes active learning in language education by enabling students to engage in genuine communication while practicing meaningful language activities. The PRIDE framework functions as a scalable project-based learning model within higher education that enables students to become responsible, autonomous learners while transforming educators into growth facilitators. The effective implementation of the framework at English Camp validates the possibility of adopting similar methods throughout various fields to deepen student involvement and foster sustained skill acquisition.

Through the combination of experiential learning methods with structured reflection, the PRIDE Model connects theoretical knowledge and practical application to prepare students for both academic accomplishments and real-world success in ever-changing global settings.

7. Acknowledgements

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Moral Lessons in Children's Literature: A Content Analysis of Ethical Themes in Charlotte's Web for Higher Education

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Abstract

This research investigates the ethical components present in E. B. White's Charlotte's Web. The study uses qualitative content analysis to explore E. B. White's Charlotte's Web as a potential educational resource for teaching moral values in university settings. The research applies normative ethical theories, including virtue ethics, deontology, and utilitarianism, to reveal key moral themes like self-sacrifice, empathy, loyalty, and moral courage present in the narrative. The research outcomes reveal the transmission of core values via character evolution alongside symbolic visuals and storytelling that evoke emotions. The research examines how children's literature functions as a tool to enhance ethical reflection and moral reasoning while supporting character education at higher education institutions. The report outlines strategies to incorporate literary ethical understanding into diverse academic programs and to develop students' critical thinking using stories as educational tools. According to the study, children's literature likes Charlotte's Web functions as an important tool for developing ethical awareness and comprehensive educational experiences in school settings.

Keywords: Charlotte's Web, Ethical Themes, Children's Literature, Moral Education

1. Introduction

The stories in children's literature serve as strong educational resources for imparting moral principles. Young readers learn about ethical ideas such as empathy and justice by engaging with familiar characters in entertaining stories, according to research by Nikolajeva (2005) and Norton (2010). These narratives drive emotional development and cognitive growth while fostering ethical development through enhanced moral reasoning.

New research highlights the importance of children's books in university settings because they support students in developing critical thinking abilities and moral contemplation. Charlotte's Web serves as an excellent educational tool for teaching university ethics because it presents fundamental ethical themes like loyalty and acceptance of mortality.

Charlotte's Web demonstrates virtue ethics and utilitarian principles through her conduct, while her dedication and sense of responsibility represent deontological ethics according to the theories of Hursthouse (1999), Kant (2002), and Mill (1863). Students gain meaningful opportunities to evaluate moral choices and contemplate ethical principles through the story's rich emotional and symbolic layers. This research investigates the potential of Charlotte's Web to enhance moral instruction and character growth via its narrative educational approach.

Rationale for Selecting Charlotte's Web

The timeless children's book Charlotte's Web (White, 1952) stands as one of literature's most cherished classics because of its emotional resonance and inclusive themes. Wilbur's friendship with Charlotte brings warmth to readers while presenting numerous moral lessons. Charlotte demonstrates exceptional moral principles through her steadfast dedication to saving Wilbur from slaughter at great personal expense by showing selflessness, loyalty, and courage (Norton, 2010). The combination of rich themes and easy readability makes Charlotte's Web an excellent resource for studying ethical stories and their use in teaching morality.

Central Ethical Themes

Altruism, life value assessment, death inevitability, and friendship power form the primary ethical messages in Charlotte's Web. Through her actions, Charlotte demonstrates selfless giving and the employment of her abilities to help others. Wilbur's journey from fear to self-reliance and emotional understanding demonstrates both ethical growth and the importance of profound relationships. Literary themes offer students a valuable framework to investigate their personal values while engaging deeply with moral principles.

1.1 Objectives

This study examines the ethical themes presented in Charlotte's Web through the application of qualitative content analysis techniques. The study conducts a systematic examination and analysis of narrative ethical themes to understand how moral values are represented through character actions and dialogue within the narrative structure. Through their exploration of ethical themes, researchers will develop educational approaches to integrate Charlotte's Web into college ethics courses and character development programs. The research shows the novel functions as a learning tool that fosters critical thinking alongside moral development and ethical reflection among university students.

1.2 Research Questions

1) What ethical themes are presented in Charlotte's Web?
2) How are these ethical themes expressed through the characters and narrative elements?
3) In what ways can Charlotte's Web be effectively used to support moral education in higher education settings?

1.3 Theoretical Framework

The research utilizes an interdisciplinary theoretical framework that integrates ethical philosophy with literary pedagogy and qualitative content analysis. Three distinct domains form the framework that facilitates the analysis of Charlotte's Web as a moral literary artifact and supports its use in ethics education. Through the incorporation of normative ethical theory, there is a structured way to interpret narrative moral themes, while content analysis methodology supplies systematic examination tools. Educational theory about children's literature's impact on moral growth is the pedagogical foundation for the research study.

1) Ethical Theories

This research employs virtue ethics in combination with deontology and utilitarianism to study the ethical dimensions presented in Charlotte's Web. These frameworks serve as essential elements of moral philosophy while providing distinct viewpoints to interpret ethical meanings in literature.

Virtue ethics focuses on building individual character and moral virtues rather than adhering to predetermined rules or evaluating consequences. Aristotle's teachings gave rise to the virtue ethics philosophical school, which modern thinkers like Hursthouse (1999) further developed by explaining that virtuous living through compassion and courage results in a morally beneficial life. Through her character in Charlotte's Web, Charlotte demonstrates the principles of virtue ethics. Her endless selflessness through her tireless efforts to save Wilbur demonstrates her embodiment of virtues like kindness along with loyalty and wisdom. Wilbur develops his character through a journey of moral and ethical growth that transforms him from a dependent individual requiring protection to one who shows emotional strength and understanding that aligns with educational standards for character development.

Immanuel Kant's deontological ethics (1785 & 2002) establish moral value from an action's inherent duty regardless of its outcomes. According to Kantian deontology, every person must adhere to principles they believe should be universally applied while respecting the inherent worth of every human being as well as fictional characters. Charlotte protects Wilbur and maintains her promise because she follows her moral duty instead of pursuing personal gain or practical benefits. By making decisions that ignore external rewards, she demonstrates her loyalty to core principles that match the concepts of deontological ethics.

According to utilitarianism, which Mill developed in 1863, actions are moral if their results lead to the greatest happiness or the least amount of suffering. Charlotte acts on Wilbur's behalf according to utilitarian principles. Through her language abilities, Charlotte turns Wilbur into an exceptional pig, which brings happiness to Wilbur, Fern, and the Zuckermans and brings relaxation to their entire community. Through her deeds that result in her demise, utilitarian ideas are highlighted because they lead to better living conditions for people around her. Students develop comparative moral reasoning skills by applying ethical lenses to understand moral messages in the story.

2) Principles of Content Analysis

The study uses qualitative content analysis to systematically explore the ways ethical messages are integrated into the literary structure of Charlotte's Web. Content analysis functions as a research method that allows all that researchers to organize and understand textual material through the detection of repeated patterns and themes along with conceptual insights (Schreier, 2012). Literary studies benefit from this approach because it supports detailed text examination alongside thematic synthesis across broader contexts.

The analytical process involves three major phases. The initial phase involves identifying analytical units like words, phrases, dialogues, narrative events, events, and character actions that may have ethical significance. The sections where Charlotte weaves her words into her web stand as principal textual elements that demonstrate ethical decision-making. The development of categories and codes depends on criteria derived from both theoretical frameworks and data analysis. There are two types of codes: Codes can originate from ethical frameworks such as "self-sacrifice," "duty," and "consequential outcomes" as well as from inductive analysis performed during textual examination. The researcher examines narrative units to identify their moral meanings through contextual analysis. Charlotte's last letter to Wilbur operates to deliver both a goodbye and to convey ethical responsibility together with her emotional heritage.

This methodology enables her to uphold a systematic examination process while dynamically adjusting to textual variations. When ethical theory is used deductively, it achieves conceptual precision, but induction uncovers hidden ethical insights in literary works. The qualitative content analysis method proves ideal for revealing various moral dimensions in literature and linking literary examination to educational practices.

3) Children's Literature in Educational Contexts

The educational significance of children's literature to teach morality and ethics has been widely recognized by people. This work provides young readers with an understandable medium through which they can examine complex moral issues via familiar storylines beyond its artistic traits. Educational stories for children help develop moral imagination as they allow young learners to explore ethical possibilities and empathize with characters in moral dilemmas, according to Norton (2010) and Tomlinson and Lynch-Brown (2018).

Children's literature features in higher education ethics and character education courses outside traditional teacher training programs. Charlotte's Web seems simple yet leads university students into deep ethical discussions. It raises fundamental questions such as: What constitutes a good life? What does it mean to be loyal? What appropriate actions should we take when faced with death and loss? These questions apply to everyone from children to adults because they address fundamental human concerns.

Ethics education that includes children's literature enables educators to develop students' ethical thinking while enhancing emotional awareness and social understanding. Literature such as Charlotte's Web functions as a mirror and a window: This type of literature acts as a reflective surface for readers to see their core principles while simultaneously opening a view into other people's life experiences and moral perspectives. Literary narratives provide students with a secure imaginative platform to examine moral uncertainties while considering alternative possibilities and sharing their ethical thinking in group interactions. Children's literature serves both aesthetic and ethical educational purposes, according to Nikolajeva (2005), because it produces readers who are both literate and morally aware.

The conceptual model demonstrates how Charlotte's Web functions as an educational tool for ethics through the combination of ethical theory and qualitative content analysis with children's literature teaching methods. The combination of multiple disciplines creates a basis for teaching methods that develop students' moral thought processes as well as critical analysis skills and emotional understanding through literary works.

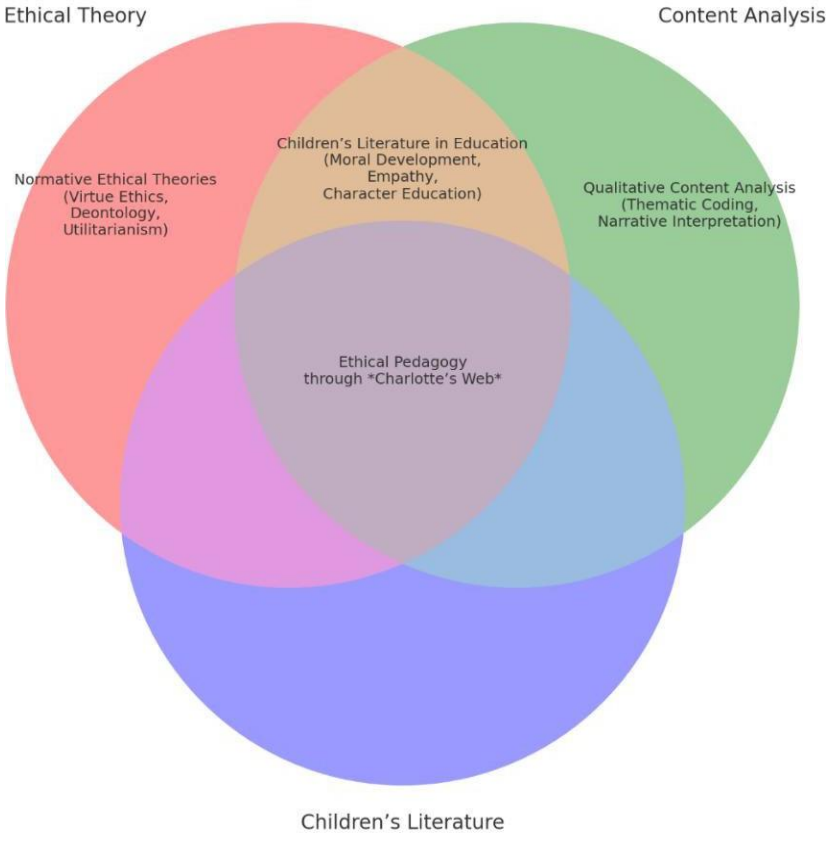

Figure 1. Conceptual Framework: Ethical Pedagogy through Charlotte's Web

The Venn diagram demonstrates where ethical theory meets content analysis methodology and children's literature. These combined elements create a teaching approach that develops ethical thinking skills via narrative examination.

2. Methodology

The investigation adopts qualitative content analysis as its research method to examine ethical themes in Charlotte's Web and explore their application to teaching ethics. The chosen research methodology aligns with interpretive research since it aims to reveal hidden moral values in a literary work and evaluate their usefulness in educational environments.

2.1 Research Design

Researchers employed a textual analysis design using Charlotte's Web (White, 1952) as their primary research source. This book was chosen for its literary depth and its reputation as a moral influence on young readers. The research approach takes an interpretivist paradigm, showing that ethical meaning emerges from the subjective interaction between the text and the reader as well as cultural, educational, and philosophical contexts.

2.2 Data Source and Unit of Analysis

The analysis focuses on key narrative aspects of Charlotte's Web, such as character actions along with their dialogues and pivotal events that contain ethical elements together with descriptive passages. The analysis focuses on scenes that highlight moral challenges as well as displays of selflessness and bravery among friends. Charlotte's choice to protect Wilbur alongside Wilbur's emotional growth and Fern's defense of the pig stand out as key ethical points for analysis.

2.3 Coding and Thematic Development

The analysis of data involved thematic coding through combined deductive and inductive methods. The priori codes developed for analysis were founded on the ethical theories of virtue ethics, deontology, and utilitarianism as outlined in the theoretical framework. The analysis incorporated themes including self-sacrifice, duty, moral courage, and consequential benefit. The close reading of the text resulted in emergent codes that revealed unexpected or context-specific themes such as child advocacy and empathy between species. The research team reviewed the text several times before conducting detailed line-by-line coding on specific excerpts. The research team classified all segments that matched ethical concepts into broader thematic groups. By using this approach, scholars were able to identify recurring moral themes and how they are portrayed in literature.

2.4 Trustworthiness and Rigor

The researcher built credibility using a thorough coding audit trail and reflexive memos to record decision-making during interpretation. The study confirmed its results by comparing them with recognized ethical theory and existing academic work on children's literature and moral education. The study strengthened its rigor by implementing transparent coding procedures alongside theoretical foundations even though literary interpretation requires subjective analysis.

2.5 Ethical Considerations

The study did not require formal ethical approval because it examined publicly available literary texts without involving human participants. The researchers demonstrated intellectual integrity by upholding ethical research standards through the proper citation of sources and honoring authorial intent during their analysis.

3. Findings and Analysis

In Charlotte's Web, the narrative structure alongside its characters presents profound moral complexity and integrates ethical themes through symbolic storytelling. The narrative development of the plot becomes significantly influenced by themes that create opportunities for ethical analysis and educational engagement. Through qualitative content analysis, five major ethical themes were identified: The study finds self-sacrifice together with loyalty and friendship as well as empathy and compassion, moral courage, and acceptance of mortality to be the five central ethical themes. This section shows how each ethical theme relates to narrative content through its specific ethical frameworks. We explore the connections between narrative content and ethical frameworks for each identified theme.

The story's central themes are represented by Wilbur's development from a defenseless piglet into a being with moral awareness. The development of Wilbur's empathy along with his ethical decision-making and personal responsibility forms the moral core of the story. Figure 2 presents the journey visually while serving as a structural framework for the subsequent thematic analysis.

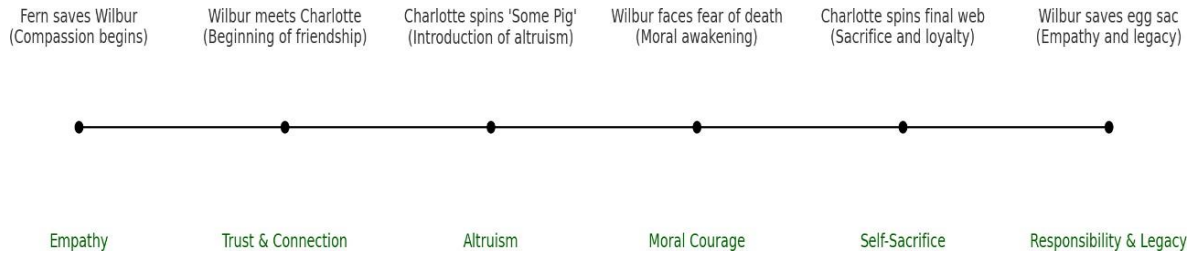

Figure 2: Wilbur's Ethical Development in "Charlotte's Web"

Figure 2 shows Wilbur's moral journey throughout the narrative with structural clarity. The next sections conduct a qualitative content analysis to explore five principal ethical themes extracted from the novel to enhance understanding. The analysis of each ethical theme connects its theoretical basis with its expression during significant events in the story.

3.1 Self-Sacrifice and Altruism (Virtue Ethics / Utilitarianism)

The novel features Charlotte's continuous self-sacrifice as its key ethical theme through her repeated actions to save Wilbur. She uses her immense physical strength to construct her web, ultimately risking her life in the process. The behavior reflects the principles of virtue ethics through its demonstration of altruistic virtue. Charlotte demonstrates moral character through her dedication to helping her friend without seeking personal benefits (Hursthouse, 1999). Her decisions can be analyzed from a utilitarian standpoint because her actions save Wilbur and create happiness for everyone around him, which leads to greater collective happiness (Mill, 1863).

Example from text: “A person who embodies both true friendship and writing talent is a rare find. Charlotte was both.”

Students can examine different moral frameworks when they study virtue-based ethics alongside consequence-based reasoning through this dual ethical approach.

3.2 Loyalty and Friendship (Virtue Ethics / Deontology)

The sustained loyalty between Charlotte and Wilbur acts as the second primary ethical theme. As a moral virtue, loyalty requires ongoing support together with honesty and unconditional presence. Charlotte displays Wilbur demonstrating his gratitude for her by taking care of her children and preserving her memory after her passing. her children and keeping her memory alive after she passes away. Charlotte follows her self-imposed responsibility to protect Wilbur according to deontological principles, which can be interpreted as virtue ethics actions (Kant, 2002).

Example from text: Charlotte says, *“You have been my friend. That in itself is a tremendous thing*.”

These educational interactions give students the opportunity to evaluate loyalty as either a moral responsibility or an inherent character quality.

3.3 Empathy & compassion (Virtue Ethics / Moral Education)

By saving Wilbur, Fern shows empathy that transcends species boundaries and questions adult reasoning through her emotionally driven ethical behavior.

The story opens with young Fern saving Wilbur and introduces the central theme of cross-species empathy. Fern challenges adult reasoning through her emotion-based moral perspective while revealing the natural compassion of children. Fern's determined efforts to prove Wilbur's life holds value show how emotional responses influence ethical choices and illustrate the importance of conscience in moral learning.

“If you were a tiny creature, would you have killed me?”

The quote presents a perfect chance for students to engage in classroom debates about animal ethics and human moral responsibilities.

3.4 Moral Courage (Virtue Ethics / Deontology)

Throughout the novel, different characters show moral courage when they face tough decisions. Wilbur goes from being a scared piglet to becoming a mature individual who shows compassion and confidence, demonstrating his growing sense of moral responsibility. In the story's ending, Wilbur shows his deep-rooted ethical transformation through his act of bringing Charlotte's egg sac home, which displays his new sense of responsibility and care.

For instance, the text states, "Wilbur stood quietly and proudly." He felt radiant.”

The current event allows us to explore courage through its dual nature of bravery and moral integrity in fulfilling ethical obligations.

3.5 Acceptance of mortality (Existential Ethics / Virtue Ethics)

The theme of acceptance of mortality falls under existential ethics and virtue ethics by confronting readers with life’s impermanence through Charlotte’s death.

The story of Charlotte’s death alongside the novel’s frank exploration of life and death introduces readers to existential inquiries about life’s fleeting nature, one’s legacy, and life's purpose. Charlotte’s Web shows death as a fundamental aspect of life while encouraging children to understand that meaning comes from the influence we create rather than the duration of our existence. Charlotte’s peaceful and stoic acceptance of her destiny represents phronesis, which stands as practical wisdom within virtue ethics.

Example from text: The hundreds who attended the fair remained unaware that the most crucial role belonged to a grey spider. The moment of her death occurred without the presence of any other person. Educational settings that promote existential reflection find this theme particularly impactful.

The following table provides a synthesized overview of five primary ethical themes discovered within Charlotte’s Web. The table shows how the five major ethical themes from Charlotte’s Web connect to their ethical theories and illustrative characters while highlighting their educational significance. This summary acts as both an analytical overview and a hands-on resource for teachers who want to incorporate moral lessons through literature into classroom instruction.

Ethical Theme	Ethical Theory	Key Characters	Pedagogical Focus
Self-sacrifice	Virtue ethics / Utilitarianism	Charlotte	Altruism, value of others’ lives
Loyalty & friendship	Deontology / Virtue ethics	Charlotte, Wilbur	Moral duties in relationships
Empathy & compassion	Virtue ethics	Fern	Moral sensitivity, conscience-based judgment
Moral courage	Deontology / Virtue ethics	Wilbur	Acting rightly despite fear or difficulty
Acceptance of mortality	Existential ethics/ Virtue ethics	Charlotte	Death, impermanence, moral legacy

Figure 3: Summary of Ethical Themes

Analysis of Charlotte’s Web shows its potential as a rich source for ethical examination. Moral development in literature emerges from narrative structure and symbolic elements as well as character development rather than just explicit moral lessons. The understanding gained from these studies encourages educators to incorporate children’s literature into university moral education programs because these narratives promote deep reflection and critical thinking about ethical dilemmas through their accessible yet meaningful storytelling techniques.

4. Pedagogical Implications

Charlotte's Web becomes a powerful ethical teaching instrument in higher education through its blend of deep ethical ideas and straightforward storytelling, which proves useful in teacher training courses and humanities studies along with interdisciplinary programs that connect literature with moral development. Integrating children's literature into university programs supports both ethical inquiry and boosts students' cognitive and emotional learning outcomes.

Charlotte's Web serves as an effective platform for discussion-based moral inquiry. The combination of structured debate, Socratic questioning, and ethical dilemmas from the story gives university students analytical tools to study the moral decisions of characters like Charlotte, Wilbur, and Fern. Students can assess the ethical principles that Charlotte's sacrifice might represent while examining Fern's dismissal of adult reasoning as a demonstration of children's innate moral sense. Students develop their abstract thinking and ethical comparison skills by applying deontological and utilitarian frameworks to literary analysis during discussions.

Affective and empathic learning promoted by the novel serves as a basic component of moral development. The emotional resonance of the story enables students to establish personal links with moral principles, which amplifies discussions on sacrifice, death, loyalty, and compassion. Students explore their ideas and challenge preconceived notions by using literary narratives as "safe spaces," which provide a distancing effect through fictional elements. When students engage in reflective journals and role-playing that includes ethical storytelling, they deepen their emotional ties to moral concepts.

The story acts as a bridge connecting various academic disciplines. Charrettes in literature and ethics education will gain value from Charlotte's Web when combined with teaching resources from education, philosophy, psychology, and animal studies. The flexible nature of this method makes it ideal for diverse academic programs focused on cultivating moral imagination to help students envision ethical outcomes and future possibilities. Students will examine real-world ethical issues from the novel through projects that address animal welfare and environmental ethics together with educator moral responsibilities.

The text stands as an exemplary model of literature-based character education, which serves as a key element in national educational policies and international global citizenship education frameworks. Future educators can learn essential instructional strategies through Charlotte's Web to develop character education lessons for primary and secondary schools. Students can learn how narrative structure combined with character development through dialogue creates effective educational lessons aimed at developing empathy and moral reasoning together with social responsibility.

The narrative language and symbolism in the novel establish a framework where literary analysis intersects with ethical contemplation. Charlotte's Web operates as both a literal and symbolic tool for moral teaching and communication and creates a lasting educational presence. Through analysis, students develop critical literacy skills to discover complex meanings in texts, which leads to connections with ethical and philosophical discussions.

Charlotte's Web serves as a celebrated literary masterpiece while simultaneously functioning as a teaching tool to enhance moral understanding. The work's enduring themes and emotional stories create an educational approach to ethics that combines mental discipline with emotional awareness and ethical development through literary pleasure.

Contemporary Connections: Applying Themes to Modern Ethics

The moral themes featured in Charlotte's Web remain relevant for analyzing modern ethical challenges even though it stands as a beloved children's book classic. Educators use modern ethical dilemmas to strengthen narrative moral lessons that help students understand abstract ethical theories better and emotionally engage with the material.

Whistleblowing and Moral Courage: Charlotte's all-encompassing courage to protect Wilbur parallels how modern whistleblowers bravely face personal dangers to expose corporate wrongdoing. Frances Haugen displayed ethical responsibility through the disclosure of Meta's (Facebook) internal data while endangering her own personal security. Through this perspective, students can evaluate moral bravery in fictional and actual examples while learning how virtue ethics connects with moral accountability responsibilities.

Animal Rights and Cross-Species Empathy: Fern decides to protect Wilbur because her deep understanding of empathy crosses the boundaries between species. This concept functions as a moral counterpart to current animal rights and veganism movements that challenge human-centered worldviews. Through animal rights dialogue, teachers can guide students to connect Fern's emotions with non-human moral value and compare conscience-based choices with utilitarian principles.

Grief Literacy and the Ethics of Loss: The respectful way Charlotte and Wilbur respond to death triggers essential discussions about mortality and how to honor those we love during worldwide crises when such conversations become especially important. Students explore existential ethical principles when they study how narratives provide guidance through grief and life's brief nature. The ability to understand emotions and bounce back from adversity grows during conversations that connect fictional narratives with real-world emotional and ethical challenges.

Charlotte's Web bridges classic literary principles with modern social issues to function as both a reflective self-assessment tool and a starting point for social discourse. Students at universities discover deeper learning connections when abstract theories relate to real-world moral dilemmas within narratives.

5. Conclusions and Recommendations

5.1 Conclusion

This study conducted a qualitative content analysis of Charlotte's Web to uncover its ethical themes and evaluate their application in university education. The comprehensive analysis of the novel revealed many deep ethical themes, including self-sacrifice and empathy as well as moral courage and acceptance of death. The narrative effectively combines character development and symbolic imagery with deeply emotional events to integrate its moral themes.

The examination of virtue ethics, deontology, and utilitarianism resulted in establishing Charlotte's Web as a useful literary tool for ethical analysis. The ethical lessons embedded in children's literature become evident through the compassionate behavior of Charlotte and Fern's moral consciousness and Wilbur's personal growth into responsible behavior. Through its research findings, literature is shown to extend beyond human values reflection and becomes an educational tool that develops ethical thinking and character while enhancing analytical engagement.

The research demonstrates multiple teaching applications for Charlotte's Web. This work enables classroom discussions about ethics while simultaneously promoting interdisciplinary education along with emotional maturity and philosophical inquiry. The study supports the idea that higher education tends to undervalue children's literature, which possesses great potential for improving ethics education and developing students who are prepared to engage responsibly and empathetically in society.

5.2 Recommendations

Following these research findings, we present recommendations aimed at educators and curriculum developers as well as directions for future research studies.

1) Integrate children's literature into ethics and education courses.

University teachers from teacher education and literature departments need to examine the possibility of including ethically meaningful children's books such as Charlotte's Web into their course plans. Literary narratives have the potential to generate thoughtful conversation and build empathy beyond what traditional instructional materials can achieve.

2) Design Literature-Based Moral Education Modules

Create educational modules and lesson plans that integrate ethical theories alongside literary analysis techniques. Students can participate in ethical discussions while writing thematic journals and comparing moral situations from literature to real-world scenarios.

3) Conduct further research across texts and cultures.

Researchers must carry out cross-cultural and age-specific analyses on children's literature to determine whether ethical themes maintain their consistency or show variation. Upcoming research should investigate student responses to moral teachings presented through literary works to determine their long-term outcomes.

4) Encourage Cross-Disciplinary Collaboration

Collaborate with experts in psychology, education, and philosophy to develop interdisciplinary approaches for ethical literary studies. Literature-based ethical teaching will advance through combined theoretical development and practical implementation enabled by this collaboration.

Through their dual approach as cultural artifacts and moral resources, children's literature enables educators and researchers to shape students' ethical understanding with stories like Charlotte's Web and train them for compassionate, wise actions with integrity in our complex modern world.

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The Study of Problems found in the workplace during Co-operative Education of students from Rajamangala University of Technology Rattanakosin Bophit Phimuk Chakkrawat Campus

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Abstract

The purpose of this research is to study the problems encountered by cooperative education students from the Faculty of Liberal Arts and the Faculty of Business Administration at Rajamangala University of Technology Rattanakosin, Bophit Phimuk Chakkrawat campus, during their work placements, as well as how the students addressed these problems. The research employed survey methods and data collection through cooperative education reports and semi-structured interviews. The results indicated two key findings. First, the problems faced by students could be categorized into four main aspects: (1) problems related to work content and work performance, (2) problems with equipment usage, (3) language-related problems, and (4) personal or life-related problems, including adjustment issues in the workplace. Second, the strategies students used to solve these problems included independent problem-solving, seeking assistance from workplace mentors, and requesting support from supervising teachers. The findings from this research can serve as useful guidelines for preparing students for the workplace. They can also inform teaching and learning management, as well as curriculum development, to better equip students for the demands of professional environments.

Keywords: Co-operative Education, Problems in Cooperative Education Practice, Cooperative Education Problem Solving Guidelines

1. Introduction

1.1 Research Background

Cooperative Education (Co-op) is an education system that combines classroom learning with practical work experience in a business in a systematic and organized manner to provide students with hands-on experience. Students are required to work in a business as full-time temporary employees for one semester. Through this program, students will have the opportunity to understand and become familiar with the real world of work, which supports learning and the development of professional and personal skills. It is an educational approach that integrates learning in academic institutions with full-time work placements in real business environments. The main purpose of the program is to prepare students in all aspects before they begin working in real organizations.

Rajamangala University of Technology Rattanakosin is one of the educational institutions that recognizes the importance of cooperative education. The university actively promotes students in each field of study to have the opportunity to participate in cooperative education. Specifically, Rajamangala University of Technology Rattanakosin, Bophit Phimuk Chakkrawat campus, which comprises two faculties—the Faculty of Liberal Arts and the Faculty of Business Administration—requires students who meet the criteria set by their respective programs within each faculty to undertake cooperative education. This is considered a graduation requirement.

Therefore, in every academic year, fourth-year students are assigned to complete a cooperative education placement for one semester. Upon completion of their program, all students are required to report their cooperative education outcomes to a committee within their department. The report is used to evaluate their performance at their workplace. Prior to the cooperative education period, each faculty provides thorough preparation for students in various aspects to ensure that students are ready to apply the knowledge and skills they have acquired at the university to real-life working situations.

Students from both the Faculty of Liberal Arts and the Faculty of Business Administration have had the opportunity to engage in cooperative education placement at various real workplaces, both within the country and abroad. However, reports from these cooperative educations have shown that most students continue to encounter difficulties in adapting to real work environments, despite the preparation provided by their faculties. This is because each workplace has different expectations and specific requirements. As a result, students often face challenges while working in real-world settings, even though they have undergone pre-cooperative education preparation.

Given the issues mentioned above, the researchers recognize the importance of understanding the problems faced by students from both faculties in the Bophit Phimuk Chakkrawat campus during their cooperative education periods. Therefore, the researcher aims to collect and analyze the problems encountered by students during their cooperative education placements over the past three academic years (2019–2021 academic year). The objective is to explore the causes of these problems and examine possible solutions. The research questions are: What types of problems do students encounter during their cooperative education placement? And how do students solve problems when they encounter problems during their cooperative education placement? The findings from this study will be beneficial for all faculties in improving their preparation processes, helping to ensure that students are more comprehensively equipped for their cooperative education experiences in the workplace.

1.2 Related theories and research studies

1) Rajamangala University of Technology Rattanakosin Bophit Pimuk Chakkrawad

Rajamangala University of Technology Rattanakosin is one of the universities under the nine Rajamangala University of Technology in Thailand. It was established as part of Thailand's efforts to expand educational opportunities for vocational students. The university traces its origins back to the College of Technology and Vocational Education, founded in 1975. In 1988, it was granted the name Rajamangala Institute of Technology to further promote vocational education, research, innovation, and academic services to society. Later, in 2005, Rajamangala University of Technology Rattanakosin was officially established under a specific legislative act. The university offers both undergraduate and graduate programs and is organized into seven faculties and three colleges. These cover diverse fields such as engineering, architecture, business administration, liberal arts, hotel and tourism, science and technology, and management innovation. The university operates across four campuses: Salaya, Bophit Phimuk Chakkrawat, Pohchang College, and Wang Klai Kangwon. (Rajamangala University of Technology Rattanakosin, 2019)

2) Cooperative education

The Cooperative Education Center, Phranakhon Si Ayutthaya Rajabhat University (2019) stated that Cooperative Education is an education system that was initially developed in England and the United States during the years 1903–1909, which, at that time, was referred to as the Cooperative Education system. In the United States, it was found that from 1960 onwards, the system continued to develop and expand further. Phranakhon Si Ayutthaya Rajabhat University (2019) also illustrated that this education system has been supported by government budgets and cooperation from enterprises, making it well-known and advanced. Currently, 33% of educational institutions worldwide use the cooperative education system and have developed it to the aspect of organizing student exchanges for international work placements. In addition, cooperative education has expanded into countries in East and Southeast Asia, where educational institutions have fully integrated cooperative education into their teaching. This is especially evident in institutions within the British educational network, which follow models similar to those in Western countries, such as the Hong Kong Polytechnic University and Nanyang Technological University in Singapore. Later, cooperative education spread to China and Thailand.

Cooperative Education is an educational system that integrates academic learning with practical work experience (Work Integrated Learning) to enhance the quality of graduates through real work experience in organizations, according to academic and professional standards, and aligned with labor market demands. Cooperative education thus plays a crucial role in preparing graduates to enter their careers, enabling them to work immediately after graduation. This educational system benefits students, universities, and employers alike. Graduates of cooperative education are therefore graduates who "know themselves, know others, and know their work" (Ubon Ratchathani University, 2017).

Cooperative Education is an educational system that emphasizes real work experience in organizations by integrating academic instruction with practical work, also known as Work Integrated Learning (WIL). This approach helps students gain an understanding of working life before graduation and develop skills relevant to their profession that meet the needs of employers. It also serves as a key guideline for students to apply theoretical knowledge to practical work in a principled and systematic manner, producing quality graduates who meet labor market demands. Furthermore, it promotes ongoing academic collaboration between organizations and educational institutions. (Supporting Education Division, Faculty of Science, Prince of Songkla University, 2023)

In summary, cooperative education is an education system that combines teaching and learning in an educational institution with real work experience in a workplace (Work-Integrated Learning or WIL). This type of education system allows students to apply the knowledge gained in the classroom to real-world work settings, while also providing opportunities to develop skills and knowledge in areas that may not be accessible in a traditional classroom environment. It enables students to discover their own abilities and interests, which can lead to a clearer and more suitable career path in the future. For some students, if they perform well and work effectively with others during their internship, there may be opportunities to continue working at the organization and eventually become full-time employees. Furthermore, this system fosters collaboration between educational institutions and workplaces in curriculum development and student preparation, ensuring that graduates meet the needs of employers and the labor market.

3) Establishment

Chollada Mongkhonwanich et al. (2021) stated that an "establishment" refers to a government or private organization or agency that shares responsibility for the operation of cooperative education with educational institutions and accepts cooperative education students to work. Similarly, Ubon Ratchathani University (2017) defined an establishment as a government or private organization or agency that collaborates with educational institutions in implementing cooperative education and accepts students for practical training.

Therefore, an establishment is a business organization that supports and contributes to the tangible development of students through cooperative education by accepting them for a one-semester training period. In addition to sharing responsibility with educational institutions by hosting students for cooperative education, establishments also play a significant role in fostering knowledge and understanding of cooperative education operations among their executives and employees. This leads to the organization's development as a source of diverse learning experiences—spanning knowledge, skills, and socialization.

From studies on problems that occur in the workplace or professional environment, including issues faced by students, it was found that experts have conducted research on a number of related topics, as follows:

Natcha Thamrongchot et al. (2012) studied the problems and obstacles in accepting undergraduate students in the Faculty of Business Administration to work in cooperative education, based on the concepts of business establishments in organizing and operating cooperative education, the qualifications of cooperative education students, and professional knowledge and skills. The study was conducted on 180 students who participated in cooperative education. The research results found the following problems and obstacles: the first aspect was the organization and operation of cooperative education—business establishments could not coordinate with educational institutions to set guidelines and prepare students before starting work. In addition, business establishments did not know the purpose of working according to the cooperative education curriculum, and they did not trust students to contact the business establishments themselves. The second aspect was the qualifications of cooperative education students—students could not make decisions to solve problems while working, lacked self-confidence, and showed a lack of meticulousness in their work. The final aspect was professional knowledge and skills—students could not communicate effectively in both Thai and foreign languages, either verbally or in writing; they were unable to analyze and plan their work properly; and they lacked proficiency in using office equipment.

Siriwan Munintrawong (2018) studied the problems related to internships and work skills of Japanese language students at Thammasat University. The research findings revealed that the top five skills students need to develop during their studies, in order of importance, are: 1) Communication skills with others, 2) Japanese language knowledge, 3) Enthusiasm, 4) Teamwork, and 5) Basic knowledge and skills for work. As for the skills that internship students showed little development in, the department should prioritize monitoring the following four skills, in order: 1) Japanese language knowledge, 2) IT skills, 3) Business knowledge, and 4) Management and problem-solving skills.

2. Methodology

The study and collection of problems encountered by students during their internships, as well as the solutions they used to address those problems, employed a mixed-methods research approach, combining both qualitative and quantitative methods. Data were collected from cooperative education reports and semi-structured interviews. The research methodology is detailed as follows:

2.1 Research Sample

The sample population in this study was selected using purposive sampling. The participants were fourth-year students from the Faculty of Liberal Arts and the Faculty of Business Administration who participated in cooperative education programs during the academic years 2019 to 2021, covering a total of three academic years.

2.2 Research Instruments

The research instruments consisted primarily of student internship reports and semi-structured interviews. These tools were used to collect and analyze data. Data were organized and presented separately by department and faculty to clearly illustrate the problems students encountered and the solutions they applied. A coding method was used to analyze open-ended responses from both the reports and the interviews.

3. Results and Discussion

3.1 Results from the analysis of problems encountered during cooperative education placements

In terms of problems found during cooperative education, it was observed that students from the Faculty of Liberal Arts experienced varying problems depending on their majors.

For Chinese language majors, the most common issue was language-related problems, accounting for 30.37%, followed by work content and performance problems at 29.36%. Problems in using various equipment made up 15.19%, while the least common issues were related to living, workplace adjustment, and personal matters, accounting for 6.8%.

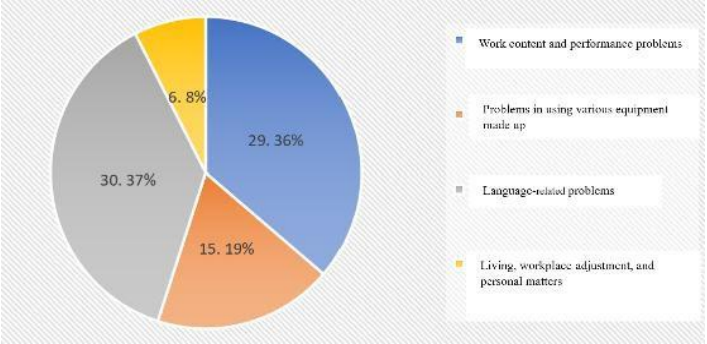

Figure 1 Problems encountered by Chinese language students during cooperative education placements

For Japanese language majors, language problems were also the most frequent, comprising 45.36%, followed by work content and performance issues at 40.32%. Equipment usage problems accounted for 28.22%, and the least common were living and personal adjustment issues, at 12.10%.

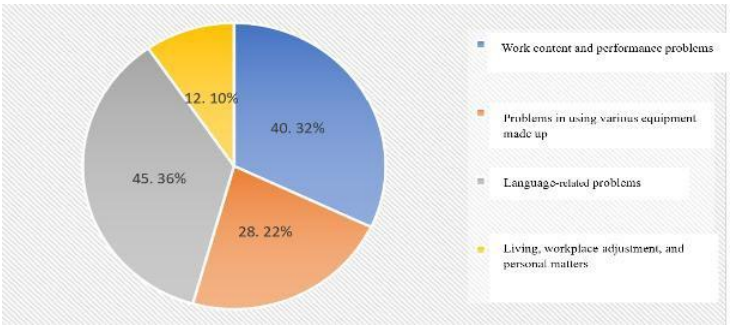

Figure 2 Problems encountered by Japanese language students during cooperative education placements

In contrast, students majoring in English for International Communication experienced the highest number of problems related to work content and performance, at 45%, followed by issues concerning living, adjustment, and personal matters at 25%. Language problems accounted for 23%, and the least common were equipment usage problems, comprising 7%.

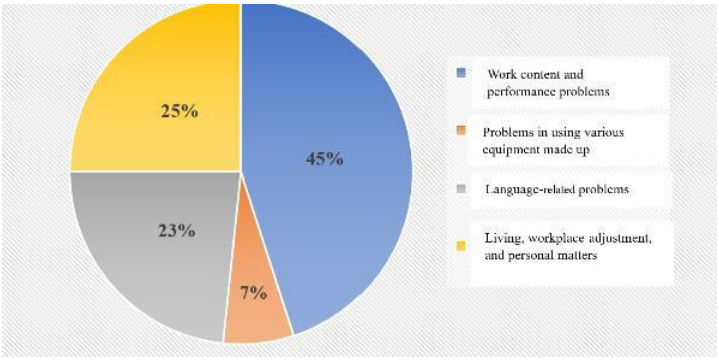

Figure 3 Problems encountered by English for International students during cooperative education placements

For students from the Faculty of Business Administration, it was found that those majoring in Accounting experienced the highest percentage of problems related to the use of various equipment (43%), followed by problems concerning work content and performance (27%). Issues related to living, adjusting to the workplace, and personal matters accounted for 23%, while language problems were the least common, at 7%.

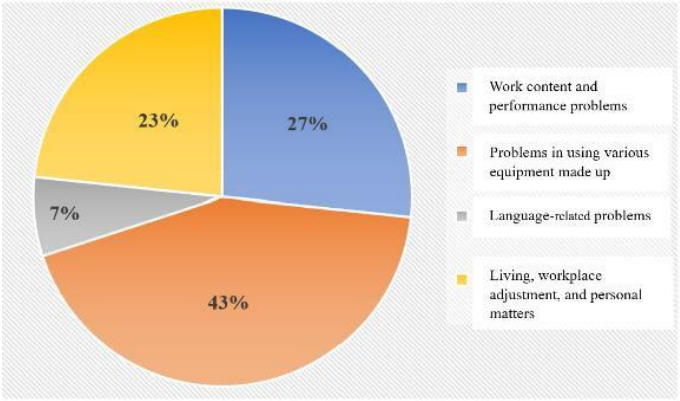

Figure 4 Problems encountered by Accounting students during cooperative education placements

Among students majoring in Management, the most frequently reported issues were related to work content and performance (34%), followed closely by equipment usage problems (33%). Problems related to living, workplace adjustment, and personal matters made up 29%, while language issues were again the least reported, at 4%.

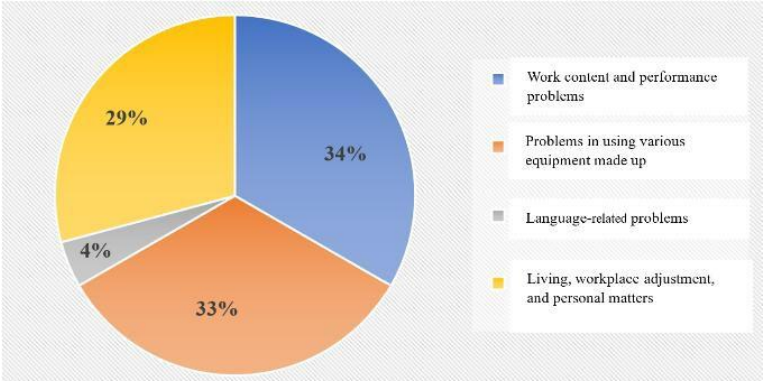

Figure 5 Problems encountered by Management students during cooperative education placements

For Marketing majors, the most prominent problems were associated with work content and performance, accounting for 52%, followed by equipment usage problems at 36%. Issues related to living, adjustment, and personal matters were reported at 7%, and language-related problems were the least common, at 5%.

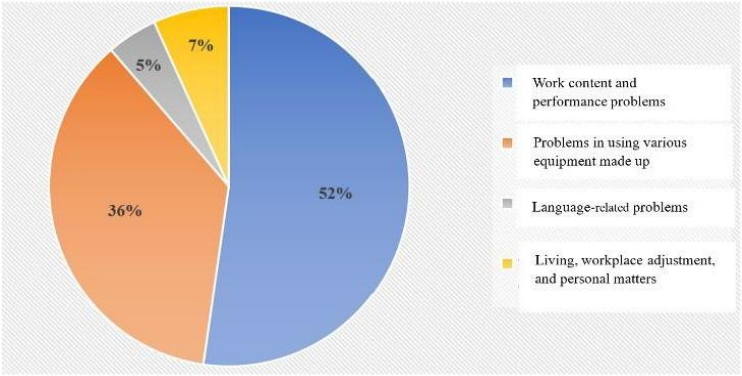

Figure 6 Problems encountered by Marketing students during cooperative education placements

3.2 Results from the Analysis of Problem-Solving Approaches in Cooperative Education placements

In cooperative education practices, after collecting data, students' problem-solving approaches can be classified into three groups based on their characteristics:

1) Independent problem-solving: Students help themselves by finding information independently.
2) Seeking assistance from workplace mentors: Students ask for help from mentors at their workplace.
3) Seeking assistance from supervising teachers: Students ask for help from their academic supervising teachers.

For Chinese language students, based on information obtained from the cooperative education report, students most commonly solved problems by helping themselves and finding information independently, accounting for 56%. The next most common approach was asking for help from a mentor in the workplace, accounting for 44%. Students in the Chinese language program did not seek help from the supervising teacher.

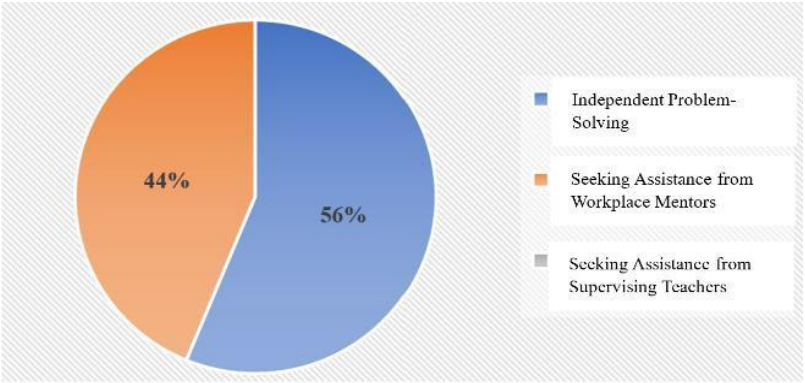

Figure 7 Methods for solving problems encountered in the cooperative education placements of Chinese language students

For Japanese language students, students most commonly solved problems by helping themselves and finding information independently, accounting for 76%. This was followed by solving problems by asking for help from a mentor in the workplace, accounting for 22%, and by asking for help from a supervising teacher, accounting for 2%.

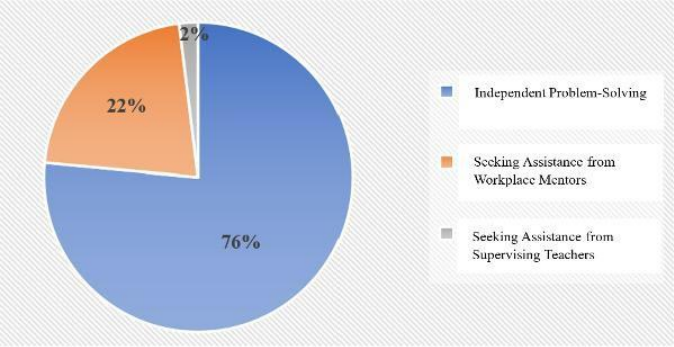

Figure 8 Methods for solving problems encountered in the cooperative education placements of Japanese language students

For students majoring in English for International Communication, students most commonly solved problems by helping themselves and finding information independently, accounting for 58%. This was followed by solving problems by asking for help from a mentor in the workplace, accounting for 42%. Students majoring in English for International Communication did not seek help from the supervising teacher.

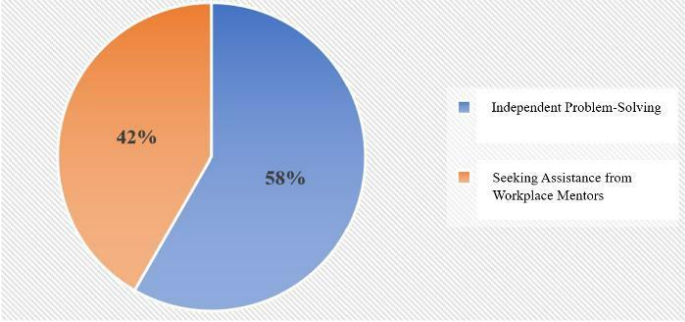

Figure 9 Methods for solving problems encountered in the cooperative education placements of English for International students

For students from the Faculty of Business Administration, it was found that Accounting students most commonly solved problems by helping themselves and finding information independently, accounting for 75%. This was followed by solving problems by asking for help from a mentor in the workplace, accounting for 25%, while Accounting students did not seek help from the supervising teacher.

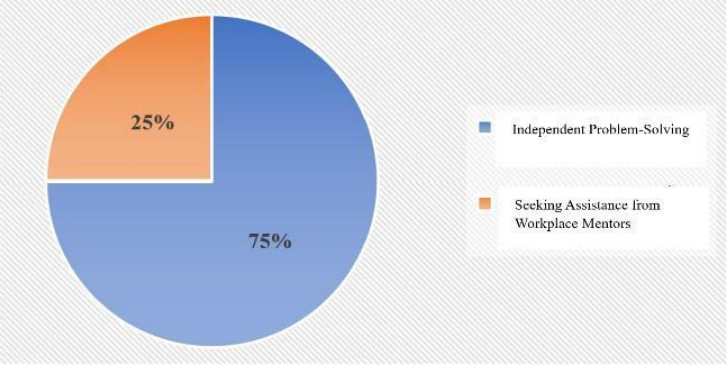

Figure 10 Methods for solving problems encountered in the cooperative education placements of Accounting students

For Management students, students most commonly solved problems by helping themselves and finding information independently, accounting for 50%. This was followed by solving problems by asking for help from a mentor in the workplace, accounting for 46%, and by asking for help from a supervising teacher, accounting for 4%.

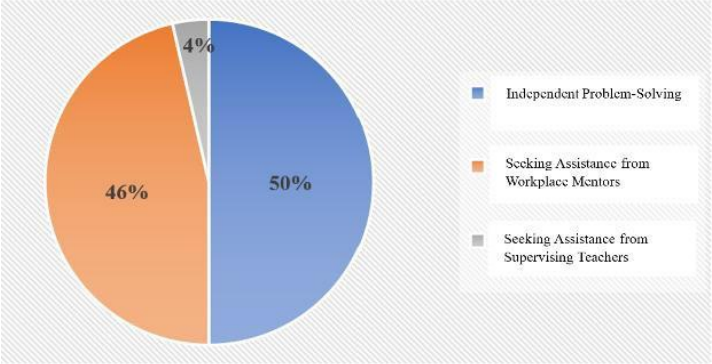

Figure 11 Methods for solving problems encountered in the cooperative education practice of Management students

For Marketing students, students most commonly solved problems by helping themselves and finding information independently, accounting for 72%. This was followed by solving problems by asking for help from a mentor in the workplace, accounting for 28%, while Marketing students did not seek help from the supervising teacher.

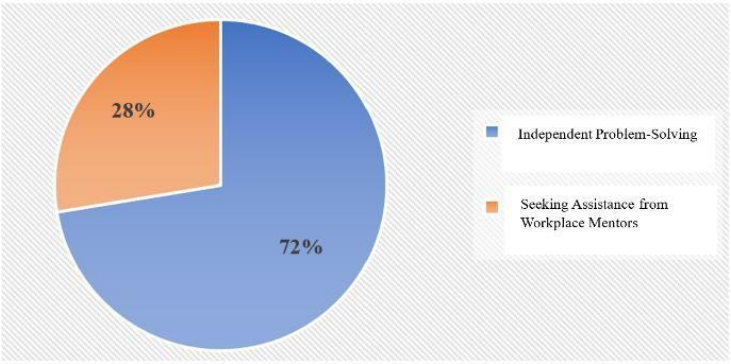

Figure 12 Methods for solving problems encountered in the cooperative education placements of Marketing students

3.3 Results of the interview

In the summary of the interview results, the representative found that the interview answers could be divided into issues similar to those identified in the cooperative education work reports. These issues mainly involved problems with work content and tasks. The interviewees pointed out that the work assigned was often difficult because it involved tasks, they had never done before or tasks not covered in their university studies, making it challenging to perform in real work settings.

Regarding the use of equipment, the interviewees noted difficulties in operating equipment they had never used prior to their work placements. Some also mentioned that the equipment at the workplace was not fully prepared for use, further complicating their tasks.

Language problems were also reported, with interviewees from both faculties facing challenges in communication. When a language problem occurred, the interviewee initially solved it by seeking help from someone with the necessary language skills or by having a mentor assist in resolving the issue. This approach helped them overcome the immediate problem. Additionally, the interviewee made use of technology to reduce communication barriers. This not only helped lessen the obstacles but also improved speaking and communication, allowing the interviewee and their contacts to better understand each other.

Additionally, students encountered issues related to living arrangements, adjusting to the work environment, and interacting with colleagues.

From the interviews, it was also found that most interviewees preferred to try solving problems on their own first, as they did not want to disturb their colleagues or supervisors. Some students also mentioned that they initially attempted to solve problems themselves because they were afraid to ask for help, fearing they might be scolded. However, many interviewees stated that they would first assess the type of problem. If it was something they believed they could handle independently, they would proceed on their own. However, if the problem seemed likely to cause damage or serious issues, they would immediately inform their supervisors.

However, the interviews also provided valuable advice for students preparing for cooperative education. Most interviewees emphasized the importance of carefully selecting a workplace—choosing one where they are likely to fit well—and thoroughly researching the job position before applying. If students are accepted into a position, it is recommended that they gather as much information as possible about the role beforehand.

4. Conclusions

The researcher discussed the results of the study on the problems encountered in the cooperative education of students at Rajamangala University of Technology Rattanakosin, Bophit Phimuk Chakkrawat area, based on the research questions. The first question asked: What types of problems do students encounter while practicing cooperative education? The research findings revealed that students faced problems in four main areas: work-related issues, issues with using various equipment, language difficulties, and challenges related to living, adjusting to work, and personal matters. Upon further analysis by faculty, it was found that work-related problems were the most prevalent across all fields, except for the accounting field, where equipment usage issues were more prominent. Based on the interview results, it appears that accounting work, which is typically office-based, requires the use of various office equipment. However, in the classroom, there is often little emphasis or practice with these tools. As a result, students may not be as comfortable using the equipment, which aligns with the findings of Natcha Thamrongchote et al. (2012), who studied the problems and obstacles in accepting students into cooperative education at the Faculty of Business Administration, Rajamangala University of Technology Phra Nakhon. Their research also found that students lacked proficiency in using office equipment.

Another interesting aspect is the issue of language problems. According to the data presented in the pie chart, students from the Faculty of Business Administration—particularly those in Accounting, Management, and Marketing—experienced only minor language problems, with most reporting issues at less than 10%. This suggests that the nature of their work typically does not require extensive language use. However, language skills are still important for this group of students. In contrast, students from three programs within the Faculty of Liberal Arts reported language problems as the second most common issue after work-related problems. This may be attributed to the nature of their studies, which focus primarily on language, requiring them to apply these skills directly in the workplace. As a result, they may face more difficulties in using language in professional contexts than their peers in the Business Administration faculty. These findings are consistent with the study by Siriwan Munintrawong (2018), who examined internship problems and work skills of students in the Japanese language program at Thammasat University. That study found that students encountered challenges specifically in tasks requiring translation and interpretation, highlighting how work that demands applied language knowledge can be a significant source of difficulty.

Regarding research question 2: How do students solve problems when they encounter issues during their internship? The study found that students across all majors generally attempted to solve problems on their own before seeking help from an advisor or consulting with a faculty supervisor. The interview data provided deeper insight into why students chose to handle problems independently at first. Some reasons included not wanting to disturb colleagues or feeling hesitant to ask for help due to fear of being scolded—especially if the issue caused from a repeated mistake. However, many students also reported that they assessed the nature of the problem before acting. If they believed the issue could be resolved independently, they would do so. Conversely, if the problem seemed likely to cause significant consequences, they would immediately notify their advisor.

Overall, the results of this study revealed the main problems that students faced while participating in cooperative education practice at the workplace. Additionally, the study highlighted the problem-solving methods that students employed when encountering these challenges. It was observed that most students attempted to solve the problems on their own before seeking assistance from their advisor or mentor. For some students who felt that the advisor could not resolve the issue, they would turn to the supervising faculty for help. Furthermore, the study emphasized the importance of selecting a suitable and well-informed workplace, as well as gathering information before starting the actual work, to benefit the students when transitioning into the real-world work environment

5. Acknowledgments

This research was successfully completed thanks to the kind support and cooperation of the department heads from both the Faculty of Liberal Arts and the Faculty of Business Administration. Their assistance in providing access to cooperative education report books—an essential source of primary data—was invaluable. The researchers would like to express sincere gratitude for their contributions. Appreciation is also extended to all supervising lecturers and students who participated in the cooperative education program for their facilitation and collaboration throughout the research process. Special thanks are due to Rajamangala University of Technology Rattanakosin for providing funding support from the fiscal year 2022 internal budget, which made this study possible.

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A Study of the Theory of Planned Behavior Influencing Consumer Purchase Intentions for Electric Vehicles in Phra Nakhon Si Ayutthaya Province

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Abstract

This study aims to examine the influence of three key factors—attitude toward the behavior, subjective norms, and perceived behavioral control on the intention to purchase electric vehicles (EVs) in Phra Nakhon Si Ayutthaya Province. The research framework is based on the Theory of Planned Behavior (TPB). The sample group consisted of 400 individuals aged between 20 and 59 years (Generation X and Y), residing in Phra Nakhon Si Ayutthaya, selected using a convenience sampling method. A questionnaire was used as the data collection tool. The data were analyzed using descriptive statistics, analysis of variance (ANOVA), and multiple regression analysis. The results revealed that most respondents were female, aged between 20–29 years, single, held a bachelor's degree, worked in private companies, and had a monthly income of less than 15,000 baht. The three main factors—attitude, subjective norms, and perceived behavioral control—were rated at a high level of agreement. Furthermore, demographic variables including age, marital status, and monthly income showed statistically significant differences in the intention to purchase EVs at the 0.05 significance level. The three psychological factors together were found to explain 59.3% of the variance in purchase intention, also statistically significant at the 0.05 level.

Keywords: Electric Vehicles, Theory of Planned Behavior, Purchase Intention, Demographics

1. Introduction

Currently, every country is inevitably facing the phenomenon of global warming — the rise in the average temperature of the Earth's surface air and oceans. This temperature continues to increase annually. According to data from the Climatic Research Unit and the National Oceanic and Atmospheric Administration (NOAA), from 1981 to 2018, the Earth's average temperature has increased by approximately 1°C. Furthermore, the concentration of carbon dioxide (CO_2) in the atmosphere reached 414.3 parts per million (ppm) in 2021, with an annual increase of about 2.4 ppm, as reported by NASA's Goddard Institute for Space Studies (GISS).

The primary causes of global warming stem from human activities such as the burning of coal and fossil fuels for transportation, along with deforestation for human benefit, which rapidly increases CO_2 in the atmosphere. These greenhouse gases accumulate in the Earth's atmosphere, trapping heat. Other contributing natural factors include variations in Earth's orbit and volcanic eruptions, both of which lead to heat retention and global temperature rise. This widespread impact threatens the natural ecosystem (Kritsada Sektakul, 2021). The more fossil fuel-powered vehicles are used, the faster global warming progresses. Although fuel prices may not be the main reason governments promote electric vehicles (EVs), concern about climate change remains a major driver. It is predicted that by 2050, emissions from the transportation sector will account for 30–50% of global greenhouse gas emissions. Hence, many governments are encouraging citizens to reduce private car usage and rely more on public transportation to minimize transportation-related pollution.

One of the most promising solutions many countries are adopting to reduce air pollution is the use of 100% electric vehicles (EVs). These innovative vehicles run solely on electricity, making them more cost-efficient in terms of maintenance and energy usage since electricity is cheaper than fossil fuels. Moreover, electric vehicles emit no exhaust gases, which are typically caused by incomplete fuel combustion, thus being environmentally friendly (Nuengruethai Rattanaporn, 2019).

In Thailand, the electric vehicle policy is one of the top priorities of the Prime Minister and the government. The policy aims to maintain Thailand's automotive production base and position the country as a hub for electric vehicle, motorcycle, and bus production. The national target is to produce 750,000 electric vehicles by 2030 (Ministry of Industry, March 2020). Electric vehicles began playing a more prominent role in Thailand around 2018, with steadily increasing sales. As of December 2019, the number of registered electric vehicles in Thailand totaled 124,085 units (Department of Land Transport), representing only 1% of all new car sales. Consumers are aware that EVs reduce reliance on fossil fuels and help protect the environment. However, many are concerned about the high purchase price, primarily due to the expensive battery component, which is significantly more costly than those in

internal combustion engine vehicles. Additionally, most EVs sold in Thailand are imported, contributing to the higher price.

Therefore, the researcher is interested in studying the application of the Theory of Planned Behavior (TPB) to examine consumers' purchase intentions regarding electric vehicles (EVs) in Phra Nakhon Si Ayutthaya Province. The findings of this research are expected to benefit automotive businesses and both government and private sector stakeholders by providing insights and strategies to better educate and inform consumers, ultimately promoting the widespread adoption of electric vehicles in Thailand for environmental sustainability.

2. Methodology

2.1 Population and Sampling

The target population for this study includes 400 individuals aged 20 to 59 years (comprising Generation Y and Generation X) residing in Phra Nakhon Si Ayutthaya Province, as detailed in Table 1.

Table 1 Population and Sampling

Age Range (Years)	Male	Female	Total	
20-24	24,703	23,717	**48,420**	Gen Y
25-29	22,318	22,690	**45,008**	
30-34	20,369	21,300	**41,669**	
35-39	22,180	23,793	**45,973**	
40-44	23,770	25,752	**49,522**	Gen X
45-49	23,369	26,605	**49,974**	
50-54	24,423	28,689	**53,112**	
55-59	22,283	26,752	**49,035**	
Total	158,712	175,581	**334,293**	

Since the exact population size is known, the sample size can be determined using Taro Yamane's formula, with a 95% confidence level and a 5% margin of error. The formula used for sample size calculation in this study is based on Taro Yamane (1967), given a known population size.

$$\boldsymbol{n = \frac{N}{1+Ne^2}}$$

Where:
n = sample size
N = population size
e = margin of error (0.05 for 5%)

$$\boldsymbol{n = \frac{334{,}293}{1+334{,}293(.05)^2}}$$

$$\boldsymbol{n = 399.52}\ \text{Individuals}$$

Therefore, the sample group consists of individuals aged between 20 and 59 years (Generation Y and Generation X) residing in Phra Nakhon Si Ayutthaya Province. The sampling method used is non-probability sampling, specifically convenience sampling. Questionnaires were distributed to 400 individuals within the specified age group who reside in Phra Nakhon Si Ayutthaya Province.

2.2 Research Instrument

The researcher collected data from the sample group using a **questionnaire** as the primary research instrument. The process of questionnaire construction consisted of the following steps:

1) Studied the development of questionnaires through academic documents, previous research, and theories related to the Theory of Planned Behavior and purchase intention of electric vehicles. This included factors such as **attitude toward behavior, subjective norms**, and **perceived behavioral control**.
2) Designed the questionnaire based on the conceptual framework to collect opinions on the various factors influencing the intention to purchase electric vehicles.
3) Revised and refined the questionnaire before submitting it for approval by the thesis advisor.
4) Conducted a **reliability test** using **Cronbach's Alpha Coefficient** to evaluate the internal consistency of the items. The coefficient ranges from 0 to 1, with values closer to 1 indicating higher reliability.
5) Final review of the questionnaire was conducted after receiving approval from the thesis advisor.
6) Distributed the questionnaire to 400 respondents.

2.3 Instrument Validation

The researcher verified the completeness and content validity of the questionnaire by presenting it to the thesis advisor. The final version of the questionnaire consisted of four parts as follows:

Part 1: Demographic Information

This section includes **closed-ended questions** designed to gather basic demographic data:

Item 1: Gender – Nominal Scale
Item 2: Age – Nominal Scale

Item 3: Marital Status – Nominal Scale
Item 4: Highest Education Level – Ordinal Scale
Item 5: Occupation – Nominal Scale
Item 6: Average Monthly Income – Ordinal Scale

Part 2: Behavioral Factors

This section includes items assessing factors that influence: Attitude toward the behavior Subjective norms (influence of reference groups) Perceived behavioral control Purchase intention for electric vehicles

Part 3: Suggestions

This section allows respondents to provide additional comments or suggestions related to the study. *All items in Parts 2 and 3 were measured using a **5-point Likert Scale**, with the following interpretation:*

5 = Strongly Agree
4 = Agree
3 = Neutral
2 = Disagree
1 = Strongly Disagree

To interpret the results, the researcher calculated class intervals using standard formulas to define score ranges and provide meaningful interpretation for each level of response.

2.4 Data Collection

The study was conducted by utilizing two sources of data as follows:

1) Primary Data

Primary data were collected directly from a sample of 400 individuals aged between 20 and 59 years (Generation X and Generation Y) residing in Phra Nakhon Si Ayutthaya Province. Data were gathered using a self-administered questionnaire developed by the researcher and distributed online via Google Forms**.** The questionnaire link was posted on various social media platforms including Facebook, Instagram, and Line**.**

2) Secondary Data

Secondary data were obtained from academic documents and related research, including theses, journal articles, academic publications, and other printed materials. Additionally, relevant information from both domestic and international internet sources was reviewed. These data were used to support the conceptual framework and provide background for the study.

2.5 Data Processing and Analysis

After collecting the completed questionnaires, the researcher verified the accuracy of the responses and performed data coding**.** The coded data were then analysed using a statistical software package, specifically the Statistical Package for the Social Sciences (SPSS)**.** This software was used to compute various statistical measures required for data analysis and interpretation.

2.6 Statistical Methods for Data Analysis

In this study, the researcher employed both **descriptive** and **inferential statistics** as follows:

1) Descriptive Statistics

Descriptive statistics were used to summarize and describe demographic characteristics of the sample group, including gender, marital status, age, highest education level, occupation, and average monthly income. These data were presented using: Frequency distributions**,** Percentage for Part 2 of the questionnaire, the researcher used: Mean (M) Standard Deviation (SD) to describe the level of opinions toward behavioral factors related to the intention to purchase electric vehicles.

2) Inferential Statistics

Inferential statistics were used to test hypotheses and analysed relationships among variables: Analysis of Variance (ANOVA) at a 0.05 significance level was used to examine relationships between independent variables such as attitude toward behavior**,** perceived behavioral control**,** and subjective norms**,** and the purchase intention of electric vehicles. Reliability Analysis was conducted using Cronbach's Alpha Coefficient to assess the internal consistency of the questionnaire. Data collected from the 400 respondents were used in this analysis, and an acceptable reliability value was considered to be not less than 0.70**.** Multiple Regression Analysis was applied to identify the strength and direction of the relationship between independent variables (predictors) and the dependent variable (purchase intention of electric vehicles).

3. Results and Discussion

3.1 Analysis of Demographic Characteristics

The analysis of the demographic characteristics of the sample group revealed the following: The majority of respondents were female, totalling 241 individuals (60.3%). Most were aged 20–29 years, accounting for 175 individuals (43.8%). The highest proportion of respondents were single, totalling 229 individuals (57.3%). Regarding education, the majority held a bachelor's degree, totaling 220 individuals (55.0%). The most common occupation was company employee, with 149 individuals (37.3%). In terms of average monthly income, the largest group earned less than 15,000 baht, with 120 individuals (30.0%).

3.2 Analysis of Respondents' Opinions on the Application of the Theory of Planned Behavior in Studying the Intention to Purchase Electric Vehicles

The results of the study revealed the following levels of respondents' opinions:

1) Attitude Toward the Behavior

The respondents' overall opinion regarding attitude toward the behavior was at a high level of agreement, with a mean score of 3.78. The highest-rated item was "Electric vehicles will be beneficial to the environment in the long term", which received a mean score of 3.88.

2) Subjective Norm

The overall level of opinion regarding subjective norms was also at a high level of agreement, with a mean score of 3.62. The highest-rated item was "Most people will value you if you use an electric vehicle in the future", with a mean score of 3.66.

3) Perceived Behavioral Control

The respondents' overall opinion on perceived behavioral control was at a high level of agreement, with a mean score of 3.62. The highest-rated item was "The price of an electric vehicle is important to you and you can afford it when you decide to adopt it", which had a mean score of 3.70.

4) Purchasing Intention

The respondents' overall purchasing intention was rated at a high level of agreement, with a mean score of 3.50. The highest-rated item in this category was "I intend to use an electric vehicle in the future", with a mean score of 3.59.

3.3 Differences in Demographic Characteristics and Related Factors

The study found significant differences between demographic characteristics and various behavioral factors, as follows:

1) Age

There were statistically significant differences among age groups in relation to all key behavioral factors: attitude toward the behavior, subjective norm, perceived behavioral control, and purchasing intention.

- Respondents aged 40–49 years showed significantly lower attitudes toward the behavior than those aged 20–29 and 30–39 years.
- For subjective norm and purchasing intention, respondents aged 40–49 and 50–59 years demonstrated significantly lower levels of agreement than those aged 20–29 and 30–39 years.
- For perceived behavioral control, respondents aged 20–29 years showed significantly higher levels of agreement than those in the older age groups (30–39, 40–49, and 50–59 years), while those aged 50–59 years had significantly lower agreement than the 30–39 age group.

2) Marital Status

Marital status showed significant differences across all behavioral factors. Specifically:

- Respondents who were single had significantly higher levels of agreement on attitude toward the behavior, perceived behavioral control, and purchasing intention than those who were married.
- Singles also expressed significantly higher agreement on subjective norm than those who were divorced/widowed/separated.

3) Average Monthly Income

Statistically significant differences were observed in all behavioral factors across income levels:

- Respondents with monthly incomes below 15,000 THB reported significantly higher attitudes toward the behavior than those earning 60,001–75,000 THB and above 75,001 THB.
- Those earning 15,001–30,000 THB and 30,001–45,000 THB also reported significantly higher attitudes than higher-income groups.
- Respondents with incomes below 15,000 THB had significantly higher agreement on subjective norms than those earning 45,001 THB and above.
- Respondents in the lower-income groups (below 45,000 THB) also demonstrated significantly higher perceived behavioral control than those in higher income brackets (45,001 THB and above).
- Even among mid-income earners (45,001–60,000 THB), perceived behavioral control was significantly higher than those earning above 75,001 THB.

4) Influential Factors on Purchasing Intention

Based on the Theory of Planned Behavior, the key factors influencing purchase intention of electric vehicles among consumers in Phra Nakhon Si Ayutthaya include:

- Attitude Toward the Behavior
- Subjective Norm
- Perceived Behavioral Control

The results revealed that these three variables significantly influenced purchasing intention at the 95% confidence level and together explained 59.3% of the variance in the intention to purchase electric vehicles. Thus, all three factors play a statistically significant role in determining consumers' purchase intentions.

4. Conclusions

The results of this study are discussed according to the research objectives as follows:

4.1 Levels of Attitude, Subjective Norm, Perceived Behavioral Control, and Purchase Intention Toward Electric Vehicles among Consumers in Phra Nakhon Si Ayutthaya

The study found that consumers generally had a high level of attitude toward electric vehicle (EV) adoption. This may be due to the increasing popularity of EVs as a modern innovation and a viable alternative to traditional cars. Many consumers perceive EVs positively and have begun to compare information about EVs, indicating growing interest and expectation that EVs can outperform conventional vehicles. Therefore, if accurate and compelling information about EV features and benefits is effectively communicated, and positive perceptions, experiences, and emotional connections are fostered, consumers' intention to purchase EVs will likely increase.

Subjective norms were also found to influence purchase intention. Although consumers showed a high level of intention to purchase EVs, it may stem from general awareness rather than actual usage experience. The decision to buy such a high-value product often depends on confidence in the product. Thus, positive recommendations from reference groups may enhance this confidence and increase consumers' purchase intention.

For perceived behavioral control, the study revealed a high level of consumer confidence and intention. As EVs are still new to many consumers, factors such as innovative technology, operational systems, pricing, and cost-effectiveness play a significant role in shaping trust. When consumers perceive that they have sufficient internal resources (e.g., knowledge, need) and external conditions (e.g., independence, opportunity), their perceived behavioral control increases—thereby strengthening their purchase intention.

4.2 Influence of Attitude, Subjective Norm, and Perceived Behavioral Control on Purchase Intention Toward Electric Vehicles

The findings confirmed that attitude toward behavior significantly influences consumers' purchase intention for EVs in Phra Nakhon Si Ayutthaya. This aligns with the research by Adnan, Nordin, and Rahman (2017), which studied EV acceptance in Malaysia and found that environmental awareness and positive attitudes, subjective norms, and perceived behavioral control significantly influenced intention.

Similarly, Shalender and Sharma (2020), who studied 326 consumers and 57 automotive sales representatives in India, found that attitude positively influenced EV acceptance and purchase intention. Tu and Yang (2019) also found that Taiwanese consumers with positive attitudes toward EVs were more likely to compare technology and cost with traditional cars, leading to higher purchase intention.

Regarding subjective norm, the results support Schmalfuß, Mühl, and Krems (2017), who found that reference groups have a strong influence on EV purchase intention, especially when close individuals introduce the product positively. Likewise, Eneizan (2019) in a study conducted in Jordan, using the Theory of Planned Behavior with 250 respondents, found that subjective norms positively influence EV adoption. Peters and Dütschke (2014) also noted that reference groups significantly influence those who have not yet experienced using EVs, a finding relevant to the current context of EV adoption in Thailand.

As for perceived behavioral control, the study's findings are consistent with Al-Amin, Ambrose, Masud, and Azam (2016), who examined Malaysian consumers and found a significant positive influence of perceived behavioral control on purchase intention. This is also supported by Asadi et al. (2020), whose study of 177 Malaysian respondents revealed that attitude, subjective norm, and perceived behavioral control all positively influenced EV purchase intention. Furthermore, Zhang et al. (2018), studying 124 respondents aged 18–55 in Beijing, found that perceived behavioral control was the most influential factor in car-sharing EV usage intention, followed by subjective norm and attitude.

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Session 2
Agricultural and Food Innovation

Influence of fertigation on growth, yield, and ratooning ability of sugarcane (*Saccharum officinarum* L.) var. Khon Kaen 3

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Abstract

This research aimed to compare the effects of drip irrigation and fertilizer application on growth, root length density (RLD), yield, and yield components of sugarcane and to evaluate their impacts on ratooning ability. To achieve this objective, sugarcane cultivation practices were conducted in sandy clay loam soil over two years. The effects of drip irrigation, drip fertigation, and rainfed conditions on growth, yield, irrigation water use efficiency (IWUE), and fertilizer nutrient use efficiency (FNUE) were compared in plant crops. The results showed that the drip fertigation treatment could produce the highest growth and yield. In the first ratoon crop (FRC), the effect of plant crop practices on the adaptation of FRC was evaluated by splitting the treatments of plant crops into two different practices: rainfed condition and drip fertigation. Growth, yield, and RLD were evaluated as indicators of the ratooning ability of the ratoon crop. The results showed that the previous crop practices for the plant crop of sugarcane did not affect the ratoon crop performance. For the FRC practice, drip fertigation exhibited the highest growth, yield, and ratooning ability. However, the RLD in both years of rainfed treatments was higher than in the drip irrigation treatments.

Keywords: Drip Irrigation, Ratooning Potential, Saccharum Officinarum, Root Distribution, First Ratoon Cane

1. Introduction

1.1 Sugarcane practice

Sugarcane (*Saccharum officinarum* L.) is a significant economic crop predominantly grown in tropical and subtropical regions. The world's largest sugarcane producers are Brazil, India, Thailand, China, and Pakistan. Thailand is the fourth-largest producer globally, with annual production reaching 1 trillion metric tons. The Northeastern region of Thailand, the country's largest sugarcane-producing area, covers approximately 0.85 million hectares, yielding 58.7 million tons of fresh produce in 2018/19, representing 43.6% of the country's total production (Office of the Cane and Sugar Board, 2019). However, the region's average yield of 68.13 tons/ha is significantly lower than its potential, highlighting the need for improved production practices. Sugarcane in the Northeast is primarily rainfed, planted towards the end of the rainy season, making it vulnerable to water deficits during the critical early growth stages (3−5 months after planting). Prolonged dry conditions hinder fertilizer uptake due to insufficient soil moisture, reducing stalk and biomass accumulation and, consequently, lowering yield and quality (Jangpromma et al., 2012). This also impacts ratoon crops, where reduced biomass accumulation in the initial crop adversely affects subsequent ratoons (Shrivastava et al., 2018). Farmers generally prefer cultivating two or more ratoon crops due to the lower costs, 25−30% less than planting new crops. However, most farmers struggle to achieve more than two ratoons, often suffering from limited productivity due to suboptimal management and environmental stressors like water scarcity and nutrient deficiencies. Inefficient crop management practices, including shallow soil depth, root stubble accumulation, low nitrogen availability, and inadequate irrigation, further reduce ratoon productivity (Rana et al., 2021).

1.2 Drip irrigation practice

Drip irrigation offers a promising solution by efficiently delivering water directly to the root zone, minimizing losses from deep percolation, runoff, and erosion. This method is particularly beneficial in dryland agriculture, ensuring efficient water and nutrient use and promoting uniform crop growth. Moreover, drip irrigation facilitates fertigation, enhancing nutrient uptake and reducing fertilizer loss, leading to higher yields, improved ratooning ability, and superior crop quality (Jayant et al., 2022; Annappa et al., 2023). Drip irrigation has demonstrated superior water use efficiency, with 74.1% efficiency and 26.3% water savings compared to conventional furrow irrigation (Annappa et al., 2024). Applying 140 kg/ha of N fertilizer via drip irrigation increased stalk and sugar production by 31% in the second ratoon crop compared to non-irrigated plots (Uribe et al., 2013). Optimizing irrigation and fertilization is therefore crucial for maximizing sugarcane yield and ratooning ability, particularly in the Northeastern region of Thailand. This study aimed to compare the effects of drip irrigation and fertilizer application on the growth, yield, root length density (RLD), and ratooning ability of sugarcane in ratoon crops.

2. Methodology

2.1 Experimental description and design

The experiments were conducted under typical field conditions over two planting seasons: 2018/19 for the plant crop (PC) and 2019/20 for the first ratoon crop (FRC) in Muang district, Nakhon Ratchasima, Thailand (14°52'37" N, 102°0'21" E). Khonkaen 3 is a high-yield sugarcane variety known for the strong ratooning ability and

excellent disease resistance (Ponragdee et al., 2011) was planted in a double-row configuration with a plant spacing of 0.3 m and row spacing of 1.6 m. The soil at the experimental site was classified as sandy clay loam, composed of 46.2% sand, 22.5% silt, and 32.3% clay, as shown in Table 1. The bulk density of the soil was 1.25 g/cm³, and the available water holding capacity (AWHC) was 1.65 mm/cm. The soil chemical properties exhibited slightly acidic pH levels (6.68 in 2018/19 and 6.77 in 2019/20), low electrical conductivity (EC=0.031 µS/cm in 2018/19 and 0.072 µS/cm in 2019/20), and organic matter (OM) content that decreased from 1.43% in 2018/19 to 1.08% in 2019/20. The availability of phosphorus was stable around 12.7-12.9 ppm, while exchangeable potassium increased from 63.0 ppm to 74.2 ppm. The amounts of rainfall in 2018/19 and 2019/20 were gauged daily at planting area 748 mm and 1,251 mm, respectively.

The experiments were conducted over two consecutive years with distinct designs. In the first year (2018/19, PC), the experiment was laid out in a randomized complete block design with four replications. Treatments included three irrigation and fertilization practices: T1: Rainfed (no irrigation), T2: Drip irrigation with soil fertilization, and T3: Drip fertigation. In the second year (2019/20, FRC), a split-plot design in RCBD with three replications was employed. Main plots comprised three previous plant crop practices residual: T1: Rainfed, T2: Drip irrigation with soil fertilization, and T3: Drip fertigation. Sub-plots included two FRC treatments: S1: Rainfed and S2: Drip fertigation.

Table 1. Physical and chemical properties of experimental soil

Physical properties		Chemical properties	(2018/19)	(2019/20)
Sand (%)	46.2	pH	6.68	6.77
Silt (%)	22.5	EC (µS/cm)	0.031	0.072
Clay (%)	32.3	OM (%)	1.43	1.08
Bulk density (g/cm^3)	1.25	Av. P (ppm)	12.7	12.9
Available water holding capacity AWHC (mm/cm)	1.65	Ex. K (ppm)	63	74.2
		Ca (ppm)	2,030	2,150
		Mg (ppm)	196	212

Note: EC (electrical conductivity), OM (organic matter), AV. P (available phosphorus), EX. K (exchangeable potassium), Ca (calcium), and Mg (Magnesium)

2.2 Water and fertilizer application

Water availability in rainfed conditions depended on the average annual rainfall. Drip irrigation was applied using drip tape with a flow rate of 2 L/h and dropper spacing of 30 cm, with water supplied based on the water balance model. Daily water consumption of sugarcane was calculated using the following equation of Allen et al. (1998)

$$ETc = ETo \times Kc$$

Where ETc = the crop evapotranspiration (mm/day),
ETo = the amount of water consumed by a standard or reference crop (mm), and an average ETo over 10 years was used in this experiment.
Kc = the crop coefficient.

Fertilizers were applied based on the nutrient balance model, with soil application occurring twice at 1 and 3 MAP. Fertilization was applied every 3 weeks, totalling six applications from 1 st to 5 th MAP. Fertilizer rates according to the nutrient balance model were calculated using the nutrient balance equation as the study of Wonprasaid et al. (2023).

$$NS = \frac{NR - (SAN - SM)}{NUE}$$

Where NS = the nutrient supply (kg/ha)
NR = the nutrient requirement for a target yield of 187.5 tons/ha
SAN = the amount of soil available nutrients obtained from soil analysis (kg/ha) in sandy clay loam soil with OM=1%, P=10 ppm, and K=60 ppm.

2.3 Data collection

1) Plant growth measurements

Growth parameters were measured at 6 MAP for PC and 6 month after harvest (MAH) for FRC. The number of stalks on a 19.2 m^2 long row section was recorded. Plant height was measured from the soil base to the leaf flag (the highest visible node). Leaf area was measured using a Li-Cor area meter Li-3100e, and leaf area index (LAI) was calculated based on ground area and leaf area.

2) Yield and yield components

Yield and yield component parameters were measured at 12 MAP for PC and 12 MAH for FRC. Yield and number of millable canes (NMC) were measured from millable canes within a 19.2 m^2 area per replication. Cane diameter was measured with a vernier caliper, and Brix (%) was determined using a refractometer.

3) Irrigation water and fertilizer nutrient use efficiency

Irrigation water use efficiency (IWUE) was calculated as the equation of (De Pascale et al., 2011)

$$\text{IWUE (tons/m}^3\text{)} = \frac{\text{FY (tons/ha)}}{\text{IW (m}^3\text{/ha)}}$$

Where FY = the yield of millable cane
IW = the irrigation water.

Fertilizer nutrient use efficiency (FNUE) was calculated by the index of partial factors of productivity of applied nutrient (Doberman, 2007) as:

$$\text{FNUE (tons/kg)} = \frac{\text{FY (tons/ha)}}{\text{NS (kg/ha)}}$$

Where FY = the yield of millable cane
NS = the amount of nutrient applied.

4) Root length density (RLD)

RLD was measured at 12 MAP for PC and 12 MAH for FRC. Root samples were taken at 0 cm, 40 cm, and 80 cm from the planting row at depths of 0-20 cm, 21-40 cm, and 41-80 cm. RLD was calculated as root length per soil volume using WinRHIZO 2013 software.

5) Germination percentage and ratooning ability

Germination percentage for FRC was assessed by examining underground stubble and estimating shoot germination, as described by Set-Tow et al. (2020).:

$$\text{Germination (\%)} = \frac{\text{Number of shoot germinate in FRC}}{\text{Number of millablecane in PC}} \times 100$$

Ratooning ability (RA) was defined as the yield potential of FRC compared to PC, calculated according to Dlamini et al.(2024a).

$$\text{RA (\%)} = \frac{\text{The productivity (yield and components of FRC)}}{\text{The productivity (yield and components of PC)}} \times 100$$

2.4 Statistical analysis

All parameters were analyzed using ANOVA with SPSS V.16 for Windows. Mean comparisons were performed using Duncan's multiple range test at a significance level of $p<.05$.

3. Results and Discussion

3.1 Growth, yield, and yield components of the planting crop

1) Growth parameters

At 6 MAP, drip fertigation significantly enhanced sugarcane growth, yielding the highest values across all parameters: 82,119 stalks/ha, a plant height of 170 cm, and LAI of 2.70 (Table 2). In contrast, the rainfed treatment produced the lowest growth metrics with 68,150 stalks/ha, a plant height of 102 cm, and an LAI of 1.30. Drip irrigation with soil fertilizer (T2) showed intermediate results, with 72,744 stalks/ha, a plant height of 157 cm, and an LAI of 2.16.

Table 2. Sugarcane growth parameters at 6 MAP under various water and fertilizer applications

Treatments	Number of stalks[1] (stalk/ha)	Plant height (cm)	LAI
T1: Rainfed condition	68,150c	102c	1.30c
T2: Drip irrigation with soil fertilizer	72,744b	157b	2.16b
T3: Drip fertigation	82,119a	170a	2.70a
F-test	**	**	**
CV (%)	12.45	10.91	15.72

[1]Means with different letters within the same column indicate significant differences at $p<.05$.

2) Yield and yield components

The effects of water and fertilizer treatments on yield and components were significant, as shown in Table 3. Drip fertigation produced the highest yield (140 tons/ha), NMC (80,613 stalks/ha), and Brix (22.3%). In contrast, the rainfed practice resulted in the highest stalk diameter (2.89 cm). Brix levels did not significantly differ among the cultivation practices.

Table 3. Sugarcane yield and yield components under different water and fertilizer applications

Treatments	Yield[1] (tons/ha)	No. of millable cane (stalks/ha)	Stalk diameter (cm)	Brix (%)
T1: Rainfed condition	81.9c	63,963c	2.89a	22.0
T2: Drip irrigation + soil fertilizer	114.4b	69,269b	2.74ab	22.2
T3: Drip fertigation	140.0a	80,613a	2.60c	22.3
F-test (0.05)	**	**	*	ns
CV (%)	14.40	10.45	11.62	9.07

[1]Means with different letters within the same column indicate significant differences at p<.05.

3) Irrigation water and fertilizer nutrient use efficiency

The IWUE results show that drip fertigation achieved the highest IWUE at 14.9 tons/m³, compared to 11.1 tons/m³ for drip irrigation with soil fertilizer (T2). This highlights the effectiveness of combining precise water delivery with nutrient management in enhancing productivity per unit of water applied, particularly in water-scarce regions. T3 also recorded superior FNUE, with efficiencies of 1.243 tons/kg for N, 1.674 tons/kg for P_2O_5, and 0.791 tons/kg for K_2O, significantly outperforming T2 and T1 (Table 4).

Table 4. Irrigation water and fertilizer nutrient use efficiency in sugarcane cultivation.

Treatments	IWUE[1] (tons/m³)	FNUE (tons/kg)		
		N	P_2O_5	K_2O
T1: Rainfed condition	5.0c	0.711c	0.960c	0.452c
T2: Drip irrigation + soil fertilizer	11.1b	1.000b	1.354b	0.639b
T3: Drip fertigation	14.9a	1.243a	1.674a	0.791a
F-test (0.05)	**	**	**	**
CV (%)	8.32	7.32	7.35	7.31

[1]Means with different letters within the same column indicate significant differences at p<.05.

4) Root length density (RLD)

Sugarcane under the rainfed treatment tended to have the highest RLD at 0 cm of planting bed depth, with values of 1.247 cm/cm³ (0-20 cm depth) and 0.080 cm/cm³ (40-80 cm depth). Drip fertigation and drip irrigation with soil fertilizer tended to have the lower RLD values, with T3 showing 0.578 cm/cm³ (0-20 cm) and 0.039 cm/cm³ (40-80 cm), and T2 showing 0.523 cm/cm³ (0-20 cm) and 0.029 cm/cm³ (40-80 cm) (Figure 1).

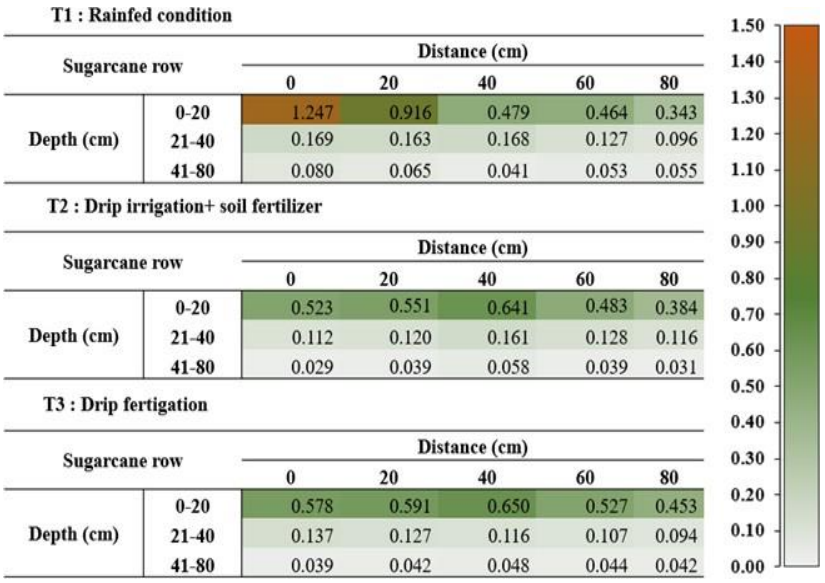


Figure 1. RLD of PC sugarcane: rainfed condition, drip irrigation+soil fertilization, and drip fertigation.

3.2 Growth, ratooning ability, yield, and yield components of the first ratoon crop

1) Germination and growth parameters

Previous crop practices significantly impacted germination rates, with T1 (rainfed) achieving the highest rate (250%), followed by T2 (223%) and T3 (202%). However, these practices did not significantly affect stalk numbers or plant height in the FRC. In contrast, the current crop practice of drip fertigation (S2) significantly increased both stalk numbers (111,213 stalks/ha) and plant height (226 cm) compared to rainfed conditions (S1), which produced 103,188 stalks/ha and a plant height of 189 cm (Table 5). Notably, no significant interaction was found between PC and FRC practices, indicating independent effects on these growth parameters.

Table 5. Sugarcane germination at 1 MAH and growth parameters at 6 MAH in FRC practice.

Treatments	Germination[1] (%)	Number of stalks (stalks/ha)	Plant height (cm)
Residual practice from PC (T)	*	ns	ns
T1: Rainfed condition	250a	110,494	212
T2: Drip irrigation + soil fertilizer	223ab	106,944	209
T3: Drip fertigation	202b	104,169	202
Crop practice in FRC (S)	Ns	*	**
S1: Rainfed condition	211	103,188b	189b
S2: Drip fertigation	239	111,213a	226a
T*S	ns	ns	ns
CV (%)	7.51	4.66	4.47

[1]Means with different letters within the same column indicate significant differences at p<.05.

2) Yield and yield components

Table 6 shows that PC practices did not significantly influence yield or yield components such as the NMC, stalk length, stalk diameter, and Brix percentage. Yield ranged from 135.0 to 137.5 tons/ha across PC treatments, with no significant differences. However, current crop practice or drip fertigation (S2) significantly increased yield (153.1 tons/ha) and the NMC (109,275 stalks/ha) compared to rainfed condition (S1) (120.6 tons/ha and 89,000 stalks/ha). Stalk length, diameter, and Brix percentage remained consistent across treatments.

Table 6. Yield and yield components of FRC.

Treatments	Yield[1] (tons/ha)	No. of millable canes (stalk/ha)	Stalk length (cm)	Stalk diameter (cm)	Brix (%)
Residual practice from PC (T)	ns	ns	ns	ns	ns
T1: Rainfed condition	137.5	99,725	290.5	2.59	22.0
T2: Drip irrigation + soil fertilizer	135.0	98,381	293.3	2.58	22.6
T3: Drip fertigation	137.5	99,294	298.1	2.50	22.0
Crop practice in FRC (S)	**	**	ns	ns	ns
S1: Rainfed condition	120.6b	89,000b	291.6	2.54	22.2
S2: Drip fertigation	153.1a	109,275a	296.2	2.58	24.2
T*S	ns	ns	ns	ns	ns
CV (%)	9.63	9.63	4.51	4.94	9.63

[1]Means with different letters within the same column indicate significant differences at p<.05.

3) Ratooning ability based on growth

Table 7 exhibits sugarcane's ratooning ability as measured by the number of stalks and the corresponding yield for the FRC. The data included comparisons across different treatment groups, highlighting the influence of previous crop practices on FRC performance. PC and FRC practices significantly influenced ratooning ability, measured by stalk numbers at 2 and 6 MAH. Rainfed exhibited the highest ratooning ability, with 349% and 154% for stalk numbers, respectively. Drip fertigation (T3) had the lowest ratooning ability, particularly 242% and 116% respectively. Drip fertigation (S2) consistently outperformed S1 across all PC practices, demonstrating its superiority in enhancing ratooning ability. The combination effect of PC and FRC practices was significant for stalk numbers; it suggested that combinations of practices can differentially affect stalk numbers at both periods of FRC.

Table 7. Sugarcane ratooning ability (RA) based on number of stalks of FRC at 2 and 6 MAH.

Treatments	RA based on number of stalks (%)	
	2 MAH	6 MAH
Residual practice from PC (T)	*	**
T1: Rainfed condition	349a	154a
T2: Drip irrigation + soil fertilizer	306ab	135b
T3: Drip fertigation	242b	116c
Crop practice in FRC (S)	**	*
S1: Rainfed condition	246a	131b
S2: Drip fertigation	352b	140a
T*S	**	ns
CV (%)	8.46	4.92

[1]Means with different letters within the same column indicate significant differences at p<.05.

4) Ratooning ability based on yield and number of millable canes.

The results in Table 8 demonstrate the significant effects of previous crop (T) and FRC practices (S) on the ratooning ability of sugarcane, measured by yield and the NMC in the FRC. Influence of previous crop practices, PC practices significantly affected ratooning ability. The rainfed condition residual effect (T1) exhibited the highest ratooning ability, with values of 134% and 144% for yield under S1 (rainfed) and S2 (drip fertigation), respectively. T1 also resulted in the highest ratooning ability for the NMC, achieving 128% under S1 and 135% under S2. Conversely, drip fertigation residual effect (T3) recorded the lowest yield values of 93% under S1 and 105% under S2, and ratooning ability based on NMC at 105% under S1 and 112% under S2. Influence of current crop practices significantly impacted ratooning ability, with drip fertigation (S2) consistently outperforming rainfed conditions (S1)

across all PC practices. Under T1, S2 achieved a ratooning ability of 144% for yield and 135% for the NMC, both higher than those under S1. Interaction effects between PC and FRC practices: T and S practices significantly influenced ratooning ability. Their interaction was significant for both yield and NMC, indicating that combinations of practices can differentially affect the stalk number and plant height of FRC.

Table 8. Sugarcane ratooning ability based on the number of stalks and yield of FRC.

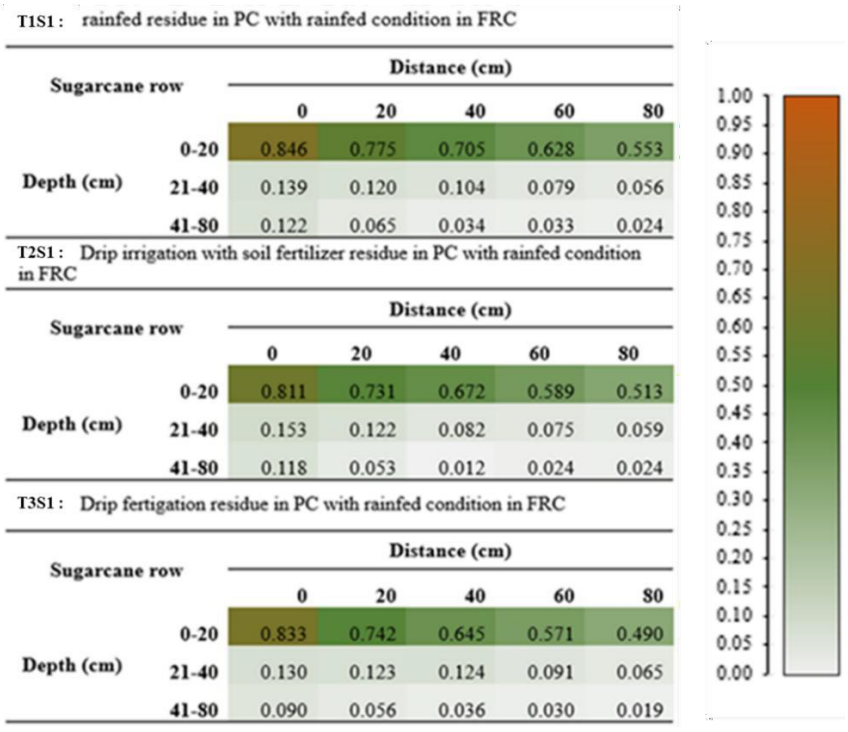
Residual practice from PC	Crop practice in FRC (S)	RA based on yield[1] (%)	RA based on the number of millable cane (%)
Rainfed condition (T1)	Rainfed condition (S1)	134ab	128ab
	Drip fertigation (S2)	144a	135a
Drip irrigation + soil fertilizer (T2)	Rainfed condition (S1)	109cd	121bc
	Drip fertigation (S2)	117bc	128ab
Drip fertigation (T3)	Rainfed condition (S1)	93d	105c
	Drip fertigation (S2)	105cd	112bc
T		**	**
S		*	**
T*S		**	**
CV (%)		15.44	18.36

[1]Means with different letters within the same column indicate significant differences at p<.05

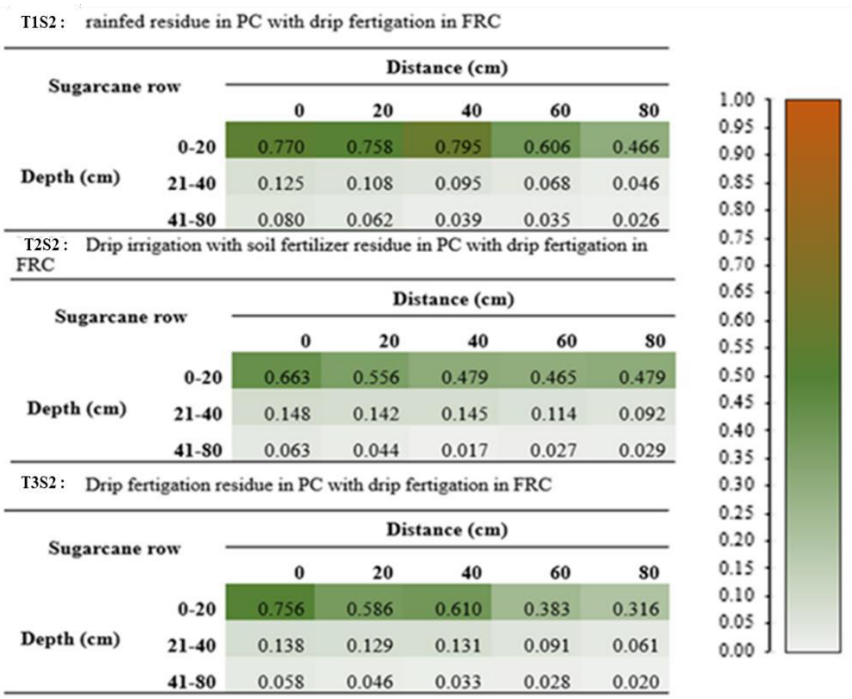
5) Root length density

Figure 2 illustrates the RLD of the FRC under different irrigation practices. The data indicate that neither the PC nor the current crop practice significantly affected the RLD of the FRC across treatments. Despite the lack of statistical significance, a noticeable trend is observed in RLD distribution across soil depths. Specifically, under rainfed conditions (Figure 2A), RLD tends to be higher at the soil surface (0-20 cm) and at deeper depths (41-80 cm) compared to drip fertigation (Figure 2B). This trend suggests that rainfed conditions may encourage a more extensive root system both at the surface and in deeper soil layers, potentially in response to water availability constraints, driving roots to explore a larger soil volume.

In contrast, drip fertigation appears to have a more uniform root distribution, with relatively lower RLD at these critical depths, likely due to this practice's more consistent water and nutrient availability. While these observations are not statistically significant, they highlight the potential influence of irrigation practices on root distribution patterns, which could affect water and nutrient uptake efficiency in sugarcane cultivation.



(A)



(B)

Figure 2 RLD of the FRC under rainfed condition (A) and drip fertigation (B) practices.

The two-year study revealed that drip-fertigated treatment produced superior growth parameters such as plant height and stalk number and LAI than the rainfed treatment. The consistent water delivery system of drip fertigation led to increased growth rates because of its ability to provide steady water supply. Drip irrigation systems delivered better soil nutrient accessibility while simultaneously improving plant nutrient absorption performance. The research of Wonprasaid and Girdthai (2014) showed that extended irrigation practices in sugarcane farming resulted in better growth and yield production and yield component development than rainfed cultivation methods. The research demonstrated that irrigation extended ratooning capacity beyond five crop cycles whereas rainfed management only reached three cycles. The research by Wiedenfeld and Enciso (2008) showed that adequate water supply led to increased sugarcane biomass, but Rodrigues et al. (2008) discovered that water scarcity negatively impacted plant growth, yield production, and product quality. The research by Khonghintaisong et al. (2018) demonstrated that water stress reduced stomatal conductance and relative leaf water content which resulted in decreased biomass accumulation and reduced crop performance. The research of Yadav and Prasad (1988) showed that sugarcane genotypes under proper irrigation developed higher LAI measurements than those under water-constrained conditions. The growth parameter LAI serves as a crucial indicator of photosynthetic capacity because it measures the plant canopy's effective light-intercepting surface. The lower LAI measurements under rainfed conditions resulted from water stress which limited leaf growth and decreased photosynthetic activities.

The environment, particularly rainfall, plays a crucial role in sugarcane growth and yield due to its unpredictability and the risk of water deficit stress. This study showed that rainfed conditions over two planting seasons resulted in lower growth, yield, and yield components than drip irrigation. Drought stress in rainfed sugarcane significantly reduced internode length, stalk germination, and biomass accumulation (Jangpromma et al., 2012). Early-stage stalk number is vital for supporting dry weight and yield at harvest (Khonghintaisong et al., 2018). The findings emphasize that drip irrigation significantly enhances sugarcane growth and yield compared to rainfed conditions. Water, as a significant component of plant cells, is essential for physiological processes, including nutrient transformation and availability, which are regulated by soil moisture levels.

Drip fertigation, using 1,601 mm of water, achieved a WUE of 74.1% and saved 26.3% more water than furrow irrigation's 2,200 mm with a WUE of 40.9% (Bhuvaneswari et al., 2020). Furthermore, with 140 kg/ha of N, precise drip fertigation increased stalk and sugar production by 31% in the second ratoon crop compared to non-irrigated conditions (Uribe et al., 2013). In rainfed conditions, limited water availability can severely constrain growth or even lead to crop failure if prolonged. Fertilizer application effectiveness was closely tied to the irrigation method. Soil-applied fertilizers resulted in lower growth and yield than fertigation, aligning with findings by Uribe et al. (2013), who noted that fertigation enhances sugarcane yield by synchronizing nutrient supply with crop demand. In this study, drip fertigation treatments showed higher growth and yield than soil fertilizer applications. Solid fertilizers are less effective without adequate soil moisture and are susceptible to being washed away by heavy rain. In contrast, fertigation allows for multiple, smaller applications, ensuring uniform nutrient distribution and reduced nutrient loss, particularly nitrogen. This method is particularly effective in shallow soils with low nitrogen availability, where

mineralized fertilizers can become immobilized in ratoon crops. Fertigation also enhances the immediate uptake of less mobile nutrients like phosphorus, increasing FNUE (Wu et al., 2016; Xie et al., 2020).

The RLD varied between rainfed and irrigated conditions, with rainfed sugarcane roots concentrating more at the plant base and extending deeper than drip-fertigation conditions. Rainfed sugarcane also tended to have taller stalks compared to fertigation treatments. Higher RLD was positively correlated with higher germination rates in second ratoon crops, indicating RLD is important for ratoon crop tillering (Chumphu et al., 2019). Effective tillering, supported by viable roots and lower bud formation on the main stems, is key to achieving higher yields (Khan & Khan, 2022). Root distribution is influenced by soil moisture and nutrient availability; sufficient water typically leads to shallower root systems, while water scarcity prompts deeper root development to access more water and nutrients (Namwongsa et al., 2019; Set-Tow et al., 2020). In addition. The remaining structures including root structures was related in initial stage of ratoon crop growth as they were the source of energy that stimulated the speed of growth and improved the vigor of the next ratoon crop (Pissolato et al., 2021).

Ratooning ability, which reflects sugarcane performance in the current ratoon crop compared to the previous crop, was found to be higher under rainfed conditions in the previous crop but improved with irrigation in the current crop. Although ratooning ability is not a direct indicator of final yield, it is a valuable metric for deciding whether to replant or continue with the ratoon crop. Sugarcane variety, location, and planting density influence ratooning ability (Xu et al., 2021). For instance, Dlamini et al. (2024b) observed that variety N25, despite high plant cane yields, experienced a sharper yield decline in ratoon crops, making it more suitable for short crop cycles. Conversely, variety N36 showed a lower yield decline, suggesting its suitability for longer ratoon cycles. Row spacing also affects ratooning ability, with different genotypes performing best at varying row spacings (Ebid et al., 2022). Dlamini et al. (2024a) identified the ratio of all ratoon crops to the plant cane yield index as the most reliable predictor of long-term ratoon performance, especially for short ratoon cycles. Moreover, the study of Qin et al. (2017) demonstrated that high germination, high tillering rate, and high stalk production were found in the sugarcane cultivar with strong RA.

4. Conclusion

The findings from this study underscore the significant benefits of drip irrigation for sugarcane growth, which demonstrate enhancements in stalk number, plant height, LAI, yield, and yield components compared to rainfed conditions. Fertigation proved more efficient than soil fertilizer application, particularly regarding IWUE and FNUE. Drip irrigation also reduced RLD in the planting bed and deeper soil layers, indicating more effective water and nutrient uptake by the plants. In the FRC, residual effects from previous crop management significantly influenced germination rates, with rainfed conditions during the PC phase resulting in higher germination percentages in the FRC. However, these initial conditions did not affect other growth parameters, yield, or yield components in FRC. Instead, current management practices during the FRC phase (S) were more critical, with drip fertigation yielding the best growth and highest yields, while rainfed conditions produced the lowest. RLD in FRC was not impacted by previous crop residual management but was tended to influence by current year practices, with higher RLD under rainfed conditions.

For RA evaluation, the results showed that both year practices (PC and FRC) influenced the RA of FRC sugarcane. For the previous crop effects the rainfed residual which tended to produce high root structure had the highest initial ratoon germination, RA based on number of stalk, NMC, and RA based on yield. For the current crop practice (S), drip fertigation practice had higher RA based on NMC, and yield than rainfed conditions. However, growth and yield productivity of the FRC did not depend on the RA. RA was only an indicator of the adaptability and high vigor of ratoon crop but the growth and productivity were much more associated with the current crop management (S). Regardless of all residual practices, the current crop fertigation practice produced a higher yield than the rainfed condition practices. Even though drip fertigation was the most effective way to produce growth and yield, but with low RA, it reduced the vigor and adaptability in the next ratoon cane if grown under non suitable condition, including, under rainfed condition. Therefore, drip irrigated sugarcane must be grown under the same practices for all ratoons to maintain growth, yield and RA.

In summary, this study highlights the importance of optimizing irrigation and fertilization management, particularly through drip fertigation, to enhance sugarcane productivity. Drip fertigation improved growth and yield while providing more efficient soil moisture and nutrient management, contributing to sustainable sugarcane production practices.

5. Acknowledgements

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Enhancing Cassava Yield and Water Use Efficiency Through Drip Irrigation Controlled by Wireless Sensors and a Water Balance Model in Clay Loam Soil

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Abstract

This study investigated the effectiveness of drip irrigation systems controlled by wireless sensors and a water balance model on the growth, yield, and water use efficiency (WUE) of cassava cultivated in clay loam soil. Four irrigation treatments were assessed: rainfed (T1), drip irrigation managed by a water balance model (T2), drip irrigation regulated by one sensor set per 1.2 ha (T3), and by three sensor sets per 1.2 ha (T4). Precision drip irrigation significantly enhanced cassava growth, nutrient uptake, biomass accumulation, and tuber yield compared to rainfed conditions. Sensor-based irrigation (T3 and T4) achieved superior WUE by reducing water input without compromising yield. Notably, T3 and T4 used approximately 369 m^3/ha less irrigation water than the timer-based treatment (T2). Increasing sensor density beyond one set/plot provided no additional agronomic advantage, highlighting the importance of cost-effective deployment. These findings highlight the potential of precision irrigation technologies to enhance cassava production and resource efficiency in regions characterized by irregular rainfall patterns.

Keywords: Manihot Esculenta, Drip Irrigation, Soil Moisture Sensor, Water Balance Model, Water Use Efficiency

1. Introduction

Cassava (*Manihot esculenta* Crantz) is an economic crop in globally, valued for its adaptability to marginal soils and resilience under drought conditions. Thailand stands among the world's leading producers and exporters of cassava, supplying global markets with products such as tapioca starch, animal feed, and bioethanol. Despite cassava's inherent tolerance to water scarcity, water availability remains a critical factor influencing its growth, yield, and overall productivity. The increasing frequency of erratic and unpredictable rainfall patterns, driven by climate change, presents significant challenges to sustainable cassava production, particularly within Thailand's predominantly rainfed agricultural systems. Conventional irrigation practices, typically based on fixed schedules or empirical judgment, often lack the precision required to address real-time fluctuations in soil moisture, crop water demand, and climatic variability. Such inefficiencies not only result in suboptimal water use but also compromise crop performance and long-term resource sustainability. Enhancing water use efficiency (WUE) through improved irrigation management is therefore essential to ensure stable and sustainable cassava yields under these variable environmental conditions. Recent advancements in precision agriculture offer promising solutions to address these challenges. Among these technologies, soil moisture sensors (SMS) have emerged as effective tools for real-time monitoring and control of irrigation systems. SMS provides continuous data on soil moisture, enabling data-driven irrigation decisions tailored to crop-specific needs (Ojha et al., 2015; Kim et al., 2020). When integrated with drip irrigation, it's highly efficient in delivering water directly to the root zone. SMSs can significantly enhance water distribution uniformity and minimize wastage, supporting sustainable agricultural practices. Precision drip irrigation systems leverage sensor data, automated controllers, and decision-support algorithms to optimize irrigation scheduling and volumes, ensuring crops receive the precise amount of water required at the appropriate time (Pereira et al., 2020). This targeted approach maximizes water productivity while reducing risks associated with over-irrigation, such as nutrient leaching, soil degradation, and root diseases (Bonilla et al., 2021). Empirical studies have demonstrated that precision-controlled drip irrigation can improve crop yields and reduce water consumption by 30–50% compared to conventional irrigation methods (Sadler et al., 2005; Vories et al., 2017). In contrast, traditional irrigation scheduling methods, such as those based on water balance models, offer a theoretical framework for estimating crop water requirements. However, these models often fail to account for dynamic field conditions, potentially leading to inefficient water application through over- or under-irrigation.

Given the critical importance of efficient water management in modern agriculture, particularly for crops like cassava that, while drought-tolerant, remain sensitive to water deficits during key growth stages. There is a clear need to evaluate the performance of precision irrigation technologies relative to conventional approaches. This study addresses this need by assessing the effectiveness of drip irrigation systems controlled by wireless sensors and a water balance model on cassava growth, yield, and water use efficiency in clay loam soil. The findings aim to inform the development of sustainable, resource-efficient irrigation strategies that enhance cassava productivity while conserving water resources in regions affected by climatic variability.

2. Methodology

2.1 Experimental site and design

The field experiment was conducted between 2019 and 2020 in Nakhon Ratchasima Province, Thailand. The experimental site featured clay loam soil, with baseline physicochemical properties assessed before planting. The 'Rayong 13' variety, recognized for its high yield potential and adaptability, was selected. A randomized complete block design was employed, consisting of four irrigation treatments with three replications each. The experimental area covered 4.8 ha, with plot dimensions standardized to ensure uniformity and minimize border effects. Standard agronomic practices for cassava cultivation in the region were consistently applied.

The four irrigation regimes were:

T1: Rainfed condition (control): No supplemental irrigation, reliant solely on natural rainfall.

T2: Drip irrigation scheduled using a conventional water balance model, with intervals calculated based on crop evapotranspiration (ETc) and effective rainfall.

T3: Drip irrigation controlled by a precision system utilizing one sensor set per 1.2 ha. T4:

Drip irrigation controlled by a precision system utilizing three sensors set per 1.2 ha.

All drip systems were equipped with pressure-compensating emitters that delivered water at a rate of 2 L/hr. For T3 and T4, irrigation thresholds were set at 70% depletion of available water holding capacity (AWHC).

2.2 Soil analysis and fertilization

Pre-planting, soil samples (0–40 cm depth) were collected across all plots to determine soil properties. A uniform fertilization was applied across treatments, guided by soil recommendations practices. Fertilizers in T2–T4 were delivered via fertigation through the drip system to enhance nutrient use efficiency. The total N-P-K rates applied were 25-25-25 kg/ha, divided into 8 times split applications over the growing season. The rainfed condition (T1) received fertilizers via conventional broadcasting methods. Field capacity (FC) and permanent wilting point (PWP) were determined using the pressure plate apparatus, and AWHC was calculated to establish irrigation thresholds for precision treatments.

2.3 Principle of soil moisture sensor

Wireless sensor networks (WSNs) serve essential functions in multiple fields. WSNs have become essential tools in precision agriculture because they track essential environmental factors, including temperature, humidity, soil moisture, and soil pH, which leads to better crop yields and quality. The implementation of WSNs helps maximize natural resource utilization through optimized irrigation practices and input application methods. A typical WSN network comprises multiple sensor nodes that collect environmental data in the deployment area and transmit it to end users (Figure 1). The networks operate with limited infrastructure, utilizing 10 to 1,000 nodes that work together to monitor specific areas. WSNs are categorized into two main types: structured and unstructured networks. The deployment of structured WSNs follows a predetermined pattern, which simplifies both setup and maintenance operations when required.

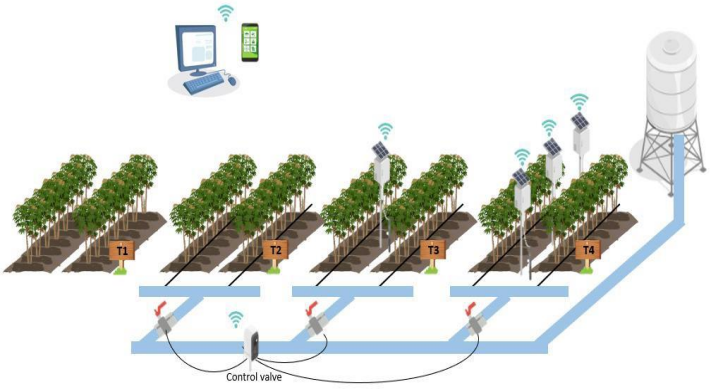

Figure 1 Diagram of irrigation treatment and wireless sensor network installation

2.4 Data collection

Data collection was performed at regular intervals throughout the cassava growth cycle, including:

1) Growth Parameters, including plant height and stem diameter, were measured at 1, 3, and 5 months after planting (MAP).

2) Leaf nutrient analysis, including percentage nitrogen, phosphorus, potassium, calcium, and magnesium in leaf dry weight were measured at 4 MAP.

3) Biomass accumulation yield and starch content

- Biomass accumulation, including dry weight (DW) of leaf, stem DW, and tuber DW (Both root and food storage part) was measured at 3, 6, 9, and 12 MAP (tuber yield).
- Yield and starch content (%) were measured at 12 MAP.

4) Soil moisture dynamic was collected daily for 3 months to show the trends of water content in irrigation treatment, and water use efficiency (WUE) were measured by the equation of De Pascale et al. (2011).

$$\text{WUE} = \frac{\text{Yield (kg/ha)}}{\text{Total water supply (m}^3\text{/ha)}}$$

2.4 Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SPSS v16.0 to assess treatment effects. Mean separations were conducted using Duncan's Multiple Range Test (DMRT) at a significant level of $p < .05$.

3. Results and Discussion

3.1 Effects of irrigation treatments on growth parameters

At 1 MAP, no significant differences in plant height were observed among treatments. However, by 3 and 5 MAP, all drip irrigation treatments (T2–T4) significantly increased plant height compared to the rainfed condition (T1) (Figure 2). T4 consistently produced the tallest plants and the largest stem diameters by 5 MAP, demonstrating the benefits of enhanced precision irrigation. Drip irrigation effectively mitigated water stress during critical growth stages, supporting superior vegetative development. These findings corroborate previous studies indicating that drought stress limits plant growth through reduced stomatal conductance and photosynthesis (Inman-Bamber & Smith, 2005; Nesreen et al., 2013; Vurayai et al., 2010). No significant differences were detected between T2 and sensor-based treatments (T3 and T4), indicating that both methods adequately satisfied cassava's water demands (~8,531 m³/ha). Increasing sensor density beyond one per 1.2 ha did not enhance growth, aligning with Kizito et al. (2008), who emphasized the need to balance precision with economic feasibility.

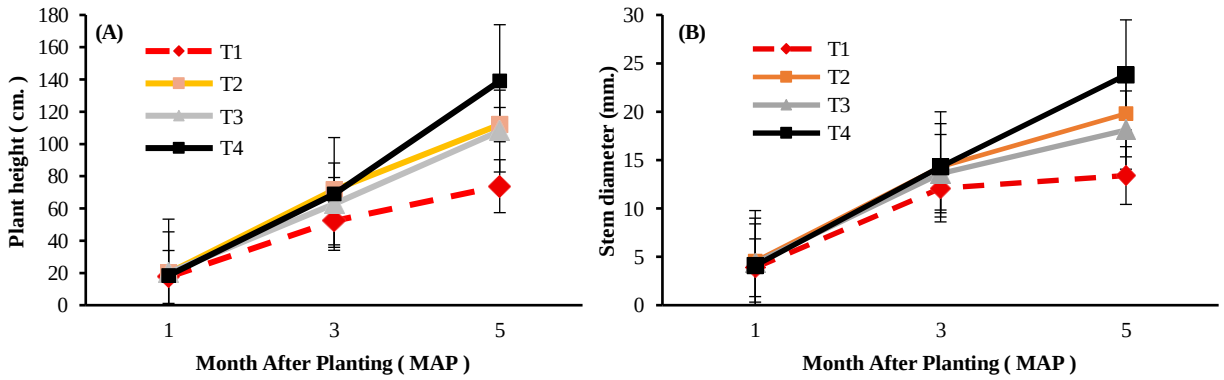

Figure 2 Effect of irrigation treatments on cassava height (A) and stem diameter (B) at 1, 3, and 5 MAP.

3.2 Effects of irrigation treatments on leaf nutrient analysis

Leaf nutrient analysis at 4 MAP revealed significantly higher concentrations of N, P, and K in all drip irrigation treatments compared to T1 (Table 1). Ca and Mg levels showed no significant variation. Improved water availability likely facilitated nutrient uptake, enhancing physiological performance. These results align with those of Rosenthal et al. (2012) and De Souza et al. (2020), who have linked optimal nutrient status with improved photosynthetic efficiency and yield.

Table 1 Effects of irrigation treatments on leaf nutrients analysis at 4 MAP.

Irrigation method	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
T1: Control	4.58b	0.16b	0.90c	0.51	0.24ab
T2: Water balance model	5.18a	0.28a	1.23b	0.58	0.25a
T3: 1 sensor set/1.2 ha	5.14a	0.28a	1.49a	0.54	0.24ab
T4: 3 sensors set/1.2 ha	5.25a	0.30a	1.51a	0.49	0.23b
P-value	*	**	**	ns	ns
CV (%)	4.76	9.50	5.35	8.87	3.09

[1] Means in the same column with different letters are significant differences at $P < 0.05$.

3.3 Effects of irrigation treatments on biomass accumulation, yield, and starch content

Drip irrigation significantly increased dry biomass accumulation at 3, 6, and 9 MAP, particularly for tubers. By 12 MAP, differences in leaf dry weight diminished, highlighting the importance of early-season water supply (Figure 3). Tuber yield was significantly higher in drip treatments, with T4 achieving the maximum yield (49 tons/ha), nearly doubling that of T1 (25.8 tons/ha). The starch content remained unaffected across treatments, suggesting water management influences yield quantity rather than quality (Table 2). These outcomes support previous findings on the benefits of drip irrigation in cassava (Phromuthai, 2014). Polthanee and Srisutham (2018) also reported that drip irrigation resulted in higher leaf, stem, tuber, and yield production of cassava than the rainfed cultivation. Samutthong and Sarobol (2006) found that irrigated cassava had a higher yield than non-irrigated cassava.

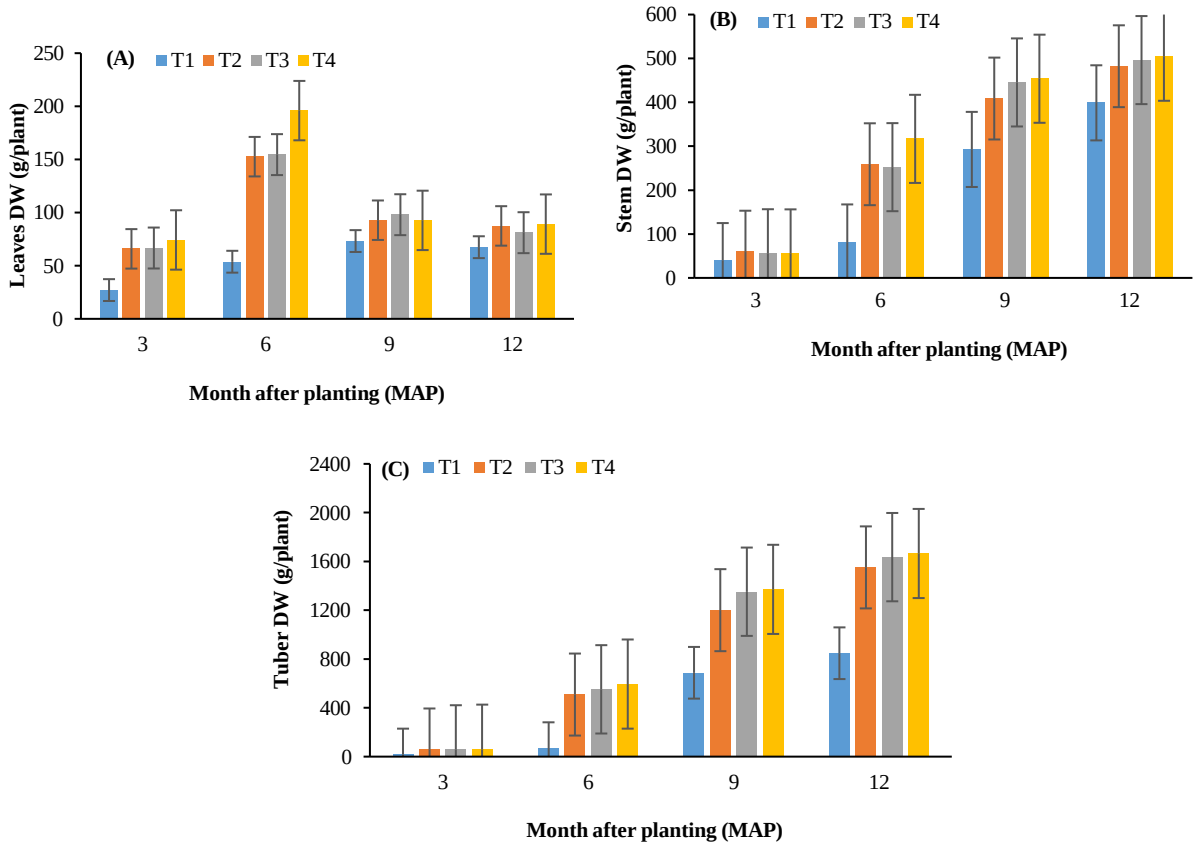

Figure 3 Effects of irrigation treatments on leaves DW (A), stems DW (B), and tubers DW (C) at 3, 6, 9, and 12 MAP.

Table 2 Effects of irrigation treatments on cassava yield and starch content at 12 MAP

Irrigation method	Yield (tons/ha)	Starch (%)
T1: Control	25.8b	26.9
T2: water balance model	47.5a	28.1
T3: 1 sensor set/1.2 ha	47.8a	26.6
T4: 3 sensors set/1.2 ha	49.0a	27.3
P-value	**	ns
CV (%)	13.01	3.91

[1]Means in the same column with different letters are significant differences at P < .05.

1.1 Soil moisture dynamics and water use efficiency

Sensor-controlled treatments (T3 and T4) maintained soil moisture within optimal ranges (30–41% AWHC), while T1 experienced frequent deficits (Figure 4). Although tuber yields were comparable among drip treatments, T3 and T4 used less water than T2, resulting in higher WUE values (13.5–13.8 kg/m³ vs. 12.1 kg/m³) (Table 3). The irrigation water volumes for T3 and T4 were equal, so one sensor per 1.2 ha was sufficient for soil moisture monitoring. The method requires sensor accuracy and uniform field conditions with flat terrain and consistent soil properties. These findings confirm that real-time soil moisture monitoring enhances irrigation efficiency without compromising productivity (Xie et al., 2020). The limitations of water balance models under variable climatic and soil conditions further emphasize the advantages of adaptive, sensor-based irrigation strategies (Fisher & Hanks, 2009; Malik et al., 2021). Zotarelli et al. (2011) investigated grass cultivation and found that moisture sensors resulted in the highest possible yield while reducing water consumption by 7–62%. The research of Tanaka et al. (2011) confirmed that the soil moisture sensor EC-5 successfully measured soil water content after rainfall and detected groundwater presence.

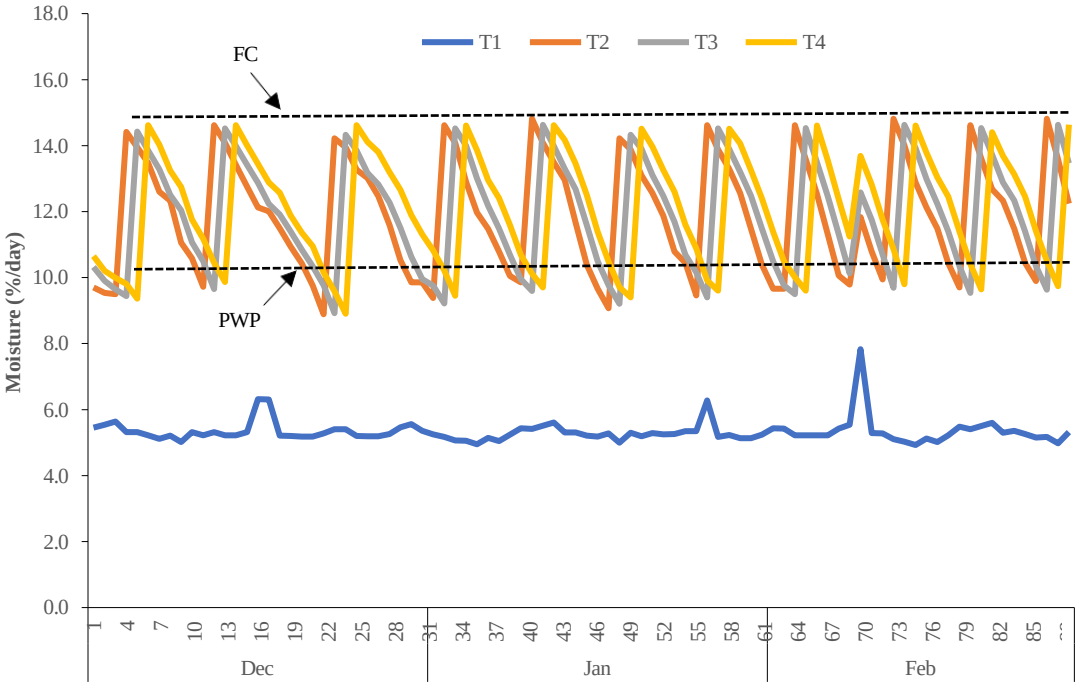

Figure 4 Effects of irrigation treatments on daily soil moisture content.

Table 3 Effects of irrigation treatments on water use efficiency.

Irrigation method	Irrigated water[1] (m^3/ha)	WUE (kg/m^3)	Rainfall (mm.)
T1: Control	0c	0b	710
T2: water balance model	3,918a	12.1a	710
T3: 1 sensor set/1.2 ha	3,537b	13.5a	710
T4: 3 sensors set/1.2 ha	3,537b	13.8a	710
P-value	**	**	
CV (%)	0.30	17.11	

[1]Means in the same column with different letters are significant differences at P < 0.05.

2. Conclusion

This study demonstrated that drip irrigation, particularly when controlled by soil moisture sensors, significantly enhances cassava growth, nutrient uptake, biomass, and yield under clay loam soil conditions. Sensor-based systems improved WUE by reducing water inputs without sacrificing yield, while increasing sensor density beyond one per 1.2 ha offered no additional benefit. Irrigation improved macronutrient uptake but did not affect starch content, indicating that water management primarily boosts yield quantity. Precision drip irrigation with soil moisture sensor integration presents a sustainable solution for improving cassava productivity and resource efficiency, especially in regions prone to irregular rainfall. Future research should focus on economic assessments, sensor deployment in heterogeneous soils, and advanced fertigation integration.

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Effects of *Dictyophora indusiata* powder and purple rice flour on the baking quality characteristics and volatile flavor of cookies

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Abstract

This study investigated the effects of incorporating *Dictyophora indusiata* powder and purple rice flour on the baking quality, nutritional composition, antioxidant properties, and flavor profile of cookies. A gradient substitution model was employed, replacing wheat flour with varying proportions of *Dictyophora indusiata* (0–5%) and purple rice flour (0–20%). The results demonstrated that the combination of these ingredients significantly enhanced the dietary fiber content (up to 3.25 g/100 g) and protein levels in the cookies, while also improving antioxidant activity, as evidenced by increased DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging rates (up to 75.8% and 72.2%, respectively). Additionally, the *in vitro* digestibility analysis revealed a reduction in starch hydrolysis and GI (glycemic index), attributed to the synergistic effects of resistant starch and fiber. However, sensory evaluation indicated that higher substitution levels led to darker coloration and altered texture, impacting overall acceptability. The study highlights the potential of *Dictyophora indusiata* and purple rice as functional ingredients for developing nutritious, low-glycemic-index baked goods, provided that substitution ratios are optimized to balance health benefits with sensory quality.

Keywords: Dictyophora Indusiata Powder; Purple Tice Flour; Cookies Quality; Antioxidant Activity; Volatile Flavor

1. Introduction

With the deepening of the concept of healthy diet, the baked food industry is facing an urgent need for nutritional and functional transformation. The traditional biscuit formulation system, which relies on refined wheat flour, has structural deficiencies such as a lack of dietary fibre and a high proportion of fast-digesting starch, making it difficult to meet consumers' expectations for low-glycemic and high-fibre foods. Current research focuses on substituting with a single ingredient, such as whole grains or edible mushrooms. While this can improve some nutritional indicators, it generally leads to texture deterioration and flavour clashes. In particular, the interaction between fibre components and the gluten network has not yet been clarified. Purple rice is rich in anthocyanins and resistant starch, and *Dictyophora indusiata* contains unique fungal polysaccharides and chitin. These two ingredients show potential for dietary fibre complementarity and functional synergy, but their combined substitution lacks a systematic study on their multidimensional effects on the baking system.

In this study, we constructed a gradient substitution model using purple rice and *Dictyophora indusiata*, innovatively integrated methods for analysing textural properties, *in vitro* digestion, and flavour, and focused on revealing the synergistic effects between non-cereal raw materials and the gluten matrix. This research breaks through key technological bottlenecks in texture control of high-fibre bakery products, provides theoretical support and practical approaches for developing new types of biscuits that are both nutritionally enhanced and palatable to consumers, and promotes the leap of functional bakery products from proof-of-concept to industrial application.

2. Materials and equipment

2.1 Reagents and materials

Raw materials: purple rice, *Dictyophora indusiata*, low gluten flour, baking powder, water, butter, salt, egg, vanilla extract.

Table 1 Experimental reagents

Name	Specification	Grade	Manufacturer
Sodium hydroxide	50 g	AR	Sinopharm Group
Glucose (GLU) assay kit	96 T	AR	Nanjing Jiancheng Bioengineering Institute
MES-TRIS buffer	500 mL	50mM PH=8.2	Strait Standard Technology Co., Ltd.
Pepsin	5 g	3200U	Sinopharm Group
Acetone	500 mL	AR	Xilong Chemical
Copper sulfate	500 g	AR	Tianjin Fangzheng
Boric acid	500 mL	AR	Xilong Chemical
Methyl red indicator (1g/L)	100 mL	AR	Xilong Chemical
Bromocresol green indicator (1g/L)	100 mL	AR	Xilong Chemical

Name	Specification	Grade	Manufacturer
Methylene blue indicator (1g/L)	100 mL	AR	Xilong Chemical
Methanol	500 mL	AR	Xilong Chemical

2.2 Instruments and equipment

Table 2 Information of experimental equipments

Instrument name	Manufacturer
Freeze concentrator	Alpha 1-2 LD plus, Germany
SB5200D ultrasonic cleaner	Ningbo Xinzhi Biotechnology Co., Ltd.
AL204 electronic balance	Mettler-Toledo Instruments (Shanghai) Co., Ltd.
THZ-100 constant temperature incubator	Beijing Yiheng Scientific Instruments Co., Ltd.
TA.XT Plus texture analyzer	Stable Micro Systems, UK
LXJ-IIB centrifuge	Shanghai Anting Scientific Instrument Factory
HH-4 digital display constant temperature water bath	Jintan Kexi Instrument Co., Ltd.
TU 1901 double-beam UV-visible spectrophotometer	Beijing Puxi General Instrument Co., Ltd.
QP2010 gas chromatography-mass spectrometer	Shimadzu Corporation, Japan
Electronic nose	Shanghai Baosheng Industrial Development Co., Ltd.

2.3 Experimental Methods

1) Cookies preparation

Dictyophora indusiata and purple rice cookies need to be prepared first: dry the *Dictyophora indusiata* and purple rice, then crush them and sieve through a 60-mesh sieve to obtain 250 μm fine powder, which is sealed for later use. Weigh 100 g of low-gluten flour according to the recipe (with *Dictyophora indusiata* substituted at 0% and 5%, and purple rice flour substituted at 0%, 10%, 15%, and 20%), 60 g of butter, 45 g of water, 60 g of sugar, 5 g of salt, 2 g of baking powder, 1 mL of vanilla extract, and 50 g of whole egg. Soften the butter, put all ingredients into a mixer, and mix at medium speed until there is no dry powder left. Then form the mixture into a dough and freeze it for 30-60 minutes to harden. Take out the hardened dough, cut it into pieces, and bake in a preheated oven at 185°C for 15 minutes until the surface turns golden brown. Then cool it down to get the finished product. When mixing the ingredients, avoid over-mixing. Adjust the freezing time according to the softness of the dough to ensure the cutting and shaping effect. The recipe is as follows:

Table 3 Purple rice and *dictyophora indusiata* cookies formula ingredients

Ingredients (g)	CON	P5	P10	P15	P20	D5P5	D5P10	D5P15	D5P20
Dictyophora indusiata	0	0	0	0	0	5	5	5	5
Purple rice	0	5	10	15	20	5	10	15	20
Low Flour	100	95	90	85	80	90	85	80	75
Butter	60	60	60	60	60	60	60	60	60
Water	45	45	45	45	45	45	45	45	45
Sugar	40	40	40	40	40	40	40	40	40
Salt	3	3	3	3	3	3	3	3	3
Baking powder	2	2	2	2	2	2	2	2	2
Vanilla extract	1	1	1	1	1	1	1	1	1
Whole egg	20	20	20	20	20	20	20	20	20

2) Determination of basic ingredients of biscuits

For the determination of dietary fibre in high-fat and high-sugar foods, samples should be extracted and degreased with petroleum ether three times (500 mL each time, shaking for 2 minutes). If the viscosity is too high, 85% ethanol should be used for degreasing three times. After degreasing, the samples should be dried at 40°C, weighed, and then the mass change factor should be calculated. For biscuits, the determination of non-sedimentable soluble dietary fibre (SDFS) is not required, so this step is omitted. cookies do not need to be desugared.

After the samples are dispersed in buffer, heat-stabilised α-amylase (at 95°C) or a combination of enzymes (incubated overnight at 37°C) combined with protease should be used to remove starch and protein. The results should be corrected for protein, ash, and blank values, and expressed as the mean of two determinations. The precision requirement is that the difference between the two determinations should be ≤ 20% of the mean value.

3) Determination of cookies baking losses

Based on the method modified from Wu et al. (2021) , the baking loss of cookies biscuits is determined by measuring the weight difference before and after baking. All data are based on the average of three repeated experiments. The formula is as follows:

$$\text{Baking Loss}(\%)=\frac{(m1-m2)}{m1}*\ 100\%$$

Where:m1 is the mass before baking; m2 is the mass after baking.

Thickness changes were measured using a vernier caliper. The initial thickness of the dough was 8 millimeters. After baking, the actual dimensions were recorded, and the percentage change was calculated. All data are based on the average of three repeated experiments. The formula is:

$$\text{Thickness}\,(\%)=\frac{(h1-h2)}{h1}*\ 100\%$$

Where: h1 is the initial thickness; h2 is the thickness after baking.

4) Texture analysis

The hardness of cookies dough and cookies is measured by the TA-XT Plus texture analyzer (SMS, UK). The settings were: P/6 for the probe, the speed was 1 mm/s pre-test, 2 mm/s during the test, and 5 mm/s post-test. Each sample was tested no less than five times.

5) Total phenolic content

The total phenolic content was determined using the method modified from Liu et al. (2021). A 2.5 g sample was mixed with 50 mL of 95% ethanol solution and sonicated at 37°C for 2 hours at 120 r/min. After centrifugation at 2600 r/min for 15 minutes, the supernatant was collected as the phenolic extract. The total phenolic content was measured using the Folin-Ciocalteu method and expressed as mg/100g.

6) Total flavonoid content

The total flavonoid content was determined using the method modified from Wu et al. (2018). A 120 µL sample was mixed with 380 µL of 60% ethanol, followed by the addition of 30 µL of 50% sodium nitrite solution. After 8 minutes, 30 µL of 10% aluminum nitrate solution was added, and the mixture was allowed to stand for 12 minutes. The absorbance was measured at 510 nm, and the total flavonoid content was calibrated using a catechin standard curve and expressed in terms of catechin equivalents (mg CE/100g DM) or (µg CE/100g DM).

7) DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed according to the national standard GB/T39100-2020, with modifications. A 20 mg sample was dissolved in 5 mL of methanol and extracted for 12 hours at 25°C. Mix 200 µL of the above sample with 2 mL of 0.025 g/L DPPH solution and incubated at 37°C for 30 minutes. The absorbance was measured at 30 nm, and the DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH Radical Scavenging Activity}\,(\%)=\frac{A_{DPPH}-A_{Extract}}{A_{DPPH}}\times 100$$

Where A_{DPPH} is the absorbance of the DPPH solution with methanol, and $A_{Extract}$ is the absorbance of the DPPH solution with the sample extract.

8) ABTS radical scavenging activity

The determination of ABTS radical scavenging ability in cookies involves dissolving ABTS and $K_2S_2O_8$ to prepare the ABTS solution. Then, 100 µL of the sample is mixed with 3.9 mL of ABTS solution and reacted for 10 minutes, after which the absorbance at 734 nm is measured (A_1). Meanwhile, 3.9 mL of phosphate buffer solution is mixed with 100 µL of the sample as a control, and the absorbance is measured (A_2). Finally, 3.9 mL of ABTS solution is mixed with 100 µL of ethanol to determine the blank absorbance (A_0). ABTS radical scavenging activity was calculated using the formula:

$$\text{ABTS Radical Scavenging Activity}\,(\%)=[1-(A_1-A_2)A_0]\times 100\%$$

9) In vitro digestibility

The *in vitro* digestion characteristics of cookies were assessed by precisely weighing 100 mg of cookies, followed by the addition of 2 mL of distilled water and 7.5 mL of sodium acetate buffer (0.2 mol/L, pH 6.0). The mixture was incubated in a water bath at 37°C for 180 minutes, with samples collected at 0, 20, 40, 60, 120, and 180 minutes. The reaction was terminated by adding anhydrous ethanol. The glucose concentration in the supernatant was determined using a glucose assay kit (GLU), and the absorbance was measured at 510 nm using a spectrophotometer to calculate the starch hydrolysis rate. Additionally, the contents of rapidly digestible starch (RDS) and slowly digestible starch (SDS), as well as resistant starch (RS), were calculated.

10) Odor analysis

The volatile organic compounds (VOCs) for cookies odor were determined using Headspace Solid Phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) and Electronic Nose Technique (ENT).

For the HS-SPME/GC-MS method, initially, 0.25 grams of cookies were mixed with a 20% NaCl solution containing an internal standard and stirred in a water bath at 50°C. The headspace adsorption was performed using an SPME fiber, followed by desorption at the GC inlet at 260°C for 5 minutes. GC separation was carried out using a DB Wax (0.25 mm×60 m×0.5µm) column with a programmed temperature increase from 40°C to 220°C at a helium flow rate of 2 mL/min. Linear retention indices were determined using a DB5MS column (0.25 mm×30 m×0.25 µm). Mass spectrometric parameters were set with an ion source at 230°C, a quadrupole at 150°C, and a scan range of m/z 35-350. Qualitative identification was achieved by matching against the NIST library combined with retention indices, and quantification was performed using the internal standard method. The sample mass, pH, and chromatograms were recorded to ensure data reliability.

For the ENT method, 2 g of crushed cookies sample was placed in a 20 mL flask and enriched at 38°C for 20 min (Herráiz-Gil, Arriba, and Escámez, 2023). The system contains 18 metal oxide sensors, each with different sensitivity to the measured gas, as listed in Table 4. The detection conditions of the electronic nose (Shanghai Bohin Industrial Development Co., Ltd., China) were set as follows: detection temperature: 25°C; humidity: 55%; injection time: 60 s; flow rate: 1 L/min; detection time: 60 s; wash time: 120 s.

11) Sensory evaluation

Combined with the 9-point preference scale method, the details of sensory evaluation are shown in the following table:

Table 4 Sensory evaluation standard

Item	Score	Scoring criteria
Colour	7-9	Colour is extremely attractive and fully consistent with desirable characteristics
	4-6	Colour is normal but lacks highlights
	1-3	Slightly dull or slightly discoloured colours
Flavour	7-9	Flavour is perfectly balanced and extremely pleasant
	4-6	Flat or slightly off-flavoured
Flavour	1-3	Flavour has a distinctly undesirable flavour
Crispness	7-9	Extremely crunchy, perfect chewing experience
	4-6	Crispness up to standard, no particular merit
	1-3	Texture too soft or too hard
Appearance	7-9	Perfectly regular and visually pleasing appearance.
	4-6	Form is complete but not refined
	1-3	The morphology is severely irregular.
Overall Acceptability	7-9	Impeccable product, strong desire for repeat consumption
	4-6	Acceptable, but not actively chosen
	1-3	Unacceptable, refuse to continue consumption
Total Score	45	

12) Data processing

Statistical analysis of the data was performed using SPSS 26.0 software. Results are expressed as x ± SD. One-way ANOVA was used to compare the mean values between multiple groups. If $p < .05$, it indicates a significant difference between groups. Origin 2021 software was used for graphing.

3. Experimental results and analyses

3.1 Effects of the addition of Dictyophora indusiata and purple rice on the nutritional composition of biscuits

The data in Table 5 showed that compared to wheat flour, purple rice provides richer minerals, protein, and dietary fibre, while *Dictyophora indusiata* mainly contributes richer ash, protein, and dietary fibre. In particular, its protein and dietary fibre contents are significantly higher than those of ordinary wheat flour. The water content of the biscuits gradually increased as the substitution levels of *Dictyophora indusiata* and purple rice flour increased, which may be attributed to the high water absorption of *Dictyophora indusiata* powder. When the substitution levels of both reached the maximum, the protein content was significantly higher than that of the control group, whereas for biscuit samples with only purple rice flour added, the increase in protein content was not significant. The starch content of *Dictyophora indusiata* was significantly lower than that of gluten flour and purple rice flour; however, due to the small amount of *Dictyophora indusiata* powder added, it did not have a significant effect on the overall starch content of the biscuits.

In Table 5, when only purple rice was added, the increase in dietary fibre content was small. When purple rice was added at 20%, the total fibre content was 2.40%, and the insoluble fibre reached 1.57 g/100 g. Purple rice is rich in cellulose, which accounts for 50-60% of its fibre content; this is mainly insoluble cellulose found in the bran layer. Purple rice also contains resistant starch, which accounts for 5-10% of its starch content, as well as soluble cellulose. Additionally, purple rice contains arabinoxylan, a hemicellulose component that is 20-30% soluble. When dissolved in water, it forms a sticky substance, which can improve the ductility of biscuit dough (Yadav, Hicks, 2015). The dietary fibre content of the biscuits was significantly enhanced when *Dictyophora indusiata* was substituted at 5%. Although the water content of *Dictyophora indusiata* itself is significantly lower than that of purple rice and low-gluten flour, the water content of the biscuits increased significantly with the addition of purple rice and *Dictyophora indusiata*. The total fibre content of the D5P20 group reached 3.25%, with insoluble fibre accounting for 67%. *Dictyophora indusiata* is rich in chitin and lignin, which significantly increased the insoluble dietary fibre content of the biscuits, while also increasing the soluble fibre content. It is also abundant in β-glucan, a major component of its cell wall, which provides abundant soluble fibre to the biscuits (Jayachandran, Xiao, and Xu. (2017). When the substitution level of purple rice was 20% and that of *Dictyophora indusiata* was 5%, the β-glucan in *Dictyophora indusiata* and arabinoxylan in purple rice synergistically formed a fibre network structure, elevating the total fibre content to 3.25 g/100 g. Thus, the combined effect of *Dictyophora indusiata* and purple rice endowed the biscuits with rich dietary fibre.

It was shown that the addition of *Dictyophora indusiata* resulted in a positive correlation between the increase in moisture content and fibre content of the biscuits. The network structure formed by the chitin and β-glucan of *Dictyophora indusiata* retained moisture during baking, while its own fibre structure remained relatively intact, suggesting that the cellulose in *Dictyophora indusiata* is heat-resistant.

Table 5 Nutrient composition table of purple rice, *dictyophora indusiata* powder and cookies with different amounts of purple rice and *Dictyophora indusiata*

Group	Moisture	Ash	Crude starch	Crude fat	Crude Protein	Total Fibre	Insoluble Fibre	Soluble fibre
Low gluten flour	12.3±0.35^{c}	1.07±0.05^{a}	71.4±0.31^{b}	2.32±0.04^{c}	7.31±0.34^{a}	2.89±0.04^{a}	1.95±0.04^{a}	0.94±0.01^{a}
Purple rice	10.9±0.22^{b}	1.45±0.03^{b}	71.4±0.16^{b}	1.98±0.05^{b}	8.93±0.29^{b}	4.08±0.07^{b}	2.79±0.05^{b}	1.29±0.05^{b}
Dictyophora indusiata	5.94±0.27^{a}	5.06±0.05^{c}	26.8±0.29^{a}	1.63±0.06^{a}	20.7±0.34^{c}	38.1±0.05^{c}	24.3±0.06^{c}	13.8±0.04^{c}
CON	7.44±0.23^{cd}	1.01±0.04^{a}	40.5±0.23^{bc}	34.2±0.05^{a}	6.52±0.19^{ab}	1.91±0.06^{a}	1.32±0.02^{a}	0.59±0.04^{a}
P5	7.41±0.18^{cd}	1.21±0.04^{b}	40.6±0.26^{c}	34.2±0.06^{a}	6.22±0.36^{a}	1.92±0.08^{a}	1.34±0.04^{a}	0.58±0.04^{a}
P10	6.83±0.36^{bc}	1.28±0.03^{bc}	40.4±0.30^{bc}	34.2±0.04^{a}	6.58±0.33^{ab}	1.96±0.08^{a}	1.33±0.05^{a}	0.63±0.04^{a}
P15	6.46±0.07^{ab}	1.31±0.05^{bc}	40.5±0.12^{bc}	34.2±0.04^{a}	6.63±0.27^{ab}	2.01±0.08^{a}	1.36±0.06^{a}	0.64±0.03^{a}
P20	6.12±0.27^{ab}	1.38±0.04^{c}	40.1±0.25^{abc}	34.2±0.04^{a}	6.76±0.34^{ab}	2.40±0.01^{b}	1.57±0.04^{b}	0.82±0.06^{b}
D5P5	7.89±0.24^{d}	1.51±0.02^{d}	40.1±0.13^{abc}	34.2±0.05^{a}	7.26±0.09^{b}	3.03±0.09^{c}	2.04±0.05^{c}	0.99±0.05^{c}
D5P10	9.17±0.18^{e}	1.54±0.05^{d}	39.7±0.34^{ab}	34.2±0.02^{a}	6.73±0.36^{ab}	3.11±0.06^{c}	2.05±0.05^{c}	1.06±0.02^{c}
D5P15	9.17±0.20^{e}	1.56±0.05^{d}	39.9±0.05^{abc}	34.1±0.06^{a}	7.14±0.25^{ab}	3.18±0.06^{c}	2.11±0.03^{cd}	1.07±0.03^{c}
D5P20	9.54±0.18^{e}	1.65±0.05^{d}	39. 6±0.29^{a}	34.1±0.05^{a}	7.30±0.27^{b}	3.25±0.03^{c}	2.18±0.01^{d}	1.07±0.03^{c}

Note: Different superscript letters for data in the same column represent significant differences in data between groups (P<.05).

3.2 Effects of the addition of Dictyophora indusiata and purple rice on the colour difference of biscuits

In Table 6, the L-value of the surface of the control group biscuits was 43.9 with medium brightness, a-value 57.7 with reddish colour and b-value 5.63 with yellowish colour. And with the increase of substitution of purple rice and *Dictyophora indusiata*, the L-value decreased significantly, indicating that the colour of the biscuits became darker. This may be due to the purple colour of purple rice and brown colour of *Dictyophora indusiata*. Meanwhile, the a-value also increased. For example, the a-value of D5P20 was as high as 68, indicating a redder surface, which might be related to the Maillard reaction of certain components in *Dictyophora indusiata* during baking.

The colour L-value of the control biscuit dough was 28.7, whereas with the addition of purple rice, the L-value decreased, and when the substitution amount was 20%, the L-value was significantly reduced taking its colour significantly darker. The addition of purple rice had a great impact on the colour of the dough, while the L-value of the dough increased slightly with the addition of *Dictyophora indusiata*, which was due to the lighter colour of *Dictyophora indusiata*, which neutralised the purple colour of the purple rice.

The biscuits were ground into powder through a grinder and sieved through a 60 mesh sieve, and the colour of the powder in the control group, the L-value was 32.4, whereas D5P20 decreased to 18.4 and became darker in colour. Taken together, the addition of purple rice and *Dictyophora indusiata* significantly affected the colour index of the biscuits, whether it was the dough or the finished biscuits, or the biscuit powder, especially the dark colour of the purple rice dominated the overall hue change.

When only purple rice was added, the dough had the lowest value of L. The black finished surface had an L value equal to 29.1 with a purplish colour, the powder had an L value equal to 14.2, also with a darker colour, whereas the values were both low, showing a greenish colour, and the b values were extremely low, with almost no yellow colour. This is due to the dark purple colour of purple rice bran, such as the anthocyanins and cellulose in purple rice absorb light leading to the colour of the dough is purple, and in the baking process, anthocyanins partially degraded Melad reaction produces black-like essence, and biscuits contain rich butter, caramelization brings reddish-yellow tones, after the biscuit crushed, the colour performance is more uniform, into a light purple.

Combined with Figures 1 and 2, after adding *Dictyophora indusiata*, the light brown colour of *Dictyophora indusiata* neutralised some of the purple colour of the purple rice, but the cellulose still resulted in a very low light-absorbing value and a low brightness of the dough. After baking, the chitin and sugars in the *Dictyophora indusiata* undergo a violent Maillard reaction at high temperatures, producing a large amount of red pigment. When the biscuits are broken into powder, the light absorption of the *Dictyophora indusiata* fibres reduces the brightness, and the products of the Maillard reaction enhance the yellow colour[8]. Figure 2 shows that the effect of purple rice on the colour of the biscuits is extremely significant and dominant, and the several evolutionary systems and sugars in the *Dictyophora indusiata* undergo a Melad reaction at high temperatures, which increases the yellow colour and ameliorates the effect of purple colour of the biscuits.

Table 6 Colour of cookies with different amounts of purple rice and *Dictyophora indusiata*

Biscuits surface	**L**	**a**	**b**	**ΔL a b**
CON	43.9±0.30^{d}	57.7±0.35^{f}	5.63±0.13^{a}	39.8±0.01^{a}
P5	22.0±0.46^{ab}	54.8±0.39^{e}	7.78±0.18^{c}	41.7±0.26^{b}
P10	19.1±1.15^{ab}	51.6±1.28^{d}	7.41±0.18^{bc}	43.4±0.75^{c}
P15	15.0±0.31^{a}	44.3±0.36^{ab}	6.85±0.25^{b}	48.9±0.42^{e}
P20	29.1±0.26^{c}	54.7±0.56^{e}	10.2±0.34^{e}	45.8±0.41^{d}
D5P5	25.4±0.17^{ab}	48.0±0.34^{c}	9.31±0.19^{d}	49.5±0.40^{e}
D5P10	22.0±0.34^{ab}	45.6±0.85^{b}	9.09±0.05^{d}	50.2±0.78^{e}
D5P15	18.7±0.37^{ab}	42.4±0.18^{a}	8.12±0.32^{c}	51.9±0.14^{f}
D5P20	38.9±1.34^{cd}	68.0±0.92^{g}	7.45±0.35^{bc}	43.0±0.68^{bc}
CON	28.7±0.25^{h}	69.2±0.17^{e}	3.67±0.02^{f}	33.6±0.30^{a}
P5	16.0±0.03^{f}	51.3±0.13^{d}	1.42±0.03^{d}	41.9±0.13^{b}
P10	12.2±0.05^{d}	44. 9±0.21^{c}	0.35±0.01^{b}	47.1±0.21
P15	9.91±0.01^{b}	42.5±0.39^{b}	1.61±0.04^{d}	49.2±0.39^{d}
P20	8.01±0.03^{a}	42.8±0.47^{b}	0.18±0.07^{ab}	48.7±0.47^{ef}
D5P5	20.2±0.08^{g}	51.3±0.31^{d}	2.71±0.07^{e}	43.3±0.31^{c}
D5P10	16.0±0.01^{f}	43.0±0.09^{b}	0.07±0.02^{a}	49.8±0.09^{f}
D5P15	13.0±0.02^{e}	43.5±0.63^{b}	0.67±0.09^{c}	48.7±0.61^{e}
D5P20	10.8±0.01^{c}	35.6±0.07^{a}	0.40±0.05^{b}	56.1±0.07^{g}
Biscuit powder	**L**	**a**	**b**	**ΔE**
CON	32.4±0.29^{f}	59.4±0.83^{f}	6.84±0.12^{c}	43.5±0.44^{a}
P5	22.9±0.28^{d}	54.8±0.75^{e}	2.31±0.23^{abc}	41.3±0.64^{b}
P10	18.9±0.18^{b}	50.7±0.45^{d}	1.79±0.17^{ab}	43.4±0.39^{b}
P15	14.5±0.03^{a}	48.9±0.21^{c}	1.56±0.16^{a}	43.8±0.20^{b}
P20	14.2±0.21^{a}	44.9±0.54^{b}	1.42±0.18^{d}	47.6±0.48^{d}
D5P5	25.2±0.11^{e}	51.5±0.94^{d}	3.31±0.11^{d}	45.4±0.77^{c}
D5P10	22.5±0.35^{d}	48.2±0.32^{c}	2.97±0.07^{bc}	47.2±0.17^{d}
D5P15	19.7±0.16^{c}	43.9±0.66^{ab}	2.02±0.26^{abc}	50.0±0.57^{e}
D5P20	18.4±0.07^{bc}	42.4±0.23^{a}	1.96±0.18^{e}	51.1±0.21^{e}

Note: Different superscript letters for data in the same column represent significant differences in data between groups (P<.05).

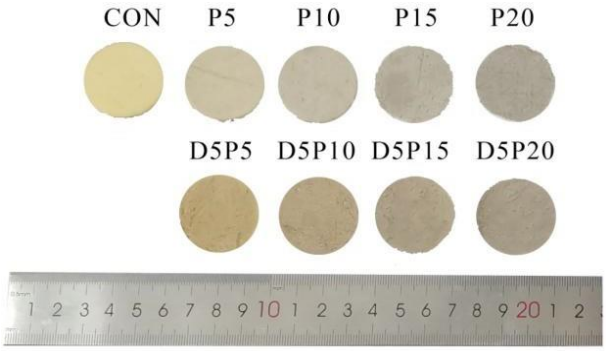

Figure 1 cookies pastry picture

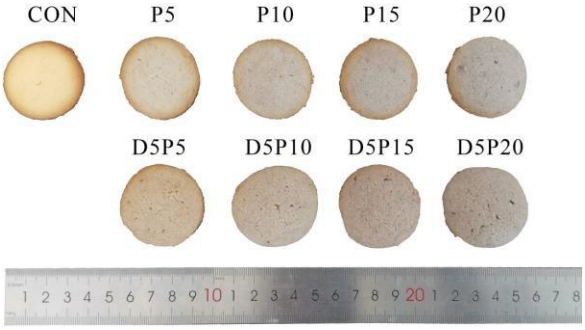

Figure 2 Finished cookies picture

3.3 Effects of Dictyophora indusiata and purple rice on physicochemical properties and baking loss of biscuits

According to the data in Table 7, the hardness of the dough increased significantly with the addition of purple rice, and when *Dictyophora indusiata* was added, the hardness of the dough increased again in comparison with the purple rice group, and the hardness of the dough in the purple rice group was higher than that of the *Dictyophora indusiata* group when the same amount of purple rice was added, and 5% *Dictyophora indusiata* was added to the same amount of purple rice, which indicated that the purple rice was the main factor influencing the hardness of the dough, and the hardness of cookies produced by the dough was higher than that of the *Dictyophora indusiata* group

after the addition of 20% *Dictyophora indusiata* and 20% *Dictyophora indusiata* to the same amount of purple rice. Purple rice produced the highest hardness of biscuits, which made the biscuits crispy and hard. It may be because the bran particles of the purple rice disrupt the internal structure of the biscuits, causing them to become brittle (Kowalski, Mikulec, and Mickowska, 2022).

In Table 7, after the addition of *Dictyophora indusiata*, the hardness of the biscuits started to decrease compared to the purple rice group, the rich soluble dietary fibres in *Dictyophora indusiata*, such as β-glucan, formed a gel during baking, which retained the moisture and volatiles, and insoluble dietary fibres, such as lignin, strengthened the internal gluten network of the biscuits, and the increase in the moisture content of the biscuits resulted in the biscuits being hard, but not brittle (Shams, Singh, Dash, 2023).

The purple rice group showed a decreasing trend in baking loss from 23.5% to 21.9% with increasing purple rice substitution. Baking loss is usually due to water evaporation and loss of other volatiles, while high fibre content may affect water holding capacity. Purple rice contains high levels of bran, which is rich in insoluble fibres such as cellulose, and these fibres may absorb water during baking and reduce water loss, thus reducing baking losses. Bran particles may also adsorb fats and oils, reducing losses due to lipid oxidation.

After the addition of *Dictyophora indusiata*, the baking loss showed a decreasing trend with the increase of purple rice substitution and low baking loss comparing to the sample group with the same percentage of purple rice, which suggests that *Dictyophora indusiata* is rich in a large amount of soluble cellulose, such as β-glucan, which is highly absorbent and may reduce water loss. High moisture content usually leads to increased baking loss, but because the soluble fibres in Dicentrarch, such as β-glucan, are extremely absorbent, they may have formed a gel that retained the water and counteracted the negative effect of high moisture content. Therefore, the strong water-holding property of *Dictyophora indusiata* may be the main factor in reducing the baking loss, while the purple rice further reduced the baking loss of the biscuits by adsorbing moisture and oil and inhibiting lipid oxidation, among other effects.

Changes in thickness: decreased with increasing proportion of purple rice, while changes in diameter, gradually increased with increasing proportion of purple rice. This may be due to the fact that cellulose and lignin in purple rice bran increased the hardness of the biscuits and limited the vertical expansion, and bran particles interfered with the gluten structure, leading to an increase in the ductility of the dough, which in turn brought about more lateral changes, resulting in an increase in diameter. With the addition of *Dictyophora indusiata*, the variation in biscuit thickness was gradually minimised, and the insoluble fibres in *Dictyophora indusiata*, such as chitin, further hardened the dough and almost completely inhibited the vertical expansion of biscuits; the variation in biscuit diameter was higher, reaching 45.0% in the D5P20 group, which could be attributed to the fact that fibres from *Dictyophora indusiata* further weakened the formation of the gluten structure, leading to a more pronounced lateral stretching.

The study showed that the addition of purple rice and *Dictyophora indusiata* significantly improved the textural properties of the biscuit dough and biscuits, and reduced the loss of biscuits during baking.

Table 7 The hardness and baking loss of cookies dough and biscuits with different amounts of purple rice and *Dictyophora indusiata*

Sample	Dough hardness (N)	Baking loss (%)	Biscuit hardness (N)	Thickness change (%)	Diameter variation (%)
CON	371±15.8^{a}	25.3±0.84^{b}	2193±13.5^{a}	33.8±3.76^{e}	29.2±0.59^{b}
P5	429±16.7^{a}	25.6±0.11^{b}	2994±19.3^{c}	30.1±2.51^{de}	20.3±0.38^{a}
P10	463±18.2^{a}	25.5±0.73^{b}	3297±28.1^{e}	25.3±1.91^{cd}	29.1±0.29^{b}
P15	482±16.3^{ab}	23.1±0.34^{ab}	3424±40.2^{f}	23.0±1.74^{c}	30.9±0.29^{c}
P20	485±11.7^{ab}	20.9±0.62^{a}	3886±13.8^{h}	20.2±2.99^{bc}	32.4±0.40^{d}
D5P5	434±16.7^{a}	23.51±0.77^{ab}	2891±55.9^{b}	16.2±0.93^{ab}	32.8±0.59^{d}
D5P10	462±15.1^{a}	22.9±0.51^{ab}	3057±38.4^{d}	14.8±0.91^{ab}	45.8±0.29^{f}
D5P15	483±20.9^{ab}	22.5±0.49^{ab}	3424±33.2^{f}	11.7±0.88^{a}	42.1±0.73^{e}
D5P20	579±22.1^{b}	21.9± 0.73^{ab}	3544±66.3^{g}	10.3±0.77^{a}	45.0±0.69^{f}

Note: Different superscript letters for data in the same column represent significant differences in data between groups (P<.05).

3.4 Effect of Dictyophora indusiata and purple rice addition on antioxidant properties of biscuits

Based on the data in Table 8, it was concluded that the ABTS free radical scavenging rate of the control group was 26.4%. The free radical scavenging rate gradually increased with the increase of the substitution of purple rice and *Dictyophora indusiata*. 72.2% was reached in the D5P20 group, and the DPPH free radical scavenging rate also gradually increased from 10.1% in the control group to 75.8% in the D5P20 group, and the contents of total phenols and total flavonoids also showed an increasing trend, which indicated that the addition of purple rice and *Dictyophora indusiata* significantly improved the antioxidant properties of the biscuits, and in particular, the *Dictyophora indusiata* which had the most significant effect.

In conjunction with Table 5, the fibres in purple rice and *Dictyophora indusiata* were partially degraded during the digestion process, releasing more classifications and flavonoids. After digestion, D5P20, had the highest values of all antioxidants indicating. There may have been a synergistic effect between the purple rice and *Dictyophora indusiata* during digestion, and the fibre used in *Dictyophora indusiata* may have helped to release more antioxidants from the purple rice.

In Table 8, the free radical scavenging power was 2.7 and 7.5 times higher than that of the control at 5% addition of *Dictyophora indusiata* and 20% addition of purple rice, respectively, and the polysaccharide compounds (β-glucan) and phenolics in *Dictyophora indusiata* played a dominant role in antioxidant (Lai, Fang, and Guo, 2023).

When only purple rice was added to the biscuits, the total phenolic content of the P20 group was elevated by 59% and the flavonoid content by 18% compared to the control group, suggesting that the anthocyanins in the purple rice may have been affected by the fibre and released in smaller amounts prior to *in vitro* digestion.

When the biscuits were digested *in vitro*, the free radical scavenging rate was significantly increased in all groups, and the total phenolic content was doubled in the D5P20 group, suggesting that the fibres of *Dictyophora indusiata* and purple rice were partially degraded in digestion and polyphenolic compounds were released. When *Dictyophora indusiata* was added, the increase in DPPH radical scavenging rate was relatively slower compared to that of the purple rice group, and the lignans in *Dictyophora indusiata* may delay the release of antioxidant components and play a certain role in slowing down the release. The anthocyanins in purple rice may be wrapped in fibre and partially inactivated during high-temperature baking, resulting in a low DPPH radical scavenging rate prior to digestion. After *in vitro* digestion, the fibre structure is destroyed, more free anthocyanins are released, and the phenols in the bound state are converted to free phenols, resulting in a significant enhancement of DPPH radical scavenging rate.

The polysaccharides in *Dictyophora indusiata*, such as β-glucan, may form a viscous gel during the gastric digestion stage, which provides a slow release of antioxidant components. In the intestinal digestion stage, by the action of digestive enzymes, the bound phenols were slowly released. The polyphenols in mushrooms, such as ferulic acid, were stable in digestion and retained for a long time during digestion, and after *in vitro* digestion, the free radical scavenging rate of ABTS in the D5P20 group increased from 72.2% to 90.4%. The addition of *Dictyophora indusiata* and purple rice significantly improved the antioxidant capacity of the biscuits.

Table 8 Antioxidant properties of cookies with different amounts of purple rice and *Dictyophora indusiata*

Before *in vitro* digestion	ABTS (%)	DPPH (%)	Total Phenol (mg GAE/100 g DM)	Total flavonoids (μg CE/100 g DM)
CON	26.4±0.02^{a}	10.1±0.06^{a}	0.27±0.01^{a}	28.7±0.02^{a}
P5	57.9±0.05^{b}	18.3±0.06^{a}	0.30±0.01^{a}	30.2±0.06^{ab}
P10	64.9±0.02^{cd}	28.0±0.05^{ab}	0.34±0.04^{ab}	31.4±0.02^{bc}
P15	69.9±0.05^{de}	32.5±0.03^{abc}	0.37±0.02^{abc}	32.7±0.08^{bc}
P20	62.0±0.02^{bc}	40.0±0.02^{abc}	0.43±0.03^{bcd}	33.8±0.09^{c}
D5P5	64.4±0.02^{bcd}	49.8±0.04^{bcd}	0.36±0.03^{abc}	36.3±0.08^{d}
D5P10	78.4±0.09^{f}	57.1±0.02^{bcd}	0.41±0.04^{bc}	37.4±0.08^{d}
D5P15	77.2±0.01^{f}	63.1±0.06^{cd}	0.46±0.04^{cd}	38.9±0.01^{d}
D5P20	72.2±0.01^{ef}	75.8±0.04^{d}	0.53±0.06^{d}	40.3±0.04^{d}
CON	43.2±0.01^{a}	33.2±0.08^{a}	0.56±0.03^{a}	59.3±0.01^{a}
P5	69.8±0.09^{b}	58.1±0.06^{a}	0.58±0.01^{a}	60.3±0.05^{a}
P10	73.1±0.02^{cd}	60.7±0.05^{ab}	0.67±0.05^{b}	60.3±0.04^{a}
P15	78.2±0.03^{de}	62.9±0.03^{abc}	0.69±0.02^{b}	60.8±0.07^{ab}
P20	83.3±0.01^{bc}	64.5±4.02^{abc}	0.77±0.01^{c}	61.3±0.01^{ab}
D5P5	76.4±0.04 ^{bcd}	82.4±0.07^{bcd}	0.81±0.04^{d}	61.8±0.05^{ab}
D5P10	82.8±0.09^{f}	87.6±0.04^{bcd}	0.9±0.04^{e}	62.8±0.03^{ab}
D5P15	88.9±0.01^{f}	87.8±0.06^{cd}	0.96±0.07^{f}	62.8±0.05^{ab}
D5P20	90.4±0.02^{ef}	88.2±0.04^{d}	1.11±0.02^{g}	65.4±0.03^{b}

Note: Different superscript letters in the same column of data represent significant differences in data between groups (P<.05).

3.5 Effect of Dictyophora indusiata and purple rice addition on in vitro digestive characteristics of biscuits

In Figure 3, the starch hydrolysis rate was the highest at all time points in the control group, indicating the fastest starch digestion in the control group. While the hydrolysis rate of starch gradually decreased with the increase of the substitution of purple rice and *Dictyophora indusiata*, especially in the D5P20 group at 180 min, the starch hydrolysis rate was the lowest and the slowest digested. The control group had the highest fast-digested starch content. With the increase of the substitution amount of the two raw materials, the content gradually decreased, and the fast-digested starch content decreased to the lowest when the addition amount reached the highest. The resistant starch, on the contrary, increased with the increase in the substitution of both ingredients, and the addition of purple rice and *Dictyophora indusiata* increased the content of resistant starch.

Regarding the glycaemic index (GI), usually high RDS leads to high GI, whereas high RS and SDS decrease GI.Therefore, the low RDS and high RS of the D5P20 group imply that it has a lower GI, and the fibres in purple rice and *Dictyophora indusiata* may encapsulate starch granules, slowing down enzyme action and thus reducing the digestion rate. Meanwhile, the presence of fibre may promote the release of antioxidants, creating a synergistic effect.

Combined with Tables 8 and 9, after digestion, the starch hydrolysis power of the biscuits in the control group was 99.3%, and the phenolic content of the digested biscuits was 1.11 mg GAE/100 g DM, while the starch hydrolysis of the control group was rapid and phenolics were released rapidly, but the total amount of the released substances was low, and the starch hydrolysis rate of the D5P20 group was 86.5%, with a decrease of 25% in the RDS, an increase of 31% in the RS, and a GI Decreased, purple rice and *Dictyophora indusiata* significantly reduced the GI of the biscuits. The addition of purple rice and *Dictyophora indusiata* significantly reduced the rate of starch hydrolysis, elevated the content of resistant starch, and significantly reduced the glycaemic index of the biscuits.

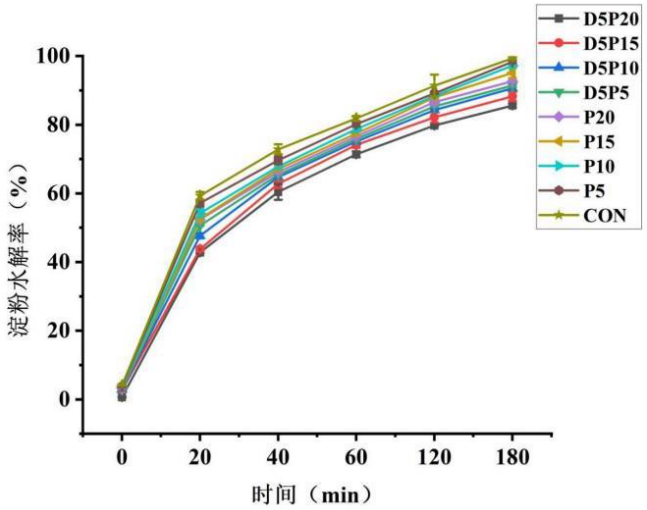

Figure 3 *In vitro* digestion characteristics of cookies with different addition amounts of purple rice and *Dictyophora indusiate*

Table 9 Starch content (g/100 g) of cookies with different amounts of purple rice and *Dictyophora indusiata*

Sample	Fast digestible starch	Slowly digestible starch	Resistant starch
CON	20.1±0.30^{e}	11.7±0.20^{a}	8.7±0.07^{a}
P5	19.5±0.27^{e}	11.7±0.25^{a}	9.4±0.19^{ab}
P10	18.4±0.11^{d}	12.4±0.26^{ab}	9.5±0.07^{abc}
P15	17.9±0.27 ^{cd}	12.9±0.19^{bc}	9.7±0.26^{bcd}
P20	17.7±0.23^{cd}	12.3±0.07^{ab}	10.0±0.1^{bcd}
D5P5	17.1±0.26^{cd}	12.5±0.18^{ab}	10.4±0.29^{cd}
D5P10	16.0±0.13^{b}	13.1±0.15^{bc}	10.6±0.15^{de}
D5P15	14.8±0.09^{a}	13.8±0.18^{c}	11.4±0.24^{e}
D5P20	15.0±0.21^{a}	13.2±0.10^{bc}	11.4±0.18^{e}

Note: Different superscript letters for data in the same column represent significant differences in data between groups (P<.05).

3.6 Effects of dictyophora indusiata and purple rice addition on the flavour substances of biscuits

The gradient substitution of purple rice and *Dictyophora indusiata* significantly altered the flavour profile of biscuits, and the changes in their content revealed a shift from traditional butter aroma to grain-wood composite aroma. Combined with Figure 5 and Table 10, the significant reduction of aldehydes, such as hexanal and nonanal, in the purple rice substitution group (P5-P20), and combined with Table 6, indicated that the antioxidant components (e.g., anthocyanins, flavonoids) in purple rice effectively inhibited lipid oxidation and alleviated the oxidative "hafting flavour"of the traditional biscuits due to the high fat content (Wang, Yang, and Liu, 2022); at the same time, the increase of terpenes d-limonene and elemene, which are unique to purple rice, relied on the increase of the addition of purple rice to give citrus aroma and woody undertones, respectively, and synergised with the caramel aroma of methyl heptenone and the creamy aroma of γ-nonanolactone to form a complex cereal aroma. The introduction of *Dictyophora indusiata* (D5P5-D5P20 group) further released pine aroma characteristic components such as β-cedrene and α-cedrene[13] , which formed a hierarchical overlay with the purple rice flavour, but the abnormal enrichment of 1-isocyanatobutane may have originated from the thermal degradation of nitrogenous polysaccharides of *Dictyophora indusiata*, and its pungent odour led to the reduction of the flavour scores of the D5P20 group in the organoleptic evaluation in Figure 7. Changes in the dynamic balance of key flavour presenting substances were particularly notable: the linear increase in vanillin and 2-acetylpyridine reinforced the baked char aroma, which corresponded to the rise in the surface a-value of the biscuits to 68.0 as a result of the enhanced Maillard reaction, but the content of ethyl caprate, the main aroma component of the esters, was significantly reduced, and the persistent loss of ethyl caprate weakened the fruity base of the traditional biscuits. This flavour reconstruction was manifested at the sensory level in the form of increased aroma complexity and enhanced feature recognition, such as a richer variety of volatiles in the D5P20 group compared with the control group, but the sensory clash triggered by the piney flavour of terpenes (e.g., τ-celestene) and the pungent odour of 1-isocyanatobutane, which may be the core causative factor leading to the overall acceptance of the D5P20 group being significantly lower than that of the ZiMi single-factor group.

By integrating the gas chromatography and electronic nose data, the remodelling of biscuit flavour characteristics by the gradient substitution of purple rice and *Dictyophora indusiata* showed a dual mechanism of changes in the content of major flavour substances and synergistic effects of special trace substances. Combined with Figures 4.4 and 4.6, at the level of the main flavour substances, ethyl decanoate, which dominates the fruity aroma of traditional biscuits, decreased linearly with the increase of the substitution ratio, and the loss of which was linked to the weakening of the response of the e-nose aromatic hydrocarbon sensor S18, which weakened the fruity-sweet odour tone of the biscuits; whereas the increase of the terpenes introduced by zi mizanami, namely, d-limonene and elemi, echoed the responses of the gas and alkane sensors S7 and S11, respectively. response, which shifted the flavour

tonality towards citrus woody notes, building a composite cereal aroma profile together with the caramel aroma of methylheptenone and the creamy aroma of γ-nonolactone. Combined with Table 10 and Figure 6, the dynamic changes of trace specific substances reveal the risks behind the increase of flavour complexity: the abnormal enrichment of 1-isocyanatobutane in the *Dictyophora indusiata* group is highly coincident with the response surge of the electronic nose amine sensor S15, and its irritating odour contrasts with the weak fluctuation of the sulphur sensor S4, suggesting that the off-flavour originates from nitrogen-containing pyrolysis products rather than sulphur compounds; whereas, the aldehydes (hexanal, hexanal, and γ-nonolactone) in the purple rice group are more important than the other aldehydes. Although the oxidative inhibitory effect of aldehydes (hexanal and nonanal contents were greatly reduced) was supported by the rise in response of the e-nose alkane sensor S11, the turpentine odour of trace terpenes such as τ-celestene and the fluctuation in response of the e-nose alicyclic hydrocarbon sensor S18 implied that the wood tones might have breached the sensory threshold. The effects of this change in flavour substance composition are manifested at the sensory level: the D5P10 group achieves an optimal blend of fruity and woody tones by balancing the ratio of ethyl decanoate to α-cedrene, which corresponds to the e-nose S18 response value of 1.51, whereas the D5P20 group's bad effects due to 1-isocyanatobutane are echoed by the e-nose S15 response value of 1.34, with 1-isocyanatobutane resulting in a decrease in flavour purity, confirming the bidirectional modulation of the overall flavour profile by specific trace substances.

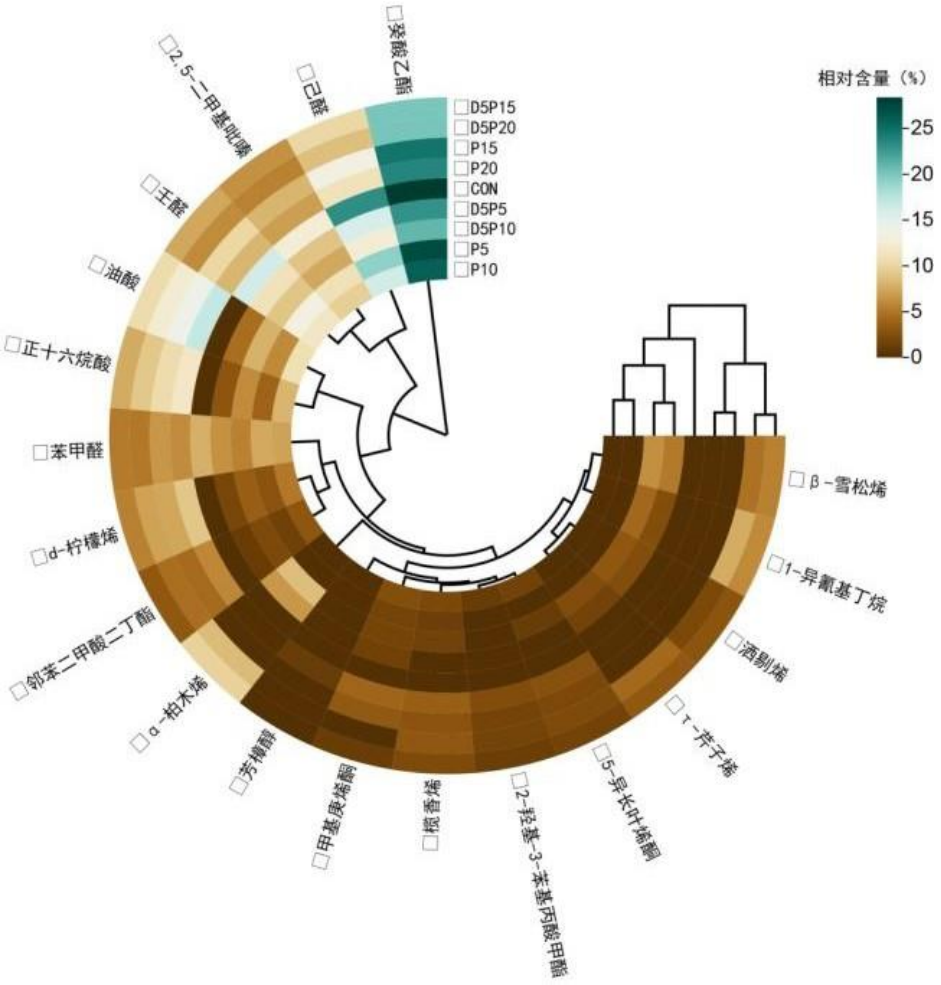

Figure 4 Heat map of relative content of total volatile compounds

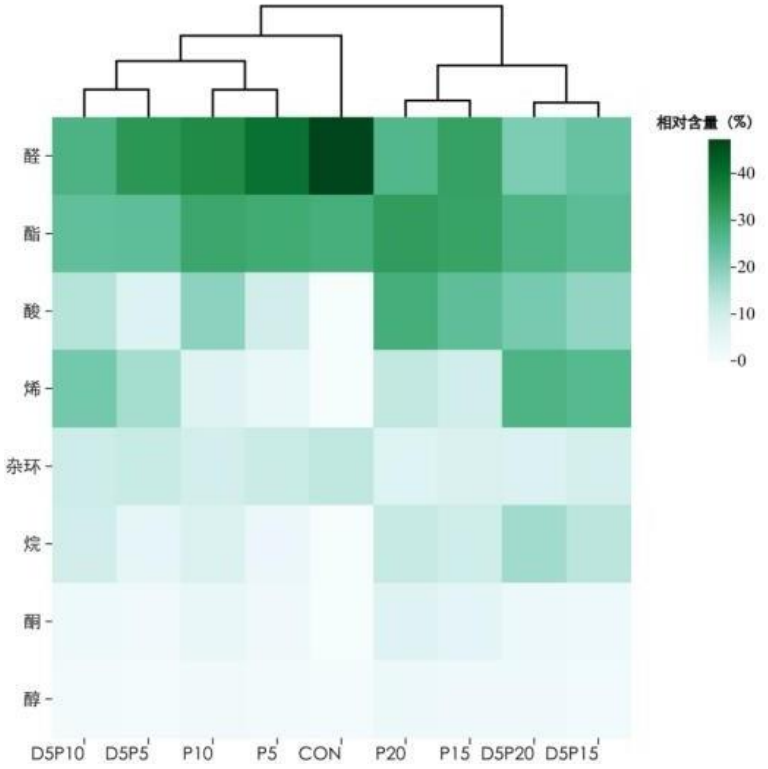

Figure 5 Heatmap of relative content of different kinds of volatile compounds

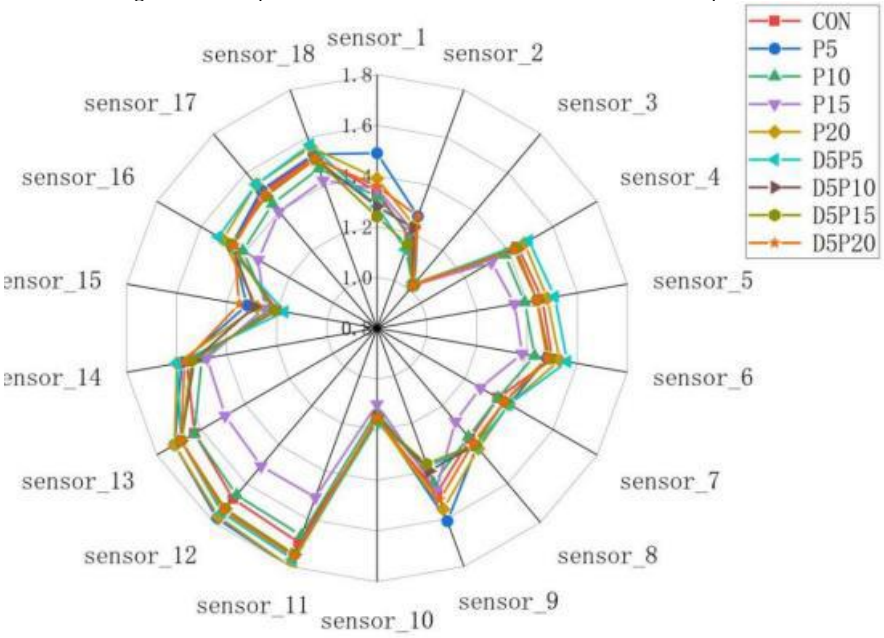

Figure 6 cookies electronic nose evaluation

Table 10 Flavour substances of cookies with different addition amount of purple rice and *Dictyophora indusiata*

Compound	Molecular formula	CSA number	CON	P5	P10	P15	P20	D5P5	D5P10	D5P15	D5P20
Methyl nonyl ketone	$C_{10}H_{20}O$	110-93-0	-	0.09	0.17	0.23	0.28	0.06	0.12	0.17	0.22
Methylheptenone	$C_8H_{14}O$	110-93-0	-	1.68	2.45	3.21	3.98	1.46	1.71	1.11	0.68
5-Isophyllenone	$C_{15}H_{24}O$	502-69-2	-	0.93	1.51	2.08	2.65	0.65	1.09	1.58	2.21
1-Isocyanobutane	C_5H_9N	111-35-5	-	-	-	-	-	2.43	4.19	6.01	7.62
2,5-Dimethylpyrazine	$C_6H_8N_2$	123-32-0	12.6	11.1	9.62	8.11	7.01	8.91	7.46	6.33	5.58
Vanillin	$C_8H_8O_3$	121-33-5	0.19	0.22	0.26	0.31	0.34	0.18	0.21	0.24	0.29
2-Acetylpyridine	C_7H_7NO	112-18-9	0.02	0.04	0.06	0.08	0.09	0.03	0.05	0.06	0.08
Sparteine	$C_{10}H_{16}$	5989-27-5	-	-	-	-	-	2.43	3.11	2.85	2.03

Compound	Molecular formula	CSA number	CON	P5	P10	P15	P20	D5P5	D5P10	D5P15	D5P20
d-Limonene	$C_{10}H_{16}$	5989-27-5	-	2.81	5.08	7.36	9.13	1.94	3.57	5.54	7.11
β-Cedrene	$C_{15}H_{24}$	502-69-2	-	-	-	-	-	5.18	6.21	5.54	4.74
α-Cedrene	$C_{15}H_{24}$	2830-06-7	-	-	-	-	-	6.64	8.54	9.82	8.46
Elemene	$C_{15}H_{24}$	515-13-9	-	1.31	2.07	2.83	3.61	0.97	1.55	2.21	3.05
τ-Celestene	$C_{15}H_{24}$	515-13-9	-	-	-	-	-	1.62	2.33	3.16	4.23
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	47-10-3	-	3.92	8.09	10.5	11.7	2.92	6.06	7.59	9.14
Oleic acid	$C_{18}H_{34}O_2$	112-80-1	-	5.98	10.9	14.2	16.8	4.53	7.92	10.6	12.4
Caproic acid	$C_6H_{12}O_2$	142-62-1	0.15	0.19	0.23	0.26	0.31	0.15	0.17	0.21	0.25
Ethyl decanoate	$C_{12}H_{24}O_2$	110-38-3	28.4	27.2	25.9	24.7	23.7	23.1	20.9	20.1	20.1
Dibutyl phthalate	$C_{16}H_{22}O_4$	84-74-2	-	1.49	3.01	4.34	5.88	1.13	2.17	3.16	4.57
Methyl caprylate	$C_9H_{18}O_2$	111-11-5	-	-	-	-	-	0.13	0.23	0.36	0.51
Ethyl butyrate	$C_6H_{12}O_2$	105-54-4	0.28	0.34	0.43	0.45	0.51	0.26	0.31	0.35	0.42
γ-Nonanolactone	$C_9H_{16}O_2$	104-61-0	0.04	0.07	0.11	0.15	0.19	0.05	0.08	0.11	0.15
Methyl 2-hydroxy-3-phenylpropionate	$C_{10}H_{12}O_3$	121-33-5	-	0.56	1.13	1.72	2.28	0.32	0.78	1.27	1.69
Hexanal	$C_6H_{12}O$	66-25-1	23.2	19.2	16.1	13.5	11.1	15.8	12.5	10.2	8.63
Nonanal	$C_9H_{18}O$	124-19-6	16.1	13.2	11.6	10.2	8.15	11.1	9.01	7.43	6.09
Benzaldehyde	C_7H_6O	100-52-7	7.81	7.47	7.15	6.66	6.07	6.32	5.59	5.22	5.08

Table 10 Flavour substances of cookies with different addition amount of purple rice and *Dictyophora indusiata*

Compound	Molecular formula	CSA number	CON	P5	P10	P15	P20	D5P5	D5P10	D5P15	D5P20
2-Heptenal	$C_7H_{12}O$	111-71-7	-	0.22	0.47	0.72	0.93	0.16	0.34	0.55	0.73
trans-2,4-decadienal	$C_{10}H_{16}O$	25152-84-5	0.06	0.13	0.21	0.26	0.32	0.12	0.14	0.19	0.25
Valeraldehyde	$C_5H_{10}O$	110-62-3	0.09	0.13	0.17	0.21	0.25	0.13	0.12	0.16	0.23
Linalool	$C_{10}H_{18}O$	78-70-6	0.09	0.34	0.62	0.85	1.08	0.24	0.43	0.65	0.88
1-Octen-3-ol	$C_8H_{16}O$	3391-86-4	0.37	0.47	0.56	0.66	0.76	0.36	0.42	0.51	0.63
Phenylethanol	$C_8H_{10}O$	60-12-8	0.56	0.65	0.75	0.85	0.95	0.52	0.59	0.68	0.81
Erythritol	$C_4H_{10}O_4$	149-32-6	-	0.04	0.08	0.11	0.15	0.02	0.05	0.08	0.12
Cyclooctanediol	$C_8H_{16}O_2$	4430-24-6	-	0.06	0.11	0.17	0.23	0.03	0.08	0.13	0.17

3.7 Sensory evaluation Analysis of results

The sensory evaluation analysis showed that the substitution of wheat flour by purple rice and *Dictyophora indusiata* had a complex effect on the sensory characteristics of the biscuits, mainly in terms of contradictions in colour, taste, texture and consumer acceptability. In Figure 7, as the amount of purple rice substitution increased (especially up to 20%), the data in Table 6 showed that the biscuits became significantly darker in colour and the surface brightness (L-value) decreased from 43.9 to 29.1 in the control group, which originated from the anthocyanins in the bran of purple rice and the light-absorbing effect of cellulose in high-temperature baking. Although the light brown colour of the *Dictyophora indusiata* partially neutralised the purple colour of the purple rice (e.g. the surface L value of the D5P20 group rebounded to 38.9), its own Maillard reaction during baking led to an abnormally high reddish hue (a-value) on the surface of the biscuits (up to 68.0 in the D5P20 group), and instead strengthened the visual characteristics of the non-traditional biscuits, which may trigger consumers' concerns about "burnt" or "abnormal biscuits". This may trigger consumers' thoughts of "burnt" or "abnormal colour".

In terms of taste, the data in Table 9 showed that the increased substitution of purple rice significantly increased the hardness of the biscuits (3886 N in the P20 group, 77% higher than the control group), and the bran particles disrupted the gluten structure to form a hard and crispy texture, which, although considered "uniquely crispy" by some of the reviewers, exceeded the taste expectations of a conventional biscuit. On the other hand, the addition of *Dictyophora indusiata* made the biscuits hard but not crispy through β-glucan gelatinisation (hardness of 3544 N in the D5P20 group, a decrease of 8.8% compared to the P20 group), and the texture was closer to that of a tough biscuit, which led to a contrast between the highest crispy scores in the P20 group and the lowest in the D5P20 group, highlighting the differential effect of the type of fibre on the texture.

Flavour ratings were polarised, with the purple rice group receiving moderate ratings for the cereal aroma from the bran, but the possible introduction of fibre roughness by the *Dictyophora indusiata* led to a sudden drop in flavour ratings for the D5P20 group, which was the main limiting factor for sensory acceptability. Notably, despite the D5P20 group's optimal performance in terms of nutritional functions (3.25% fibre content and 7.5-fold improvement in antioxidant properties) and health attributes (reduced GI), it had the lowest overall acceptability score, significantly lower than the control group, reflecting that consumer acceptance of high-fibre health biscuits is still constrained by traditional sensory expectations. This contradiction was further exacerbated by the swelling characteristics: the insoluble fibre of *Dictyophora indusiata* almost completely inhibited vertical swelling (only 10.3% change in D5P20 thickness), whereas the elevated lateral ductility (45% change in diameter) resulted in a flattened biscuit morphology, which differed significantly from the fluffy appearance of the traditional biscuits.

Therefore, the core contradiction in sensory evaluation lies in the conflict between the abrupt changes in colour and texture brought about by nutritional fortification and consumers' inherent perception of golden, crispy and sweet biscuits.

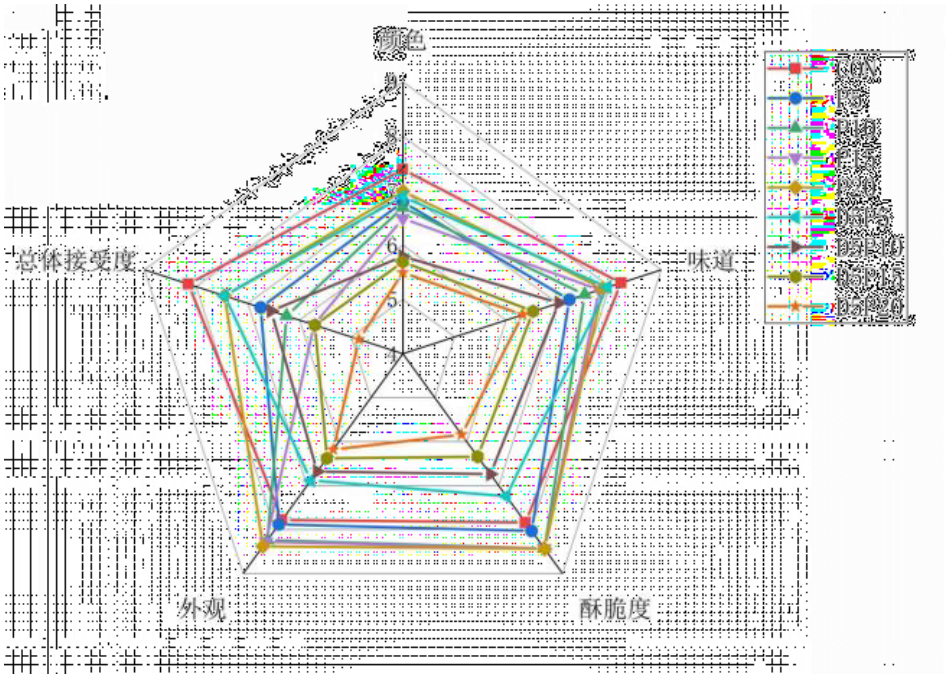

Figure 7 Sensory evaluation diagram of cookies with the same amount of purple rice and *Dictyophora indusiate*

4. Conclusions

The replacement of wheat flour by purple rice and *Dictyophora indusiata* provides a breakthrough direction for the nutritional upgrading of biscuits. The rich insoluble fibre and resistant starch in purple rice, together with the β-glucan and chitin of *Dictyophora indusiata*, formed a synergistic and synergistic dietary fibre network, which not only increased the total fibre content to more than twice that of the traditional biscuits, but also significantly enhanced the protein content and antioxidant activity, and at the same time, realised the potential of blood glucose regulation by inhibiting the rapid digestion of starch, which lays a scientific foundation for the development of functional low- GI bakery products. Although there were limitations in sensory evaluation, such as dark colour (the natural colour of purple rice anthocyanins) and high hardness, the purple rice group maintained acceptable crunchiness and cereal flavour with moderate substitution (e.g., P20), and the gel characteristics of *Dictyophora indusiata* gave the biscuits a unique and tough texture, which demonstrated the possibility of differentiating from traditional biscuits in terms of market positioning. The study confirms that by precisely controlling the substitution ratio (e.g. a medium ratio of 10% purple rice and 5% *Dictyophora indusiata*), the conflict between colour and texture can be effectively mitigated, which has a broad application prospect in the future in the fields of healthy snacks and special food. This exploration not only breaks through the nutritional defects of traditional biscuits, which are high in sugar and fat, but also provides an innovative example of the functional use of natural ingredients.

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Natural Colorant from *Chara corallina* and Application in Ma Muang Bao Gummy Jelly

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Abstract

This experiment investigated the application of natural colorant, which was stabilized chlorophyll from *Chara corallina*. Ma Muang Bao gummy jelly product was used for this application. The result showed that the basic recipe for Ma Muang Bao gummy jelly production consisted of 20 grams of mango, 20 grams of water, 10 grams of gelatin, 10 grams of glucose syrup, 39. 2 grams of sugar and 0.8 grams of citric acid. Muang Bao gummy jelly supplemented with 0.5% colorant powder from *Chara coralline* was suitable. The obtained products had a preference score for each characteristic in the range of 7. 94- 8. 46 (like moderately to like very much). For the analysis of physicochemical quality of Ma Muang Bao gummy jelly supplemented with colorant powder from *Chara corallina*, it was found that moisture, ash, protein, fat, fiber, carbohydrate and energy contents were 41.74%, 2.35%, 11.98%, 0.025%, 0.054%, 43.90% and 223.74%, respectively. The total acid content was 0.14% and the water activity (a_w) was 0.64. While the lightness (L*), greenness (-a*) and yellowness (b*) were 46.14, -0.82 and 22.71, respectively. Microbiological quality showed total viable counts less than 25 CFU/ g, while yeast and mold counts were less than 10 CFU/ g. Additionally, it can be concluded that the natural colorant (stabilized chlorophyll) from *Chara corallina* has the potential for application in food products.

Keywords: Natural Colorant, Chara Corallina, Gummy Jelly, Ma Muang Bao

1. Introduction

Natural colorants have recently gained increasing attention due to the increasing awareness for the safety of synthetic colors. Plants, including algae, are an important source of natural colorants. Large algae are generally divided into Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) based on their production of pigments. The differences between algal pigments are environmental adaptations required to increase the efficiency of light capture for photosynthesis at different depths. The pigments of macroalgae can be divided into three types: chlorophyll, carotenoids, and phycobilins. Chlorophyll and carotenoids are water-insoluble pigments, while phycobilins are a group of water-soluble proteins (Dawes, 1998; Lee, 2008)

Food safety research is increasingly important and relevant today because of its impact on consumer health and government costs in treating patients caused by unsafe food intake. This research designed an experiment using *Chara corallina* as a source of natural colorant, as this algae has been consumed in some areas of the South of Thailand for a long time. These algae are eaten fresh like other local vegetables. Local people believe that this algae can cure diabetes and stomach diseases. However, there are no research reports showing the medicinal properties of this algae. Preliminary studies have shown that the amount of chlorophyll in the cells of *Chara corallina* is higher than that of other freshwater algae. The high amount of chlorophyll makes it interesting to use as a natural colorant. It is known that the use of chlorophyll as a natural pigment has been used for a long time. (Shahid et al., 2013) Many researchers have studied the benefits of pigments to develop them for human use, especially in food, pharmaceutical industries and other industries. Manufacturers have tried to produce natural pigments, such as chlorophyll and some carotenoids, to replace synthetic dyes that are known to be carcinogenic. Chlorophyll can stimulate the recovery of damaged tissue, boost immunity, improve blood circulation and digestion, and treat serious diseases. Furthermore, chlorophyll has been developed for the treatment of cancer using photodynamic therapy (PDT) because of its photosensitizing properties. (Park, et al., 1989; Agostinis et al. 2001; Lordan et al. 2011) Several natural colorants are commercially used and approved for use in the United States and the European Union, such as anthocyanin (from grape skin extract, grape pigment extract, berry or carrot and cabbage juice), carotenoids (from *Bixa orellana* L.), β-carotene (from carrots, carrot oil, corn endosperm and bell pepper), chlorophyll (from alfalfa (*Medicago sativa*)), curcuminoids (from turmeric), betalain (from beetroot powder), carminic acid (from cochineal extract) and caramel from heating of sugar (Sigurdson et al., 2017).

This research aimed to study the application of stabilized chlorophyll powder from *Chara corallina* as colorants in Ma Muang Bao ((*Mangifera indica L.*). gummy jelly. *Mangifera indica* L. was the local mango of the South of Thailand. The results of this study can be used in the food industry or other related industries. Additionally, the impact will generate income for communities in areas where *Chara corallina* is used, and the natural colorants will increase food safety for consumers.

2. Methodology

2.1 Preparation of colorant powder from Chara corallina

Chara corallina were washed thoroughly with tap water, then the algae were blanched in boiling water for 2 minutes, and ground the sample finely with a multi-purpose blender. 70 grams of blanched and ground algae were used and reacted with 0.3% $NaHCO_3$ salt solution. The reaction temperature was 90 °C and the reaction time was 10 minutes. Metal-chlorophyll complexes were extracted using a modified method of Rahayuningsih et al. (2018) and Salama & Moharram (2007). The obtained algae sample, 10 ml, were extracted with 25 ml of 96% ethanol in an erlenmeyer flask shaker. The flasks were closed with aluminum foil and placed in a shaker water bath at 60 °C and the samples were shaken at 135 rpm for 45 min. After that, the obtained solution was processed through encapsulation with a spray dryer using maltodextrin as wall material. The colorant powder was stored in a tightly closed container waiting to be used.

2.2 The application of colorant from Chara corallina in food

The application of colorants from *Chara corallina* was used in Ma Muang Bao gummy jelly product. Gummy jelly is the product that is popularly colored to make it more appetizing.

1) Physicochemical composition of Ma Muang Bao

The physicochemical compositions of Ma Muang Bao were studied as following:

- Proximate composition (moisture, ash, lipid, protein and carbohydrate content including crude fiber) according to the method of AOAC (2000)
- Total titratable acids according to the method of AOAC (2000)
- Water Activity (a_w) using Water Activity Meter (Aqulab series 3, Decagon Devices, Inc., USA)
- Color value using Color Meter (HunterLab, model ColorFlex EZ, USA)

2) Ma Muang Bao gummy jelly basic recipe

Ma Muang Bao gummy jelly was produced by modifying the basic gummy jelly production formula of Garcia (2000). The appropriate basic recipe for Ma Muang Bao gummy jelly was studied, the formulas are shown in Table 1.

Table 1 Different formulas of Ma Muang Bao gummy jelly used in the basic formula study

Ingredient	Formula 1	Formula 2	Formula 3
Ma Muang Bao (g)	0.7	0.8	0.9
Water (g)	20.0	20.0	20.0
Gelatin (g)	10.0	10.0	10.0
Glucose Syrup (g)	10.0	10.0	10.0
Sugar (g)	39.3	39.2	39.1
Citric acid (g)	0.7	0.8	0.9

***Mangifera indica* L. preparation**

Cold water was prepared for soaking the mango. 1 teaspoon of salt and 1 tablespoon of lime juice were added to 3 liters of water in the big bowl. 1 kilogram of ice was used to make cold water. Mixed everything well. Young mangoes were peeled and soaked in the prepared water. One young mango was cut into 4 pieces and soaked them back in the water. The mangoes were soaked for about 20 minutes and rinsed with clean water twice. After the mangoes were drained, they were ready for use.

Preparation of Ma Muang Bao gummy jelly

The prepared mangoes were soaked in 10% NaCl solution with a ratio of mango to brine (w/w) of 1:1 for 30 minutes. After the time was up, drained the water and blended until smooth. The water (in the formula) was divided into 2 parts: Part 1: The water was mixed with finely blended mango, sugar, glucose syrup and citric acid. Mixed ingredients were cooked over medium-low heat and simmered until the temperature reaches 120 °C. Part 2: The water was mixed with gelatin. After that, parts 1 and 2 were mixed together. The thick mixer was scooped out and dropped it into the mold. Then the samples were refrigerated at 4 °C for 24 hours before removing from the mold.

The experimental design consisted of 3 treatments according to the formula for preparing Ma Muang Bao gummy jelly. The appropriate formula was selected by sensory evaluation with 35 panellists using 9-point hedonic scale. Sensory characteristics such as appearance, color, odor, taste, texture and overall acceptance were considered. The RCBD experiment was designed and the means were compared using the Duncan's New Multiple Rang Test (DMRT).

3) The appropriate amount of colorant powder from Chara corallina use in gummy jelly

The appropriated amount of colorant powder from *Chara corallina* using in gummy jelly was studied with the appropriate formula from the previous study. Three levels of colorant powder were evaluated: 0.0, 0.5, 1.0 % of all ingredients. The appropriate treatment was selected by sensory evaluation with 35 panellists using 9-point hedonic scale. Sensory characteristics such as appearance, color, odor, taste, texture and overall acceptance were considered. The RCBD experiment was designed and the means were compared using the Duncan's New Multiple Rang Test (DMRT).

4) Quality of Ma Muang Bao gummy jelly supplemented with colorant powder from Chara corallina

Quality of Ma Muang Bao gummy jelly supplemented with colorant powder from *Chara coralline* was evaluated as following:

- Proximate composition (moisture, ash, lipid, protein and carbohydrate content including crude fibre) according to the method of AOAC (2000)
- Total tritratable acids according to the method of AOAC (2000)
- Water Activity (a_w) using Water Activity Meter (Aqulab series 3, Decagon Devices, Inc., USA)
- Color value using Color Meter (HunterLab, model ColorFlex EZ, USA)
- Total viable count according to the method of BAM (2001)
- Yeast and mold according to the method of BAM (2001)

3. Results and Discussion

3.1 Physicochemical composition of Ma Muang Bao

Physicochemical compositions of Ma Muang Bao were evaluated, the results are shown in Table 2.

Table 2 Physicochemical compositions of Ma Muang Bao

Composition	Amount
Moisture (%)	90.58 ± 0.03
Ash (%)	0.28 ± 0.05
Protein (%)	0.31± 0.02
Fat (%)	0.08 ± 0.02
Fiber (%)	0.75 ± 0.17
Carbohydrate (%)	7.99 ± 0.15
Total titratable acids (%)	0.25 ± 0.00
Water Activity (a_w)	0.99 ± 0.00
Color value	
L*	71.88 ± 0.02
a*	-3.28 ± 0.01
b*	34.32 ± 0.02

Physicochemical compositions of Ma Muang Bao were evaluated, it was found that the moisture, ash, protein, fat, fiber, carbohydrate and total acid content were 90.58, 0.28, 0.31, 0.08, 0.75, 7.99 and 0.25%, respectively. The water activity (a_w) was 0.99, the lightness (L*) was 71.88, the redness (a*) was -3.28 and the yellowness (b*) was 34.32. Yuthachit (1998) studied physicochemical compositions of Ma Muang Bao, the result was as follows: 90.36% moisture content, 3.27% total acid in the form of citric acid, 6.51 % total soluble solids (°Brix), 4.34% total sugar, 2,96% reducing sugar, firmness was 7.08 Newton, density was 983.1 ± 0.02 kg/m3, L a b was 31.06, -1.48 and 9.27, respectively. Songwanphuak et al. (2012) studied the amount of active compounds and antioxidant activity in 13 species of Thai mango, the result showed that Ma Muang Bao was found to have the highest total phenol content. The antioxidant activity study by DPPH method found that it was in the group of mangoes with high antioxidant activity, while the analysis by FRAP method found that it was in the group of mangoes with moderate antioxidant activity.

3.2 Ma Muang Bao gummy jelly basic recipe

Ma Muang Bao gummy jelly basic recipe was examined with 3 different formulas, the results are shown in Table 3

Table 3 Sensory characteristics preference scores of Ma Muang Bao Gummy Jelly

Sensory characteristics	Mean of hedonic score		
	Formula 1	Formula 2	Formula 3
Color	7.83 ± 0.38^{b}	8.11 ± 0.57^{a}	7.80 ± 0.40^{c}
Odor	6.71 ± 0.45^{c}	7.43 ± 0.55^{a}	6.86 ± 0.49^{b}
Taste	7.20 ± 0.47^{b}	8.57 ± 0.49^{a}	7.17 ± 0.51^{c}
Texture	7.31 ± 0.52^{b}	8.20 ± 0.40^{a}	7.29 ± 0.51^{c}
Overall acceptance	7.14 ± 0.35^{c}	8.26 ± 0.44^{a}	7.20 ± 0.47^{b}

Remark: -The different letters within the same column indicate significant differences between treatments ($p<.05$) according to Duncan's New Multiple Range Test (DMRT).

From Table 3, hedonic scores for color, odor, taste, texture and overall acceptance of Ma Muang Bao gummy jelly formula 2 were significantly higher than formulas 1 and 3 ($p<.05$). Therefore, the suitable formula for Ma Muang Bao gummy jelly production was Formula 2, which consisted of 20 grams of mango, 20 grams of water, 10 grams of gelatin, 10 grams of glucose syrup, 39.2 grams of sugar and 0.8 grams of citric acid. Takeungwongtrakul et al. (2020) developed gummy jelly products from strawberry syrup, it was found that the optimal formula consisted of 43% strawberry syrup, 40% sugar, 10% gelatin powder, 9% glucose syrup, and 1% ascorbic acid. The production process was as follows: Strawberry syrup was poured into a pot and heated at 45°C for 5 minutes. Glucose syrup, sugar, ascorbic acid and gelatin powder were mixed and stirred well, then boiled for 5 minutes to obtain a concentration of 78 ± 0.01°Brix. The mixed solution was poured into the mold and refrigerated for 24 hours. The formulas of three commercial gummy jelly, namely Jolly Bears, Zoo Gummy and Dole were observed for their ingredients. Jolly Bears contained 39% glucose, 35% sugar, 12% fruit juice, gelling agent (edible gelatin INS 428), acidity regulator INS 330, INS 331(iii), certified artificial colors INS 102, INS 110, INS 122, INS 124, INS 133, artificial flavoring agent and no preservatives. Zoo gummy contained 45% sucrose, 28% glucose syrup, 14% gelling agent (gelatin), 1.5% acidity

regulator (citric acid), artificial flavoring agent and artificial colors INS 102, INS 110, INS 122, INS 133. Dole's gummy jelly consisted of 60% mixed fruit puree, 18.16% water, 13.96% sugar, 1% malted rice syrup, natural orange flavor, 5% food additives, glycerin (INS 422), carrageenan (INS 407), locust bean gum (INS 410), citric acid (INS 330), natural orange flavor, potassium citrate (INS 332(ii)) and vitamin c (INS 300).

3.3 The appropriate amount of colorant powder from Chara corallina used in gummy jelly

The appropriate amount of colorant powder from *Chara corallina* used in Ma Muang Bao gummy jelly was examined with 3 different levels (0. 0.5 and 1.0), the results are shown in Table 4

Table 4 Sensory characteristics preference scores of Ma Muang Bao gummy jelly with different levels of colorant powder from *Chara corallina*

Sensory characteristics	Mean of hedonic score		
	0% colorant powder	0.5% colorant powder	1.0% colorant powder
Color	7.06 ± 0.48^{c}	8.37 ± 0.54^{a}	7.86 ± 0.32^{b}
Odor	7.09 ± 0.55^{b}	7.94 ± 0.71^{a}	7.20 ± 0.52^{b}
Taste	7.26 ± 0.44^{b}	8.46 ± 0.60^{a}	7.17 ± 0.51^{b}
Texture	7.31 ± 0.52^{b}	8.29 ± 0.45^{a}	7.23 ± 0.75^{b}
Overall acceptance	7.03 ± 0.17^{c}	8.46 ± 0.50^{a}	7.29 ± 0.51^{b}

Remark: -The different letters within the same column indicate significant differences between treatments ($p<.05$) according to Duncan's New Multiple Range Test (DMRT).

Table 4 indicates that hedonic scores for color, odor, taste, texture and overall acceptance of Ma Muang Bao gummy jelly supplement with 0.5% colorant powder from *Chara corallina* were significantly higher than Ma Muang Bao gummy jelly supplement with 0 and 1% colorant powder from *Chara corallina* ($p<.05$). Therefore, the appropriate amount of colorant powder (or chlorophyll powder) from *Chara corallina* added to Ma Muang Bao gummy jelly product was 0.5%. For the application of chlorophyll in food, natural colorants from chlorophyll can be used as food additives and have potential health benefits (Minh et al., 2019). The study of Jayasinghe et al. (2016) investigated the extraction of chlorophyll and carotenoids from *Ulva lactuca*) and *Sargussum wighttiu* with acetone, methanol and water, while picobiliprotein was obtained from *Gracilaria verrucosa* ground with ice-cold potassium phosphate buffer. Then it was applied in jelly-type desserts and evaluated for its microbiological, sensory and stability qualities. The results of the study showed that yield of chlorophyll, carotenoids and crude phycoerythin were 45%, 31% and 33%, respectively. These pigments had a shelf life of more than six months in 5% citric acid at room temperature. While jelly candies retained their natural color for more than 30 days at room temperature, with a color loss of 30%, the same amount as with synthetic colors. Agar jelly prepared with natural food colorings provided significantly higher calcium (120 mg/L) and potassium (550 mg/L) concentrations compared to jellies prepared using synthetic colorings. In addition, jellies using natural colors also contain high amounts of protein (10.2-12.0%), carbohydrate (10.8-12.3%), and fat (1.16-1.93%). These natural colors are highly nutritious, indicating their potential as dietary supplements.

3.4 Quality of Ma Muang Bao gummy jelly supplemented with colorant powder from Chara corallina

The Quality of Ma Muang Bao gummy jelly supplemented with colorant powder from *Chara corallina* was evaluated, the results are shown in Table 5.

Table 5 Physicochemical quality and microbiological quality of Ma Muang Bao gummy jelly supplemented with colorant powder from *Chara corallina*

Quality	**Amount**
Physicochemical quality	
Moisture (%)	41.74 ± 0.33
Ash (%)	2.35 ± 0.17
Protein (%)	11.98 ± 0.47
Fat (%)	0.02 ± 0.00
Fibre (%)	0.05 ± 0.00
Carbohydrate (%)	43.90 ± 0.10
Energy (Kcal)	223.74 ± 2.29
Total tritratable acids (%)	0.14 ± 0.00
Water activity (a_w)	0.64 ± 0.02
Color value	
L*	46.14 ± 0.02
a*	-0.82 ± 0.01
b*	22.71 ± 0.02
Microbiological quality	
Total viable count (CFU/g)	< 25
Yeast and mold (CFU/g)	< 10

For the analysis of physicochemical quality of Ma Muang Bao gummy jelly supplemented with colorant powder from *Chara corallina*, it was found that moisture, ash, protein, fat, fiber, carbohydrate and energy contents were 41.74%, 2.35%, 11.98%, 0.025%, 0.054%, 43.90% and 223.74 Kcal, respectively. The total acid content was 0.14% and the water activity (a_w) was 0.64. While the lightness (L*), greenness (-a*) and yellowness (b*) were 46.14, -0.82 and 22.71, respectively. Microbiological quality showed total viable counts less than 25 CFU/g, while yeast and mold counts were less than 10 CFU/g. Karapairaw (2018) studied the physical, chemical and nutritional properties of

the developed formula of Torch ginger gummy jelly compared with the basic formula, it was found that basic formula of Torch ginger gummy jelly contains moisture, ash, protein, fat, fiber, carbohydrates and total sugars of 13.01, 7.36, 7.47, 0.02, not found, 75.56, and 74.96 percent, respectively. While total energy was 2934.33 cal/gram, water activity value (a_w) value was 0.76. Lightness (L*) value, redness (a*) value and yellowness (b*) value were 31.09, 11.59 and 9.39, respectively. The developed formula of Torch ginger gummy jelly contained 18.28% moisture, 1.62% ash, 9.26% protein, 0.02% fat, not found fiber, 65.87% carbohydrate and 65.78% total sugar, respectively.

4. Conclusions

Application of stabilized chlorophyll from *Chara corallina* as natural colorant in food product was investigated. Ma Muang Bao gummy jelly product was used for this application. The experimental results demonstrated the potential of natural colorant powder from *Chara corallina* for application in food products. Then this natural colorant will increase food safety for consumers.

5. Acknowledgements

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Efficacy of Probiotic Yeasts in Controlling Vibrio parahaemolyticus and Vibrio harveyi in Whiteleg Shrimp (Litopenaeus Vannamei)

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Abstract

Vibrio parahaemolyticus and *V. harveyi*, are the causative agent of disease are causative agents of disease in shrimp aquaculture. In this work, we isolated yeasts from intestine of wild shrimp possessing inhibitory activity against *V. parahaemolyticus* and *V. harveyi*. Sixteen of 48 yeast isolates inhibited *V. parahaemolyticus* and *V. harveyi*. YP8, YP12, YP15, YP16, YP18, YP20, YP24, YP26, YP30 and YP32 were ten yeast isolates with high inhibitory activity with inhibition zones ranging from 9.00 ± 0.07 to 21.80 ± 0.14 mm. The toxicity test of nine yeast isolates (excluding YP32) revealed no harmful effects on whiteleg shrimp (*Litopenaeus vannamei*) after 120 h. In addition, five isolates (YP26, YP24, YP20, Y18 and Y8) showed high survival rates in the shrimp intestine. Polymerase chain reaction targeting the internal transcribed spacer region was used to identify yeast species, which was validated by sequencing. *Saccharomyces cerevisiae*, *Candida tropicalis*, *Wickerhamomyces anomalus*, *Yarrowia lipolytica* and *Candida glabrata* were shown to have 99-100 % identity to GenBank sequences. These findings indicate that *Y. lipolytica*, *S. cerevisiae* and *W. anomalus* are suitable for use as probiotics to control *V. parahaemolyticus* and *V. harveyi* in shrimp aquaculture due to their strong inhibitory activity, safety to shrimp, and ability to survive in the shrimp intestine.

Keywords: Yeast, Inhibitory Activity, Vibrio Parahaemolyticus, Vibrio Harveyi, Whiteleg Shrimp

1. Introduction

Shrimp aquaculture requires the integration of biotechnology and microbiology into shrimp aquaculture requires integrating biotechnology and microbiology for improved quality and quantity of shrimp products is a crucial step toward obtaining improved quality and quantity of shrimp products, such as the inclusion of various substances to feed formulations to improve shrimp growth (Sarkar and Bhaskara Rao, 2016). In addition, shrimp diseases incidence can be reduced by managing water, soil, stock, nutrition, and the environment (Ringø et al., 2014). Bacterial infections are caused by a variety of parameters in shrimp aquaculture (Lazado et al., 2015). Both *Vibrio parahaemolyticus* and *Vibrio harveyi* are gram negative bacteria in the genus Vibrio, family Vibrionaceae and order Vibrionales. *V. parahaemolyticus* is indeed one of the significant pathogenic agents that pose a threat to the viability of the aquaculture industry, particularly shrimp farming (Pui et al., 2014). *V. harveyi* is a bacterium that has been associated with diseases in a wide range of warm water fish and invertebrates, with particular significance in shrimp culture (Zhang et al., 2020). The antimicrobial drugs and disinfectants have had limited success in preventing disease. Antibiotic overuse contributes greatly to the creation and spread of antibiotic-resistant bacteria (Seethalakshmi et al., 2021). Moreover, the accumulation of antibiotics in shrimp products is a significant concern, especially in the context of international markets (Kumar et al., 2016). The use of probiotics in shrimp culture has gained popularity for several reasons, including their environmental friendliness and contribution to sustainable aquaculture practices. Probiotics have the ability to improve water quality, host nutrition, reduce disease incidence, increase host survival, and enhance immune response in aquaculture (Bhassu et al., 2024). Many yeasts are capable of producing bioactive compounds like as glucans, nucleotides, polysaccharides, carotenoid pigments, lipids, proteins, vitamins (Ceseña et al., 2021), amino acids, polyamine, astaxanthin, protease and killer toxin (Mirzaei et al., 2021). The β-glucans and mannoproteins of yeast have potential role in the immunity of the host (Navarrete and Tovar-Ramírez, 2014). Their ability to bind to the intestinal mucosa. Additionally, the absence of demonstrated yeast mediated transmission of antibiotic resistance (Nayak, 2011).

This study focused on isolating yeasts from natural shrimp, evaluating their inhibitory effects against *Vibrio parahaemolyticus* and *Vibrio harveyi*, assessing their toxicity to shrimp, determining their survival in the shrimp intestine, and identifying the yeast strains.

2. Methodology

2.1 Sample collection and yeasts isolation

Whiteleg shrimp were collected from Sikao markets in Sikao district, Trang province, Thailand. The shrimp intestines were cultured in Sabouraud dextrose broth (SDB) containing antibiotic (gentamicin) and incubated at 30 ºC for 48 h. The streaked Sabouraud dextrose agar (SDA) plates containing gentamicin were incubated at 30 ºC for 48 h. After incubation, different yeast colonies were chosen from SDA. The selected yeast colonies were re-streaked on SDA to obtain pure yeast isolate.

2.2 Investigation of inhibitory activity of yeast against V. parahaemolyticus and V. harveyi using modified agar well diffusion technique

The selected yeasts were streaked on SDA and incubated at 30 ºC for 48 h. The agar pieces were made from SDA using a sterile pasteur pipette. The selected yeasts were pierced onto the agar piece and incubated at 30 ºC for 48 h. *V. parahaemolyticus* and *V. harveyi* were cultured in Mueller Hinton broth (MHB) with shaking at 150 rpm at 30 ºC for 3 h. After adjusting the bacterial suspension to 0.5×10^8 CFU/ml, it was distributed on Mueller Hinton agar (MHA). The pit agar of bacterial lawn was then taken out, yeast seeded agar pieces were put into the pits of the bacterial lawn and incubated at 30 ºC for 24 h. The diameter of the inhibition zones was measure. The experiment was carried out in triplicate.

2.3 Evaluation of yeast toxicity to shrimp

Whiteleg shrimp with an average weight of 5 g were collected from a shrimp farm in Sikao District, Trang Province, Thailand. The shrimp were transferred to experimental tanks and acclimated for five days. Each tank measured 30 × 40 × 30 cm and contained 15 shrimp. The selected yeasts were cultured in SDB with shaking at 150 rpm at 30 ºC for 48 h. The cell pellet of yeast was collected by centrifugation at 8,000 rpm for 5 min and suspended in phosphate-buffered saline (PBS). The suspension was adjusted to amounts of 10^8 CFU/ml and then 20 µl of the suspension was injected into each shrimp (n=15/isolate). Shrimp mortality was recorded daily for five days to determine survival rates. The experiment was conducted in duplicate.

2.4 Investigation of the survival of yeast in the shrimp intestine

Whiteleg shrimp with an average weight of 5 g were transferred to shrimp tanks (30 × 60 × 30 cm) at a density of 30 shrimp/tank. Selected yeast isolates were cultured in SDB with shaking at 150 rpm at 30 °C for 48 h. After centrifugation at 8,000 rpm for 5 min, the yeast cell pellets were collected and suspended in PBS. The suspension was then mixed with shrimp feed and adjusted to 10^8 CFU/g of feed, followed by coating the mixture with fish oil.

All shrimp were fed three times a day (07:00, 14:00, and 19:00) with commercial shrimp feed at 8% of body weight/day. The water temperature in the shrimp tanks was maintained at 30 °C. Dissolved oxygen levels were controlled through continuous aeration. To maintain water quality, 50% of the water in each tank was replaced daily with fresh seawater. Remained feed and feces were removed daily through siphon. The feeding experiment was conducted in duplicate. The number of yeast cells in the shrimp intestine was determined every 24 h for 10 days using the spread plate technique on SDA.

2.5 Identification of yeast

Yeast genomic DNA was extracted to identify the selected yeasts. The species identification was performed by PCR targeting the internal transcribed spacer (ITS) region using primer ITS1F (5'TCCGTAGGTGAACCTGCGG3') and ITS4R (5'TCCTCCGCTTATTGATATGC3') (Cárdenas et al., 2012). The ITS region was amplified in 20 µl reaction volumes comprising 2 µl of extracted DNA, 4 µl of 5x buffer, 10.3 µl of milliQ water, 1.6 µl of 2.5 mM dNTP mixture, 1 µl of 10 µM of each primer and 0.1 µl of 5 U/µl *GoTaq* DNA polymerase. For the cycling conditions, the initial denaturation was done at 95 ºC for 5 min, followed by 35 cycles of 94 ºC for 1 min, 55 ºC for 2 min, 72 ºC for 2 min and a final extension at 72 ºC for 10 min. The PCR product was observed after 60 min of electrophoresis in 1% agarose gel. The PCR products were purified and sequenced. The obtained sequences were compared with the GenBank database using nucleotide blast.

2.6 *Statistical analysis*

Data were analyzed using one-way ANOVA, and results were reported as replicate means and standard deviations, with comparisons made using Duncan's multiple range test. A p-value of less than 0.05 was considered statistically significant

3. Results and Discussion

3.1 Yeast isolation by modified agar well diffusion technique

From the intestine of whiteleg shrimp, 48 yeast isolates were isolated. 16 of the 48 yeasts isolated showed inhibitory activity toward *V. parahaemolyticus* with inhibition zones ranging from 6.90 ± 0.35 and 13.80 ± 0.92 mm in diameter. While the inhibitory activity against *V. harveyi* with inhibition zones that ranged between 6.83 ± 0.21 and 21.80 ± 0.14 mm in diameter (Tables 1 and 2). Interestingly, ten out of 16 yeast isolates designated as YP8, YP12, YP15, YP16, YP18, YP20, YP24, YP26, YP30 and YP32 displayed high inhibitory activity against *V. parahaemolyticus* and *V. harveyi* with inhibition zones that ranged between 9.00 ± 0.07 and 21.80 ± 0.14 mm in diameter. In particular, YP26 and YP24 isolates exhibited high inhibitory activity against *V. harveyi* which was higher than *V. parahaemolyticus*. On SDA, most yeast isolates against *V. parahaemolyticus* and *V. harveyi* formed butyrous, cream-colored colonies (Figures 1a and 1b). Under the microscope, ellipsoidal and ovoid cells were observed. (Figures 1c and 1d).

Table 1 Isolation of yeasts against *V. parahaemolyticus* and *V. harveyi.*

Sources	**No. yeast isolates**	**No. isolates active against *V. parahaemolyticus***	**No. isolates active against *V. harveyi***
Sikao market 1	15	5	5
Sikao market 2	12	4	4
Sikao market 3	21	7	7
Total	48	16	16

Table 2 The inhibitory activity of 16 yeast isolates toward *V. parahaemolyticus* and *V. harveyi*.

Yeast isolates	Inhibitory activity of yeasts (mm)	
	V. parahaemolyticus	***V. harveyi***
YP4	7.23 ± 0.85^{e}	7.30 ± 0.99^{h}
YP7	6.90 ± 0.35^{e}	7.10 ± 0.07^{h}
YP8	10.20 ± 0.71^{bc}	10.50 ± 0.71^{de}
YP10	7.03 ± 0.14^{e}	7.33 ± 0.49^{h}
YP12	9.00 ± 0.07^{cd}	10.10 ± 0.28^{ef}
YP14	7.33 ± 0.21^{e}	7.20 ± 0.07^{h}
YP15	9.10 ± 0.28^{cd}	9.53 ± 0.42^{gh}
YP16	10.50 ± 0.35^{b}	10.40 ± 0.35^{e}
YP18	11.10 ± 0.71^{b}	9.23 ± 0.14^{g}
YP20	12.53 ± 0.64^{a}	14.50 ± 0.49^{c}
YP22	7.00 ± 0.14^{e}	6.83 ± 0.21^{h}
YP24	12.93 ± 0.14^{a}	18.10 ± 0.35^{b}
YP26	13.80 ± 0.92^{a}	21.80 ± 0.14^{a}
YP30	10.03 ± 0.78^{bc}	11.23 ± 0.64^{d}
YP32	11.01 ± 0.42^{b}	14.60 ± 0.00^{c}
YP34	7.80 ± 0.28^{de}	7.10 ± 0.07^{h}

Note: Values are presented as means ± SD, and different letters (a, b, c, d, e, f, g, h) indicate significant differences ($p < .05$).

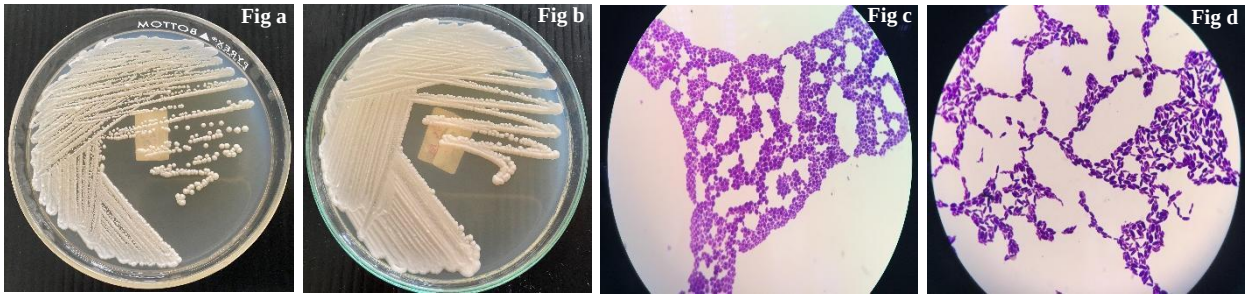

Figure 1 Colonies of yeast on SDA plates (a, b) and yeast cells observed under the microscope at 100× magnification using light microscopy (c, d).

3.2 *Investigation of the toxicity of yeast on shrimp*

According to this study, isolates YP8, YP12, YP15, YP16, YP18, YP20, YP24, YP26, and YP30 did not affect shrimp after 120 h. However, shrimp treated with isolate YP32 showed a survival rate of 93.33% after 120 h (Table 3).

Table 3 The toxicity of 10 yeast isolates on shrimp.

Yeast isolates	Survival rate of shrimp (%) (n = 15)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
YP8	100^{a}	100^{a}	100^{a}	100	100^{a}
YP12	100^{a}	100^{a}	100^{a}	100	96.7 ± 4.7^{a}
YP15	100^{a}	100^{a}	100^{a}	96.7 ± 4.7^{a}	96.7 ± 4.7^{a}
YP16	100^{a}	100^{a}	100^{a}	96.7 ± 4.7^{a}	96.7 ± 4.7^{a}
YP18	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
YP20	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
YP24	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
YP26	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
YP30	100^{a}	100^{a}	100^{a}	100^{a}	96.7 ± 4.7^{a}
YP32	100^{a}	100^{a}	100^{a}	96.7 ± 4.7^{ab}	93.3 ± 0^{b}

Note: Values are presented as means ± SD, and different letters (a, b) indicate significant differences ($p < .05$).

3.3 The survival of yeast in the shrimp intestine

The numbers of yeast in shrimp intestine, yeast isolates YP26, YP24, YP20, YP18 and YP8 were 6.2 × 10, 4. 9 × 10, 1.1 × 10, 0.6 × 10 and 0.3 × 10 CFU/ml after 1 day, respectively. After 10 days, the numbers increase to 2.9 × 10^4, 2.1 × 10^4, 1.8 × 10^4, 6.8 × 10^3 and 3.4 × 10^3 CFU/ml, respectively. In contrast, yeast isolates YP12, YP15 and YP30 were not detected after 1 days, while YP16 and YP32 were not detected after 3 days (Figure 2).

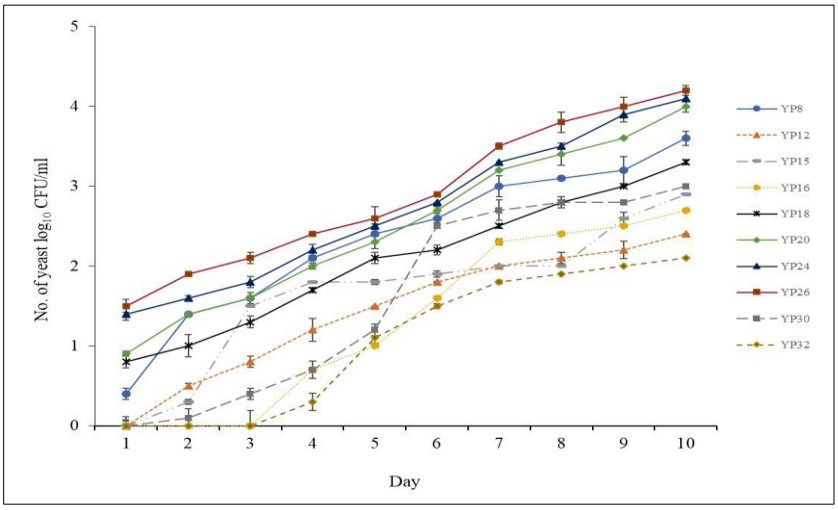

Figure 2 The survival of yeast in the shrimp intestine

3.4 Yeast identification

Saccharomyces cerevisiae, *Candida tropicalis*, *Wickerhamomyces anomalus*, *Yarrowia lipolytica*, and *Candida glabrata* were the four yeast genera and five species identified with 99-100 % identity to GenBank sequences using PCR targeting the ITS region (Table 4)

Table 4 Identification of 10 yeast isolates.

Yeast isolates	**ITS region**	**% Identity**
YP8	*Saccharomyces cerevisiae*	99
YP12	*Candida tropicalis*	99
YP15	*Candida tropicalis*	99
YP16	*Candida tropicalis*	99
YP18	*Wickerhamomyces anomalus*	99
YP20	*Saccharomyces cerevisiae*	99
YP24	*Saccharomyces cerevisiae*	100
YP26	*Yarrowia lipolytica*	99
YP30	*Wickerhamomyces anomalus*	100
YP32	*Candida glabrata*	100

The four yeast isolates identified, *Candida tropicalis* (YP12, YP15, YP16) and *Candida glabrata* (YP32) have been reported as human pathogens. They are the two most common pathogens responsible for candidemia (Yang et al., 2022). Hematological disease patients with *C. tropicalis* bloodstream infections had a high mortality rate. (Yang et al., 2023). It is rare for *C. glabrata* to cause significant illness in people. Nevertheless, *C. glabrata* is a pathogenic organism that colonizes epithelial surfaces including the mouth, gastrointestinal system, vagina, skin, and present in stool (Hassan et al., 2021).

The five identified yeast isolates, *Y. lipolytica* (YP26), *S. cerevisiae* (YP8, YP20, YP24) and *W. anomalus* (YP18) are of particular interest because they exhibited high inhibitory activity against *V. parahaemolyticus* and *V. harveyi*, caused no harm to shrimp, and were able to survive in the shrimp intestine. *Y. lipolytica* has a high protein content and an entire amino acid profile. It has been demonstrated to have a probiotic effect in addition to containing a high-quality single cell protein (Worland et al., 2020). *Y. lipolytica* naturally secretes a number of extracellular metabolites, such as lipases and esterases (Fabiszewska et al., 2024). This yeast can activate non-specific immunological parameters as well as the antioxidant immune mechanism in Pacific red snapper leukocytes from the head, kidney, and spleen, making it a potential immunostimulant (Alamillo et al., 2017). *Y. lipolytica* has been use to enhance the survival and growth of the pearl oyster (*Pinctada mazatlanica*) (Aguilar et al., 2010), as well as to stimulates non-specific immunity in Pacific red snapper (*Lutjanus peru*) (Alamillo et al., 2017). Likewise, the cell wall of *S. cerevisiae* can synthesize the essential substance β-glucan, which stimulates non-specific immunity (Lei et al., 2021). Ayiku et al. (2020) reported the potential of probiotic feed derived from *S. cerevisiae* as an immunostimulant for shrimp infected with *V. harveyi*. Moreover, the use of *W. anomalus* in the diets of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) has been shown to improve growth performance (Agboola et al., 2021). Many yeasts can produce bioactive substances (Mirzaei et al., 2021), including β-glucans and mannoproteins (Navarrete and Tovar-Ramírez, 2014). Yeasts are used as alternative feed ingredients in aquaculture because of their nutritional value (Ceseña et al., 2021). They can be incorporated into aquaculture diets and are generally obtained through biotechnological processes or as by-products of the agri-food industry (Aguirre-Guzman et al., 2023).

4. Conclusions

In this work, *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* exhibited high inhibitory activity against *Vibrio parahaemolyticus* and *V. harveyi*. Additionally, these yeasts were non-toxic and able to survive in the shrimp intestine, demonstrating their potential effectiveness in shrimp aquaculture.

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Session 3
Science and Engineering Technology

The Cytotoxicity and Alkaline Phosphatase Activity of Water-Soluble Extract from the Abalone Nacre on the Preosteoblast

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Abstract

Nacre, a calcium carbonate-based biomaterial, has traditional pharmaceutical applications in bone regeneration. This study explored the osteoinductive potential of nacre from abalone Haliotis diversicolor, specifically its water-soluble extract (HD-WSM), on MC3T3-E1 mouse osteoblasts. HD-WSM was extracted and tested for cytotoxicity using the AlamarBlue Assay. Results indicated HD-WSM was non-cytotoxic within a 50-250 µg/ml concentration range, with cell viability remaining consistent with controls after 48 hours. A slight increase in cell proliferation was observed at 150-250 µg/ml after six days, leading to the selection of 100 and 200 µg/ml for further analysis. Alkaline phosphatase (ALP) activity, a marker of osteoblast differentiation, was measured using an ALP Assay Kit. HD-WSM significantly enhanced ALP activity in a dose-dependent manner across 50-250 µg/ml concentrations, starting from day three. The untreated cells also showed increasing ALP levels, reflecting natural osteoblast proliferation. The results demonstrate that HD-WSM promotes both cell proliferation and ALP activity in MC3T3-E1 cells, suggesting its potential as an osteoinductive material.

Keywords: Cytotoxicity, Alkaline Phosphatase, Nacre, Preosteoblast

1. Introduction

Nacre, or mother of pearl, is a biological ceramic. It consists of calcium carbonate platelets embedded in an organic matrix with a highly controlled structural organization. Mollusk nacre is a biocompatible, biodegradable, and osteoinductive biomaterial. Chips, powder, and extracts from oyster and mussel nacre demonstrate strong evidence of osteoblast stimulation and bone mineralization both in vitro and in vivo (Green et al., 2014). Studies have demonstrated that nacre attracts and activates bone marrow stem cells and osteoblasts (Green et al., 2014, 2015). In vivo studies show that mollusk nacre from oysters and mussels induces osteogenesis and bone mineralization in ectopic bone environments. In bone graft tests, nacre fragments or powder can be grafted into the bones of sheep (Rousseau et al., 2012), guinea pigs (Asvanund and Chunhabundit, 2012), rats (Liao et al., 2002), and rabbits (Lamghari et al., 2001). In patients with bone defects, fresh woven bone fused with the nacre implant, facilitated by osteoclasts and osteoblasts (Green et al., 2014, 2015). Nacre implantations stimulated osteogenesis and integration of the nacre into the host bone without fibrous tissue interposition or a significant inflammatory reaction (Asvanund and Chunhabundit, 2012; Lamghari et al., 2001; Liao et al., 2002; Rousseau et al., 2012). While nacre is tolerated in vivo, its slow degradation and resorption may hinder its use in calcified tissues requiring rapid self-renewal (Lamghari et al., 2001). Although the nacre organic matrix constitutes only 1-5% w/w of the mollusk shell, it plays a critical role in shell formation. This includes the synthesis of transient amorphous minerals, their crystallization, the selection of calcium carbonate polymorphs (calcite or aragonite), and the organization of crystallites into complex shell textures (Marin et al., 2008). The nacre organic matrix can be separated into water-soluble (WSM) and water-insoluble (WISM) fractions. WISM biopolymers determine the framework structure of the shell layer, while WSM modulates calcium ion deposition at nucleation sites (Marin et al., 2008; Espinosa et al., 2011). The nacre biogenic composite, particularly the WSM, has been considered a promising biomaterial for bone repair due to its osteogenic potential. Researchers suggest that WSM contains signaling molecules that can be released in a physiological medium and activate osteoblasts. Therefore, these signaling molecules may serve as homologous components between nacre and bone tissue (Lao et al., 2007; Rousseau et al., 2008). This project aimed to determine whether the water-soluble matrix of abalone *Haliotis diversicolor nacre* (HD-WSM) contains signal proteins that promote the stimulation and differentiation of MC3T3-E1 osteoblasts. Cytotoxicity and alkaline phosphatase activity were investigated.

2. Methodology

2.1 Extraction and purification of nacre water-soluble matrix

The HD-WSM was extracted from powdered nacre, by a non-decalcifying process. One hundred grams of the nacre powder was suspended in 200 ml of autoclaved deionized water for 24 hours at room temperature, with continuous stirring. The suspension was then centrifuged for 30 min at 4000 rpm. The supernatant was called the HD-WSM (Mouriès et al., 2002). The HD-WSM was purified and concentrated by Vivaspin sample concentrators (Amersham Pharmacia Biotech) prior to being run onto SDS-PAGE.

2.2 Cell culture

To maintain and propagate the cells, MC3T3-E1 Subclone 4 ATCC ® CRL-2593, was cultured in MEM-alpha supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, and 1% penicillin/streptomycin (Hyclone) in a humidified incubator at 37°C and 5% CO_2. The cells were subcultured every three days in the presence of 0.25% trypsin. MC3T3-E1 cells were plated out at 10^3 cells/well of 96 well plates, or 10^5 cells/well of 6 well plates

and after reaching the 70% confluency level, cells were treated with HD-WSM in various concentrations for 28 days. The medium was changed every third day.

2.3 Cell proliferation and viability assay

To estimate the effect of HD-WSM on proliferation and viability of MC3T3-E1 cell, the assay of Alamarblue® cell viability reagent (Thermo Scientific) was performed. Resazurin, the active ingredient of AlamarBlue® reagent, is a non-toxic, cell-permeable compound that is blue in color and virtually non-fluorescent. Upon entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding cells.

After completing the incubation, alamarBlue® reagent was added 10 µL to 100 µL sample, followed by 2 hours of incubation at 37ºC. The resulting fluorescence is read on a plate reader or spectrophotometer (Model 680 Microplate Reader; Bio-Rad, Hercules, CA, USA). The proliferation rate of the cells was calculated according to the following formula: (ODsample - ODblank) / (ODcontrol - ODblank).

2.4 Alkaline phosphatase activity

At the end of the treatment period, the cells were washed with PBS and lysed. Alkaline phosphatase (ALP) activity in the cell lysates was measured in 0.2 M bicarbonate buffer pH 10, 0.05% Triton X-100, 4 mM MgCl2 and 2 mM p-nitrophenolphosphate by incubating the lysates for 60 min at 25 °C, protected from light. The reactions were stopped by adding 1 M NaOH. The absorbance at 405 nm was measured. Activities were normalized to the cell protein content, as measured by the Bradford method (BioRad), using standard protein provided in the Alkaline Phosphatase Assay Kit (ab83369, Abcam).

$$ALP\ activity\ (U/mL) = (\ AV)/T$$

where A = amount of pNP generated in samples calculated from a standard curve (µmol)
V = volume of sample added in the assay well (mL)
T = reaction time (in minutes)

3. Results and Discussion

3.1 HD-WSM is non-cytotoxic to preosteoblasts MC3T3-E1

To determine the experimental doses of HD-WSM for evaluation of bone differentiation in MC3T3-E1, the cytotoxicity of HD-WSM was determined by AlamarBlue Assay (Thermo Fisher Scientific). The HD-WSM was added to culture media at the concentration of 0, 50, 100, 150 and 200 ug/ml for 24 and 48 hours. Initial findings indicated that HD-WSM, across the concentration range of 50 to 250 µg/ml, did not exhibit cytotoxic effects on MC3T3-E1 cells after 24 and 48 hours of treatment. Cell viability and proliferation rates remained comparable to those of untreated control cells, confirming its non-cytotoxic nature during acute exposure.

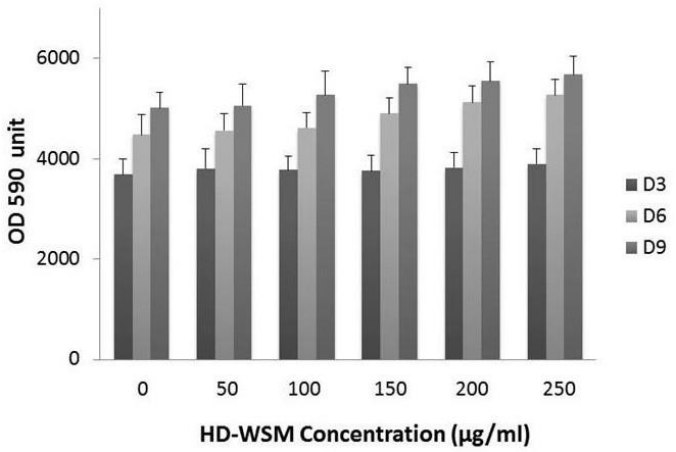

Figure 1 Cell viability assays of MC3T3-E1 cells. Effect of HD-WSM on cell viability percentage as measured by AlamarBlue® Cell Viability Reagent (Thermo Fisher Scientific) indicating non-toxic nature of WSM for MC3T3-E1 cells. The experiment was performed at concentration of 0, 50, 100, 150, 200 and 250 ug/ml for 3, 6 and 9 days.

Extending the observation period to 3, 6, and 9 days (Figure 1) further supported the sustained non-cytotoxic profile of HD-WSM. At Day 3 (D3), OD 590 unit values across all concentrations were largely similar, reinforcing the initial lack of cytotoxicity. By Day 6 (D6), concentrations of HD-WSM between 150 and 250 µg/ml demonstrated a weak, yet discernible, enhancement in cell proliferation compared to the control and lower concentrations. This subtle pro-proliferative effect appeared to persist and slightly strengthen at Day 9 (D9) for these higher concentrations, suggesting a potential long-term benefit on cell growth.

Based on these results, HD-WSM was confirmed to be non-cytotoxic to MC3T3-E1 preosteoblasts within the tested range. The observed weak enhancement in cell proliferation at higher concentrations (150-250 µg/ml) after extended exposure presents a promising indication for its potential osteogenic properties. Consequently, concentrations of 100 µg/ml and 200 µg/ml were judiciously selected for further bioactivity studies. This selection ensures that subsequent experiments are conducted within a safe, non-toxic range while encompassing concentrations that demonstrated a potential positive effect on cell proliferation, allowing for the exploration of dose-dependent responses on bone differentiation markers.

3.2 HD-WSM enhances alkaline phosphatase activity (ALP) in MC3T3-E1 cells.

To investigate the potential of HD-WSM to enhance early osteoblast differentiation, alkaline phosphatase (ALP) activity was evaluated in MC3T3-E1 preosteoblasts over 3, 6, and 9 days of treatment. ALP activity, an established early marker of osteoblast differentiation and in vitro bone formation, was quantified using an Alkaline Phosphatase Assay Kit (ab83369, Abcam).At the commencement of the experiment (D0), ALP activity was minimal across all groups. In the untreated control group (0 µg/ml), a natural increase in ALP activity was observed over time, rising from approximately 0.1 U/ml at D0 to 0.8 U/ml at D6, and further to 1.7 U/ml by D9, consistent with the intrinsic differentiation potential of proliferating MC3T3-E1 cells. Treatment with HD-WSM significantly enhanced ALP activity in a dose- and time-dependent manner (Figure 1). By Day 3 (D3), concentrations of HD-WSM as low as 50 µg/ml initiated a noticeable increase in ALP activity compared to the untreated control. This enhancement became markedly stronger at higher concentrations and longer incubation periods. For instance, at 200 µg/ml, ALP activity reached approximately 2.3 U/ml by D3, increasing substantially to 5.2 U/ml at D6, and peaking at over 7.3 U/ml by D9. Comparatively, at D9, ALP activity in the 200 µg/ml HD-WSM group was nearly 4.3 times higher than that of the untreated control. A similar dose-dependent trend was observed, with 150 µg/ml and 100 µg/ml also showing substantial increases in ALP activity compared to lower concentrations and the control group at each time point. Data are presented as average ± SD.

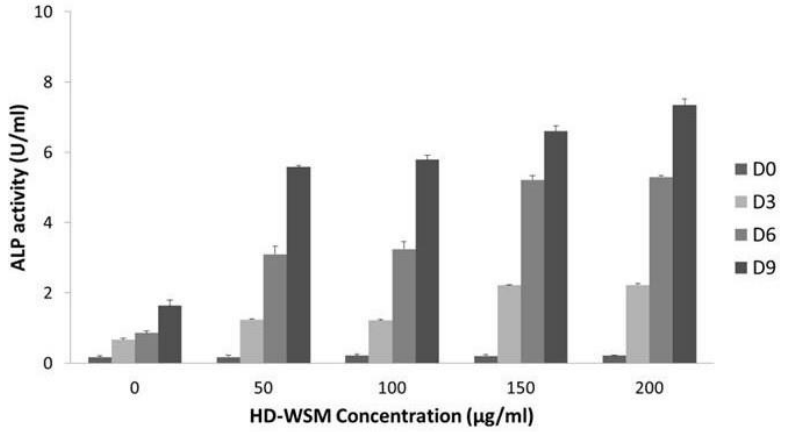

Figure 2 Alkaline phosphatase activity (ALP) of MC3T3-E1 cells. The experiment was performed at concentration of 0, 50, 100, 150, and 200 µg/ml for 3, 6 and 9 days. ALP activity was measured by Alkaline Phosphatase Assay Kit (Abcam) indicating HD-WSM enhances ALP in MC3T3-E1 cells.

This experiment aimed to assess the effect of HD-WSM on the early osteoblast differentiation marker, ALP activity, in MC3T3-E1 preosteoblasts. ALP activity is widely recognized as a crucial indicator of early osteoblast maturation, with its upregulation preceding matrix mineralization during in vitro bone formation. The observed dose- and time-dependent increase in ALP activity upon HD-WSM treatment strongly suggests that this extract positively influences early osteoblast differentiation. While the untreated control group exhibited an expected baseline increase in ALP activity due to the inherent proliferative and differentiative capacity of MC3T3-E1 cells, the dramatic elevations seen with HD-WSM treatment signify a distinct inductive effect. From as early as Day 3, HD-WSM concentrations starting from 50 µg/ml demonstrated a strong enhancing effect on ALP, which was amplified with increasing concentrations, reaching its highest level at 200 µg/ml by Day 9.

These findings are particularly significant when considered in conjunction with the preceding cytotoxicity study, which confirmed the non-cytotoxic nature of HD-WSM across the tested concentration range. This ensures that the observed increase in ALP activity is a genuine pro-osteogenic effect, rather than a consequence of cellular stress or altered cell viability. The sustained increase in ALP activity over 9 days further indicates that HD-WSM promotes not only the initiation but also the progression of early osteoblast differentiation.

4. Conclusions

Based on the comprehensive findings from both the cytotoxicity and alkaline phosphatase (ALP) activity experiments, a clear conclusion can be drawn regarding the initial characterization of Haliotis divericolor-water soluble extract (HD-WSM) on MC3T3-E1 preosteoblasts.

Firstly, the cytotoxicity assessment unequivocally demonstrated that HD-WSM is non-cytotoxic to MC3T3-

E1 cells across the entire concentration range tested (50-250 µg/ml) and over an extended period of up to 9 days. Cell viability and proliferation rates remained comparable to untreated controls, ensuring that any subsequent biological effects observed are genuine and not confounded by cellular distress or death.

Secondly, the investigation into ALP activity, a pivotal early marker of osteoblast differentiation, revealed that HD-WSM significantly enhances this enzymatic activity in a dose- and time-dependent manner. This strong upregulation of ALP, observed as early as Day 3 and escalating with increased concentration and duration of treatment, provides compelling evidence of HD-WSM's osteoinductive properties. The observed effects were substantial, exceeding the natural increase in ALP activity seen in proliferating osteoblasts.

In conclusion, the combined results from these two foundational experiments indicate that HD-WSM is a safe and promising agent that actively promotes early osteoblast differentiation. Its non-cytotoxic nature, coupled with its robust ability to enhance ALP activity, strongly positions HD-WSM as a compelling candidate for further research into its potential applications in bone regeneration and tissue engineering.

5. Acknowledgements

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Sitting Posture Detection and Prevention of Office Syndrome Using MediaPipe Technology

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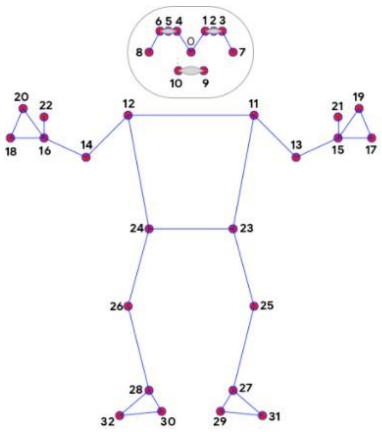
Abstract

With the increasing prevalence of office syndrome caused by prolonged sitting and poor posture, this paper proposes a real-time posture detection system aimed at improving ergonomic practices in the workplace. The system leverages the MediaPipe Framework, an advanced deep learning-based tool for human pose estimation, to monitor and assess upper body posture. This study aims to assess the accuracy and responsiveness of a MediaPipe-based system in detecting incorrect sitting postures associated with office syndrome. The proposed system continuously tracks posture throughout work hours, thereby reducing the likelihood of developing musculoskeletal pain. The research process began with the development of a posture detection algorithm that utilized MediaPipe's pose estimation model to calculate angular deviations based on landmarks for the shoulder and head. This was followed by the implementation of the system, real-time testing, and performance evaluation under simulated office conditions. Through an experiment with 10 volunteers, selected to represent a manageable and diverse group for initial validation. The system's accuracy was evaluated by comparing the calculated angles with the actual angles in three key positions: neutral, left-side tilt, and right-side tilt. The results showed that the system performed with high accuracy in the neutral position, with a Mean Absolute Error (MAE) close to 0%, but had a higher MAE of approximately 20% in the tilted positions. The system demonstrated an average processing time of 0.20 seconds per frame, which corresponds to approximately 5 frames per second, indicating its potential for real-time posture monitoring. This study contributes to the development of efficient workplace health technologies that promote better posture and reduce the risk of office syndrome.

Keywords: Posture Detection, Human Pose Estimation, MediaPipe Framework, Office Syndrome

1. Introduction

The rapid advancement of digital technologies in the workplace has greatly transformed the nature of office-based work. Tools for data storage, accounting, procurement, and other essential business functions are now predominantly computer-based, offering significant convenience and efficiency to office employees. However, this technological shift has also introduced unintended health consequences, particularly as employees increasingly spend over seven hours a day interacting with computers and other digital devices. While technology has enhanced productivity, it has inadvertently contributed to a rising concern: musculoskeletal pain among office workers.



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9. mouth_left
10. mouth_right
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12. right_shoulder
13. left_elbow
14. right_elbow
15. left_wrist
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20. right_index
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22. right_thumb
23. left_hip
24. right_hip
25. left_knee
26. right_knee
27. left_ankle
28. right_ankle
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30. right_heel
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32. right_foot_index

Figure 1 MediaPipe Pose Landmarks (Kim et al., 2023)

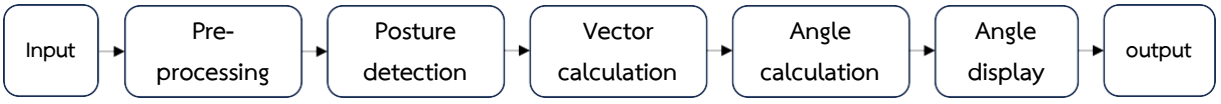

Figure 2 Posture Detection Process Flow

Recent studies have highlighted the widespread occurrence of musculoskeletal disorders among office employees, especially those engaged in sedentary work. Research by (Lazko et al., 2021) indicates that 44.2% of female office employees report experiencing wrist pain (radiocarpal), 40.4% suffer from neck pain, and 38.5% do not

report pain, suggesting a significant issue with workplace ergonomics. This pain is often attributed to factors such as prolonged sitting, incorrect posture, and insufficient physical activity throughout the workday. Musculoskeletal pain, resulting from poor ergonomic practices, is a key contributor to what is increasingly recognized as "office syndrome" a collection of conditions that affect the musculoskeletal and nervous systems due to long periods of poor posture and sedentary behavior.

The rise in the office syndrome has led to significant concerns not only about the well-being of employees but also about the long-term impact on productivity. The root causes of these disorders include prolonged sitting, lack of movement, poor ergonomics, and inadequate posture. A lack of proper ergonomic practices, such as sitting with poor posture or without appropriate breaks, can lead to pain in the wrist, neck, shoulders, and back, which, in turn, can escalate into more severe conditions like chronic pain or repetitive strain injuries (RSI). As such, addressing these issues has become a key challenge in improving workplace health and preventing long-term physical harm to employees.

Several technological solutions have been proposed to address this growing problem. (Paliyawan et al., 2014). introduced a novel approach for preventing office syndrome by detecting prolonged sitting using a model that utilizes Kinect cameras and data mining classification. The Kinect camera can monitor body posture and movements, alerting employees to the need to adjust their positions or take breaks. Similarly, (Srahongthong, et al.,2023) developed an application that uses augmented reality (AR) to provide personalized treatment programs for office syndrome. The AR system uses a user questionnaire to propose therapy programs tailored to individual needs, such as specific exercises or stretches to alleviate pain and prevent further injury.

In another approach, (Amin et al., 2024) examined the effectiveness of an app-based neck exercise program grounded in the McKenzie protocol, which was shown to reduce pain intensity by 46%. This program offers six neck movements designed to help relieve tension and reduce discomfort. Likewise, (Lee et al., 2017) proposed a series of stretching exercises aimed at reducing neck and shoulder pain, demonstrating the positive impact of such routines when practiced consistently over four weeks.

Additionally, (Kim et al., 2023) leveraged deep learning models such as MediaPipe to optimize human pose detection for applications that monitor the movements of individuals, providing real-time alerts in cases of falls or injuries at home. MediaPipe, a tool developed for accurate pose tracking, uses pre-trained deep-learning models to monitor and analyze human movements, providing valuable insights for both injury detection and prevention.

Despite these promising advancements, most solutions still focus on postural correction in specific instances, rather than providing a comprehensive, real-time solution that proactively helps employees avoid developing musculoskeletal pain in the first place. There is a growing need for an integrated system that combines the principles of ergonomics with real-time feedback to detect and correct sitting posture before it leads to discomfort or injury.

This research aims to fill that gap by developing and testing a real-time sitting posture detection system based on MediaPipe, an advanced deep learning-based tool that specializes in human pose detection. The proposed system will provide continuous feedback to office workers, allowing them to adjust their posture during work hours and reduce the likelihood of developing office syndrome. By incorporating ergonomic principles into the detection and analysis of sitting posture, this system will help promote healthier work habits and minimize the risk of musculoskeletal pain. In doing so, this research contributes to the growing body of work in workplace health technology, offering a proactive approach to managing and preventing office syndrome.

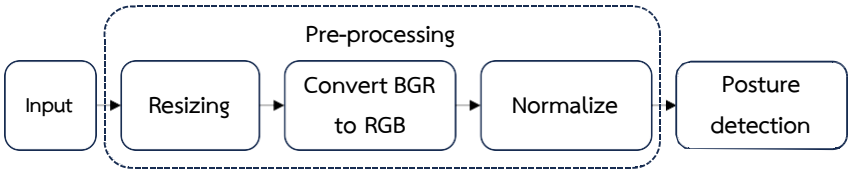

Figure 3 Pre-processing Flow

Furthermore, this paper will explore the theoretical underpinnings of ergonomic design in office work, the specific role of posture in preventing musculoskeletal disorders, and the potential benefits of integrating deep learning technologies like MediaPipe into real-time posture detection systems. The study will also evaluate the effectiveness of such a system in a workplace setting, examining both the technological feasibility and the potential for widespread adoption to improve employee health and productivity.

2. Methodology

2.1 System Design

The proposed system utilizes the MediaPipe Framework, an open-source solution developed by Google, to detect and analyze upper-body posture in real time. MediaPipe supports detection of 33 key landmarks across the human body, which are used for posture classification as illustrated in Figure 1. The system is designed to operate efficiently on standard computing devices, ensuring responsive performance during prolonged work sessions. The overall process follows four key stages: Pre-processing, Posture Detection, Vector Calculation, and Angle Calculation, as shown in Figure 2.

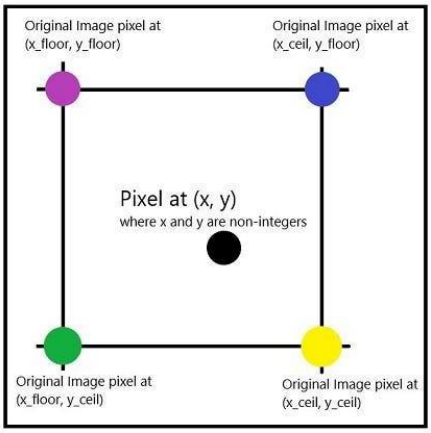

Figure 4 Linear Interpolation [7]

2.2 Pre-processing

The input image is first resized to 640x480 pixels to optimize processing speed without significant loss in quality. The resizing is achieved using the Bilinear interpolation method, which balances the preservation of image quality with the need for improved processing efficiency. Bilinear interpolation works by considering four neighboring pixels surrounding the target point. These four pixels, typically arranged in a rectangle, are selected based on their proximity to the target point. The algorithm then performs linear interpolation first along one axis (typically the x-axis) and then along the second axis (typically the y-axis). This approach ensures that the resizing process is computationally efficient while maintaining an acceptable level of image detail, thereby allowing for smooth and timely ergonomic tracking according to Figure 3 and 4. Then, the color format is converted from BGR (default in OpenCV) to RGB, which is compatible with MediaPipe. Lastly, pixel values are normalized to the [0, 1] range by dividing each by 255. This standardization ensures consistent input for pose estimation.

2.3 Posture Detection

At this stage, the MediaPipe framework is utilized to identify specific anatomical landmarks crucial for upper-body posture analysis, including the nose (landmark 0), left shoulder (landmark 11), and right shoulder (landmark 12). These landmarks are tracked in three-dimensional space (x, y, z), enabling the system to monitor head and shoulder alignment in real-time and provide reliable spatial data for subsequent angle and vector calculations.

Figure 5 Pose Estimation and Angle Calculation

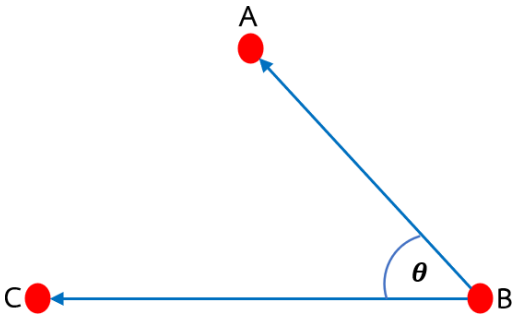

Figure 6 Corresponding Vector

MediaPipe's pose detection process operates through two key stages: person detection and landmark estimation. Initially, a lightweight bounding box detector identifies the presence and position of a person within the image frame. Once the individual is localized, a pose landmark model estimates the 3D coordinates of 33 key points across the body, including the head, shoulders, elbows, wrists, hips, knees, and ankles. These coordinates are continuously updated for each frame and normalized for image dimensions to ensure consistency.

Figure 7 Actual Angle Measurement

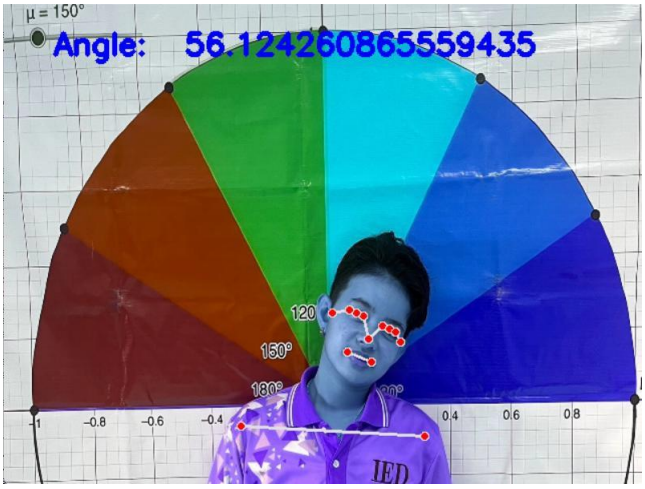

Figure 8 Angle Tracking Result

2.4 Vector and Angle Calculation

To determine head posture, vectors are computed between key landmarks as illustrated in Figure 5 and 6. Specifically, point A represents the nose (landmark 0), while points B and C represent the left shoulder (landmark 11) and right shoulder (landmark 12), respectively. Two vectors are constructed: vector AB from the left shoulder to the nose, and vector BC from the left shoulder to the right shoulder. The angle between these vectors is calculated using the dot product formula, which provides a geometric measure of the orientation between the two vectors following to equation (1) to (5) respectively.

$$Dot\ product = AB_x \cdot BC_x + AB_y \cdot BC_y + AB_Z \cdot BC_z \quad (1)$$

$$|AB| = \sqrt{AB_x^2 + AB_y^2 + AB_z^2} \quad (2)$$

$$|BC| = \sqrt{BC_x^2 + BC_y^2 + BC_z^2} \quad (3)$$

$$cos\ \theta = \frac{dot\ product}{|AB| \cdot |BC|} \quad (4)$$

$$\theta = cos^{-1}(cos\ \theta) \quad (5)$$

To ensure that the calculated angles are consistent and independent of the image size or resolution, normalization is applied. Each coordinate (x, y) from the pose landmarks is divided by the corresponding width and height of the input image, respectively. This results in dimensionless values in the range [0, 1], which allows the angle computation to be invariant to scale. This is particularly important for real-time posture detection across different camera setups and environments.

2.5 Posture Classification Thresholds

Head posture classification is based on the angular deviation between the vector from the left shoulder to the nose (vector AB) and the vector from the left shoulder to the right shoulder (vector BC), as computed in the previous section. The resulting angle serves as a geometric indicator of head tilt direction.

To interpret these angles, thresholds were empirically defined through experimental calibration. A neutral

posture is identified when the measured angle remains within ±10° of a reference baseline, typically centered 90° in the vector configuration. If the angle decreases significantly (less than 70°), the posture is classified as a left- side tilt, indicating the nose is closer to the left shoulder. Conversely, an angle greater than 110° indicates a right-side tilt, where the nose is positioned closer to the right shoulder. These threshold values were validated using manual protractor measurements during the experiment and aligned with ergonomic literature, which identifies 15°–20° of lateral tilt as a common boundary for detecting non-neutral posture (Chapman et al., 2021; Mingels et al., 2016). This classification system allows the posture detection algorithm to reliably differentiate between normal and incorrect sitting positions associated with office syndrome risk.

3. Results and Discussion

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^{n} |\theta_i^{measured} - \theta_i^{actual}| \quad (6)$$

Where $\theta_i^{measured}$ = the angle detected by the system
θ_i^{actual} = the manually measured angle
n = the number of observations

For the evaluation of the proposed system, an experimental setup was established in a controlled environment with a lighting level of approximately 300 LUX to simulate typical office conditions. The experiment involved 10 volunteers, comprising 6 females and 4 males, to assess the system's effectiveness in real-world scenarios. The volunteers were asked to perform standard office tasks while their posture was continuously monitored by the system. The data collected from these tests were used to evaluate the accuracy, responsiveness, and usability of the posture detection system, as well as its impact on promoting ergonomic practices.

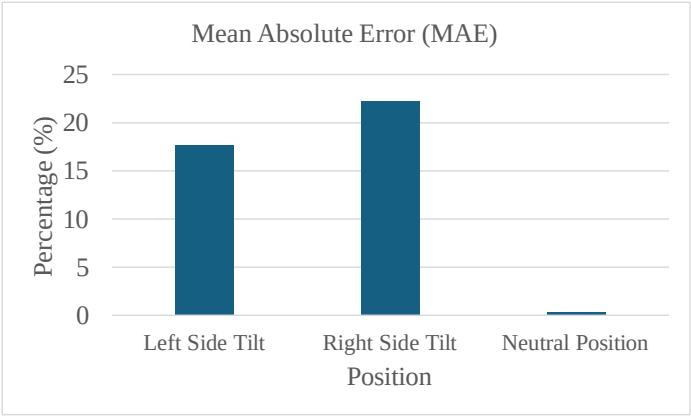

Figure 9 Mean Absolute Error (MAE) for Different Head Positions

To evaluate the accuracy of the proposed system, an experiment was conducted by comparing the actual angles (Figure 7) with the calculated angles (Figure 8) derived by the system. The experiment was divided into three parts: the first part involved measuring the angle in the neutral position, where the volunteer maintained a neutral head posture facing directly forward. The second part assessed the system's performance in detecting the angle during a left side tilt, where the volunteer tilted their head to the left. The final part measured the angle with the right-side tilt, where the volunteer tilted their head to the right. Each part of the experiment was conducted 20 times by 10 volunteers (6 females and 4 males) to ensure robust data collection. The calculated angles for each tilt position were compared with the actual angles to determine the system's accuracy and its ability to detect head movements in different directions. To validate the accuracy of the system, the actual angles were measured using a standard manual protractor application, which was placed and aligned along the participant's head and shoulder axis during each posture. These measurements served as reference angles for comparing against the automated detection angles calculated by the MediaPipe's pose estimation model according to Figure 8. The results were analyzed to assess the precision of the system in accurately detecting and calculating head posture.

To quantify the accuracy of the system, the Mean Absolute Error (MAE) was used as the primary metric for evaluating posture angle detection. For each trial, the angle calculated by the system using MediaPipe landmarks was compared to the corresponding actual angle, which was measured manually using a standard protractor aligned with the participant's head and shoulder axis. The absolute error for each instance was computed as the absolute difference between the measured angle and the actual reference angle. These individual errors were then averaged across all repetitions for each posture type—neutral, left tilt, and right tilt—to determine the final MAE, according to the equation (6).

The results of the Mean Absolute Error (MAE) analysis are presented in Figure 9, which shows the error percentages for three different head positions: Neutral Position, Left Side Tilt, and Right-Side Tilt. In the Neutral

Position, the system demonstrated a very low MAE, close to 0%, indicating that the calculated angles closely match the actual angles when the head is in a neutral, straight-forward posture. This suggests that the system is highly accurate in detecting the posture in this position. However, as the head tilts to the left or right, the MAE increases, with both the Left Side Tilt and Right-Side Tilt showing an error of approximately 20%. These higher error values suggest that the system's accuracy decreases as the head tilts away from the neutral position. The increased MAE in tilted positions may be attributed to challenges in detecting and differentiating smaller changes in posture, or potential variations in individual head movements during tilting. Overall, while the system performs optimally in the neutral position, further refinement may be needed to improve accuracy in detecting head tilts.

Additionally, the processing time for the proposed system, executed on an **11**th Gen Intel® Core™ i**7**-1165G7 processor at 2.80 GHz, was evaluated. The system processed each frame with an average time of 0.20 seconds per frame, corresponding to approximately **5** frames per second (fps). This processing time demonstrates the system's capability to perform real-time posture detection efficiently, providing timely feedback for posture correction during typical office tasks.

4. Conclusions

This study presented the development of a real-time posture detection system utilizing the MediaPipe Framework to address the growing issue of office syndrome associated with prolonged sitting and poor ergonomic habits. The system was evaluated for its ability to detect head posture across various positions, demonstrating high accuracy in identifying neutral postures, with moderate deviations observed during head tilts.

Despite these limitations, the system's consistent performance in neutral alignment and its low-latency operation underscores its potential for practical use in workplace environments. The findings also highlight the importance of further refinement, particularly in improving sensitivity to subtle postural deviations.

In addition, the analysis revealed that factors such as individual body structure, user height, camera angle, and involuntary movements can affect detection accuracy. These insights support the need for adaptive calibration strategies and personalized posture baselines to enhance system robustness across diverse users.

Overall, the proposed system offers an efficient and scalable approach for promoting ergonomic awareness and reducing musculoskeletal risks in office settings. Future work will aim to improve detection reliability, extend posture analysis to full-body tracking, and incorporate intelligent feedback mechanisms for real-time posture correction and user engagement.

5. Acknowledgements

This work was financially supported by the Rajamangala University of Technology Srivijaya (RUTS).

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Real-Time pH Monitoring and Automated Control System for Sustainable Aquaculture

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Abstract

Fluctuations in pH levels can have a detrimental impact on the health, productivity, and ecosystem balance of aquatic organisms. Maintaining a stable pH within the optimal range is essential to ensure water quality and fish well-being. This study presents the design and validation of a real-time automated pH monitoring and control system to support sustainable aquaculture practices by regulating water pH using 0.2% (W/V) vinegar solution and 5% (W/V) dolomite solution. The system features real-time pH monitoring via a pH sensor and utilizes the ESP32 microcontroller to automate pH adjustments, with data logged to Google Sheets for remote tracking. The research process was conducted in two phases: The first phase involved determining the appropriate quantities of each solution for safe and effective pH regulation through incremental dosage trials, ensuring that the pH change did not exceed 2 units per day. The second phase evaluated the system's performance in actual control scenarios by adjusting the pH in a 1500 ml water container from acidic and basic conditions toward neutral, and by measuring the required time and solution saturation dynamics. The results show that the system can efficiently adjust the pH from acidic to neutral or from basic to neutral, with the required application of 4 ml of dolomite solution and 2 ml of vinegar solution per cycle. The time required for pH adjustment was approximately 1 day for changing pH from 4 to 7 and 1.5 days for adjusting pH from 8.5 to 7. These findings validate the effectiveness of the proposed system in controlling pH levels in aquaculture and demonstrate its potential for large-scale applications in water quality management.

Keywords: pH Control System, Aquaculture, Real-Time Monitoring, IoT-based Monitoring, Vinegar Solution, Dolomite Solution

1. Introduction

Fish farming has quickly become one of Thailand's most important agricultural industries, driven by the rising demand for domestic and international fish. As the global population grows and the need for sustainable seafood increases, the fish farming sector continues to expand, with various farming systems being employed to meet these needs. These systems include monoculture, extensive, and semi-intensive farming methods, each offering unique benefits and challenges based on the objectives of the operation (Soltan, 2016). Among these, monoculture has gained popularity due to its efficiency in managing feeding schedules and monitoring fish growth and health. The highly controlled environment of monoculture allows for higher production in a shorter time, making it an appealing choice for both small and large-scale operations.

However, while monoculture offers significant advantages in terms of productivity, it also comes with a range of challenges that must be addressed to ensure sustainability over the long term. One of the most pressing issues associated with monoculture farming is water quality degradation. The high density of fish in these systems puts considerable strain on the water, leading to the accumulation of waste products like ammonia and organic matter. This can create a harmful environment for the fish, with poor water quality often causing stress, diseases, and slower growth rates. Additionally, the buildup of nutrients in the water can disrupt the balance of the ecosystem, diminish biodiversity and interfere with natural biological processes. Furthermore, monoculture farming tends to rely heavily on large quantities of feed and chemicals, which not only leads to inefficient use of natural resources but also contributes to environmental pollution. Therefore, while monoculture is a highly productive and profitable method of fish farming, it is crucial to address these challenges to ensure that the system remains ecologically balanced and sustainable in the long run.

One of the most important factors influencing the success of fish farming is water quality, specifically the pH level. The pH of water affects the health and growth of fish, and maintaining a stable pH within an optimal range is crucial for maximizing productivity. Various factors, including ammonia levels, water temperature, and dissolved oxygen, interact to determine the overall water quality. Among these, pH levels play a critical role in fish health, and it has been widely recognized that water pH should remain between 6.5 and 8.5 for optimal fish growth (Craig et al., 2008). However, pH levels in natural waters tend to fluctuate, with pH typically around 5.6 due to dissolved carbon dioxide, which forms carbonic acid. During daylight hours, photosynthesis by aquatic plants removes carbon dioxide from the water, causing a rise in pH. Conversely, in the evening when photosynthesis decreases, carbon dioxide accumulates, leading to a decrease in pH. This diurnal variation can be problematic in fish farming, where maintaining a stable pH is critical for fish well-being.

To address this challenge, various strategies have been proposed for controlling and balancing the pH of water in aquaculture systems. One such method involves the use of vinegar solutions to lower the pH. Studies have shown that apple cider vinegar (ACV) and coconut sap vinegar (CSV) can significantly improve shrimp growth performance by maintaining a stable pH of approximately 7.9 in the water (Jamis et al., 2018). In situations where the water becomes too acidic, limestone has been used successfully to raise the pH to a more favorable level (Queiroz et

al., 2004), with improvements observed within two weeks of application. These solutions, combined with technological advancements such as the Internet of Things (IoT), offer an integrated approach for real-time monitoring and management of water quality.

The integration of IoT-based systems in aquaculture has revolutionized the ability to monitor water quality in real time. IoT sensors can continuously track parameters such as pH, temperature, and dissolved oxygen, and send this data to a central control system. This allows for precise adjustments to be made automatically to maintain optimal water quality. Sodium carbonate and calcium carbonate are commonly used to buffer the water and prevent drastic fluctuations in pH levels (Abinaya et al., 2019). In addition to these methods, acidic liquids can be applied to neutralize high pH levels, particularly when the pH exceeds 7.5 (Budiman et al., 2019).

Despite the availability of these methods, there is still a need for more efficient and automated systems that can effectively balance the pH levels in fish farming environments. This work aims to develop a comprehensive pH monitoring and control system for fish farming, utilizing vinegar and dolomite solutions to adjust pH levels. The proposed system will automatically dispense vinegar solutions when the pH exceeds 8 and use dolomite solutions to raise the pH when the water becomes too acidic. By integrating real-time monitoring and automated control, this system will help maintain water quality within the optimal range for fish farming, thereby improving fish health, growth, and overall productivity.

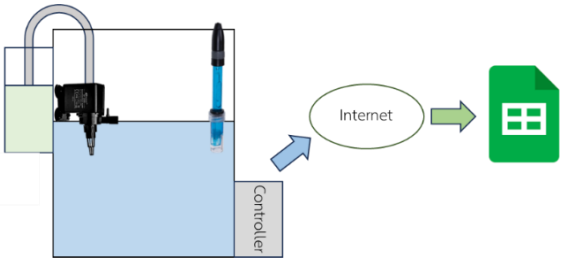

Figure 1 Proposed system installation

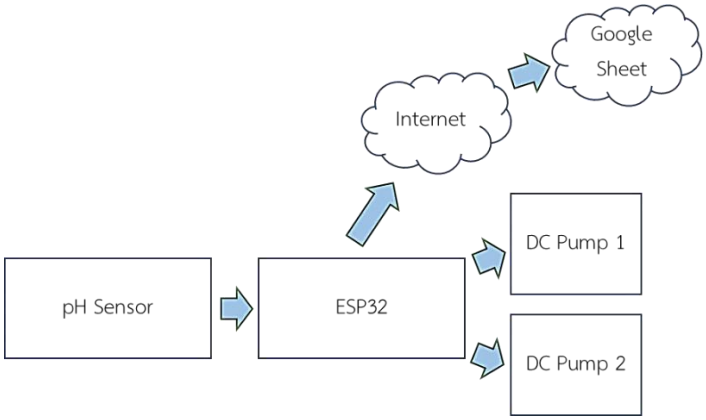

Figure 2 Proposed system block diagram

2. Methodology

In the proposed system, water pH is regulated using a 0.2% (W/V) vinegar solution as an acidifying agent and a 5% (W/V) dolomite solution as an alkalizing agent, with real-time monitoring and data logging performed via Google Sheebts through the ESP32 microcontroller. The 0.2% (W/V) vinegar solution was prepared by dissolving 2 grams of vinegar in 1 liter of water. Given that commercial vinegar typically contains 5% acetic acid, this results in an approximate acetic acid concentration of 0.01% (100 mg/L) in the working solution. During system operation, 2 ml of this vinegar solution was added to a 1,500 ml water tank, resulting in an acetic acid concentration of approximately 0.133 mg/L in the tank. This value is significantly lower than reported LC_{50} values for acetic acid (typically ranging from 45 to 1,000 mg/L), indicating the solution's safe application in aquatic environments. The 5% (W/V) dolomite solution was prepared by dissolving 5 grams of dolomite powder in 100 ml of water, yielding a mineral concentration of 50 g/L. For each alkalizing cycle, 4 ml of this solution was dispensed into the 1,500 ml water tank, resulting in an added mineral content of approximately 133 mg/L. This dosage is also well below known toxicity thresholds and was selected to gradually buffer the pH toward neutral without adversely affecting aquatic organisms.

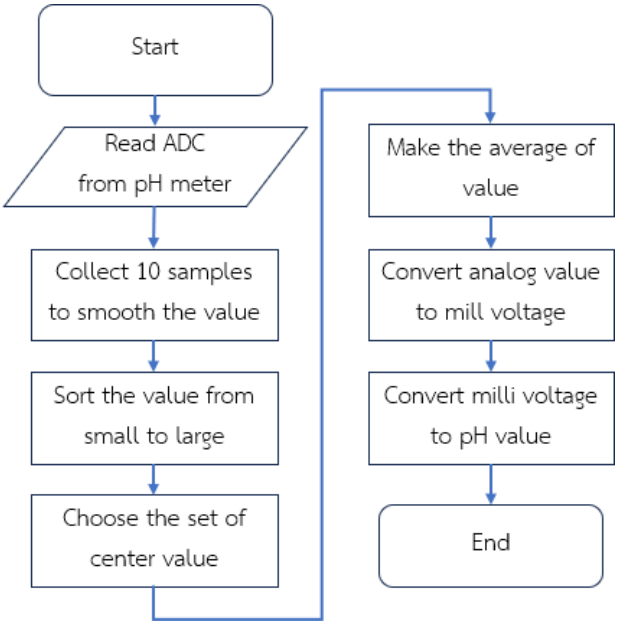

Figure 3. Data Acquisition

Figure 1 illustrates the setup of the proposed system. The system includes a pH sensor, and an oxygen pump installed inside the tank. The pH sensor continuously measures the water's pH level, while the oxygen pump ensures adequate water circulation. The dispensers for the acid and base solutions, along with their controllers, are positioned on the side of the tank.

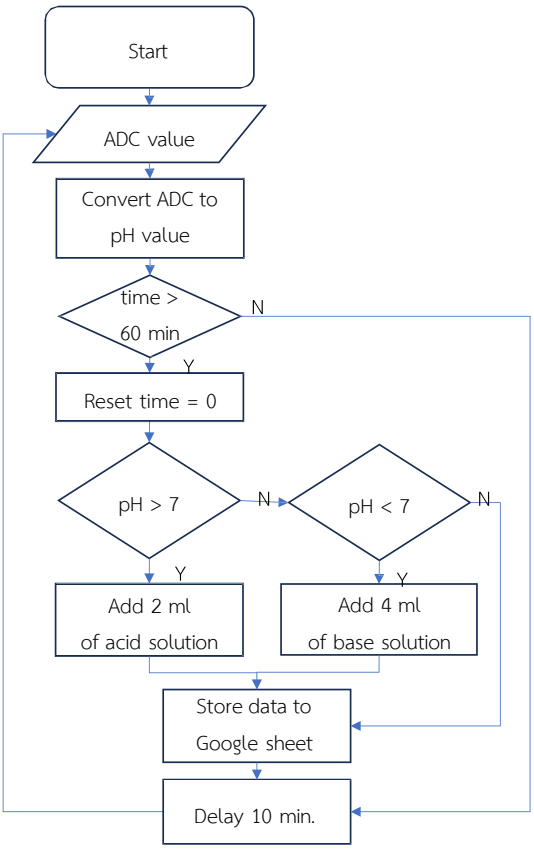

Figure 4 Control System Design

Figure 2 presents the block diagram of the proposed system, outlining the interactions between the pH sensor, controller, pumps, and the Google Sheets interface. The pH sensor serves as the input device to capture the water's pH

readings, which are then transmitted to the ESP32 controller. Based on these readings, the controller determines when to activate the DC pumps (DC pump 1 and DC pump 2) to correct any pH imbalances. The system is designed to adjust the pH by dispensing either vinegar or dolomite solution, depending on whether the water is too acidic or basic. Additionally, the pH values are logged in real-time to Google Sheets via the controller's internet connection, providing remote monitoring and tracking of the pH levels.

2.1 Data Acquisition

The pH measurement and control system begin according to Figure 3 by acquiring the analog signal from the pH sensor, which is then converted into a digital value by the ESP32 microcontroller's ADC using the equation (1). To enhance accuracy and reduce noise, 10 samples are collected, sorted from smallest to largest, and the center values are averaged. The digital value is subsequently converted back to the analog voltage using equation (2). Finally, the analog voltage is mapped to a pH value using the linear calibration formula in equation (3) where m is the slope and b is the intercept derived from sensor calibration. This method ensures accurate real-time pH monitoring, allowing for precise control over water quality in aquaculture environments.

2.2 Control Design

The control system of the proposed design is illustrated in Figure 4. The proposed pH control system utilizes real-time monitoring and automated pH adjustments to maintain optimal water quality in aquaculture. The system is equipped with a pH sensor that continuously measures the water's pH, which is then processed by an ESP32 microcontroller. Based on the measured pH, the system activates either a 0.2% (W/V) vinegar solution or a 5% (W/V) dolomite solution to adjust the pH levels, ensuring they stay within the desired range. The pH values and adjustment data are logged in Google Sheets, enabling remote tracking and real-time monitoring. The system employs a controlled dispensing mechanism that ensures pH changes do not exceed 2 units per day, and each solution is applied in precise amounts based on the pH level. A critical feature of the system is that after each adjustment, a minimum of 60 minutes is allowed to pass before the process continues, ensuring that the pH has stabilized before further changes are made. This automated approach, coupled with data logging and time checking, ensures efficient and accurate pH regulation, contributing to better water quality management in aquaculture.

$$\text{Digital value} = \left(\frac{\text{Analog input voltage}}{5.0}\right) \times 4095 \quad (1)$$

$$\text{pH} = m \times V_{analog} + b \quad (2)$$

Table 1 pH Difference of Dolomite Solution at Various Quantities

Quantity of solution	pH value (before adding solution)	pH value (after adding solution)	pH difference
8 ml	6.83	7.11	0.28
6 ml	6.82	7.07	0.25
4 ml	6.71	6.82	0.11
3 ml	6.32	6.39	0.07
2 ml	6.69	6.73	0.04

Table 2 pH Difference of Vinegar Solution at Various Quantities

Quantity of solution	pH value (before adding solution)	pH value (after adding solution)	pH difference
8 ml	6.87	6.43	0.44
6 ml	6.82	6.53	0.29
4 ml	6.84	6.63	0.21
2 ml	6.84	6.72	0.12

3. Result

The pH control system was developed and tested through a two-part experiment. The first part involved solution analysis, where vinegar and dolomite solutions were selected as the pH control agents. The goal of this phase was to determine the appropriate quantities of each solution required for effective pH regulation.

The mock container, holding 1500 ml of water, was used for the experiment. The quantity of solution added was adjusted based on the principle of pH adjustment in aquaculture, where the pH change should not exceed 2 units per day. As shown in Table 1, it was determined that the maximum allowable quantity of 5% (W/V) dolomite solution to be added to the container at any given time is 4 ml. Similarly, as indicated in Table 2, the maximum allowable quantity of 0.2% (W/V) vinegar solution is 2 ml per application.

In the second part of the experiment, we investigated the saturation time of pH changes when each solution was applied. The results revealed that the 5% (W/V) dolomite solution reached its saturation point in approximately 10 minutes, as illustrated in Figure 1. Similarly, the 0.2% (W/V) vinegar solution also reached its saturation point within the same timeframe of 10 minutes, as shown in Figure 5.

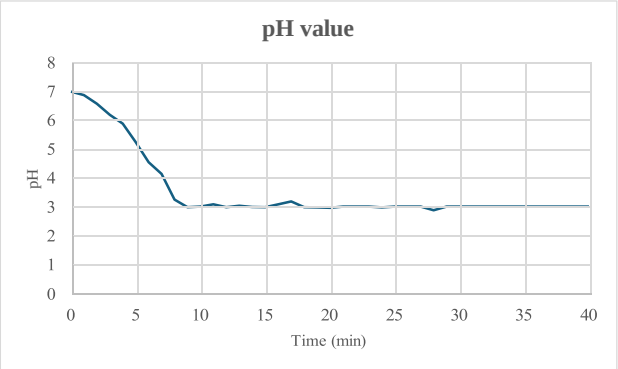

Figure 5 saturation time of pH value of vinegar solution

These findings provide valuable data for optimizing the pH control system and ensuring that both dolomite and vinegar solutions are used effectively without exceeding the maximum allowable pH changes for aquaculture environments.

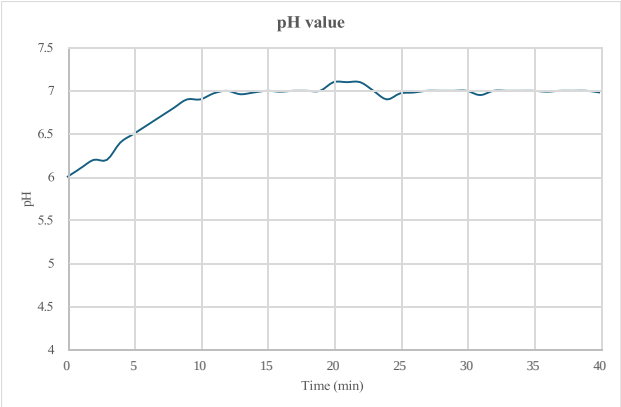

Figure 6 saturation time of pH value of dolomite solution

The second phase of the experiment aimed to evaluate the effectiveness of the pH-controlling system. A test container, holding 1500 ml of water, was equipped with an oxygen pump to facilitate water circulation. To adjust the pH from acidic to neutral, a 5% (W/V) dolomite solution was used, while a 0.2% (W/V) vinegar solution was employed to adjust the pH from basic to neutral. Each experiment was repeated three times for consistency.

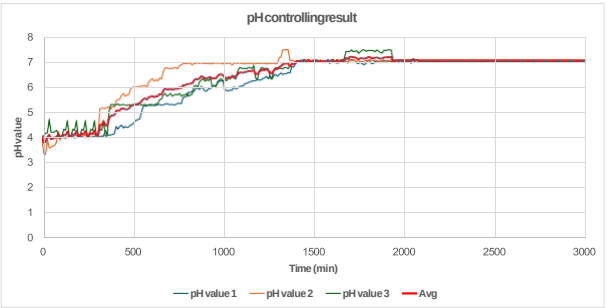

Figure 7 effectiveness of the pH-controlling system

As shown in Figure 6, the average time required to adjust the pH from 4 to 7 was approximately 1500 minutes, or about 1 day. Similarly, Figure 7 illustrates that the average time required to adjust the pH from 8.5 to 7 was approximately 2000 minutes, or 1 day and a half.

These results demonstrate the efficiency of the pH-controlling system in restoring water to a neutral pH within a reasonable time frame for both acidic and basic conditions.

4. Conclusion

The proposed pH control system demonstrated effective and efficient regulation of pH levels in aquaculture environments. By utilizing a combination of 0.2% (W/V) vinegar solution and 5% (W/V) dolomite solution, the system successfully adjusted the pH from both acidic and basic conditions to neutral, within the optimal range. The experiment confirmed that the maximum allowable quantities of each solution (4 ml of dolomite and 2 ml of vinegar) did not exceed the recommended pH change of 2 units per day, ensuring water quality stability.

The system's performance was further validated by testing its saturation time for pH changes. Results showed that both the vinegar and dolomite solutions reached their saturation points in approximately 10 minutes, highlighting the system's quick response. Additionally, the time required to adjust pH from 4 to 7 and from 8.5 to 7 was approximately 1 day and 1.5 days, respectively, indicating the system's efficiency in maintaining stable pH levels over a reasonable period.

These findings suggest that the integration of real-time pH monitoring with automated pH adjustment can significantly improve control over water conditions in aquaculture. Future developments will focus on enhancing the system's scalability and efficiency for broader applications in various water quality management scenarios.

Although the experiments were conducted in a fixed volume of 1500 ml, the system design is inherently modular and suitable for scale-up. Adapting the system to larger tanks or ponds would require proportional calibration of dosing volumes, optimization of sensor positioning, and enhancements in water circulation and mixing. However, in larger-scale deployments, additional factors such as buffering capacity, non-uniform pH gradients, and slower reaction kinetics must be considered. Future work will focus on evaluating the system's performance in larger aquaculture environments and under variable field conditions. Investigations will include scalability assessment, adaptive control strategies, and integration with multi-parameter water quality monitoring to support broader applications in sustainable aquaculture and environmental management.

5. Acknowledgment

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Uncovering the Role of Fe in Geopolymers via Synchrotron XANES and EXAFS: Insights from Fly Ash, Bagasse Ash, and Calcium Carbide Residue Systems

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Abstract

Geopolymers made from industrial waste like fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR) are being explored as sustainable alternatives to Portland cement. This study focuses on how adding 5 wt% of iron oxide (Fe_2O_3) affects the internal structure of these geopolymers. We used advanced synchrotron techniques—X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS)—to understand how iron behaves inside the material. All samples with added iron showed an absorption edge around 7120 eV, confirming that iron mainly exists in the +3 oxidation state (Fe^{3+}). It can be noticed that the way iron atoms were arranged depended on the type of raw materials. In samples with high fly ash, iron seemed to bond more neatly inside the structure. In contrast, samples with more calcium carbide residue had more disorder and signs of separate iron oxide phases. These changes were supported by XRD and compressive strength results, which showed that better iron incorporation led to better structure and performance. Our findings suggest that the raw material composition strongly affects how iron fits into the geopolymer and influences the material's strength. This study highlights the value of synchrotron techniques in designing better geopolymers and deepens our understanding of how iron interacts in different systems.

Keywords: Geopolymer, Iron Incorporation, XANES, EXAFS

1. Introduction

Geopolymers are **a** type of inorganic binder formed through the alkali activation of aluminosilicate materials, offering a more sustainable option compared to ordinary Portland cement (OPC). These materials not only show high strength and chemical resistance, but also help lower CO_2 emissions thanks to their low-temperature processing and the use of industrial by-products (Davidovits, J, (1991); Provis, J L and van Deventer, J S J, (2009)). Common raw materials used as geopolymer precursors include fly ash (FA), a residue from coal combustion; bagasse ash (BA), produced from sugarcane waste; and calcium carbide residue (CR), a by-product rich in calcium hydroxide from acetylene gas production. When combined, these materials can produce blended geopolymer systems that balance silica, alumina, and calcium, forming hybrid gel structures like N–A–S–H and C–A–S–H (Wang, Q et al., 2022; Amin, M N et al., 2022).

Although the roles of silica- and calcium-rich precursors in geopolymer chemistry are well documented, the effect of transition metals—especially iron (Fe)—is still not fully understood. Iron is commonly found in waste materials and can also be added intentionally as Fe_2O_3. Depending on the chemical environment, iron may either bond with the aluminosilicate structure or stay as a separate crystalline phase. This behavior can influence the type of gel that forms and affect the final strength and durability of the geopolymer matrix (Lemougna, P N et al., 2013; Tchakouté, H K et al., 2017).

To explore how Fe behaves inside the geopolymer structure, this study uses synchrotron-based X-ray absorption techniques—X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). These methods are very sensitive to oxidation states and local atomic bonding and are ideal for studying Fe distribution in disordered geopolymer matrices (Parsons, J G et al., 2002; Newville, M, 2004). Samples with various FA–BA–CR proportions, both with and without 5 wt% Fe_2O_3, were examined to see how different raw material combinations influence Fe bonding.

This study provides new understanding of how iron interacts with geopolymer gels, and shows the potential of advanced synchrotron techniques to help develop stronger and more sustainable construction materials.

2. Methodology

2.1 Raw Materials

Fly Ash (FA): From Mae Moh power plant, rich in Si and Al
Bagasse Ash (BA): From Surin Sugar Co., moderate reactivity
Calcium Carbide Residue (CR): From Thai Acetylene Co., high in $Ca(OH)_2$
Fe Source: Commercial Fe_2O_3, added at 5 wt%

2.2 Mix Design

Seven geopolymer paste formulations were prepared using different ratios of fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR) as aluminosilicate precursors. Each mixture was prepared in both a control version (without Fe) and a modified version with 5 wt% Fe_2O_3 added as an iron source. The 5 wt% Fe_2O_3 dosage was selected

based on preliminary studies and literature indicating that this level of iron oxide can influence structural incorporation without causing excessive crystallization or segregation. Previous studies such as Tchakouté et al. (2017) and Lemougna et al. (2013) used similar or slightly lower amounts for investigating Fe coordination in geopolymer systems. The alkaline activator consisted of 5 molar sodium hydroxide (NaOH) and sodium silicate (Na_2SiO_3), mixed in a ratio of 1:0.5 by volume. The binder-to-solution ratio was maintained at 1:0.75 for all mixtures to ensure consistency in workability. After thorough mixing, the pastes were cast into molds and cured at ambient temperature. These formulations were designed to evaluate the influence of precursor composition and Fe addition on the structure and properties of the resulting geopolymers. The mix proportions of the geopolymer samples are presented in Table 1, while Table 2 displays the XRF-derived oxide compositions (wt%) of the FA–BA–CR-based mixes with 5 wt% Fe_2O_3 addition.

Table 1 The mix proportions of the geopolymer samples

Samples	Ratio of starting materials			Fe_2O_3 (%)	5 M NaOH	Na_2SiO_3
	FA	BA	CR			
0.16FA0.16BA0.67CR	0.16	0.16	0.67	5	0.5	0.25
0FA0BA1CR	0.00	0.00	1.00	5	0.5	0.25
0.67FA0.16BA0.16CR	0.67	0.16	0.16	5	0.5	0.25
0.33FA0.33BA0.33CR	0.33	0.33	0.33	5	0.5	0.25
0.16FA0.67BA0.16CR	0.16	0.67	0.16	5	0.5	0.25
0FA1BA0CR	0.00	1.00	0.00	5	0.5	0.25
1FA0BA0CR	1.00	0.00	0.00	5	0.5	0.25

Table 2 XRF-derived oxide composition (wt%) of FA–BA–CR-based geopolymer mixes with 5 wt% Fe_2O_3 addition

Samples	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	K_2O
0.16FA0.16BA0.67CR	20.61	3.56	**7.76**	65.03	0.54	1.70	0.80
0FA0BA1CR	4.10	1.58	**5.07**	88.94	0.00	0.30	0.00
0.67FA0.16BA0.16CR	33.56	9.24	**14.93**	35.02	0.54	5.25	1.46
0.33FA0.33BA0.33CR	37.12	5.53	**10.45**	41.10	1.09	3.10	1.60
0.16FA0.67BA0.16CR	57.19	3.82	**8.66**	23.28	2.17	2.35	2.54
0FA1BA0CR	77.26	2.10	**6.87**	4.92	3.26	1.60	3.49
1FA0BA0CR	30.01	12.94	**19.42**	28.92	0.00	7.39	1.31

2.3 Characterization Techniques

A combination of laboratory and synchrotron-based techniques was employed to analyze the phase composition, iron coordination, and mechanical properties of the geopolymer samples.

1) X-ray Diffraction (XRD)

XRD was used to identify crystalline phases in the geopolymer matrix. The patterns were recorded in the 2θ range of 10°–70°, using Cu Kα radiation. Phases such as quartz, calcite, portlandite, and hematite were identified to evaluate reaction products and residual precursors.

2) X-ray Absorption Spectroscopy (XAS)

XAS experiments, including X-ray Absorption Near-Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS), were conducted at the Fe K-edge to investigate the oxidation state and local coordination of iron in the geopolymer matrix. Measurements were performed in fluorescence mode at the Synchrotron Light Research Institute (Thailand). Data analysis was carried out using the Demeter software package (Athena and Artemis).

3) Compressive Strength Testing

Compressive strength was measured on cubic geopolymer specimens (50 × 50 × 50 mm) at 28 days. A universal testing machine was used with a loading rate of 1 mm/min. Strength values were compared between Fe- modified and control samples to assess the effect of iron incorporation.

3. Results and Discussion

3.1 Fe Oxidation State and Local Coordination (XANES)

X-ray absorption near-edge structure (XANES) analysis was conducted to investigate the oxidation state of Fe and its coordination behavior in geopolymer samples containing 5 wt% Fe_2O_3. All samples exhibited a distinct Fe K-edge absorption feature at approximately 7120 eV, which is characteristic of iron in the +3 oxidation state (Fe^{3+}). This confirms that the added Fe_2O_3 remained predominantly in its trivalent form throughout the alkali activation process.

Although the edge position remained consistent across all compositions, significant differences were observed in the intensity of the white line—the peak immediately following the absorption edge. Samples with higher fly ash content, such as 1FA0BA0CR-Fe and 0.67FA0.16BA0.16CR-Fe, exhibited lower white line intensities, suggesting a more ordered Fe coordination environment. In contrast, CR-rich compositions such as 0.16FA0.16BA0.67CR-Fe and 0FA0BA1CR-Fe showed higher white line intensities, which may indicate a more distorted or disordered local structure around the Fe atoms.

These variations reflect the influence of precursor composition on Fe incorporation. High FA systems, rich in reactive silica and alumina, likely promote the integration of Fe into the aluminosilicate framework. Meanwhile, calcium-rich systems (CR-dominant) tend to hinder polymerization and may result in Fe existing as isolated species or secondary phases (Zhang, G and Provis, J L (2020), Ngnintedem, D L V et.al(2002).

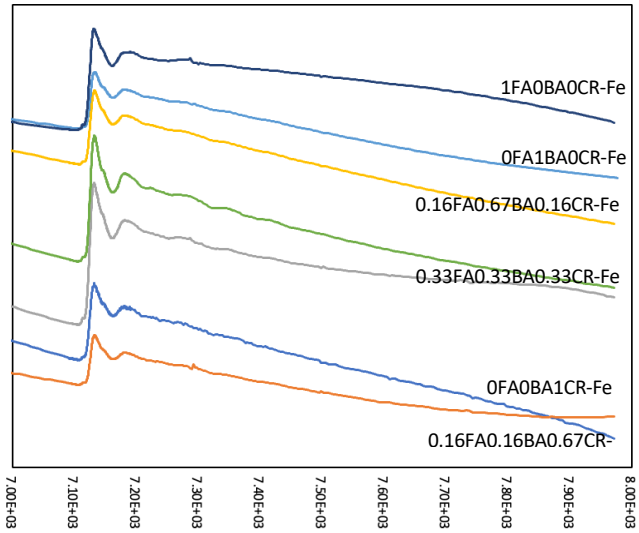

Figure 1 The Fe K-edge XANES spectra of geopolymer samples made with different amounts of fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR), all containing 5 wt% Fe_2O

3.2 Fe Coordination Environment (EXAFS)

Extended X-ray absorption fine structure (EXAFS) analysis was performed to evaluate the local atomic arrangement around Fe atoms in the geopolymer matrix. The Fourier-transformed EXAFS (FT-EXAFS) spectra in R-space revealed clear differences in Fe–O coordination among the various precursor combinations.

All samples exhibited a primary FT peak in the range of 1.5–2.2 Å, corresponding to the first coordination shell of Fe–O bonds. The amplitude and sharpness of this peak varied depending on the mix composition. In fly ash-rich systems such as 1FA0BA0CR-Fe and 0.67FA0.16BA0.16CR-Fe, the Fe–O peaks were sharper and more intense, indicating a more ordered and symmetric local coordination environment. This suggests that Fe was better incorporated into the aluminosilicate gel framework, potentially by substituting for Al in the tetrahedral structure.

In contrast, samples with high calcium carbide residue, such as 0.16FA0.16BA0.67CR-Fe and 0FA0BA1CR-Fe, showed broader and less intense Fe–O peaks. These features point to a more disordered coordination environment, which may result from Fe existing outside the gel network, either as amorphous species or as segregated iron oxide phases. The reduced second-shell features in these samples further support the lack of long-range order, consistent with weak Fe integration.

These EXAFS observations align with the XANES results, reinforcing the conclusion that the degree of Fe incorporation is strongly influenced by the precursor composition. High FA content facilitates more stable Fe–O coordination, while high CR content leads to increased disorder and potential phase separation.

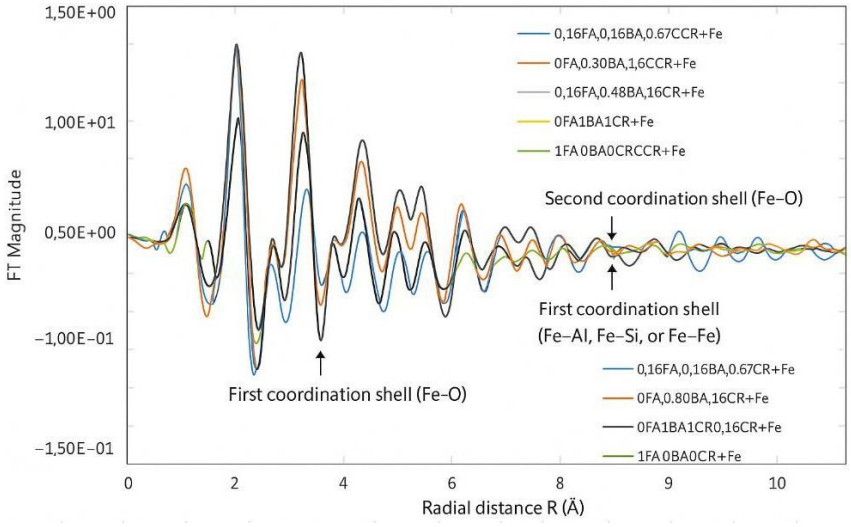

Figure 2 The Fourier-transformed EXAFS (FT-EXAFS) spectra at the Fe K-edge for geopolymer samples with 5 wt% Fe_2O_3, made using different amounts of fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR)

3.3 Phase Composition (XRD)

X-ray diffraction (XRD) analysis was conducted to identify the crystalline phases present in the geopolymer samples with and without Fe_2O_3 addition. All diffractograms exhibited a broad hump between 25° and 35° 2θ,

indicative of the amorphous aluminosilicate gel typically formed in geopolymer systems. Superimposed on this amorphous background, several crystalline phases were identified, including quartz (Q), calcite (C), portlandite (H), and dolomitic calcite (M), primarily originating from unreacted raw materials.

In CR-rich samples such as 0.16FA0.16BA0.67CR and 0FA0BA1CR, strong diffraction peaks of portlandite ($Ca(OH)_2$) and gaylussite ($Na_2Ca(CO_3)_2$) were observed. These phases indicate incomplete reaction of calcium hydroxide from CR, which may hinder the formation of a continuous gel network. Upon addition of Fe_2O_3, new peaks appeared at ~33.2° 2θ, corresponding to hematite (Fe_2O_3). These peaks were most pronounced in Fe-modified CR-rich systems (e.g., 0.16FA0.16BA0.67CR-Fe and 0FA1BA0CR-Fe), suggesting limited Fe incorporation and a tendency toward phase segregation.

In contrast, samples with higher FA content, such as 1FA0BA0CR-Fe and 0.67FA0.16BA0.16CR-Fe, showed broader and weaker hematite reflections. This implies that Fe was more successfully incorporated into the amorphous geopolymer matrix rather than forming distinct crystalline phases. These trends are consistent with the EXAFS findings, which revealed more ordered Fe coordination environments in FA-rich systems [11].

The absence of additional crystalline aluminosilicate phases (e.g., zeolites or feldspathoids) suggests that the majority of geopolymerization products remained in an amorphous state. This was further supported by the prominence of the amorphous hump in all XRD patterns.

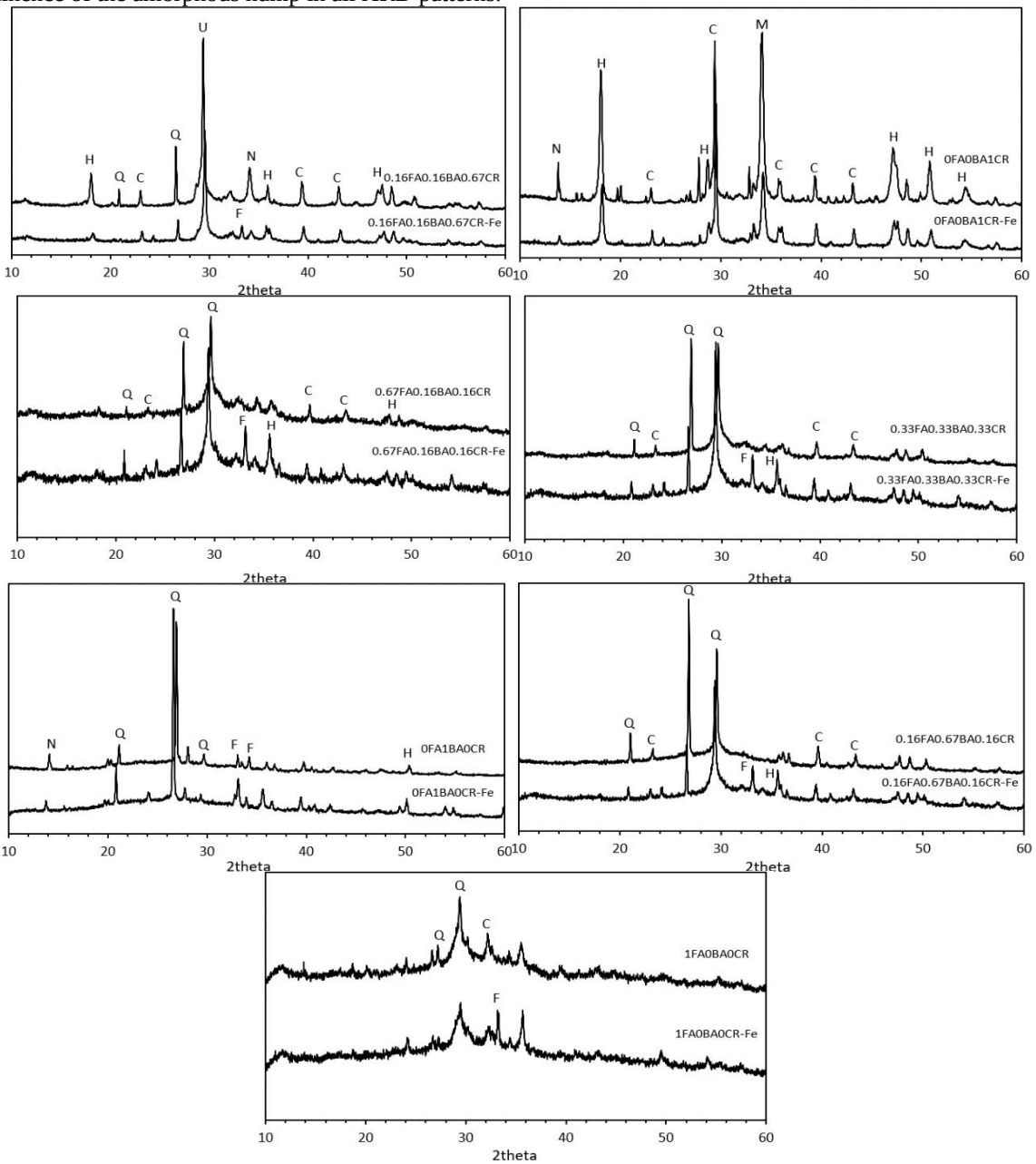

Figure 3 presents the XRD patterns of geopolymer samples synthesized from various proportions of fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR), with and without 5 wt% Fe_2O_3 addition

H: $Ca(OH)_2$ Portlandite C: $CaCO_3$ Calcite N: $Na_2Ca(CO_3)_2$ Gaylussite Q: SiO_2 Quartz U: SiO_2 Quartz

M: $(Mg_{0.03}Ca_{0.97})(CO_3)$ Calcite S: Ca_3SiO_5 Hatruite F: Fe_2O_3 Hematite

3.4 Mechanical Performance (Compressive Strength)

The compressive strength at 28 days showed clear differences based on both the raw material mix and whether Fe_2O_3 was added. Fly ash–rich samples gave the best strength results. For example, the mix with 100% fly ash (1FA0BA0CR) reached about 43 MPa without Fe. But when Fe_2O_3 was added, the strength dropped to around 25 MPa. This might be because iron, while well integrated into the structure, slightly disrupted the gel network that holds the material together.

On the other hand, samples with high calcium carbide residue (like 0FA0BA1CR) had very low strength, below 10 MPa. Adding Fe to these mixes made the strength even worse. XRD results showed leftover portlandite and clear peaks of hematite, meaning that Fe did not enter the gel but instead formed separate crystals, which weakened the material.

Blends with balanced amounts of FA, BA, and CR (like 0.33FA0.33BA0.33CR and 0.67FA0.16BA0.16CR) gave medium to good strengths (around 25–35 MPa). Adding Fe to these balanced mixes didn't hurt the strength much and sometimes slightly improved it. EXAFS and XANES results showed that Fe was only partly included in the structure in these cases, and the gel stayed stable.

In summary, Fe helps or hurts depending on the mix. It works better when there's enough silica and alumina (from FA), but not when there's too much calcium (from CR).

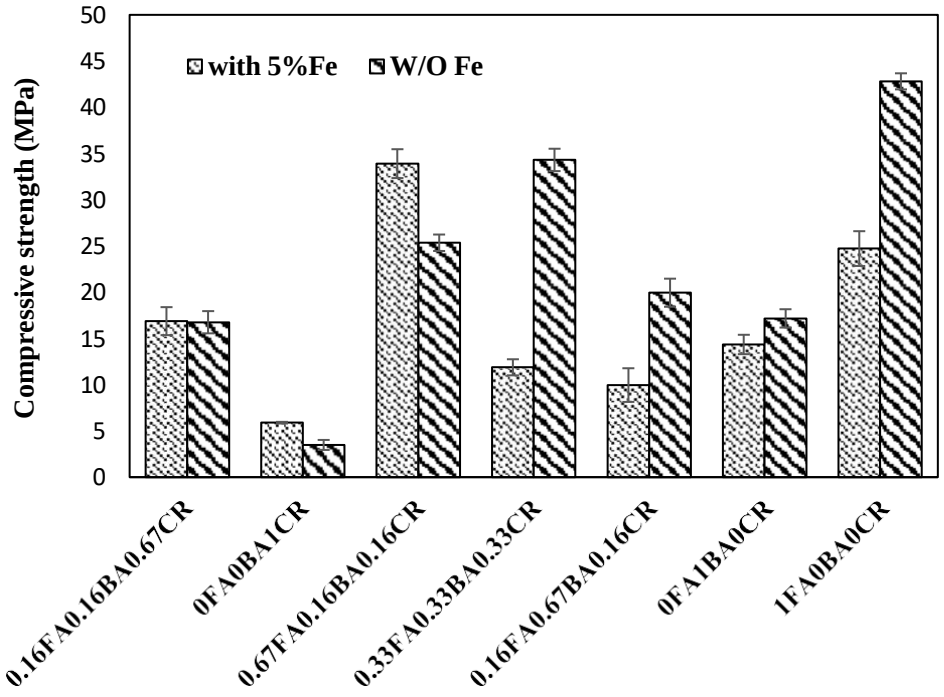

Figure 4 the 28-day compressive strength of geopolymer samples made from different amounts of fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR), with and without 5% Fe_2O_3

3.5 Cross-Technique Correlation

The results from all techniques—XANES, EXAFS, XRD, and compressive strength—show a clear link between the local structure of iron and how strong the geopolymer becomes.

In fly ash–rich samples, Fe was better incorporated into the gel network. XANES showed low white line intensity (meaning less disorder), and EXAFS showed sharp Fe–O coordination peaks (meaning more order). These samples also had weak or no hematite peaks in XRD and gave high compressive strength. This suggests that when Fe is well integrated into the aluminosilicate structure, it does not harm strength—and may even help slightly.

In contrast, CR-rich mixes showed the opposite. XANES white lines were strong, EXAFS peaks were broad, and XRD showed clear hematite crystals. These samples had the lowest strength. The data suggest that Fe was not part of the gel but formed separate phases that disrupted the structure.

Balanced mixes (with moderate FA, BA, and CR) gave medium strength. Fe was only partly incorporated, and the structure stayed stable. The XRD, XANES, and EXAFS results for these mixes showed intermediate behavior—some Fe coordination, mild hematite peaks, and moderate disorder.

Overall, Fe can support or weaken the geopolymer depending on the composition. It works best when FA is high and CR is limited, which allows Fe to become part of the amorphous gel. When calcium is too high, Fe tends to separate out and reduce performance (Tchakouté, H K et.al, 2016).

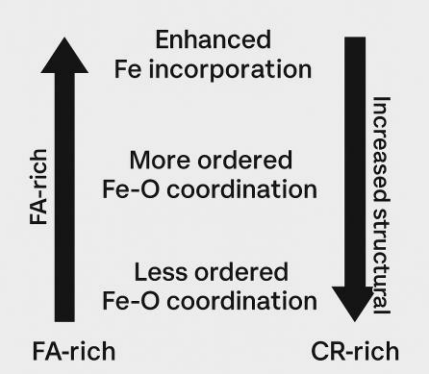

Figure 5 Cross-Technique Correlation

4. Conclusions

This study explored how iron (Fe) affects the structure and strength of geopolymers made from fly ash (FA), bagasse ash (BA), and calcium carbide residue (CR). Using synchrotron-based XANES and EXAFS techniques, we found that Fe remained in the +3 oxidation state in all samples, but its local coordination environment varied depending on the mix design.

In FA-rich systems, Fe was better integrated into the amorphous aluminosilicate gel, as shown by lower white line intensities and more ordered Fe–O environments. These systems maintained higher compressive strength. In contrast, CR-rich compositions showed more crystalline hematite phases and disordered Fe coordination, leading to weaker strength due to poor gel formation and incomplete polymerization.

Overall, the results demonstrate that the role of Fe in geopolymer systems is highly dependent on the precursor blend. Fly ash helps stabilize Fe in the gel network, while excessive calcium from CR can hinder Fe incorporation and reduce performance. These insights support the potential of using Fe as a structural modifier in geopolymer systems, provided that precursor balance is carefully optimized.

5. Acknowledgements

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Influence of Pozzolanic Materials in Ternary Blend Systems on Strength and Chloride Resistance of Concrete

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Abstract

Pozzolanic materials (fly ash, meta kaolin, and silica powder) were investigated to partially replace cement based on their impact on the strength and chloride resistance of the concrete. The compressive strength was investigated in the range 240–350 kg/cm^2 with a water-to-binder ratio (w/b) of 0.40 for the best performance, while varying the amount of cement replaced by ternary blended systems using cement with the main quantities by weight of cement being fly ash (65%), kaolin (10–15%), and silica powder (2.5 or 5%) compared to the controlled sample and cementitious mixtures. The concrete mix included high volume water-reducing agent (superplasticizer Type F) of a special water-reducing type. Along with tests of the mechanical properties of compressive strength, splitting tensile strength, flexural strength, and also chloride ion movement test electrolysis using the chloride migration test. The test results showed that the ternary blended systems of the concrete containing the pozzolanic materials at w/b equal to 0.40 had the best mechanical properties. The concrete mixture with replacement of cement with fly ash and metakaolin with alumina oxide had a greater effect on compressive strength than adding silica. The tensile strength and flexural strength were better in the mixture of fly ash and silica powder, as was the electrophoretic movement of chloride ions in the concrete, with better binding of chlorine. However, the physical properties of the metakaolin and silica powder, such as their particle distribution and high specific surface area, resulted in a more rapid pozzolanic reaction that increased the CSH and CAH, and increased the opacity of the concrete, resulting in overall improved mechanical properties of the concrete.

Keywords: Pozzolans, Compressive Strength, Flexural Strength, splitting Tensile Strength, Chloride Migration

1. Introduction

Traditionally, most general structures use reinforced concrete based on the expectation that the structure should be durable under normal conditions. In fact, most designs do not consider resistance. However, reinforced concrete structures containing steel that comes into contact with seawater or brackish water or is used for construction in coastal areas, including underground structures, can be damaged by environmental factors that affect the mechanical properties of the concrete. This results in the structural strength being continuously reduced, making this a problem that must be addressed as a high priority. Maintenance and repair can help to extend the life of the structure so that it can be used for a long time. In the past, many researchers have studied the development and improvement of the properties of concrete to make it stronger and denser (Cheng et al., 2017; Mindess et al., 2003, Neville A.M, 1996) by modifying the materials used. Concrete improvement has commonly used pozzolanic materials, such as fly ash and kaolin (meta kaolin), which when mixed in the concrete in appropriate amounts will cause a pozzolanic reaction, resulting in more CSH and CAH compounds in the concrete and producing concrete having a denser internal structure and higher strength (Gopalan M.K., 1993 and Sangsuwan C., Sujjavanich S, 2020). However, such studies focused mainly on improving the compressive strength and density of the concrete [Ichikawa T., 2009]. In addition, there have been reports on the properties associated with resistance to chloride penetration to prevent corrosion of the steel reinforcement in concrete, especially in a marine environment involving contact of concrete with sea or brackish water, where the life span is reduced and may include resistance to alkaline silica reaction (ASR) (Arya C et al., 1996; Sangsuwan C., Sujjavanich S, 2012; Prince W., Gagné R., 2001). The current research studied resistance to chloride permeation in concrete mixed with pozzolanic materials (fly ash and silica powder) with the aim of identifying the most appropriate concrete mixture based on testing compressive strength, splitting tensile strength, flexural strength, and the permeability of chloride solution in the concrete.

2. Materials and Methods

2.1 Materials

Pozzolanic material, a waste material from industrial plants, and natural materials were used as a partial replacement for cement. The ternary blended systems used Ordinary Portland cement type 1 (OPC) and fly ash (FA) as the main contents (65%), with metakaolin (MK) varying between 10% and 15% and silica powder (SP) at 2.5 or 5%, as well using control samples. The tested mechanical properties consisted of: compressive strength, split tensile strength, flexural strength, and a chloride migration test of the concrete.

1) Cement

The OPC had a specific gravity of 3.13, with details provided in Table 1.

2) Pozzolans

FA, MK, and SP were used, with the FA from power plants in Lampang province Thailand, the MK from Metamax Co., Ltd, imported from the United States, and the SP from the Herosign Marketing Co., Ltd, imported from China, as shown in Table 1.

3) Aggregate

Fine aggregate was obtained from river sand that had passed through sieve number 4 with mixed sizes according to the ASTM C 33 standards and a fineness modulus (F.M.) of 2.58, absorption 2.15 (%), surface moisture of 3.95% and specific gravity of 2.65. The coarse aggregate consisted of crushed limestone in the size range 10–19 mm, with mixed sizes according to ASTM C 33 standards, and an F.M. value of 2.85, water absorption equal to 1.00, 0.65% surface moisture, 2.85 specific gravity, and a density of 1,680 kg/m^3.

4) Admixture

Special water reducer type HRWR Admixture (Superplasticizer) of Sika (THAILAND) Limited.

Table 1 Chemical compositions of raw materials.

Oxide	Chemical Composition, %										
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	MnO_2	SO_3	$Na2O_{eq}$	LOI
Cement	17.2	4.95	3.89	61.23	0.73	0.057	0.48	0.047	2.14	0.373	2.01
Fly ash	35.21	18.54	12.25	16.24	2.14	1.158	1.98	-	2.98	2.461	0.475
Metakaolin	49.58	41.22	0.287	0.018	<0.00	0.24	0.12	-	0.057	0.319	-
Silica powder	99.72	0.074	0.010	0.0094	0.0033	0.004	0.005	-	-	0.007	-

2.2 Mixture Proportions

The influence was studied of using pozzolanic materials (FA, MK and SP) in partial replacement of cement. The manufactured concrete was designed to have a compressive strength in the range 350–400 kg/cm^2. Different concrete samples were investigated using three levels or cement replacement (0.40, 0.45 and 0.50). The main component was the OPC and FA (65%), with MK levels of 15% or 10% and SP levels of 5% or 2.5% by weight of cement. The following terms were used to define the respective samples: OPC/FA20/MK15, OPC/FA25/MK10, OPC/FA20/SP5, and OPC/FA25/SP2.5.

2.3 Sample Preparation and Test Methods

Test samples were prepared for the concrete mix proportions used in the design test according to the recommendations of ACI 211.1-1, with water-to-binder (w/b) ratios of 0.40, 0.45, or 0.50 and a cement- to-sand-to-coarse aggregate ratio equal to 1:2:4 by volume. The materials used to produce 1 m^3 of concrete are provided in Table 2.

Table 2 Mix proportions of ternary blended concrete (kg/m^3).

Mix	w/b	HRWR (%)	OPC (kg/m^3)	FA (kg/m^3)	MK (kg/m^3)	SP (kg/m^3)	Coarse Aggre. (kg/m^3)	Fine Aggre. (kg/m^3)	Water
OPC	0.40	-	440	-	-	-	721	1,066	176
OPC/FA20/MK15		0.5	286	88	66	-			
OPC/FA25/MK10		0.5	286	110	44	-			
OPC/FA20/SLP5		0.75	330	88	-	22			
OPC/FA25/SLP2.5		0.75	319	110	-	11			
OPC	0.45	-	440	-	-	-	721	1,066	198
OPC/FA20/MK15		0.5	286	88	66	-			
OPC/FA25/MK10		0.5	286	110	44	-			
OPC/FA20/SLP5		0.75	330	88	-	22			
OPC/FA25/SLP2.5		0.75	319	110	-	11			
OPC	0.50	-	440	-	-	-	721	1,066	220
OPC/FA20/MK15		0.5	286	88	66	-			
OPC/FA25/MK10		0.5	286	110	44	-			
OPC/FA20/SLP5		0.75	330	88	-	22			
OPC/FA25/SLP2.5		0.75	319	110	-	11			

Sample preparation and curing of concrete were performed according to ASTM C192. After mixing the concrete samples were tested for collapse, according to ASTM C143. After the concrete had hardened for 24 hours, it was removed from the mold and immersed in water for 28 days. The compressive strength of the hardened concrete was measured, using the procedures in ASTM C39. Cube-shaped samples of (10x10x10 cm) were prepared and cured for 28 days before testing. The splitting tensile strength test was measured, using the standard test method (ASTM C494). Cylindrical-shaped samples (20 cm height and 10 cm diameter) were produced and immersed in water for 28 days before testing. The flexural strength test was in accordance with the standard test method (ASTM C78-94) using a small simple beam (10×10×60 cm) with third-point loading. UTM (1500 kN) equipment was used for the test. The chloride migration test method was in accordance with the standard test (JSCE-G571-2003).

The chloride migration test by cylindrical concrete samples (4 inches diameter) were made using the ternary blended mixtures and the control mixture, as shown in Table 2. Each concrete sample was soaked in water for 28 days. Then, the concrete was cut into slices (2 inches thick) and installed in the testing device. The movement of chloride

ions was determined based on electrolysis using the chloride migration test and Nernst Planck's equation (modified Fick's second law) (Polder R.B, 1996) from the JSCE -G571-2003 standard. The sample was soaked in distilled water for 24 hours and then an electric potential difference of 12 V was applied and the amount of chloride ions in the solution was measured every 5 days using a chloride analyzer.

Other studies have investigated the coefficient of chloride diffusion in concrete for a steady state (steady state diffusion) (Goto S, and Roy, D.M., 1981), which may be quite slow and time consuming, especially for high performance concrete. For this reason, an effective method is applied to accelerate the movement of chloride ions in hardened concrete by means of "electrical conduction" or "migration" [El-Belbol S.M, and Buenfeld, N.R., 1988 and McGrath P.F and Hooton R.D., 1996]. The test uses a direct current electric potential difference to apply power to the positive and negative terminals on either side of the test specimen. Chloride ions penetrate the concrete sample and are deposited in the solution at the anode. Chloride permeability can be determined based on the steady-state chloride flux, the conduction current, or the steady-state chloride permeability.

With non-steady state diffusion and conduction, Nernst Planck's second law or Fick's second law is used to describe the transport of chloride ions in the accelerated chloride test, as shown in Equation 1:

$$\frac{dC}{dt} = -\frac{dj}{dx} = D\left[\frac{d^2C}{dx^2} - \frac{zF\Delta V}{RTl} * \frac{dC}{dx}\right] \quad (1)$$

The exact analytical solution for Equation 2 is:

$$C = \frac{Co}{2}\left[e^{\alpha x} * erfc\left(\frac{x+\alpha Dt}{2\sqrt{Dt}}\right) + erfc\left(\frac{x-\alpha Dt}{2\sqrt{Dt}}\right)\right] \quad (2)$$

where: $$\alpha = \frac{zF\Delta V}{RTl}$$

and Z = the ion valence, F = Faraday's constant, ΔV= the potential drop across the sample, R = the gas constant, T = the temperature in Kelvin, l = the sample length, C = the pore solution concentration at any depth and time t, and C is the pore solution concentration at the surface [McGrath P.F, and Hooton R.D., 1996].

For the case of a steady state conduction flux, where the diffusion component is small, Equation 3 simplifies to:

$$J_{cl} = D_e \frac{z_{cl} F C_{cl} (\Delta E - \Delta E_c)}{RTl} \quad (3)$$

Where J_{Cl} = the flux of chloride ions in a steady state (mol/(cm^2 /year) , D_e = effective diffusion coefficient (cm^2 /year), R = the gas constant (8.31 J/(mol K)), Z_{Cl} = charge of chloride ion (-1), L = the length (mm), F = Faraday's constant (96,500 C/mol), C_{Cl} = the chloride ion concentration (mol/l), ΔE - ΔE_C= the potential gradient (V), and T = time

For the test, a cylindrical concrete sample was prepared with a diameter of 4 inches and incubated for 28 days. After that, the concrete was cut to measure the movement of chloride ions based on electrolysis using the chloride migration test using Nernst Planck's (Modified Fick's second law) [14] from the JSCE–G571-2003 standard. Each sample was immersed in distilled water for 24 hours, then an electric potential difference of 15 V was applied, as shown in Figure 4 (c), and the chloride content was measured in the solution every 5 days using a chloride analyzer, as shown in Figure 1 (a) and (b).

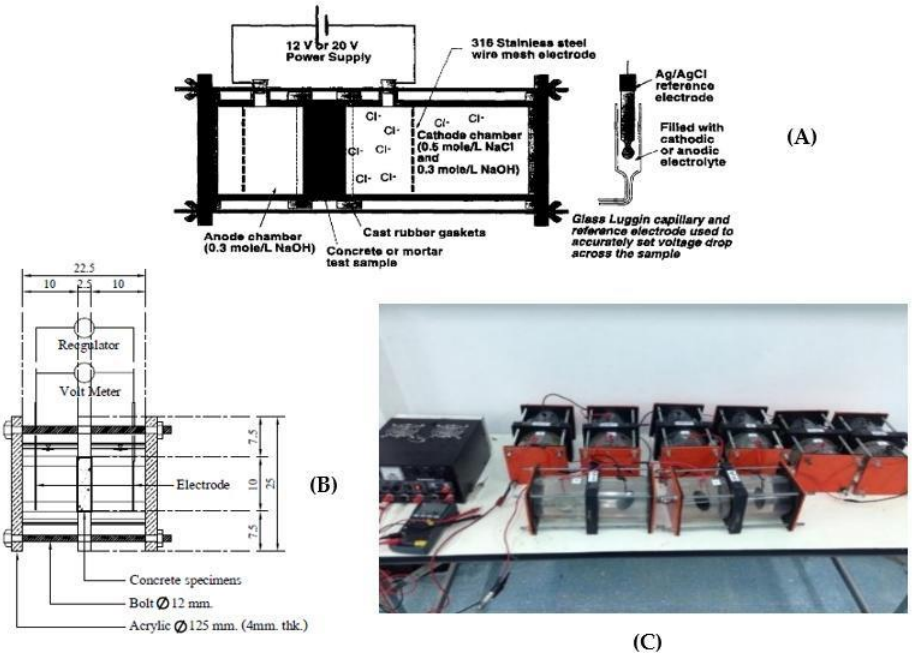

Figure 1 (a) shows amount of chloride released from the concrete to accumulate in solution based on chloride migration test, (a) [McGrath P.F, and Hooton R.D., 1996] and (c) Equipment and specimens for testing chloride ion mobility based on electrolysis.

3. Results and Discussion

3.1 Compressive Strength

Figure 2 shows the results of the compressive strength of concrete after 28 days that was equal to 450 kg/cm^2. Using the lowest ratio (w/b = 0.40) produced better results than from 0.45 or 0.50, with the cement with the highest compressive strength being for M/PFA20/MK15 (580 kg/cm^2) that was 26% more than for the control, 16% more than for M/PFA25/MK10, and more than M/PFA20/SP5 and M/PFA25/SP2.5 by 10% and 18%, respectively. Thus, the mixtures including meta kaolin (10–15%) had 5–9% higher compressive strength than from using an increased amount of silica powder (2.5–5%), which were considered to little different in practice, similar to the mixtures with increased fly ash content (20–25%) not being very different in compressive strength. According to the research reported [10], it affects the improvement of concrete properties in terms of reaction sensitivity and the characteristics of increasing the amount of cementitious CSH and CAH from the pozzolanic reaction, resulting in a micro filler effect decreasing the number of gaps. and slowing the movement of the solution making the concrete more dense, with increased mechanical properties and durability [Li G., 2004 and Shafiq N et al., 2015].

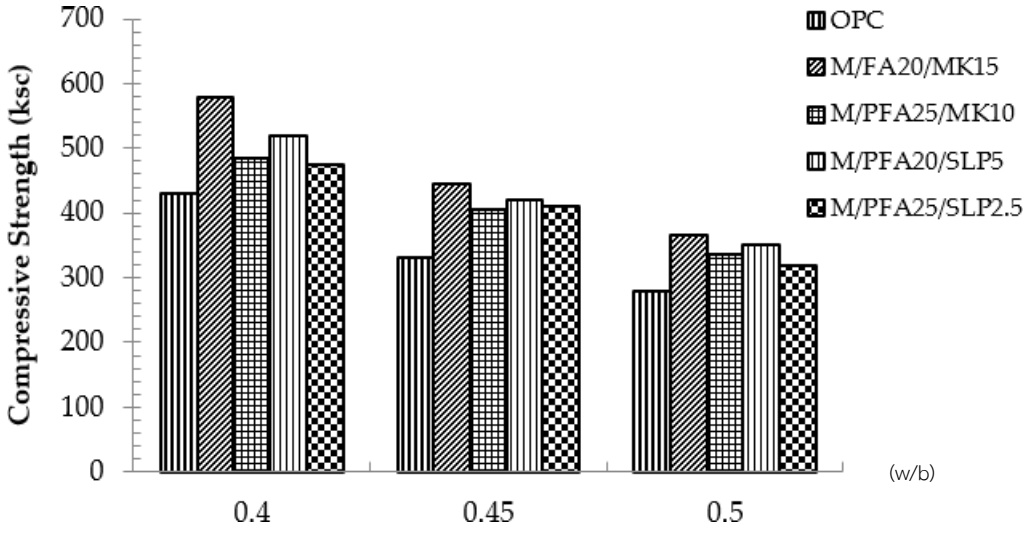

Figure 2. The relationship between compressive strength of control concrete and concrete mixed with tri-interlocking pozzolanic materials (w/b =0.40, 0.45, and 0.50).

3.2 Splitting Tensile Strength and Flexural Strength

From Figure 3, the split tensile strength of concrete after 28 days in the control concrete mix was 24 kg/cm^2 using the lowest water-to-binder ratio (w/b = 0.40), which was a better result than from using either 0.45or 0.50. The hemispherical tensile strength of the M/PFA20/MK15 mix was 35 kg/cm^2, being 31% higher than the control concrete mix. It was observed that the control mixture had a lower tensile strength than every mixture involving the pozzolanic materials, with values in the range 14–31%. From Figure 4, the results of the flexural strength of concrete after 28 days showed that the control concrete mix had a flexural strength of 60 kg/cm^2, using a low water-to-binder ratio. The highest flexural strength was with w/b = 0.40. The results for the flexural strength of the control mixture showed a similar trend to the compressive and tensile strengths of the hemi section. The M/PFA20/MK15 tri-admixture had the highest flexural strength of 69 kg/cm^2 that was 13% higher than for the control concrete mix, with every tri-adhesive mixture producing the same results with the flexural strength being greater than that of the control mixture, with similar values in the range 10–13%.

However, the mixture containing 10–15% MK developed higher compressive strength than from using 2.5–5% SP. The compressive strength of MK is 5–9% greater than that of silica powder which is not considered to much different in practice. The mixtures with 20–25% fly ash content and ternary blended had very different compressive strength values. Various researchers have reported the improved properties of concrete in terms of reactivity and other characteristics due to the increased amount of cementitious C-S-H and C-A-H from the pozzolanic reaction, resulting in a micro filler effect, filling in voids and slowing the movement of the solution as the concrete is denser, with increased mechanical properties and durability. In addition, SP contains a relatively high amount of SiO_2 and a high specific surface area, which are beneficial to the pozzolanic reaction, causing increased CSH with a low Ca/Si ratio and greater compression power [Shafiq N et al., 2015 and Qureshi L.A et al., 2020].

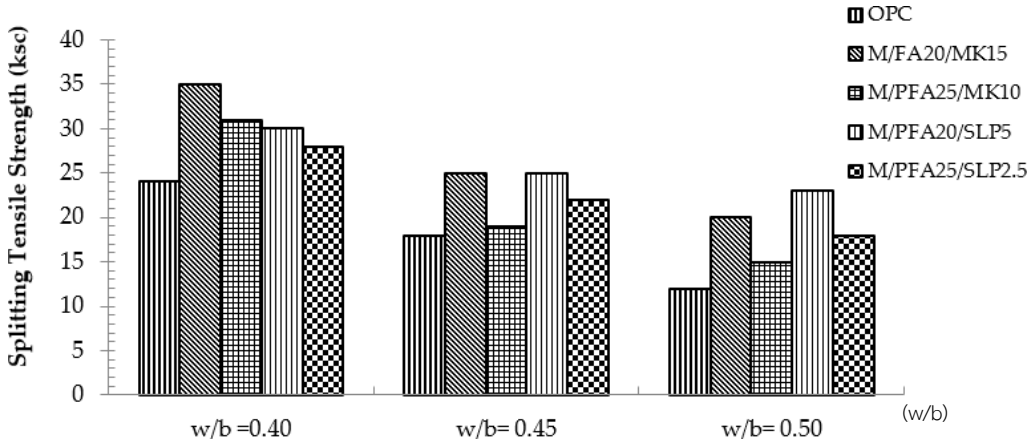

Figure 3 The relationship between tensile strength of control concrete and concrete mixed with pozzolanic materials (w/b =0.40, 0.45, or 0.50).

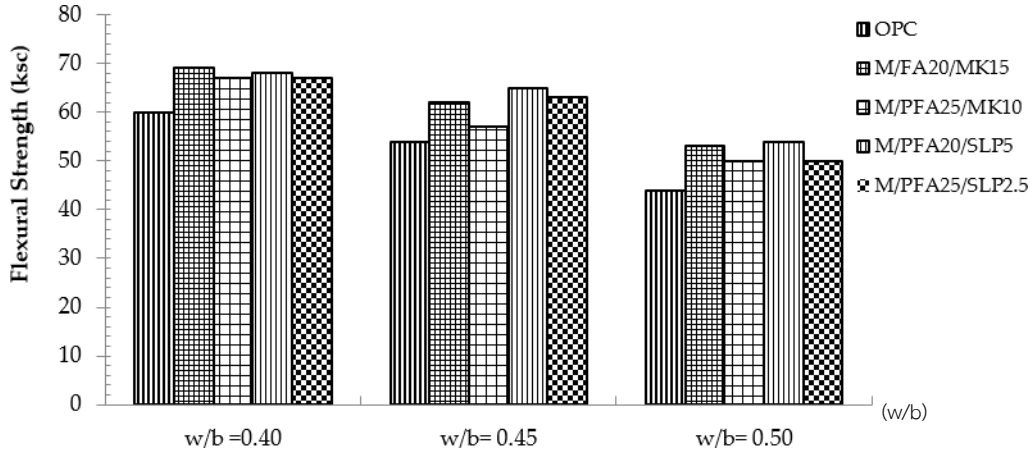

Figure 4 The relationship between flexural strength of control concrete and concrete mixed with pozzolanic materials (w/b =0.40, 0.45, or 0.50).

3.3 Chloride Migration Test

The results of the chloride migration test in terms of the flow rate (Flux, J_{Cl}) of chloride ions and the diffusion coefficient of the concrete in the test were used as data to predict the reduction of chloride content based on electrochemical methods to compare with the laboratory experimental results.

From Figure 1, considering the movement of chloride ions based on electrolysis in concrete aged 28 days, the w/b ratio of 0.40 produced the best cumulative movement of chloride content compared to w/b =0.45 and 0.50. The mixture of control concrete at an initial age of 5 days had an accumulated chloride amount of 200 mg/l, which continued to increase until 35 days of age, with an accumulated chloride amount of 1,300 mg/l. In contrast, at age 5 days, of the three cementitious mixtures, the lowest decrease in permeability by chloride ion content was for the mixture M/PFA25/SLP2.5 (50 mg/l), reduced by 25% compared to the control mixture, while the other mixtures tended to decrease similarly. The greatest decrease in chloride permeability was for the 35-day-old mixture of M/PFA25/SLP2.5 with reductions of 12, 14, 18, and 27% compared to the control mixture: M/PFA20/SLP5, M/PFA25/MK10, and M/PFA20/MK15, respectively.

These results suggested that the ternary blended mixtures M/PFA25/SLP2.5 and M/PFA20/SLP5 were more effective in reducing the amount of chloride permeability in ternary blended concrete than the other tested material mixes. The SiO_2 content in the SP from the pozzolanic reaction changed some calcium hydroxide in the concrete to cementitious materials (C-S-H, C-A-H), helping to improve various properties of the concrete, such as water permeability and durability [Ustabaş İ, and Kaya, A., 2018] In addition, the small kaolin particles helped to improve the quality of the concrete by filling small spaces (the microfiller effect), reducing porosity, and making the concrete more opaque, resulting in higher concrete strength and durability [Malhotra V et al., 2000 and Hasan Z.A et al., 2021]. The optimal amounts of fly ash, metakaolin, and silica powder to replace cement to improve concrete properties were approximately 2.5–5% by weight.

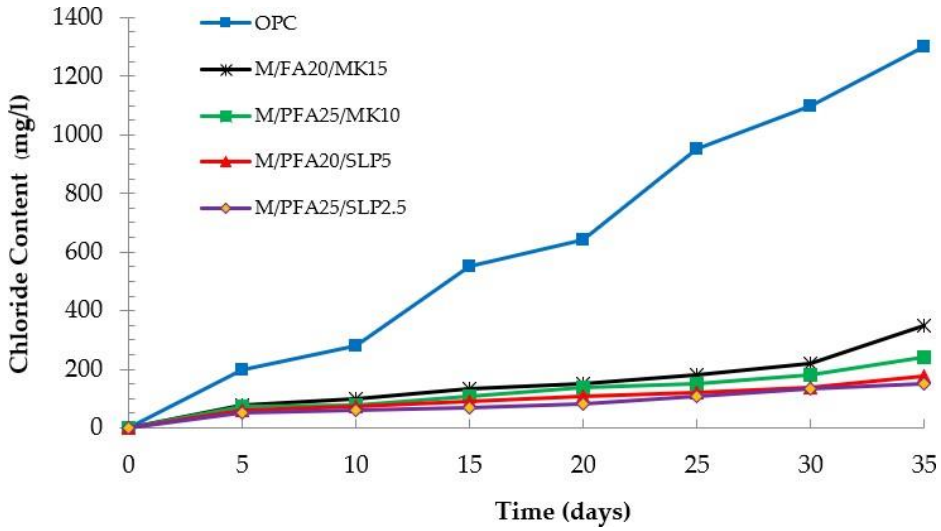

Figure 5. Amount of chloride accumulated in (NaOH) solution of ternary blended concrete and control samples based on chloride migration test (w/b =0.40).

4. Conclusions

The waste materials were investigated from an electric plant for use in natural concrete works by incorporating fly ash, metakaolin and silica powder as SCM use mixes with concrete. In addition, the effects were investigated of the SCM ternary blended systems on the freshness and hardness properties of concrete and mortar cement. The main conclusions drawn are provided below.

In addition, every ingredient of the ternary blended system used a different amount of HRWR (superplasticizer), especially the mixture containing silica powder that contained 0.75% more HRWR water-reducing agent than the mixture containing kaolin that contained 0.50% because the silica powder had a higher specific surface area, resulting in more water absorption than the mixtures containing metakaolin.

The mechanical properties (Compression strength, Flexural strength and split tensile strength) were all positively affected when the cement was partially replaced with 20% FA, 15% MK and 5% SP. The addition of 2.5-5% SP slightly increased the mechanical strength of the concrete in contrast with the partial replacement of cement with FA and MK that had greater silica and alumina oxide contents than silica, which improves the mechanical properties of concrete, resulting in higher strength.

Using the three coordinated ingredients of M/PFA25/SP2.5 and M/PFA20/SP5 was more effective in reducing the amount of chloride permeability in the three-cement concrete system than any of the other mixtures. Due to the amount of SiO_2 in the silica powder from the pozzolanic reaction, some calcium hydroxide in the concrete was changed to cementitious materials (CSH, CAH), helping to improve various properties of the concrete such as water permeability and durability. In addition, metakaolin clay improved the quality of the concrete by inserting kaolin particles into small voids (micro filler effect), reducing porosity and making the concrete more opaque, resulting in the concrete having greater power and durability.

The studied binary blended systems produce very similar pozzolanic reaction results, especially metakaolin yields more alumina than silica and produces more C-S-H and C-A-H products in the mortar. The CaO/SiO_2 ratio was low, resulting in an increased number of nuclei and higher opacity. This C-S-H gel partially fills the pores of the voids, causing the cement paste to have an increased density in the Interfacial Transition Zone (ITZ).

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Electrochemical Behavior and Characterization of Nanochitosan-2, 4-dihydroxybenzaldehyde as a Sensor for the determination of Copper(II) ion

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Abstract

Chitosan nanoparticle-2,4-dihydroxybenzaldehyde was synthesized via the reaction between the 2-amino groups of nanochitosan and 2,4-dihydroxybenzaldehyde, yielding 75%. Chemically modified rotating gold disk electrodes incorporating chitosan nanoparticle-2,4-dihydroxybenzaldehyde were prepared and employed as sensors for the detection of copper(II) ions. The electrochemical characterization of copper (II) at the modified electrodes was investigated using cyclic voltammetry. Optimal performance was achieved with a paste composition of 74% (w/w) graphite powder, 5% (w/w) chitosan, and 21% (w/w) paraffin oil in a 0.2 M sodium acetate solution at pH 7, using a scan rate of 0.8 V/s, a deposition time of 40 s, and an equilibration time of 25 s. A linear response was observed in the range of 1 to 10 mg/L, with a correlation coefficient of 0.98 and a detection limit of 0.176 mg/L.

Keywords: Copper, Sensor, Nanochitosan, Modified Electrode

1. Introduction

Copper is an important element in biological systems. It is an essential micronutrient involved in at least thirty enzymatic processes. However, it becomes toxic above a certain concentration. The World Health Organization (WHO) and the European Water Quality Directive recommend that the concentration in drinking water should not exceed 2 mg/L (Dalibor et al., 2011).

In recent years, the application of electrochemical methods using chemically modified electrodes has attracted significant interest in various areas of research and development, particularly for biosensors and electroanalytical techniques (Martínez-Huitle et al., 2010). Modified electrodes are those that incorporate chemically active species, achieved through the immobilization of a modifying agent on the surface of a base electrode. This process allows for the control and customization of the physicochemical properties at the electrode–solution interface, thereby enhancing the reactivity and selectivity of the base sensor. Electrode modification can be performed in various ways using different materials (Souza et al., 1997), such as carbon paste electrodes modified with DTPT (3,4-dihydro-4,4,6-trimethyl-2(1H)-pyrimidine thione) (Abbaspour and Moosavi, 2002), and nanocomposites based on polyaniline (PANI) and single-walled carbon nanotubes (SWCNTs) modified with ethylenediaminetetraacetic acid (EDTA) chelating ligands (Megha et al., 2018) for the potentiometric determination of Cu(II).

Chitosan has been identified as an effective reactant for the chemical extraction and determination of metals. The amino groups ($–NH_2$) of chitosan can react with aldehydes or ketones to form imines, commonly known as Schiff bases (Nuriye et al., 2012). It has been reported that chitosan-based Schiff bases exhibit excellent chelation ability with heavy metal ions. Multidentate Schiff bases have been widely used as ligands because they readily bind to metal ions through the formation of highly stable coordination compounds. Additionally, these compounds have recently been utilized as ionophores in metal ion sensors (Kucukkolbasi et al., 2013).

Modified carbon paste electrodes have gained increasing attention in recent years due to their desirable properties, such as ease of preparation and suitability for electrochemical detection

In this study, a chitosan-modified rotating gold disk electrode was developed and applied for the determination of trace levels of copper ions (Cu^{2+}).

2. Methodology

2.1 Apparatus and Electrochemical Measurements

The electrochemical performance of the prepared electrode for the detection of copper(II) ions was evaluated using cyclic voltammetry (CV). All measurements were conducted at room temperature using a VA 797 Computrace Electrochemical Analyzer (Metrohm). A conventional three-electrode system was employed, consisting of a rotating gold disk electrode modified with the sensing material as the working electrode, an Ag/AgCl (3 M KCl) electrode as the reference electrode, and a platinum wire as the auxiliary electrode.

2.2 Preparation of Chitosan Nanoparticles (CN)

Sodium tripolyphosphate (TPP) was dissolved in deionized water, while chitosan was dissolved in 1% (v/v) acetic acid. Chitosan nanoparticles (CN) were produced by the dropwise addition of 2 mL of TPP solution into 10 mL of chitosan solution. The gelation process was carried out under constant magnetic stirring, followed by ultrasonication at room temperature. The resulting nanoparticles were separated by centrifugation at 15,000 rpm and 4 °C for 30 minutes. The supernatant was removed, and the CN were rinsed with deionized water and subsequently freeze-dried (Nuriye et al., 2012).

2.3 Preparation of Chitosan Nanoparticles – 2,4-dihydroxybenzaldehyde

One gram of chitosan nanoparticles (CN) was added to 100 mL of methanol, and the mixture was continuously stirred to obtain a homogeneous solution. Subsequently, an equimolar amount of 2,4-dihydroxybenzaldehyde—relative to the nitrogen content in CN—was added to the mixture. The reaction mixture was refluxed for 48 hours. The resulting chitosan Schiff base (CNSB) was separated by centrifugation at 15,000 rpm and washed several times with ethanol. After drying in a vacuum oven at 50 °C for 24 hours, a yellow powder of CNSB was obtained (Nuriye et al., 2012).

2.4 Preparation of Chitosan Nanoparticle-2,4-dihydroxybenzaldehyde modified electrode

The modified electrode was prepared by manually mixing 73.7% graphite powder with 5.3% chitosan nanoparticle–2,4-dihydroxybenzaldehyde, followed by the addition of 21% paraffin oil in a mortar. The mixture was thoroughly blended for approximately 10 minutes to obtain a homogeneous paste. After homogenization, the paste was packed into the cavity of the working electrode body. The electrode surface was polished with weighing paper until a shiny appearance was observed. It was then used directly for voltammetric measurements without any preconditioning. When not in use, the electrodes were rinsed with distilled water and stored in a refrigerator at +4 °C (Kucukkolbasi et al., 2013).

2.5 Analytical Procedure

A volume of 25 mL of sodium acetate solution (0.2 mol/L) and 3 mL of copper standard solution (1×10^{-3} mol/L) were added to the measuring vessel. A three-electrode system was used, consisting of the modified electrode as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode. Nitrogen gas was purged through the solution for 3 minutes to eliminate dissolved oxygen. The electrochemical behavior of the modified electrode was investigated using cyclic voltammetry (CV). A series of Cu (II) standard solutions were measured under the optimized working conditions described above.

3. Results and Discussion

3.1 Particle Size of Chitosan Nanoparticle (CN)

The particle size of chitosan nanoparticles (CN) was measured using a zeta potential analyzer. The average particle size was determined to be 28.4 nm (Fig. 1), which is smaller than that reported in previous research (33 nm) (Kucukkolbasi et al., 2013).

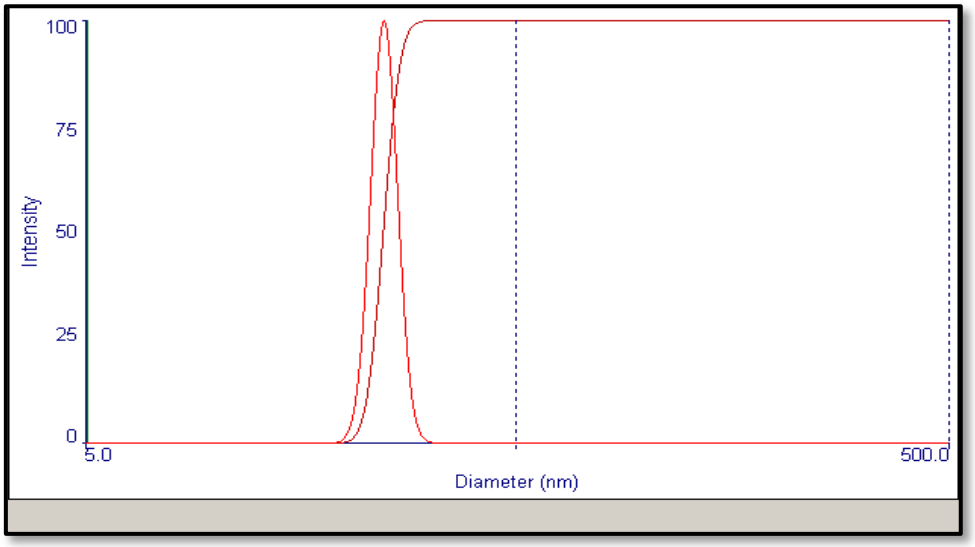

Figure 1 The size distribution by intensity of Chitosan Nanoparticle (CN)

3.2 Electrochemical behaviors of copper (II)

The electrochemical behavior of copper (II) ions on the nanochitosan–2,4-dihydroxybenzaldehyde modified rotating gold disk electrode was investigated using cyclic voltammetry.

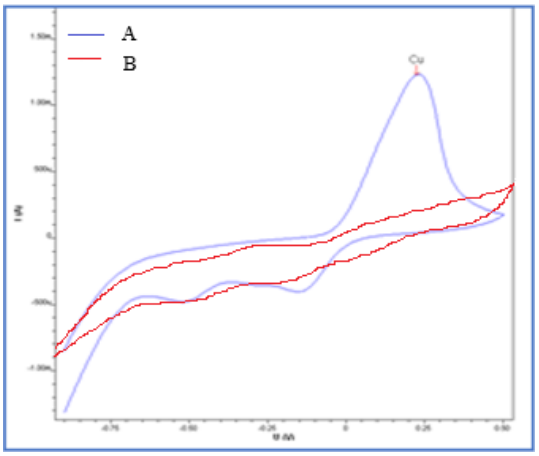

Figure 2 Cyclic voltammograms of copper (II) at (A) nanochitosan–2,4-dihydroxybenzaldehyde modified rotating gold disk electrode and (B) unmodified (bare) rotating gold disk electrode, versus Ag/AgCl in sodium acetate buffer (pH 7.0)

Redox Reactions of Cu(II) on the Nanochitosan–2,4-Dihydroxybenzaldehyde Modified Rotating Gold Disk Electrode. The redox processes of copper on the modified electrode surface are as follows:

$$Cu(s) \rightarrow Cu^{2+}(aq) + 2e^{-} \quad E^{o} = +0.24 \text{ V}$$
$$Cu^{2+}(aq) + e^{-} \rightarrow Cu^{+}(aq) \quad E^{o} = -0.48 \text{ V}$$
$$Cu^{+}(aq) + e^{-} \rightarrow Cu(s) \quad E^{o} = -0.22 \text{ V}$$

The electrochemical behavior of the prepared electrode was examined over a potential range from –1.50 to +0.50 V (vs. Ag/AgCl) using a scan rate of 0.8 V s^{-1}. Measurements were conducted in a copper (II) standard solution using sodium acetate (NaAc) buffer at pH 7.0. The oxidation peak of Cu (II) at the modified electrode was significantly higher than the reduction peaks. These results indicate that the modified electrode facilitates the preconcentration of Cu (II) and substantially enhances the sensitivity for Cu (II) detection. The peak current obtained using the modified electrode was markedly higher than that observed with a bare electrode. This enhanced performance can be attributed to the strong interaction between the Cu (II) ions and the nanochitosan–2,4-dihydroxybenzaldehyde, forming stable coordination complexes. Overall, the nanochitosan–2,4-dihydroxybenzaldehyde modified rotating gold disk electrode demonstrates high efficiency and sensitivity for the determination of Cu (II) ions.

3.3 Optimization of analytical conditions

1) pH and Supporting Electrolyte

The effect of pH on the Cu(II) signal was investigated in the range of 3.0 to 10.0 in the presence of 1000 mg/L Cu(II). The peak current increased as the pH increased from 3 to 7, then decreased beyond pH 8 (Figure 3). At lower pH (pH < 5) and higher pH (pH > 8), the modifier lost its ability to complex with Cu(II), resulting in a decrease in peak current. This behavior is attributed to the degradation of the nanochitosan–2,4- dihydroxybenzaldehyde structure, which compromises its ability to immobilize Cu(II) ions. The optimal pH was found to be 7.0, and this condition was used in all subsequent experiments.

The choice of supporting electrolyte significantly affects the voltammetric response of the sensor. The effect of various supporting electrolytes—namely HCl, $NaNO_3$, KNO_3, and sodium acetate (NaAc)—on the stripping peak currents of Cu(II) was evaluated at pH 7.0. Among these, the best voltammetric response was observed when using NaAc as the supporting electrolyte.

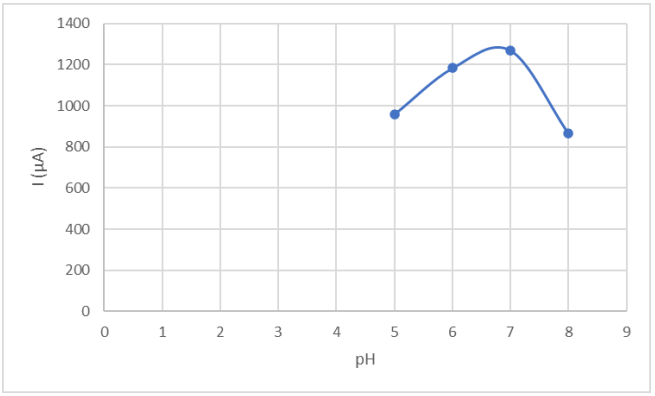

Figure 3 Relationship between peak current and pH for the copper (II) solution

2) Deposition time and Equilibration time

Deposition time significantly influences the determination of Cu (II). The peak currents were investigated over a range of 0–70 s in the presence of 1000 mg/L Cu (II). As expected, the peak current increased with longer deposition times. However, beyond 40 s (Figure 4), the signal began to decrease. This phenomenon is likely due to an initial increase in surface area caused by modified electrode dispersion, followed by a reduction in conductivity as the surface becomes blocked. At higher Cu (II) concentrations, the metal ion uptake rate increases, leading to the rapid saturation of the binding sites on the modified electrode (Dalibor et al., 2011). An optimal deposition time of 40 s was selected for use in the experimental procedures.

Equilibration time was evaluated in the range of 5–30 s, also in the presence of 1000 mg/L Cu (II). The peak current increased as equilibration time was extended, reaching a maximum at 25 s (Figure 5). This equilibration time was used in all subsequent experiments.

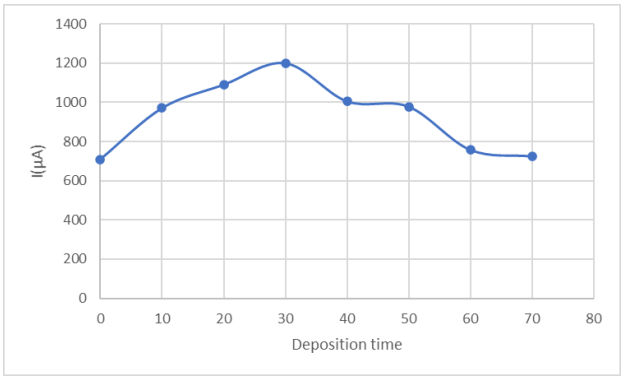

Figure 4 Relationship between peak current and deposition time for the copper (II) solution

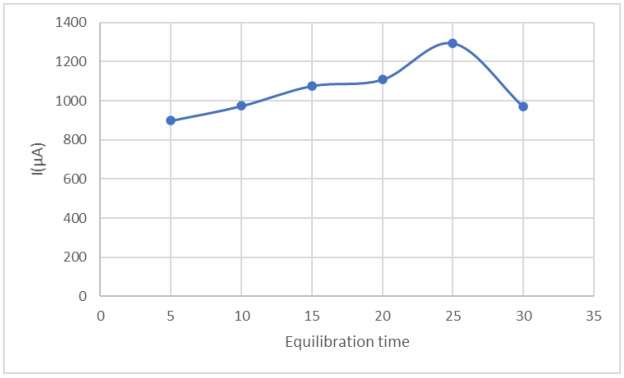

Figure 5 Relationship between peak current and equilibration time for the copper(II) solution

3) Scan rate

The influence of scan rate on the Cu (II) signal was investigated in the range of 0.1–1.0 V/s in the presence of 1000 mg/L Cu (II). The peak current increased with the scan rate, reaching a maximum at 0.8 V/s (Figure 6). Therefore, a scan rate of 0.8 V/s was used in all subsequent experiments.

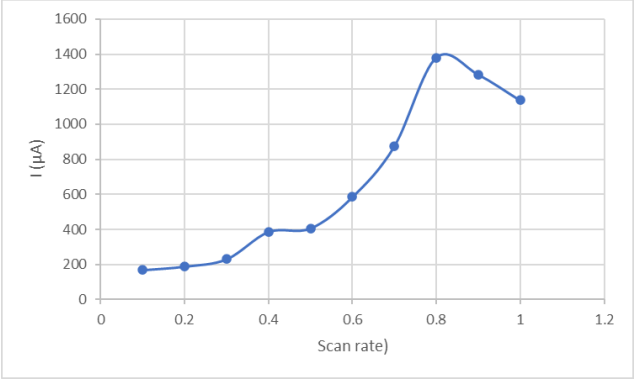

Figure 6 Relationship between peak current and scan rate for the copper(II) solution

4) Ion Interference

Experimental results showed that cadmium and lead ions interfered with copper determination. The copper current decreased as the concentration of interfering metal ions increased, indicating an inverse relationship. In other words, higher concentrations of heavy metal ions led to a significant reduction in the measurable copper signal, as shown in Table 1.

Copper could still be accurately analyzed in the presence of cadmium and lead ions when their concentrations were below 0.01 mg/L. However, at concentrations above 0.01 mg/L, the measured signal values were lower than the actual copper concentrations, resulting in inaccurate analysis.

Table 1 Relative Signal of Copper Standard Solution in the Presence of Different Concentrations of Interfering Ions

Ion interfering	Concentration (ppm)	Relative signal (%)
Cd^{2+}	0.01	97.10
	0.10	90.96
	1.00	58.29
	10.00	10.29
Pb^{2+}	0.01	98.02
	0.10	86.68
	1.00	65.36
	10.00	14.22

5) Linear Range and Detection Limit

The peak current exhibited a linear relationship with Cu (II) concentration in the range of 1 to 10 mg/L, with a correlation coefficient of 0.98. The detection limit (3σ) of Cu (II) using the developed method under optimized conditions was determined to be 0.176 mg/L.

4. Conclusion

In this study, an optimal paste composition was identified, consisting of 74% (w/w) graphite powder, 5% (w/w) nanochitosan, and 21% (w/w) paraffin oil. Nanochitosan was successfully synthesized via ionotropic gelation of chitosan and tripolyphosphate, and subsequently functionalized with 2,4-dihydroxybenzaldehyde to form Schiff base-modified nanoparticles. The synthesized compound was effectively used as a modifier for the rotating gold disk electrode. The major achievement of this work lies in the development of a simple and selective electrochemical sensor for trace-level determination of copper (II) ions using nanochitosan–2,4-dihydroxybenzaldehyde-modified electrodes.

5. Acknowledgements

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Enhancement of Electrical Energy Generation using Banana-Based Electrolytes for Science Education

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Abstract

Renewable energy represents a viable alternative in response to the global decline in fossil fuel resources. Batteries function by storing chemical energy and converting it into electrical energy through electrochemical reactions. In this study, a microbial fuel cell kit was designed and developed as an educational tool aligned with STEAM-based learning for upper secondary school students. Banana pseudostem sap (BPS) was investigated as a potential natural electrolyte for constructing a simple biobattery using zinc and copper electrodes. The primary objective of the experiment was to enhance electrical output by optimizing the fermentation time of the BPS electrolyte. The acidity of the fermented electrolyte, indicated by pH levels, was found to play a critical role in electrical performance. Experimental results demonstrated that a seven-day fermentation period produced the highest output, with a maximum voltage of 0.99 V, electric current of 2.74 mA, and power output of 2.71 mW, corresponding to the lowest pH value of approximately 4.86. These findings suggest that banana-based biobatteries can generate measurable electrical energy, offering a cost-effective and environmentally sustainable approach to green energy storage, particularly in educational and low- resource settings.

Keywords: STEAM Learning, Electrical Energy, Banana Pseudostem Sap (BPS), Electrochemical and Renewable Energy

1. Introduction

The combustion of fossil fuels has been shown to exert profound and far-reaching impacts on both the environment and human health (Cao et al., 2006; Perera et al., 2019). In contrast, renewable energy—derived from naturally replenishing sources—plays a critical role in achieving a sustainable future by reducing environmental degradation, fostering economic development, and enhancing energy security (International Energy Agency, 2007; Patel, 2021). Batteries, in general, are electrochemical devices that convert stored chemical energy into electrical energy through redox reactions. Among various types, biobatteries represent a promising green technology that utilizes plant-based fluids or organic waste to generate environmentally friendly electricity (Golberg et al., 2010; Muske et al., 2007). These systems typically involve copper and zinc electrodes serving as the cathode and anode, respectively. Organic acids function as electrolytes, facilitating the flow of electrons from zinc to copper and thereby producing an electric current (Purnima et al., 2024; Sorey et al., 2012). In the present study, a microbial fuel cell kit was employed as an instructional tool to demonstrate the generation of electrical energy from banana pseudostem sap (BPS). The experiment investigated the influence of fermentation time on the electrical output, with the aim of enhancing students' understanding of renewable bioelectrochemical energy through hands-on learning experiences.

2. Methodology

2.1 Microbial Fuel Cell Kit Assembly

A cost-effective microbial fuel cell (MFC) educational kit was constructed for the experiment. Zinc (Zn) and copper (Cu) electrodes were selected as the anode and cathode, respectively. Each electrode measured 20 mm in width, 2 mm in thickness, and 150 mm in length (W × T × L).

2.2 Electrolyte Preparation

The electrolyte was derived from post-harvest banana pseudostems (BPS), considered agricultural waste. Samples were collected from local farms in Thanyaburi, Pathum Thani Province, Thailand. To ensure cleanliness, the banana trunks were washed three times with distilled water to remove residual dirt. The pseudostem sap was then extracted and prepared in 500 mL volumes, which were stored in sealed glass containers for subsequent fermentation.

2.3 Experimental Procedure

For each trial, 500 mL of the prepared BPS was transferred into the electrolyte compartment of the microbial fuel cell kit. Measurements of temperature, pH, voltage, and electric current were recorded daily at 9:00 AM over a seven-day fermentation period (as illustrated in Figure 1). Each experiment was conducted in triplicate under identical conditions to ensure reproducibility. The power output was calculated using the following equation:

$$P = IV \quad (1)$$

Where P = power (W)
V = voltage (Voltage)
I = current (Ampere)

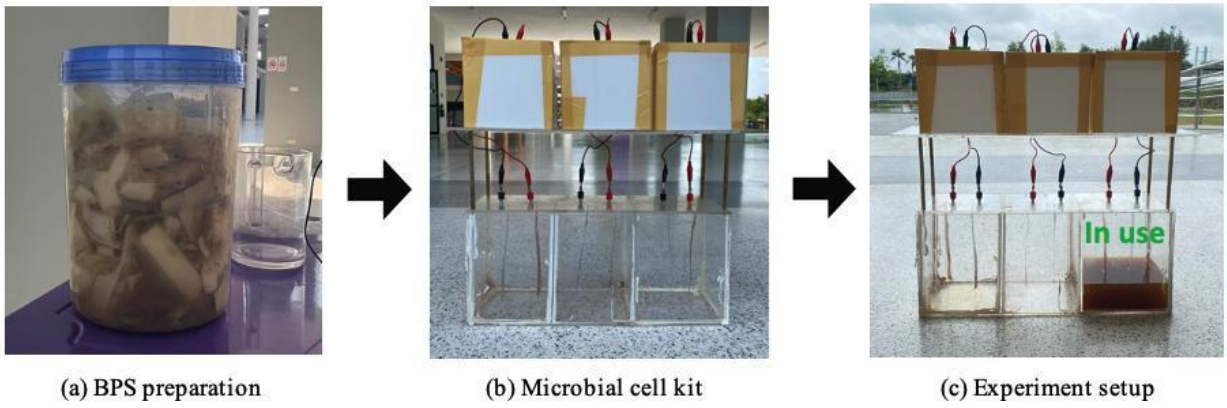

Figure 1 (a) BPS preparation (b) Microbial cell kit (c) Experiment setup

3. Results and Discussion

3.1 Temperature and pH level at fermentation time

The average ambient temperature during the experimental period was approximately 28°C, which lies within the optimal range for yeast fermentation. Temperature is a critical factor influencing microbial activity, particularly that of yeast, which typically thrives between 20°C and 30°C. Maintaining an appropriate temperature range enhances the metabolic efficiency of probiotic microorganisms, thereby facilitating a more effective fermentation process (Walker, 1998; Lee, 2008). The pH values observed throughout the fermentation process exhibited a gradual decline over time, indicating an increase in acidity. The lowest pH value recorded was 5.02 on the seventh day of fermentation, corresponding to the highest level of acidity. This acidification trend is consistent with microbial fermentation activity, as the proliferation of yeast and other microorganisms often contributes to acid production through metabolic byproducts (Vatansever et al., 2017), as summarized in Table 1.

The biobattery system employed in this study operates based on fundamental redox reactions occurring between two dissimilar metal electrodes immersed in an organic electrolyte. Zinc (Zn) and copper (Cu) electrodes were utilized as the anode and cathode, respectively. The electrolyte medium, derived from fermented banana pseudostem sap (BPS), contains organic acids that facilitate ion transport and contribute to the overall conductivity of the system. During operation, the zinc electrode undergoes oxidation by releasing electrons and dissolving into the electrolyte as zinc ions:

Anode (oxidation): $Zn\,(s) \rightarrow Zn^{2+}\,(aq) + 2e^{-}$

These electrons then flow through an external circuit to the copper electrode, where a reduction reaction takes place. Hydrogen ions (H^{+}), derived from organic acids present in the BPS, accept the electrons and form molecular hydrogen:

Cathode (reduction): $2H^{+}\,(aq) + 2e^{-} \rightarrow H_2\,(g)$

This electron flow from the zinc to the copper electrode constitutes the electric current observed in the circuit. The overall cell reaction can be summarized as:

$$Zn\,(s) + 2H^{+}\,(aq) \rightarrow Zn^{2+}\,(aq) + H_2\,(g)$$

The efficiency of the electrochemical cell is influenced by factors such as the acidity (pH) of the electrolyte, electrode surface area, and fermentation duration of the banana sap, which alters the concentration of organic acids (Lee, 2008; Vatansever et al., 2017).

Table 1 Temperature and pH data of electrolyte fermentation time

Fermentation Day	pH	Temperature
1	5.82	29
2	5.75	28
3	5.64	27
4	5.45	27
5	5.24	28
6	5.18	29
7	5.02	29

3.2 Electrical energy

In this experiment, a pair of dissimilar metal electrodes—zinc (Zn) and copper (Cu)—were employed, functioning as the anode and cathode, respectively. During the electrochemical reaction, the zinc electrode undergoes oxidation, releasing electrons and dissolving into the electrolyte as zinc ions (Zn^{2+}). Simultaneously, the copper electrode serves as the site of reduction, where it receives electrons through an external circuit, thereby facilitating the generation of an electric current.

Table 2 Electrical energy measurement of voltage, current and power from Banana pseudostem sap (BPS)

Fermentation Day	Electrical energy		
	Voltage (V)	Current (mA)	Power (mW)
1	0.55	1.25	0.69
2	0.68	1.28	0.87
3	0.75	1.65	1.24
4	0.84	1.95	1.64
5	0.92	2.35	2.16
6	0.95	2.65	2.52
7	0.99	2.74	2.71

Table 2 presents the electrical energy output generated from banana pseudostem sap (BPS) over a seven-day fermentation period. The results indicate a progressive increase in electrical performance correlating with the fermentation duration. This trend is attributed to the rising acidity of the electrolyte, which plays a pivotal role in enhancing the electrochemical activity and facilitating effective energy generation within the system.

Table 2 summarizes the electrical performance metrics—voltage (V), current (mA), and power output (mW)—obtained from the biobattery system using banana pseudostem sap (BPS) as the electrolyte over a fermentation period of seven days. The results reveal a progressive increase in both current and power output from Day 1 through Day 7. The current increased from 1.25 mA on Day 1 to a peak of 2.74 mA on Day 7. Correspondingly, the power output rose from 0.69 mW to 2.71 ± 0.05 mW, demonstrating a consistent enhancement in energy generation efficiency with prolonged fermentation time. This increase in electrical performance correlates strongly with the declining pH of the BPS, indicating increased acidity due to microbial fermentation. The lowest pH value recorded was 5.02 on Day 7, supporting the hypothesis that increased proton availability enhances ionic conductivity and facilitates redox reactions at the electrodes.

1) Electric Voltage value of Banana pseudostem sap (BPS)

The voltage generated from the fermented banana pseudostem sap was measured daily over a seven-day period. The results revealed a consistent upward trend in voltage output as the fermentation progressed. The lowest recorded voltage was approximately 0.55 V on Day 1, while the highest voltage, reaching approximately 0.99 V, was observed on Day 7. This increase in voltage corresponds with the extended fermentation time, suggesting a positive correlation between microbial activity and electrochemical potential. The detailed voltage progression is illustrated in Figure 2.

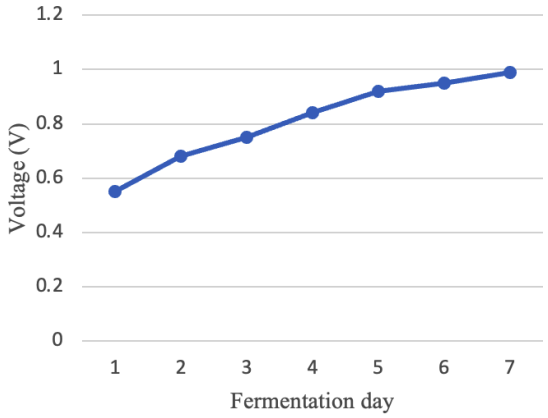

Figure 2 Value of electric voltage of Banana pseudostem sap (BPS)

The voltage output recorded throughout the seven-day fermentation process exhibited a distinct upward trajectory, as depicted in Figure 2. The measured voltage increased progressively from 0.55 V on Day 1 to a peak of 0.99 V on Day 7, reflecting a continuous enhancement in the electrochemical potential of the system over time. This trend can be primarily attributed to the gradual accumulation of organic acids—most notably lactic and acetic acids—resulting from microbial metabolic activity within the banana pseudostem sap (BPS). As the fermentation progressed, the concentration of hydrogen ions (H^+) within the electrolyte increased, leading to a marked decrease in pH. This heightened proton availability significantly improved the ionic conductivity of the medium, which in turn facilitated more efficient electron transfer between the zinc (anode) and copper (cathode) electrodes. This phenomenon is consistent with the Nernst equation, which describes how variations in ionic concentration influence the electrochemical cell potential. Furthermore, the acidic environment enhanced the kinetics of the redox reactions occurring at the electrodes. At the anode, zinc underwent oxidation to produce Zn^{2+} ions and electrons, while at the cathode, hydrogen ions were reduced to molecular hydrogen (H_2). These reactions were more effectively sustained in low-pH conditions, thereby maximizing the electrical output. Overall, the observed correlation between fermentation duration, electrolyte acidification, and voltage generation underscores the pivotal role of biochemical conditions in optimizing the performance of BPS-based biobatteries.

2) Electric current value of Banana pseudostem sap (BPS)

The electric current generated from the banana pseudostem sap (BPS) was measured over a seven-day fermentation period. The results revealed a steady increase in current output corresponding with fermentation duration. The lowest current, approximately 1.25 mA, was recorded on Day 1, while the highest value, reaching 2.74 mA, was observed on Day 7. This trend indicates a direct correlation between microbial activity and current generation, as illustrated in Figure 3.

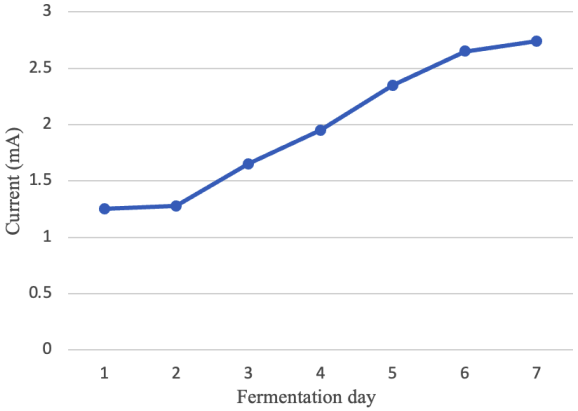

Figure 3 Value of electric current of Banana pseudostem sap (BPS).

The electric current generated from banana pseudostem sap (BPS) exhibited a progressive increase over the seven-day fermentation period. The current rose from an initial value of 1.25 mA on Day 1 to a peak of 2.74 mA on Day 7, indicating a continual enhancement in electrochemical activity in conjunction with microbial development. This increasing trend in current output is primarily attributed to the metabolic activity of indigenous microorganisms present within the BPS. Throughout fermentation, microbial species such as *Lactobacillus* spp. and *Acetobacter* spp. metabolize complex polysaccharides and organic substrates into simpler metabolites, predominantly short-chain organic acids, including lactic and acetic acid. These fermentation-derived acids incrementally elevate the concentration of hydrogen ions (H^+) in the electrolyte, leading to a corresponding reduction in pH. The accumulation of H^+ ions enhances the ionic conductivity of the electrolyte, thereby improving the mobility of charge carriers between electrodes. This improvement reduces internal resistance within the electrochemical cell, facilitating more efficient electron transfer through the external circuit. The redox reactions governing this process can be described as follows:

· Anodic reaction (oxidation): $Zn\,(s) \rightarrow Zn^{2+}\,(aq) + 2e^-$

· Cathodic reaction (reduction): $2H^+\,(aq) + 2e^- \rightarrow H_2\,(g)$

Within this redox system, the zinc electrode functions as the electron donor, while hydrogen ions serve as the electron acceptors at the copper electrode. As fermentation progresses and acidity intensifies, the availability of protons at the cathode interface increases, subsequently accelerating the reduction kinetics and enhancing overall current output. This synergistic interplay between microbial acidogenesis and electrochemical processes underscores the pivotal role of fermentation in optimizing biobattery performance. The sustained increase in current not only reflects the dynamic maturation of microbial populations but also highlights the direct influence of biochemical transformations on the electrochemical efficiency of the system.

3) Power value of Banana pseudostem sap (BPS)

The power output generated from the fermented banana pseudostem sap substrate over a seven-day period exhibited a notable upward trend. The lowest power value, approximately 0.69 mW, was recorded on Day 1, while the highest value, reaching 2.71 mW, was observed on Day 7. This increase in power output corresponds closely with the concurrent rise in both voltage and current as fermentation progressed. These findings highlight the direct influence of extended fermentation time on electrochemical performance, as illustrated in Figure 4.

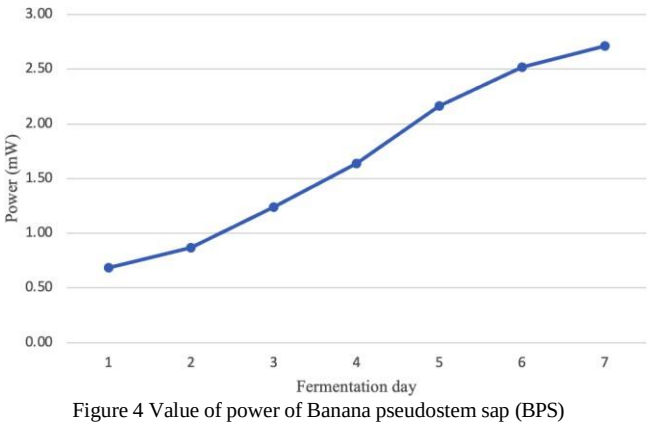

Figure 4 Value of power of Banana pseudostem sap (BPS)

The experimental results indicate that fermentation duration plays a crucial role in determining the electrical performance of the biobattery system. An extended fermentation period was positively correlated with increased values of both voltage and current, reflecting enhanced electrochemical activity over time. This improvement is primarily attributed to microbial metabolism, wherein naturally occurring microorganisms decompose fermentable sugars into organic acids. These biological transformations release free electrons and ions, which are key contributors to electrical energy generation (Sigalingging et al., 2022).

Among the critical physicochemical factors, electrolyte acidity exerts a substantial influence on the efficiency of electricity generation. Increased acidity results in a higher concentration of free hydrogen ions (H^+), which significantly enhances the ionic conductivity of the electrolyte. A more conductive medium facilitates faster ion migration and more efficient charge transfer between electrodes, thereby reducing internal resistance and increasing current output. Furthermore, elevated H^+ availability at the cathode accelerates reduction reactions, contributing to a greater overall energy yield (Sigalingging et al., 2022; Supratomo, 2019). Consequently, the progressive acidification of the medium during fermentation emerges as a key determinant in optimizing biobattery performance.

4. Conclusion

A hands-on science experiment was conducted to demonstrate the generation of electrical energy from banana waste through electrochemical reactions, specifically designed for senior high school students. The results confirmed that banana pseudostem sap (BPS), derived from agricultural waste, can be effectively converted into electrical energy. The system achieved a maximum voltage of approximately 0.99 V and an electric current of 2.74 mA, with both parameters exhibiting a consistent increase over the seven-day fermentation period. The observed enhancement in electrical performance is closely linked to fermentation-induced acidification, which increases the concentration of hydrogen ions (H^+) in the electrolyte. This rise in acidity improves ionic conductivity and facilitates redox reactions at the electrodes, thereby increasing current and voltage outputs. These findings underscore the feasibility of utilizing BPS as a sustainable and environmentally friendly energy source for low-power applications. Moreover, the experiment highlights the educational and technological potential of agricultural waste in advancing renewable energy innovations.

5. Acknowledgments

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Probiotic-Driven Hair Care Using Combining Herbal Dyes with a Microbiome-Modulating Conditioner

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Abstract

Conventional chemical hair dyes frequently cause adverse effects, including hair damage and scalp irritation, driving consumer interest towards safer, natural alternatives. This study successfully developed an eco-friendly hair care system combining natural herbal dyes (*Clitoria ternatea*, *Curcuma longa*, *Lawsonia inermis*) with a restorative probiotic-enriched conditioner (*Zingiber officinale* prebiotic, *Lactobacillus* spp. probiotics) as a safer alternative to conventional chemical treatments, which often cause hair damage and scalp irritation. Optimized bleaching as the first step prepared and then hair dyeing with butterfly pea achieved the highest initial color intensity (72%), though colorfastness was limited. The ginger-based conditioner supported probiotic viability and improved hair softness and smoothness without observed toxicity. While the green hair care system was refrigerated, its stability was better promising than room temperature, though its long-term preservation (more than 3 months) requires further improvement.

Keywords: Eco-friendly Hair Probiotics, Herbal Hair Dye, Herbal Hair Conditioner Formulation, Scalp health improvement.

1. Introduction

Hair dyeing with conventional chemical formulations presents significant drawbacks, notably causing damage to the hair structure, leading to issues like brittleness, dryness, and breakage (He et al., 2023). Furthermore, these chemical agents, such as ammonia and hydrogen peroxide, can induce scalp irritation and allergic reactions, manifesting as inflammation or rashes (Handa et al., 2012; He et al., 2023; Palaniappan et al., 2024). These adverse effects extend beyond physical discomfort, impacting overall well-being and self-esteem (Smith et al., 2018). Recognizing these issues, a growing segment of health-conscious consumers is actively seeking safer, herbal-based alternatives for hair coloring (Park et al., 2012; Brandwein et al, 2016; Jhamb et al., 2023). While natural hair dyes derived from botanical sources typically offer less vibrant and long-lasting color compared to their synthetic counterparts, they possess a crucial advantage: the absence of known carcinogenic and allergenic substances commonly found in chemical dyes (Brandwein et al., 2016). Regular applications of these natural colorants can contribute to improved color uniformity and potentially enhance user confidence (Kumari et al., 2024). Moreover, many of the botanicals utilized in these formulations offer additional therapeutic benefits for hair health (Siu-Yin et al., 2019). These include mitigating hair loss, managing thinning or prematurely graying hair, and promoting healthy hair growth (Xu et al., 2016; Choi et al., 2024; Patel et al., 2015). As recently concerned to the users, the development of safer hair care practices also necessitates attention to post-dyeing treatments as complementary to the dyeing process, functioning in maintaining and restoring hair health. Conditioners typically contain ingredients like proteins, silicones, vitamins, and plant oils that help smooth the cuticle, retain moisture, and improve strength and resilience (Fernandes et al., 2023; Leite &Campos, 2018).

Recently works have expanded to include probiotics—beneficial live microorganisms such as *Lactobacillus* spp.—within cosmetic and scalp care formulations (Yin et al, 2024; Siu et al., 2019; Clayaud et al., 2013; Park et al., 2012; Pagac et al., 2024). These microorganisms, naturally topically present on the skin and scalp, can help maintain a healthy microbial balance, potentially preventing infections and improving overall hair and scalp condition (Theodorou et lk et al., 2024; Sash et al, 2024). To further enhance the efficacy of probiotics, prebiotics—non-digestible compounds that foster the growth of beneficial microbes—can be incorporated (Bermudez-Brito et al., 2012). Certain herbal extracts, such as *Zingiber officinale* (ginger), can serve as natural prebiotics, providing nourishment for probiotics and contributing additional anti-inflammatory and antimicrobial properties beneficial to scalp health (Tan et al., 2020; Habeebuddin et al., 2022). the dyeing properties of aqueous extracts from *Clitoria ternatea* (butterfly pea) (Hariadi et al., 2018), *Curcuma longa* (turmeric) (Suryawanshi et al., 2017), and *Lawsonia inermis* (henna) (Singam et al., 2020) have been long used for natural herbal dyes for hair dyeing as ecofriendly and non-toxic approach compared to common chemical hair dyes ((Cui et al, 2020; Packianathan et al., 2010; Adeel et al., 2018)

Therefore, from overall, this research objective was to develop and evaluate an innovative, natural hair care system composed of two synergistic components: a herbal hair dye and a probiotic-enriched conditioner. Specifically, the study investigated the dyeing properties of aqueous extracts from *Clitoria ternatea* (butterfly pea), *Curcuma longa* (turmeric), and *Lawsonia inermis* (henna). Simultaneously, a restorative conditioner was formulated, incorporating probiotics derived from yogurt (*Lactobacillus* spp.) and a prebiotic-rich *Zingiber officinale* (ginger) extract. The overarching aim was to determine the feasibility of this dual system as a safer, efficacious, and sustainable alternative to conventional chemical hair treatments, addressing consumer demand for non-toxic personal care products. Key evaluation parameters included dye performance, conditioning effects, toxicity on biological models, and preliminary stability under various storage conditions.

2. Methodology

An integrated approach to improving hair and scalp health through hair dyeing and conditioning involves the following steps, presented in sequential order below.

2.1 Preparation and Determination of Herbal Extract Concentration for Dyeing

This step outlines the process of obtaining and preparing the herbal extracts intended for hair dyeing and determining the appropriate concentrations for effective coloration to obtain and evaluate the bioactive compounds from selected Thai herbal plants—butterfly pea (Clitoria ternatea), turmeric (Curcuma longa), and henna (Lawsonia inermis)—these herbs were utilized as natural hair dyes due to their pigment and antioxidant properties. The herbs were individually weighed (butterfly pea: 0.15 g; turmeric and henna: 4 g each), boiled with 50 ml of distilled water for 1 hour, and filtered to obtain clear aqueous extracts. Each filtrate was further diluted with 10 ml of distilled water. Photographic documentation was made for extraction, filtration, and dilution steps. The concentration of each extract was calculated using a standard formula using the following formula:

$$\text{Concentration (mg/ml)} = \frac{\text{Weight of dry herbal material (mg)}}{\text{Final volume of extract (ml)}}$$

2.2 Hair Preparation and Bleaching Process

Hair bleaching is the essential preliminary step undertaken to ensure a controlled and accurate evaluation of the herbal dyes' efficacy. This crucial preliminary step details the preparation of hair samples to ensure uniform dye uptake and evaluation. To achieve this, the natural melanin pigment within the hair samples must first be removed. This decolorization creates a uniform, light canvas, enabling optimal absorption of the herbal colorants and allowing for a clear assessment of their dyeing properties, uninfluenced by the hair's original shade. Hair samples were standardized for size, cleanliness, and condition. Each bundle (2 g) was divided into two groups: Group A (treated with perchloric acid ($HClO_4$) at concentrations of 2%, 5%, and 12%) and Group B (treated with hydrogen peroxide and ammonia (H_2O_2, 6–12%) and ammonia (NH_3, 6M). These bleaching procedures includes two distinct bleaching methods:

1) Bleaching with Perchloric Acid

This specific procedure using perchloric acid was to remove natural hair pigment. Hair samples were treated with varying concentrations of perchloric acid ($HClO_4$) to investigate the effect of a strong acid on melanin bleaching in hair fibers, providing a comparison with conventional bleaching methods. Three treatment groups were prepared: (1) 2% $HClO_4$ diluted in 250 mL of distilled water, (2) 5% $HClO_4$ diluted in 240 mL of distilled water, and (3) 12% $HClO_4$ used without dilution. The hair samples were fully immersed in each solution and left at room temperature (approximately 25°C) for 30 minutes. After treatment, the samples were rinsed with distilled water and air-dried before further analysis.

2) Bleaching with Hydrogen Peroxide and Ammonia

In the alternative, more conventional bleaching method, hair samples were treated with a combination of ammonia (NH_3) and varying concentrations of hydrogen peroxide (H_2O_2). This method aimed to replicate a standard commercial bleaching procedure, allowing for a direct comparison with the bleaching effects of strong acid. According to most referred by commercialized hair dyeing usage, four treatment groups were selectively prepared using H_2O_2 at concentrations of 6%, 9%, or 12%, each mixed with 6 M NH_3. The hair samples were fully immersed in each mixture and treated for four different durations: 5, 15, 30, and 45 minutes at room temperature (approximately 25°C). After treatment, the samples were rinsed thoroughly with distilled water and air-dried before further analysis.

2.3 Hair Dyeing with Herbal Extracts

This section details the application of the prepared herbal extracts onto the bleached hair samples to achieve coloration. The extracted dyes were applied to both bleached and unbleached samples to assess their dyeing effectiveness. Hair samples were immersed in herbal extracts for 5, 10, 30, and 45 minutes, then rinsed, shampooed, and dried. The optimized method involved bleaching with H_2O_2 + NH_3 for 15 minutes and dyeing for 30 minutes, repeated three times for enhanced retention.

2.4 Development of Post-Dyeing Herbal Hair Conditioner with Probiotics

The formulation of a conditioner designed to be used after the herbal dyeing process, incorporating both herbal extracts and probiotics for hair and scalp health. With a natural conditioner developed to restore hair health after dyeing, the herbal and probiotic elements to nourish and protect the hair and scalp involve several sub-steps:

1) Preparation of Probiotic Culture

Details the cultivation and preparation of the probiotic microorganisms to be included in the conditioner. Probiotic bacteria were isolated from 0.5 ml of concentrated yogurt (~10^6–10^7 CFU/ml) via centrifugation at 4,000 rpm. The pellet was re-suspended in distilled water. Nutrient broth (NB) was prepared with: peptone 10 g, meat extract 8 g, yeast extract 4 g, glucose 4 g, sodium acetate 5 g, Tween 80 1 g, K_2HPO_4 2 g, $MgSO_4$ 0.04 g. NB (5 ml) was dispensed into 10 test tubes, to which 1 ml of each herbal extract and 0.5 ml of probiotic culture were added and serially diluted. Tubes were incubated at 37°C for 24 hours, and bacterial growth was confirmed microscopically.

2) Preparation of Herbal Extract for Use in Conditioning Solution

The process of extraction and preparation was carried out to yield specific herbal extracts possessing beneficial properties suitable for the conditioning formulation. To prepare the ginger extract for a conditioning cream, 100 g of thinly sliced ginger (Zingiber officinale) was soaked in 70% ethanol for 72 hours, filtered, and subsequently dried by evaporation at 55°C, yielding 2.13 g of dry extract per 50 ml of processed liquid. This extract was then incorporated into a hair conditioning cream designed for use after herbal hair dyeing, which was formulated by melting and combining coconut oil and beeswax with pre-warmed aloe vera and the ginger extract. A water phase was gradually added to this oil phase under stirring to create an emulsion. After cooling to 40°C, probiotic biomass (obtained from centrifuged yogurt), vitamin E, and essential oil were added. The final conditioner was stored in sterile, dark containers at 4°C and is intended for application to damp hair and scalp after shampooing, where 2–5 ml is gently massaged in, left for 3–5 minutes, and then rinsed with warm water.

3) Developing Hair Conditioner Formulation

The process of combining the probiotic culture, herbal extracts, and other ingredients was to create the final hair conditioner product. According to previous data used in our lab (data not shown), the formulation consisted of the following ingredients:

Ingredient	Function	Amount
Coconut oil (cold-pressed)	Base oil; moisturizes and conditions hair	20 ml
Ginger extract (Zingiber officinale)	Stimulates scalp, adds shine, antifungal	10 ml
Aloe vera gel (100% pure)	Soothes scalp, hydrates hair	10 ml
Probiotic biomass (Lactobacillus spp.)	Scalp microbiome balance, hair strength	5 ml
Beeswax	Emulsifying agent, added texture	3 g
Vitamin E oil	Antioxidation, prevents oxidative damage	5 drops
Distilled water	Solvent	50 ml

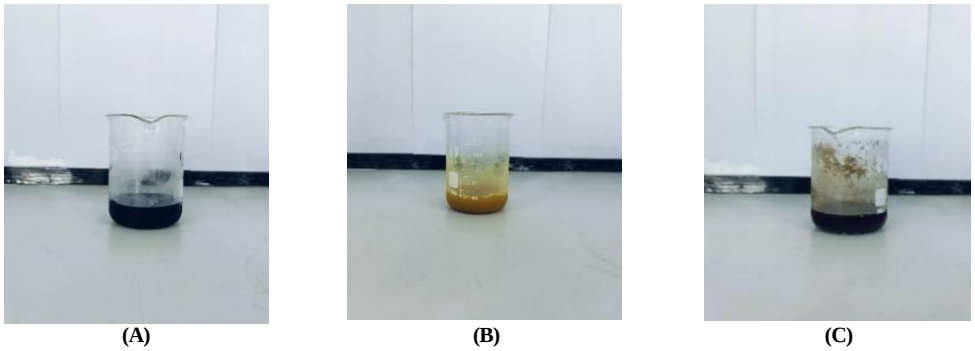
3. Results and Discussion

To establish a hair dyeing and conditioning system that effectively combines aesthetic appeal with the maintenance of optimal hair and scalp health, this research prioritized the development of a novel formulation and centered on the integration of pro-prebiotic principles derived from carefully selected herbal extracts. Recognizing the increasing consumer preference for natural and safer alternatives in personal care, the initial phase of the study focused on identifying and rigorously evaluating specific botanical types.

The inherent color-imparting properties suitable for hair and subsequent details in the findings in formulating the optimal conditions for this natural hair care system was developed as a post-dyeing hair conditioning product enriched with prebiotics and natural herbal extracts. The restoration and maintain hair health following coloration was the key of this innovative protocol together with the involved integrating probiotic and prebiotic components of bioactive plant-based extracts were from these processes are presented as follows:

3.1 Herbal Extracts for Hair Dyeing and Conditioning

The formulation of a natural hair dyeing and conditioning system prioritized the incorporation of carefully selected herbal extracts, specifically ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and butterfly pea flower (*Clitoria ternatea*). These botanicals were chosen for their established antioxidant, antimicrobial, and hair-conditioning properties. For dyeing and conditioning applications, aqueous extracts of butterfly pea (*Clitoria ternatea*), turmeric (*Curcuma longa*), and henna (*Lawsonia inermis*) were prepared. The extraction process involved individually boiling each herb (1 g/100 ml distilled water), followed by drying the resulting liquid extracts at 55°C. The yields from 50 ml of extract were: butterfly pea – 0.15 g, turmeric – 4.0 g, and henna – 4.0 g. As illustrated in Fig 1, the redissolved herbal extracts demonstrated effective dyeing capabilities upon application to hair. Butterfly pea imparted a dark brown to black hue, turmeric produced a brown tint, and henna yielded a deep brown coloration. The visual characteristics of the initial aqueous extracts are shown in Figure 4.1: (a) butterfly pea – deep indigo, (b) turmeric – bright yellow-orange, and (c) henna – brown.



(A) **(B)** **(C)**

Figure 1 The appearance and color of three herbal extracts at the concentration of 1 g/100 mL after boiling in water. (A) Butterfly Pea (*Clitoria ternatea*) (B) Turmeric (*Curcuma longa*), and (C) Henna (*Lawsonia inermis*).

3.2 Hair Bleaching and Dyeing with Herbal Extracts

Before dyeing hair with herbal extracts, a crucial initial step is bleaching, which removes the natural melanin pigment to create a uniform, light base for accurate color absorption and evaluation. The study evaluated two chemical agents for their bleaching efficacy: perchloric acid ($HClO_4$) at 2–12% concentrations and a mixture of hydrogen peroxide (H_2O_2, 6–12% (v.v)) with 12% (v/v) of 6 M ammonia (NH_3) were chosen to remove the natural melanin pigment present in the hair by eliminating the inherent color. As visually demonstrated in Figure 2, perchloric acid failed to significantly lighten the hair and induced undesirable dryness, brittleness, and a rough texture, rendering it unsuitable for cosmetic application. In contrast, the hydrogen peroxide and ammonia mixture effectively lightened hair color, particularly at concentrations between 6% and 12%. A 15-minute exposure time was determined to be optimal, providing a balance between noticeable lightening and minimizing hair damage, making it the preferred method for preparing hair for subsequent herbal dyeing. The necessity of a bleaching step prior to herbal dyeing raises an important consideration when comparing it to conventional chemical dyeing processes. Chemical dyes often utilize ammonia to swell the hair cuticle, allowing smaller synthetic dye molecules to penetrate the hair cortex and react with the hair structure to create a permanent color change. The bleaching step in this study, using hydrogen peroxide and ammonia, similarly opens the hair cuticle and removes the natural melanin. This pre-treatment theoretically enhances the hair surface's receptivity, improving the adhesion of larger natural pigment molecules from herbal extracts. However, unlike the chemical reactions of synthetic dyes within the hair cortex, the herbal dyes primarily coat the outer layers of the hair shaft. This difference in mechanism likely contributes to the observed poorer colorfastness of the herbal dyes. The bleaching process, while aiding initial color uptake of the herbal extracts by providing a clean, light base and a slightly opened cuticle, does not facilitate the same level of chemical bonding or deep penetration achieved by synthetic dyes.

Following the successful bleaching process using hydrogen peroxide and ammonia, three herbal extracts – Butterfly Pea (*Clitoria ternatea*), Turmeric (*Curcuma longa*), and Henna (*Lawsonia inermis*) – were applied to the lightened hair samples. All three extracts effectively imparted brown tones, with Butterfly Pea exhibiting the most intense coloration (72%), followed by Turmeric (68%) and Henna (65%). These color changes were visually apparent and corroborated by color measurements taken from photographs of the treated hair (as shown in Figure 2), confirming the dyeing capabilities of these natural extracts on bleached hair. However, a significant limitation was observed: the colorfastness of these herbal dyes was notably lower compared to conventional commercial dyes. After repeated washing, the colors faded considerably, indicating a weaker binding affinity to the hair shaft. Consequently, for individuals opting for these herbal coloring methods, regular reapplication, potentially on a weekly basis, is recommended to maintain color vibrancy. This reduced long-term durability presents a key challenge in the sustained use of these natural dyes for hair coloring.

A. Hair before bleaching
B. Hair bleached with 2% (v/v) $HClO_4$
C. Hair bleached with 5% (v/v) $HClO_4$
D. Hair bleached with 12% (v/v) $HClO_4$

Figure 2 Appearance of hair bleached with $HClO_4$ at concentrations of 2%, 5%, and 12% (v/v)

A. Hair sample unbleached (control)
B. Hair bleached with H_2O_2 and NH_3 for 5 minutes
C. Hair bleached with H_2O_2 and NH_3 for 15 minutes
D. Hair bleached with H_2O_2 and NH_3 for 30 minutes
E. Hair bleached with H_2O_2 and NH_3 for 45 minutes

Figure 3 Appearance of hair bleached with H_2O_2 and NH_3 at different times: 5, 15, 30, and 45 minutes.

3.3 Hair Dyeing with Herbal Extracts

Having established the optimal hair preparation method using X% hydrogen peroxide and Y% ammonia bleaching, the subsequent phase of this study involved assessing the dyeing capability of three herbal extracts: *Clitoria ternatea* (butterfly pea), *Curcuma longa* (turmeric), and *Lawsonia inermis* (henna). As visually documented in Figure 4, the application of each extract to the pre-lightened hair samples resulted in noticeable color transformations, yielding various shades of dark brown. Colorimetric analysis, alongside visual comparisons through photographic documentation (Figure 4.5), further corroborated these findings. Notably, the turmeric extract imparted the most intense coloration (registering 72% relative to untreated black hair), closely followed by henna (at 68%) and butterfly pea (at 65%).

While these herbal dyes clearly demonstrated their capacity to impart visible color to the bleached hair substrate, a significant drawback was the observed colorfastness, which proved to be considerably lower than that exhibited by conventional commercial dyes. The treated hair displayed rapid fading after only a few washing cycles, suggesting a less robust interaction and binding of the natural pigment molecules to the hair shaft in comparison to their synthetic counterparts. Consequently, to maintain color vibrancy and ensure consistent coverage, frequent reapplication, estimated at approximately weekly intervals, would be a practical necessity for individuals opting for these herbal coloring methods. Although these herbal extracts represent a promising direction for the development of natural and potentially safer alternatives to chemical hair dyes, their current performance appears to lack the long-lasting durability that characterizes synthetic formulations. This underscores the need for further investigation into strategies aimed at enhancing the color retention properties of these natural dyes.

a. Hair dyed with *Clitoria ternatea* extract

b. Hair dyed with *Curcuma longa* extract

c. Hair dyed with *Lawsonia inermis* extract

Figure 4 Appearance of hair color after dyeing with herbal extracts.

3.4 Development of Hair Conditioning Cream Formulation with Probiotics and Herbal Extracts

This part of the study focused on developing a hair conditioning cream enriched with probiotic microorganisms, with the goal of promoting both hair and scalp health through a more natural and biologically supportive approach. In the initial stage, suitable probiotic strains were selected based on their ability to survive within the cream formulation and remain active during use—an essential requirement that was successfully met (data not shown). Probiotic survival in cosmetic formulations is crucial as it ensures the delivery of live and functional microorganisms to the scalp. Factors like water activity, pH, and the presence of preservatives can significantly impact their viability.

To evaluate their suitability in more detail, the selected probiotics were incorporated into the cream base and monitored for their survivability. The results revealed that the microorganisms maintained a high survival rate—ranging from 70% to 90%—under the formulation conditions (though specific data are not presented here). These findings suggest that the cream's environment, including its ingredients and pH level, was well-suited to support the viability and function of the beneficial bacteria. Common probiotic strains used in cosmetics often belong to the *Lactobacillus* and *Bifidobacterium* genera, known for their skin and scalp benefits. Furthermore, the findings confirmed that the chosen probiotic strains were highly compatible with the hair conditioner. Their presence in the formulation was not merely passive; they remained active at an efficacy rate of approximately 70–80%, indicating that they could meaningfully contribute to the overall conditioning and scalp-care benefits of the product. Probiotic efficacy in hair and scalp care can manifest through various mechanisms, including balancing the scalp microbiome, reducing inflammation, and potentially improving hair follicle health. This demonstrates that it is indeed possible to integrate living, health-promoting microorganisms into a topical cosmetic formulation without compromising their viability or beneficial effects.

3.5 Probiotics and Prebiotic Herbal Extracts in Hair Conditioner

To develop a bioactive hair conditioning cream aimed at promoting scalp and hair health through a balanced scalp microbiome, probiotics derived from yogurt were incorporated. Recognizing that probiotic survival is crucial for their beneficial effects, the formulation was enriched with ginger (*Zingiber officinale*) extract as a natural prebiotic. Prebiotics, non-digestible compounds typically found on the hair and scalp, selectively stimulate the growth and/or activity of beneficial microorganisms, providing a nutrient source for a healthy microbial ecosystem. Probiotic cultures of the *Lactobacillus* genus, known for their beneficial effects on the scalp, were prepared from freshly re- inoculated yogurt and diluted 1:10 in PBS containing 0.1–0.5% ginger extract. These cultures, intended to function in this way, demonstrated strong microbial growth (7–8 log units at OD600) and high cell viability under microscopic observation (Figures 5) after 24 hours of incubation at 37°C. This confirms that ginger extract effectively supports *Lactobacillus* survival, highlighting its potential as a prebiotic in this formulation. Beyond its prebiotic function, ginger extract also offers inherent antioxidant and antimicrobial properties that can further contribute to a healthy scalp environment. Additional tests showed that diluted herbal dye extracts (*Clitoria ternatea* (butterfly pea), *Curcuma longa* (turmeric), and *Lawsonia inermis* (henna)) at a 1:1000 dilution did not negatively impact probiotic activity, indicating their compatibility with the microbial growth. Overall, these results confirm the viability and activity of probiotics within the ginger-based formulation, supporting the development of a natural conditioner designed to nurture the hair and scalp ecosystem.

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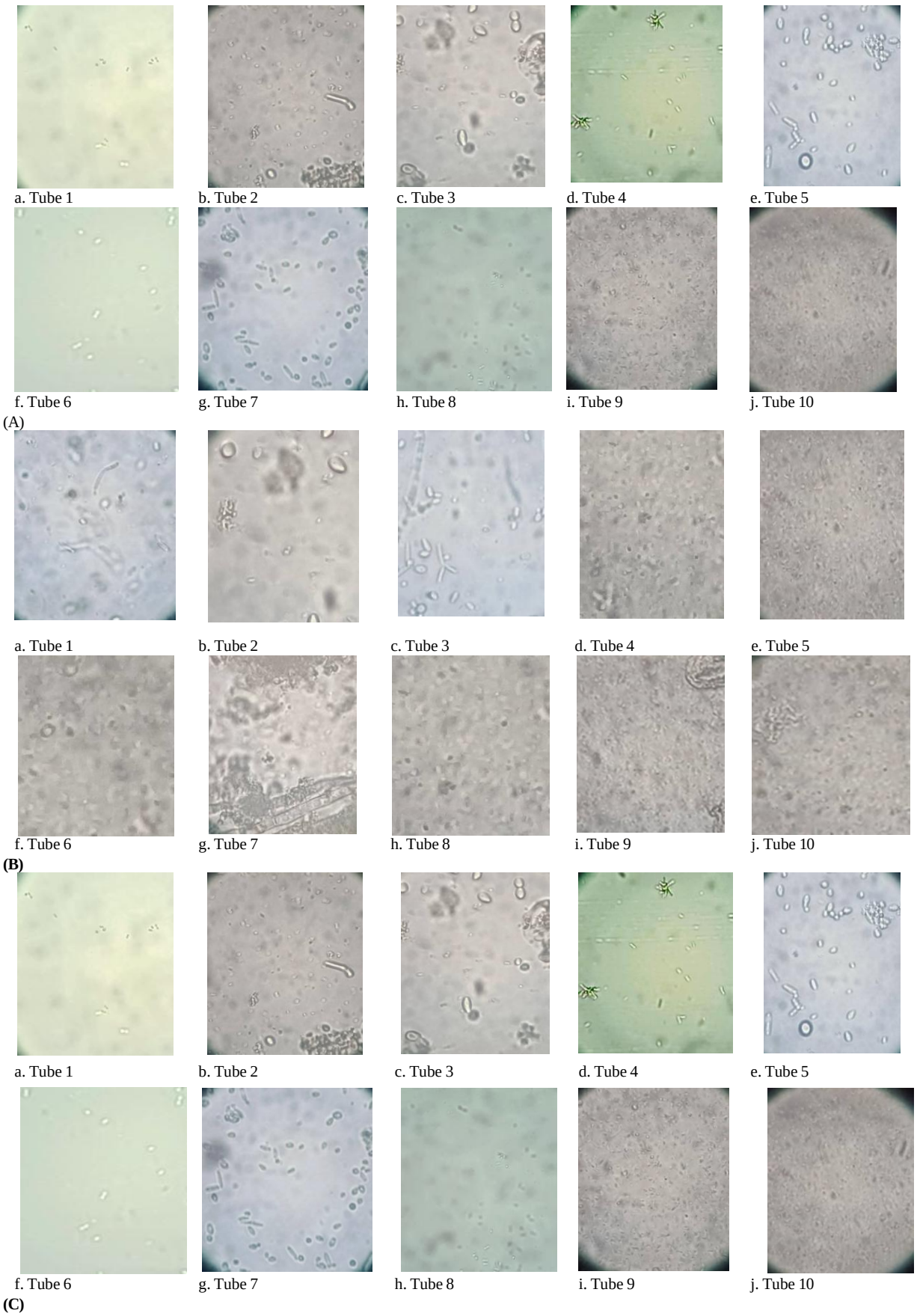
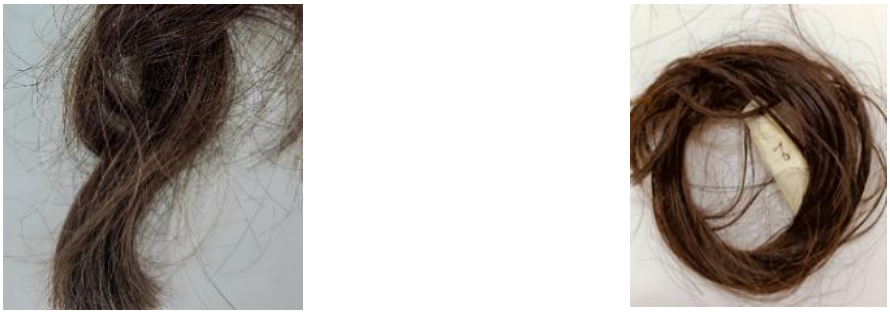


Figure 5 Microorganisms under a microscope in the hair conditioning cream formula combined with (A) *Clitoria ternatea* extract (1 mg/mL), (B) *Curcuma longa* extract (1 mg/mL), or (C) *Lawsonia inermis* extract (1 mg/mL). Tubes a-j represent samples from tubes 1 to 10, with dilutions ranging from 1:10 to $1:10^{10}$.

3.6 Evaluating Probiotic Conditioner for Herbally-Dyed Hair

A natural and effective post-treatment conditioner, composed of herbal extracts, oils, and probiotics, was developed with the goals of reducing hair damage, enhancing softness, and maintaining scalp balance after the dyeing process. This formulation was specifically designed to restore and nourish hair following the application of herbal dyes. After applying the probiotic-enriched hair conditioning cream to hair previously dyed with herbal extracts, the treated hair exhibited noticeably improved softness and smoothness. Both visual and tactile assessments indicated that its texture was comparable to that of hair treated with commercially available conditioners (Figure 6).

While these initial results are promising, the probiotic-based formulation currently lacks the diversity of functional ingredients commonly found in commercial products, which often include a wide range of additives for targeted hair benefits. To broaden its appeal and efficacy, further research is needed to explore alternative formulations and additional bioactive components. This would help improve product performance and better address the varying needs of consumers.



a. Hair dyed with herbal extracts and treated with commercial conditioner

b. Hair dyed with herbal extracts and treated with probiotic-enriched conditioner

Figure 6 Comparison of hair dyed with herbal extracts and treated with either a probiotic-enriched conditioner or a commercial conditioner

Overall, these findings indicate the potential of a hair care system integrating natural herbal dyes and a probiotic-enriched conditioner. The herbal dyes, while effective in imparting color, demonstrated limitations in long-term color retention. Conversely, the probiotic-based conditioner showed promising results in improving hair softness and smoothness post-dyeing, suggesting its potential to mitigate some of the adverse effects associated with hair treatments. However, further optimization of both the dyeing and conditioning formulations is warranted to enhance longevity, broaden efficacy, and ultimately provide a comprehensive and appealing natural alternative to conventional chemical hair treatments.

4. Conclusion

The findings from this study provide valuable insights into the potential of eco-friendly hair care formulations that combine natural herbal dyes and probiotics. To fully realize the commercial and functional potential of this formulation, further research is needed to enhance key aspects such as the stability of probiotic activity through techniques like encapsulation (Habeebuddin et al., 2022; Natarajan et al., 2025). Additionally, improving texture consistency and storage stability under various conditions will be crucial for product optimization (Maurya et al., 2021). Long-term studies on the formulation's effectiveness across different hair types (Yin et al., 2024) and consumer satisfaction surveys will help ensure its broad market appeal. Moreover, integrating biodegradable or recyclable packaging can improve the formulation's environmental sustainability, aligning it with the growing consumer demand for green, eco-conscious cosmetics (Nipurte et al., 2022).

5. Acknowledgement

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Session 4
Architecture and Creative Works

Knowledge Set about Online Media, Gingerbread House Architecture to Promote Tourism

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Abstract

This research aims to study the architecture of Gingerbread houses, Case Study: Bangkok Metropolitan Region and prepare a knowledge set of the media of the architecture of the Gingerbread houses in order to promote tourism among young generation and Thai tourists. The research starts from 1) the collection of data obtained from related documents and research, 2) the surveys of actual locations, 3) the interviews and observations by using questionnaires as a tool for surveying data. The sample group includes 40 second-year students of the Faculty of Engineering and Architecture, Rajamangala University of Technology Suvarnabhumi Nonthaburi Campus and young Thai tourists aged 15-30 years, 4) the photography and video recording of the architecture of the Gingerbread houses, 5) the preparation of the knowledge set of online media of the Gingerbread houses, 6) the collection of data, 7) the analyses of data applying SPSS for qualitative analysis, and 8) the conclusion, discussion and suggestions. The Gingerbread houses in this study are as follows: 1) Gingerbread House Monk Cells, 2) Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis, 3) Ekanak Museum, Bangkok Metropolis, 4) Diamond Residence, Bowon Niwet Temple, Bangkok Metropolis, 5) Moon Residence, Bowon Niwet Temple, Bangkok Metropolis, and 6) Ruean Phra Thanesuan. The research result shows that the knowledge set of the architectural media of Gingerbread houses for promoting tourism has a link to the website. https://www.gingerbreadhouseonline.com. Links to YouTube: 1) Gingerbread Houses – Abbot Cell, Monk Cells and Panthawakarn School Building, Suan Phlu Temple, Bangkok Metropolisouses, https://youtu.be/R-_hP4c7RK4, 2) Gingerbread Houses – Moon Residence, Diamond Residence and Boonyarattawet Monk Cell, Bowon Niwet Temple, Bangkok Metropolis, https://youtu.be/T1OshjjB9gs, 3) Gingerbread Houses – Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis, https://youtu.be/rS01EcVfFMk, 4) Gingerbread Houses – Ekanak Museum, Bansomdejchaopraya Rajabhat University, https://youtu.be/Ua0BxT2ToJU, 5) Gingerbread Houses - Ruean Phra Thanesuan, Sanam Chan Palace, Nakhon Pathom Province, https://youtu.be/epircwkK_nw. Link to E-Book of Gingerbread Houses: 1) Knowledge Set of Gingerbread Houses, https://my.eboox.cc/JOT/L1/, 2) Abbot Cell, Monk Cells and Panthawakarn School Building, Suan Phlu Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L2/, 3) Chandra Residence, Diamond Residence and Boonyarattawet Monk Cell, Bowon Niwet Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L3/, 4) Ekanak Museum, Bansomdejchaopraya Rajabhat University, Bangkok Metropolis, https://my.eboox.cc/JOT/L4/, 5) Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L5/, and 6) Ruean Phra Thanesuan, Sanam Chan Palace, Nakhon Pathom Province, https://my.eboox.cc/JOT/L6/. The Evaluation of E-Book and Knowledge Set of Gingerbread House Architecture Online Media for Promoting Tourism. For media, the overall quality is at a very good level, the mean of which equals 3.74. Upon considering each area, the qualities of all areas (image, sound, techniques and methods) have been found to be at a very good level. The area with the highest mean is the techniques and methods, equaling 3.93. The second area is the image with the mean of 3.71, and the mean of the sound is 3.56 respectively. When reckoning each area, the results are as follows: Image: The qualities of each area are at a very good level, the mean of which is 3.71. Upon considering the mean of each area, the highest mean has been found to be the interesting still image with the mean of 3.98, and the quality is at a very good level. The second area is the quality of the still image with the mean of 3.89, and the quality is at the very good level. The next area is the appropriateness of the illustration with the mean of 3.69, and the quality is at a very good level. The next area is the clarity of the meaning of the still image with the mean of 3.62, and the quality is at a very good level. In addition, the mean of the appropriateness of the still image is 3.38, and the quality is at a good level respectively. Sound: The overall quality is at a very good level with the mean of 3.56. Upon reckoning the mean of each area, the area with the highest mean has been found to be the clarity of the audio description with the mean of 3.78, and the quality is at a very good level. The second area is the appropriateness of the music with the means of 3.33, and the quality is at a good level respectively. Techniques and Methods: The qualities of the overall areas are at a very good level with the mean of 3.93. Upon considering the mean of each area, the qualities of all areas have been found to be at a very good level, and the highest mean is the creation of knowledge and direct experience with the mean of 3.93. The next area is the appropriateness of visual and audio sequences with the mean of 3.89 respectively. According to students' responses, more students are interested in visiting the Gingerbread houses. The students have seen the splendid and magnificence of the Gingerbread houses, as the Gingerbread houses are the heritage of Thailand.

Keywords: Knowledge Set, Online Media, Gingerbread House, Promote Tourism

1. Introduction

The Gingerbread pattern was an architectural pattern that flourished during the two reigns mentioned above. Now the pattern and some ancient buildings disappeared. Therefore, data were collected, studied and explained to provide a clearer perspective. Appropriate illustrations were arranged to help readers for better understanding.

The Gingerbread pattern is assumed to be a pattern influenced by the Western nations, which were most prosperous during the era of Queen Victoria. Countries all over Europe and Asia were under the rule of the British Empire. During the colonial era, Siam had to improve and change the entire system, resulting in politics, economy, society, traditions and culture that the Siamese people were accustomed to having to adjust their minds in order to maintain it. Outside of sovereignty, King Chulalongkorn inherited the royal policy of his father in developing the country to be prosperous in every aspect to be equal to the Western nations. What indicates how much Siam has developed according to the Westerners is the beauty of architecture, constructions, buildings, houses, public utilities, and conveniences in life that imitate the Western way of life. The beauty of the Gingerbread fretwork was in the old days of the capital city, decorated in Dusit Palace since King Chulalongkorn, Rama V, traveling to Europe and neighboring countries, including to Malay. The popularity began to spread to royal palaces, religious sites, government officials' houses, wealthy houses and shophouses. Evidence of buildings and houses decorated with this type of pattern was very limited because the fretwork of the Gingerbread pattern was created from wood materials decorated with old architectural buildings. Some ancient buildings were not less than 100 years old.

Patravadee Siriwan's research is as follows:

1) Study the pattern of the architecture of the Gingerbread houses. Case Study: Bangkok Metropolitan Region in 2016.
2) Study the pattern of the Gingerbread fretwork. Case Study: Bangkok Metropolitan Region.
3) Study the physical characteristics of the architecture of Gingerbread houses in Bangkok Metropolis in 2021.

The researcher has had the following conclusions. The architecture of the Gingerbread houses and the Gingerbread fretwork is splendid, exquisite, magnificent and impressive, being attractive to spectators. It is the uniqueness of the Thai craftsmen needing conservation and promotion to young generation. The research team has been aware of the importance of the Gingerbread houses; therefore, the online knowledge set on the architecture of the Gingerbread houses has been produced, aiming to promote tourism among young generation aged 15-30 years. In addition, the Gingerbread houses are the Thai heritage admired by young generation and tourists, and they are splendidly, exquisitely and magnificently built by the Thai craftsmen.

2. Methodology

1) Collection of Data from Relevant Research Documents
2) Survey of the Actual Location (Field Study): The Gingerbread houses studied are as follows:
   2.1) Gingerbread Monk Cells, Suan Phlu Temple, Bangkok Metropolis
   2.2) Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis
   2.3) Ekanak Museum, Bangkok Metropolis
   2.4) Diamond Residence, Bowon Niwet Temple, Bangkok Metropolis
   2.5) Moon Residence, Bowon Niwet Temple, Bangkok Metropolis
   2.6) Boonyarattawet Monk Cell, Bowon Niwet Temple, Bangkok Metropolis
   2.7) Ruean Phra Thanesuan, Sanam Chan Palace, Nakhon Pathom Province
3) Interviews and observations Using Questionnaires as a Tool for Data Collection

The sample group for this study comprises the 40 second-year students of the Faculty of Engineering and Architecture, Rajamangala University of Technology Suvarnabhumi, Nonthaburi Campus. Additionally, questionnaires and observation forms have been utilized.

4) Photography and Video Recording of the Architecture of Gingerbread Houses for Field Survey
5) Create a knowledge set about online media on the architecture of the Gingerbread houses.
6) Collect data.
7) Analyze data. SPSS has been used for analyzing qualitative data and evaluating the online knowledge set on the architecture of the Gingerbread houses in order to promote tourism, and the observation forms have been used to evaluate the architecture of the Gingerbread for promoting tourism.
8) Summary of Results, Discussion and Suggestions

3. Results and Discussion

Research Result and Knowledge Set of Gingerbread House Architecture for Promotion of Tourism

Link to the Website

https://www.gingerbreadhouseonline.com

Links to YouTube

1) Gingerbread Houses – Abbot Cell, Monk Cells and Panthawakarn School Building, Suan Phlu Temple, Bangkok Metropolis, https://youtu.be/R-_hP4c7RK4

2) Gingerbread Houses – Moon Residence, Diamond Residence and Boonyarattawet Monk Cell, Bowon Niwet Temple, Bangkok Metropolis, https://youtu.be/T1OshjjB9gs

3) Gingerbread Houses - Gold Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis, https://youtu.be/rS01EcVfFMk

4) Gingerbread House - Ekanak House Museum, Rajabhat Bansomdejchaopraya University, Bangkok, https://youtu.be/Ua0BxT2ToJU

5) Bread House - Phra Thanesuan House, Sanam Chandra Palace, Nakhon Pathom Province, https://youtu.be/epircwkK_nw

Links to E-Book of Gingerbread Houses

1) Knowledge Set of Gingerbread Houses, https://my.eboox.cc/JOT/L1/

2) Abbot Cell, Monk Cells and Panthawakarn School building, Suan Phlu Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L2/

3) Moon Residence, Diamond Residence and Boonyarattawet Monk Cell, Bowon Niwet Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L3/

4) Ekanak Museum, Bansomdejchaopraya Rajabhat University, Bangkok Metropolis, https://my.eboox.cc/JOT/L4/

5) Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis, https://my.eboox.cc/JOT/L5/

Ruean Phra Thanesuan, Sanam Chan Palace, Nakhon Pathom Province, https://my.eboox.cc/JOT/L6/

Evaluation Results of E-Book for Tourism Promotion: Knowledge Set on Online Media of Gingerbread House Architecture for Tourism Promotion

The evaluation results of the E-Book are to promote tourism and the knowledge set of online media of Gingerbread architecture, promoting tourism. For media, the overall quality is at a very good level with the mean of 3.74. Upon considering each area, the overall areas have been found to be at a very good level (image, sound and techniques and methods), and the quality is at a very good level, and the highest mean is the technique and method with the mean of 3.93. The second area is the image with the mean of 3.71 and sound with the mean of 3.56 respectively. When considering each area, the results are as follows:

In terms of image, the overall quality of each area is at a very good level with the mean of 3.71. Upon considering mean of each area, the area with the highest mean is the size of the still interesting image with the mean of 3.98, and the quality is at a very good level. The second area is the quality of the still image with the mean of 3.89, and the quality is at a very good level. The next area is the appropriateness of the illustration with the mean of 3.69, and the quality is at a very good level. The next area is the clarity of the meaning of the still image with the mean of 3.62, and the quality is at a very good level. In addition, the appropriateness of the still image has the mean of 3.38, and the quality is at a good level respectively.

In terms of sound, the overall quality of each area is at a very good level with the mean of 3.56. Upon considering the mean of each area, the area with the highest mean is the clarity of the audio description with the mean of 3.78, and the quality is at a very good level, followed by the appropriateness of the music with the mean of 3.33, and the quality is at a good level respectively.

In terms of techniques and methods, the overall quality of each area is at a very good level with the mean of 3.93. Upon considering the mean each area, the qualities of all areas are found to be at a very good level, creating knowledge and direct experience with the mean of 3.96, followed by appropriateness of the presentation format with the mean of 3.93, and the appropriateness of the image and sound with the mean of 3.89 respectively.

For the results of the evaluation of the knowledge set of the Gingerbread house architecture to promote tourism, students are able to understand correctly and completely with the mean of 2.85. Upon considering the mean of each area, the students are able to understand correctly and completely in every area. The area with the highest mean is the meaning and location of Gingerbread fretwork with the mean of 2.98, followed by the Gingerbread fretwork with the mean of 2.91, followed by the type of the buildings in Bangkok Metropolis decorated with the Gingerbread fretwork, and the location of the buildings that are popularly decorated with the Gingerbread fretwork with the mean of 2.76 respectively.

Suggestions: QR codes for the 6 Gingerbread houses should be created to promote tourist attractions and provide the knowledge of the Gingerbread houses, as the Gingerbread houses are the heritage of Thailand, and the Gingerbread houses and the Thai craftsmen’s skills should be conserved, and the narration on YouTube should be improved.

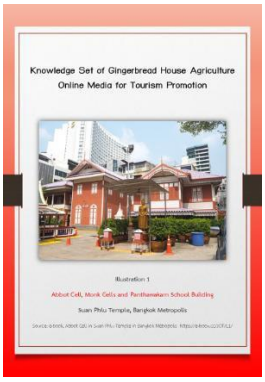

Figure 1: Abbot Cell, Monk Cells and Panthawakarn School Building, Suan Phlu Temple, Bangkok Metropolis

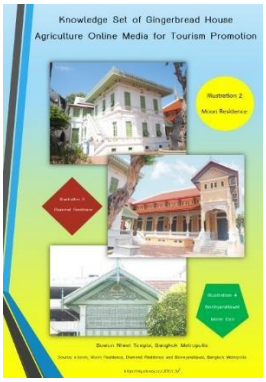

Figure 2: Moon Residence, Diamond Residence, Boonyarattawet Monk Cell, Bangkok Metropolis

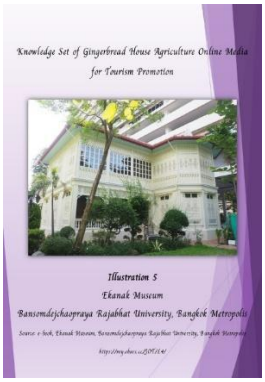

Figure 3: Ekanak Museum, Bansomdejchaopraya Rajabhat University, Bangkok Metropolis

https://my.eboox.cc/JOT/L2/
https://youtu.be/R-_hP4c7RK4
https://www.gingerbreadhouseonline.com

https://my.eboox.cc/JOT/L3/
https://youtu.be/T1OshjjB9gs
https://www.gingerbreadhouseonline.com

https://my.eboox.cc/JOT/L4/
https://youtu.be/Ua0BxT2ToJU
https://www.gingerbreadhouseonline.com

Figure 4: Golden Teak Museum, Thewarat Kunchorn Temple, Bangkok Metropolis

Figure 5: Ruean Phra Thanesuan, Sanam Chandra Palace, Nakhon Pathom Province

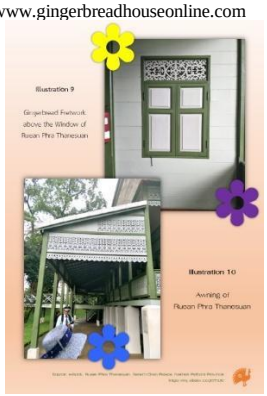

Figure 6: Ruean Phra Thanesuan, Sanam Chandra Palace, Nakhon Pathom Province

https://my.eboox.cc/JOT/L5/
https://youtu.be/rS01EcVfFMk
https://www.gingerbreadhouseonline.com

https://my.eboox.cc/JOT/L6/
https://youtu.be/epircwkK_nw
https://www.gingerbreadhouseonline.com

https://my.eboox.cc/JOT/L6/
https://youtu.be/epircwkK_nw
https://www.gingerbreadhouseonline.com

4. Conclusions

4.1 Summary of Results

The architecture of the Gingerbread pattern in Bangkok Metropolitan Region can be classified into the following issues:

1) Buildings with Gingerbread Pattern
   1.1) Traditional Thai style buildings include:
      1. Gingerbread Monk Cells, Suan Phlu Temple
      2. Golden Teak Museum, Thewarat Kunchorn Temple
      3. Ekanak Museum
      4. Diamond Residence, Bowon Niwet Temple
      5. Moon Residence, Bowon Niwet Temple
      6. Boonyarattawet Monk Cell, Bowon Niwet Temple
      7. Ruean Phra Thanesuan, Sanam Chan Palace, Nakhon Pathom Province

2) Three groups of materials have been used for building Gingerbread houses as follows:
   2.1) Buildings Made Entirely of Wood
      1. Gingerbread Monk Cells, Suan Phlu Temple
      2. Ruean Thanesuan, Sanam Chan Palace, Nakhon Pathom Province
      3. Boonyarattawet Monk Cell, Bowon Niwet Temple
   2.2) Half Concrete Half Wood Buildings
      1. Ekanak Museum
   2.3) Brick and Mortar Buildings
      1. Golden Teak Museum, Thewarat Kunchorn Temple
      2. Diamond Residence, Bowon Niwet Temple
      3. Moon Residence, Bowon Niwet Temple
3) Three Drawing Plans Used for Building Gingerbread Houses

3.1) Rectangular Drawing Plan includes Gingerbread monk cells, Suan Phlu Temple, Ruean Thanesuan, Diamond Residence, Moon Residence, Boonyarattawet Monk Cell, Bowon Niwet Temple.

3.2) Square Drawing Plan connected with octagonal room in the end consists of Ekanak Museum.

3.3) Independent Drawing Plan comprises Golden Teak Museum, Thewarat Kunchorn Temple.

4) Three Types of Gingerbread Roof

4.1) Manila roof includes Gingerbread monk cells, Suan Phlu Temple

4.2) Mixed hipped gable roof consists of Golden Teak Museum in Thewarat Kunchorn Temple, Ekanak Museum, Ruean Phra Thanesuan, Moon Residence in Bowon Niwet Temple.

4.3) Gable roof comprises Gingerbread monk cells in Suan Phlu Temple, Diamond Residence in Bowon Niwet Temple and Boonyarattawet Monk Cell in Bowon Niwet Temple.

5) The total number of floors is as follows:

5.1) Two-story buildings include Gingerbread monk cells in Suan Phlu Temple, Golden Teak Museum Building, Ekanak Museum, Diamond Residence in Bowon Niwet Temple, Moon Residence in Bowon Niwet Temple and Boonyarattawet Monk Cell in Bowon Niwet Temple.

5.2) One-story building comprises Ruean Phra Thanesuan, Nakhon Pathom Province

6) The Popular Colors of Gingerbread Houses

6.1) Yellow buildings with red roofs include Golden Teak Museum, Thewarat Kunchorn Temple and Ekanak Museum.

6.2) Repainted buildings consist of red, white, red roof, such as Gingerbread monk cells in Suan Phlu Temple.

6.3) Green and white buildings comprise Ruean Phra Thanesuan and Moon Residence in Bowon Niwet Temple in Bowon Niwet Temple.

6.4) Orange building with orange roof includes Diamond Residence in Bowon Niwet Temple.

7) Decorative elements found in every building are as follows:

Fretwork Grille, Stair Balustrade, Stair Railing, Sun Fin, Balcony Railing, Eave above Doors, Stairs, Gable, Door Frame, Window Panel, Awning

8) Six Gingerbread Patterns

8.1) Artificial Flower Pattern Found in Ekanak Museum, Golden Teak Museum in Thewarat Kunchorn Temple, Chan Residence in Bowon Niwet Temple, Diamond Residence in Bowon Niwet Temple, Boonyarattawet Monk Cell in Bowon Niwet Temple, Ruean Thanesuan

8.2) Club Pattern Found in Golden Teak Museum in Thewarat Kunchorn Temple

8.3) Cross Pattern Found in Golden Teak Museum in Thewarat Kunchorn Temple

9) The Current Uses of Gingerbread Houses

9.1) Adjusted to Be a Museum Opened to the Public: Golden Teak Museum in Thewarat Kunchorn Temple and Ruean Thanesuan

9.2) Applied as Office Buildings and Government Offices (Not Museum): Gingerbread Monk Cells in Suan Phlu Temple, Ekanak Museum, Diamond Residence in Bowon Niwet Temple, Moon Residence in Bowon Niwet Temple and Boonyarattawet Monk Cell in Bowon Niwet Temple

10) Awarded Gingerbread Houses

10.1) Award for Outstanding Conservation from the Association of Siamese Architects under Royal Patronage: Gingerbread Monk Cells in Suan Phlu Temple

10.2) Award for Outstanding Architectural Conservation 2012 in the Category of Institutional and Public Buildings from the Association of Siamese Architects under Royal Patronage: Ekanak Museum

Recommendations

1) Policy: The curriculum of tourism by conserving the architecture of the Gingerbread houses should be supported in order to promote tourism for the architecture of the Gingerbread houses among young generation aged 15-30 years, admiring the Thai craftsmen. Schools and universities should visit the Gingerbread houses, so that young generation can admire the splendid of the architecture of the Gingerbread houses.

2) The Gingerbread houses should be promoted in order to provide young generation with the information of the Gingerbread houses and tourists for benefits in the future.

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