



Oscillatoria sp.



Pseudanabaena sp.



Planktolyngbya sp.



Merismopedia sp.



Scenedesmus sp.



Monorapidium sp.



Nitzschia sp.



Pleurosigma/Gyrosigma sp.



Navicula sp.



Protoperidinium sp.



Ceratium sp.



Prorocentrum sp.



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Editorial

The ASEAN Journal of Scientific and Technological Reports (AJSTR) Vol. 25 No. 2 (April-June 2022) ISSN 2773-8752 is the third issue under AJSTR. This issue published eight worth-reading research articles. These exciting research articles were reviewed and answered by experts from various universities and institutions. We sincerely hope that some of the research papers will help guide and motivate our active researchers to produce and create more valuable research shortly. The AJSTR has served our energetic readers and customers on the international level.

For this reason, all selected and accepted research articles will be written and organized in English. Furthermore, the new international editorial board of AJSTR has also been set up and started to administrate and manage all the journal's business simultaneously. From now on, the AJSTR and a new editorial team are ready to organize, manage, publish, and deliver all good quality articles written in well-organized English to the world of academic society. I would like to introduce AJSTR editorial board members as below.

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A Walking Route to Travel in Lopburi City

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Abstract: This article was a part of a research paper to present a walking route to travel in Lopburi City. It was to be the shortest distance of path and use the least travel time. The research methodology used the survey of 10 real locations together with viewing satellite maps and diagrams from Google.go.th/maps for graphing and weighting of edges (distance; time). The result analysis used the basic ideas of graph theory by finding the path that has all the vertex and the ideas of matrix by finding the adjacency matrix for verification using Gephi program. The result of the research showed that a suitable walking route to travel in Lopburi City was to be as follows: Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Phrasi Rattana Mahathat → Wat Bandai Hin → Wat Inthra → Wat Nakhon Kosa → Phra Kal Shrine → Phra Prang Sam Yot (and reverse direction) with a total distance of 2,090 meters in 25 minutes.

Keywords: Graph theory; The adjacency matrix; A path; Gephi

Citation:

Suthisung, N.; Tangcharoen, S. A Walking Route to Travel in Lopburi City. *ASEAN J. Sci. Tech. Report.* **2022**, 25(2), 1-9. <https://doi.org/10.55164/ajstr.v25i2.245572>.

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1. Introduction

Lopburi Province has a wide variety of tourist attractions, both natural attractions, and historical attractions, and historical tourism is very popular in all parts of Thailand nowadays. People were especially fond of watching movies or dramas and going on historical tours often took pictures that correspond to the stories from real historical places. Lopburi Province has many historical attractions known in the past as Lawo City, which in the reign of King Narai the Great who lived and built the remaining buildings to be seen today. In addition, there are many historical attractions in Lopburi such as Phra Kan Shrine, Phra Prang Sam Yot, Ban Wichayen, Wat Puen, Prang Khaek Temple, King Narai the Great Museum, Wat Phra Sri Rattana Mahathat, Wat Nakhon Kosa, Wat Bandai Hin and Wat Inthra, etc. The historical attractions in Lopburi are not far from each other and can be visited within a day's walk. If Lopburi has publicized it to the public in advance, it will be helpful to make decisions about choosing and traveling to all places. This article presents a suitable walking route for exploring the history of Lopburi. Which is the best walking path? How far is it and how long does it take to walk?

2. The purpose of this research is to find suitable walking route for historical tourism in Lopburi Province.

3. Background

3.1 Ten cultural and heritage attractions in Lopburi city



Fig 1 (A). Phra Kan Shrine



Fig 1 (B). Phra Prang Sam Yot



Fig 1 (C). Ban Wichayen or Ban Luang) Royal House for ambassadors)



Fig 1 (D). Wat Puen



Fig 1 (E). Prang Khaek Temple



Fig 1 (F). Wat Phra Sri Rattana Mahathat



Fig 1 (G). King Narai the Great Museum



Fig 1 (H). Wat Nakhon Kosa



Fig 1 (I). Wat Bandai Hin

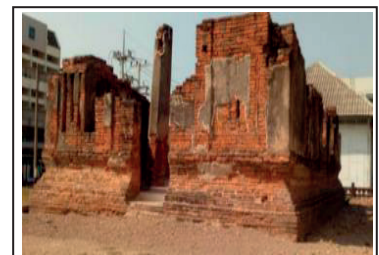


Fig 1 (J). Wat Inthra

Fig 1 (A) to Fig 1 (J) cited on Punthupun [1] as follows:

Fig 1 (A): Phra Kan Shrine is well known to the people of Lopburi Province.

Fig 1 (B): Phra Prang Sam Yot is an archaeological site and one of the important historical sites of Lopburi.

Fig 1 (C): After people watched the drama "Buppesanniwad", Ban Wichayen became popular.

Fig 1 (D): Wat Puen is an ancient temple in Lopburi.

Fig 1 (E): Prang Khaek Temple is considered to be one of the oldest Khmer castles in Lopburi.

Fig 1 (F): Wat Phra Sri Rattana Mahathat had been renovated many times both during the reign of King Narai the Great, King Ramesuan and His Majesty the Great Emperor.

Fig 1 (G): King Narai the Great Museum formerly known as a museum. In these buildings, antiques from prehistoric times to the Rattanakosin period are displayed, found in Lopburi and

nearby provinces as well as displaying various utensils that represent the way of life of the people in the central basin.

Fig 1 (H): A religious site that overlaps many times was originally a Khmer temple. There is a pagoda in the Lopburi style, around the 17th century in front.

Fig 1 (I): From the evidence that appears, Wat Bandai Hin is assumed that this temple was definitely built during the reign of King Narai.

Fig 1 (J): Wat Inthra is an ancient site located within the inner city wall.

3.2 Basic concepts of graph theory

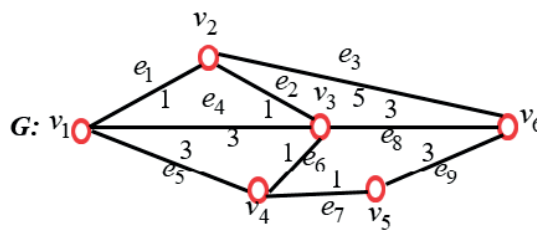
We apply the graph theory [2,3,4,5] to brief basic ideas as follows:

Graph G consists of finite sets $V(G)$ that is not an empty set and a set $E(G)$ can be an empty set.

The member of $V(G)$ is denoted by v_i called the vertex, the member of $E(G)$ is denoted e_i as the edge, for each by i ; $i \in \{1, 2, 3, \dots, k\}$

If then $|V(G)| = n$ the graph G is an ordered graph n , that is, the graph G has a number of n points.

If then $|E(G)| = m$ the graph G is a graph with size m , that is, the graph G has m lines. From the figure, for example, graph G :



$$V(G) = \{v_1, v_2, v_3, v_4, v_5, v_6\}$$

$$E(G) = \{e_1, e_2, e_3, e_4, e_5, e_6, e_7, e_8, e_9\}$$

$$|V(G)| = 6$$

$$|E(G)| = 9$$

The degree of point v_i in graph G is the number of lines that hit the point v_i in graph G

Weight of edge in each line of the graph G , there is a real number denoted by these real numbers called "Line weight" is written as $w(e_i)$

The weight of the graph G is denoted by $w(G)$; $w(G) = \sum_{e_i \in E(G)} w(e_i)$

If u and v are defined as points in the graph G

Walk $u-v$ in Graph G is a sequence of points and lines, where $u-v: u=u_0, e_1, u_1, e_2, u_2, e_3, \dots, u_{k-1}, e_k, u_k = v$ of line $e_i = u_{i-1}u_i \in E(G)$ for each by i ; $i \in \{1, 2, 3, \dots, k\}$

Length of walk $u-v$ is the number of lines in the walk $u-v$

Trail $u-v$ as in graph G as walk $u-v$ as in graph G that do not have the same lines in the same walking path

Path $u-v$ as in graph G as walk $u-v$ as in graph G no duplicate point $u-v$

Circuit as in graph G , it is a closed walkway in the graph G (A closed trail is a walk that goes back to the starting point).

Cycle as in graph G is cycling in graph G which has no duplicate point except the starting point and the last point are the same point substituted by C which

$C: u=u_0, u_1, u_2, \dots, u_{k-1}, u_k = u; u_i \neq u_j$ for each i, j by $k \geq 3$ and $1 \leq i < j \leq k$

Distance between u and v in graph G is the shortest length of $u-v$ compared to the length of the path $u-v$ all in graph G substituted by $d_G(u, v)$ as graph G :

3.3 Algorithm of Graph to about shortest walk

I. Munier et al. [6] suggested that the steps involved in Kruskal's Algorithm to generate a minimum spanning tree (MST):

Step 1: Sort all edges in increasing order of their edge weights.

Step 2: Pick the smallest edge.

Step 3: Check if the new edge creates a cycle or loop in a spanning tree.

Step 4: If it doesn't form the cycle, then include that edge in MST. Otherwise, discard it.

Step 5: Repeat from step 2 until it includes $|V| - 1$ edges in MST.

II. Abhilasha [7] suggested that the steps involved in Prim's Algorithm to generate a minimum:

Step 1: Determine the arbitrary starting vertex.

Step 2: Keep repeating steps 3 and 4 until the fringe vertices (vertices not included in MST) remain.

Step 3: Select an edge connecting the tree vertex and fringe vertex having the minimum weight.

Step 4: Add the chosen edge to MST if it doesn't form any closed cycle.

Step 5: Exit

III. Gray & Ping [3] introduce a path is a trail in which neither vertices nor edges are repeated we have compared three algorithms to find the shortest walk in the analysis a walking route to travel in Lopburi City.

3.4 The ideas of matrix

A matrix is a representation of data in the form of numbers or numbers. It consists of columns (vertical) and rows (horizontal). The matrix shows the relationship between two variables [8-9].

The adjacency matrix of graph G substituted by $A(G)$ is the matrix size $n \times n$ by

$$A(G) = [a_{ij}]_{n \times n} \text{ and } a_{ij} = \begin{cases} 1 & \text{If } v_i \text{ close to } v_j \\ 0 & \end{cases}$$

If the graph G is written in the form of an adjacency matrix by adding weights to the adjacent points, it can be written as follows:

$$A(W(G)) = \begin{matrix} & \begin{matrix} v_1 & v_2 & v_3 & v_4 & v_5 & v_6 \end{matrix} \\ \begin{matrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{matrix} & \begin{bmatrix} 0 & 1(1) & 1(3) & 1(3) & 0 & 0 \\ 1(1) & 0 & 1(1) & 0 & 0 & 1(5) \\ 1(3) & 1(1) & 0 & 1(1) & 0 & 1(3) \\ 1(3) & 0 & 1(1) & 0 & 1(1) & 0 \\ 0 & 0 & 0 & 1(1) & 0 & 1(3) \\ 0 & 1(5) & 1(3) & 0 & 1(3) & 0 \end{bmatrix} \end{matrix}$$

3.5 Gephi

Gephi is a free and open-source, graph network, display program that supports real-time data and graph display.

3.5.1 The Gephi method is divided into three parts as follows: 1) Community detection isolates data into related groups using modularity. 2) Centrality is the most centralization of a social network by means of Degree Centrality, Betweenness Centrality, and Closeness Centrality. 3) Find the shortest path between two nodes under the graph, that is, in the shortest path, the sum of the weights on each edge adds up to the shortest of all paths.

3.5.2 The Gephi features consist of three modes: 1) Mode 1 Overview is the mode of displaying the graph based on the input data in Mode 2 and selecting the shortest distance operation from two nodes and other operations using the mouse to click on. 2) Mode 2 Data Laboratory is an Excel-like data entry mode where point values (node) and edge are entered. This mode can process data to be used to find Degree Centrality, Betweenness, Centrality, and Closeness Centrality. And 3) Mode 3 Preview is a mode that shows the physical characteristics of the data such as size, color of points and size, color, weight of lines, etc [10].

4. Research Methodology

4.1 Research Instruments

4.1.1 Satellite map: google.co.th/maps to be displayed in a graph

4.1.2 The concept of graph theory uses paths (walks with no duplicate walking points) to find walkways that pass through every point. And three algorithms use to compare in finding the shortest walk in the analysis of walking. The adjacency matrix concept is used to determine the weight of a line (distance and time) to find the shortest path and the shortest time from the adjacent point. In addition, the Gephi program is used to check the resulting graph that the direction and path of travel are correct or not including checking the weight of the line (distance and time).

4.2 Research Method: The research process is divided into 6 phases:

4.2.1 Study the research documents of tourist attractions in Lopburi.

4.2.2 Explore the real location of the walking trails for sightseeing in Lopburi.

4.2.3 Calculate the distance and time it takes to walk for sightseeing in Lopburi by comparing it with google.co.th/maps.

4.2.4 Walking path for tourism in downtown Lopburi was analyzed by using the paths graph theory and adjacency matrix together with the Gephi examination.

4.2.5 Summarize suitable walking routes in Lopburi.

4.2.6 Public relation of A Walking Route to Travel in Lopburi City to The Public Relations Office Lopburi Province

(The researcher has submitted a request for public relation of a walking route to travel in lopburi city to The Public Relations Office Lopburi Province. And it has published walking routes for tourism in Lopburi a via Facebook fanpae on August 20, 2020.)

4.3 Data Collection Method

4.3.1 Show a satellite map of 10 tourist attractions in Lopburi from google.co.th/maps in schematics and satellite maps.

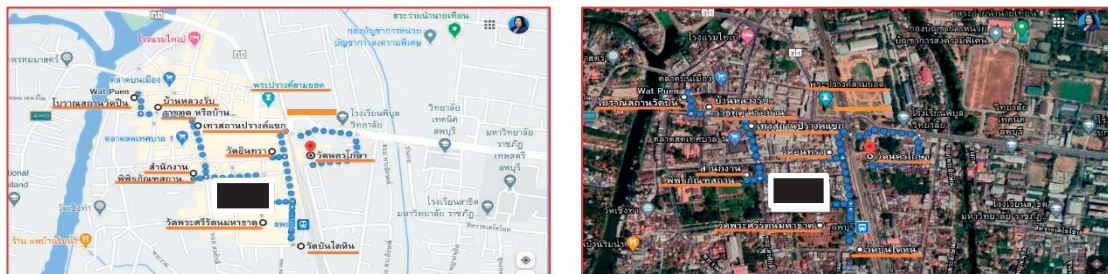


Figure 2. Satellite map of tourist attractions in Lopburi [11]

4.3.2 Measure the distance and time it takes to walk for sightseeing in Lopburi from google.co.th/maps as shown in Figure 1 together with the measurement of distance and time from the actual location.

4.3.3 Create a graph of the location of 10 attractions in Lopburi City and a route showing distance and time (meters; minute).

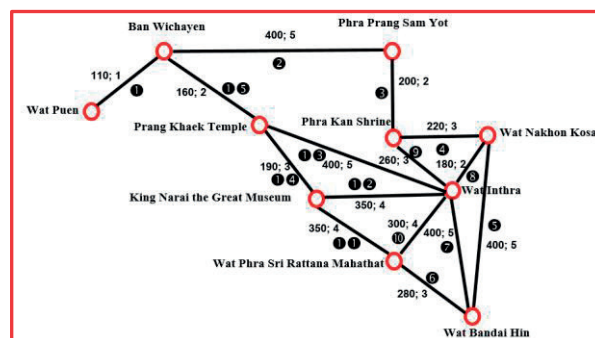


Figure 3. Graph showing walking routes for tourism in Lopburi (Distance (m); time (min))

4.4 Analysis

Wathanasiripong et al. [12] conducted research by visiting 9 temples on a trip to Ayutthaya. The Khruskhal's algorithm was then applied to the minimum spanning tree to find the travel route with the shortest distance to complete the worship trip.

● Use the path analysis of the graph with every places walking by Kruskal's Algorithm and Prim's Algorithm to find the shortest path is as follows.

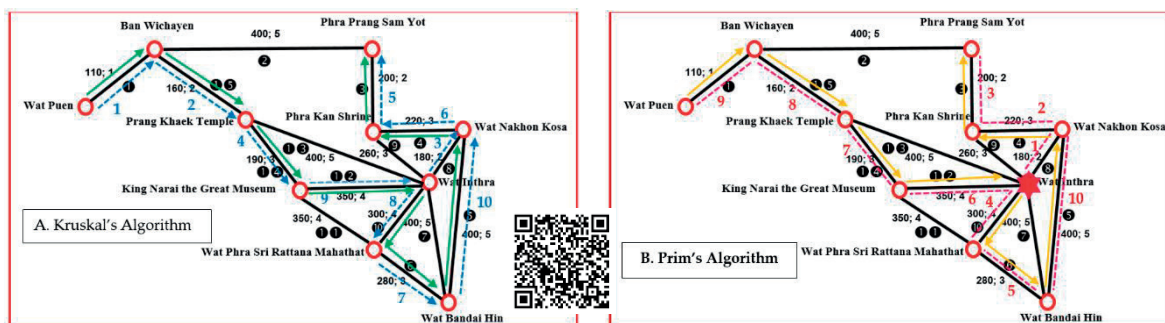


Figure 4. Graph showing walking routes for tourism in Lopburi by Kruskal's Algorithm (A) and Prim's Algorithm (B)

Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Inthra → Wat Phra Sri Rattana Mahathat → Wat Bandai Hin → Wat Nakhon Kosa → Phra Kan Shrine → Phra Prang Sam Yot

With distance $110 + 160 + 190 + 350 + 300 + 280 + 400 + 220 + 200 = 2,210$ m.

● Use the path analysis of the graph with every walking way and the adjacency matrix that considers adjacent points to find the shortest path is as follows.

4.4.1 A suitable walking route for traveling in Lopburi without losing general meaning. The starting point at Wat Puen has the following path:

1. *Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Phra Sri Rattana Mahathat → Wat Bandai Hin → Wat Inthra → Wat Nakhon Kosa → Phra Kan Shrine → Phra Prang Sam Yot*

With distance $110 + 160 + 190 + 350 + 280 + 400 + 180 + 220 + 200 = 2,090$ m.

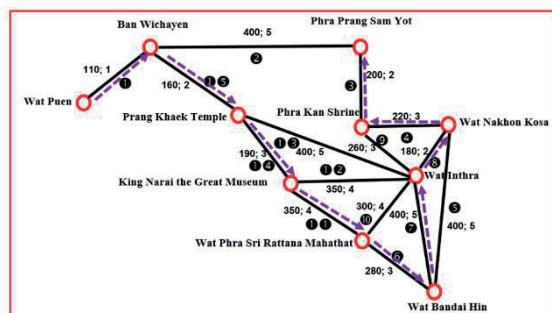


Figure 5. Graph showing walking routes for tourism in Lopburi by a path's Algorithm

2. *Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Phra Sri Rattana Mahathat → Wat Bandai Hin → Wat Nakhon Kosa → Wat Inthra → Phra Kan Shrine → Phra Prang Sam Yot*

With distance $110 + 160 + 190 + 350 + 280 + 400 + 180 + 260 + 200 = 2,130$ m.

Therefore, path 1 of the walking graph by a path for tourism in Lopburi, 10 places with all walking lines. It is the shortest path with a distance of 2,090 meters and considering the adjacency matrix can be shown as follows:

	Puen	Wichayen	Phra Pang	Prang Khaek	Phra Kal	Nakorn Kosa	Museum Lopburi	Wat Inthra	Mahathat	Bandai Hin
Puen	0	1(110)	0	0	0	0	0	0	0	0
Wichayen	1(110)	0	1(400)	1(160)	0	0	0	0	0	0
Phra Pang	0	1(400)	0	0	1(200)	0	0	0	0	0
Prang Khaek	0	1(160)	0	0	0	0	1(190)	1(400)	0	0
Phra Kal	0	0	1(200)	0	0	1(220)	0	1(260)	0	0
Nakorn Kosa	0	0	0	0	1(220)	0	0	1(180)	0	1(400)
Museum Lopburi	0	0	0	1(190)	0	0	0	1(350)	1(350)	0
Wat Inthra	0	0	0	1(400)	1(260)	1(180)	1(350)	0	1(300)	1(400)
Mahathat	0	0	0	0	0	0	1(350)	1(300)	0	1(280)
Bandai Hin	0	0	0	0	0	1(400)	0	1(400)	1(280)	0

4.4.2 The suitable walking route for traveling in Lopburi in terms of time without losing general meaning the starting point at Wat Puen has the following path:

1. Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Phra Sri Rattana Mahathat → Wat Bandai Hin → Wat Inthra → Wat Nakhon Kosa → Phra Kan Shrine → Phra Prang Sam Yot for $1 + 2 + 3 + 4 + 3 + 5 + 2 + 3 + 2 = 25$ minutes

2. Wat Puen → Ban Wichayen → Prang Khaek Temple → King Narai the Great Museum → Wat Phra Sri Rattana Mahathat → Wat Bandai Hin → Wat Nakhon Kosa → Wat Inthra → Phra Kan Shrine → Phra Prang Sam Yot for $1 + 2 + 3 + 4 + 3 + 5 + 2 + 3 + 2 = 25$ minutes

Therefore, the walking route graph for tourism in Lopburi province, 10 places, all places, all routes the shortest path takes 25 minutes. The distance in path 1. was chosen because it is the shortest. And consider the adjacency matrix as follows:

	Puen	Wichayen	Phra Pang	Prang Khaek	Phra Kal	Nakorn Kosa	Museum Lopburi	Wat Inthra	Mahathat	Bandai Hin
Puen	0	1(1)	0	0	0	0	0	0	0	0
Wichayen	1(1)	0	1(5)	1(2)	0	0	0	0	0	0
Phra Pang	0	1(5)	0	0	1(2)	0	0	0	0	0
Prang Khaek	0	1(2)	0	0	0	0	1(3)	1(5)	0	0
Phra Kal	0	0	1(2)	0	0	1(3)	0	1(3)	0	0
Nakorn Kosa	0	0	0	0	1(3)	0	0	1(2)	0	1(5)
Museum Lopburi	0	0	0	1(3)	0	0	0	1(4)	1(4)	0
Wat Inthra	0	0	0	1(5)	1(3)	1(2)	1(4)	0	1(4)	1(5)
Mahathat	0	0	0	0	0	0	1(4)	1(4)	0	1(3)
Bandai Hin	0	0	0	0	0	1(5)	0	1(5)	1(3)	0

4.4.3 Verification using Gephi

4.4.3.2 Checking the graph

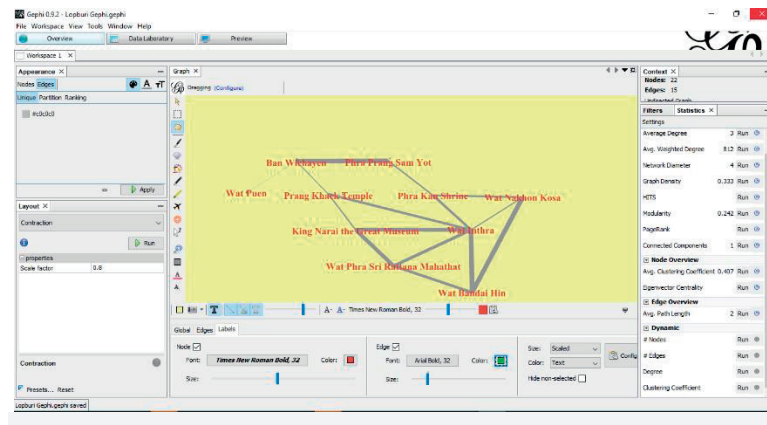
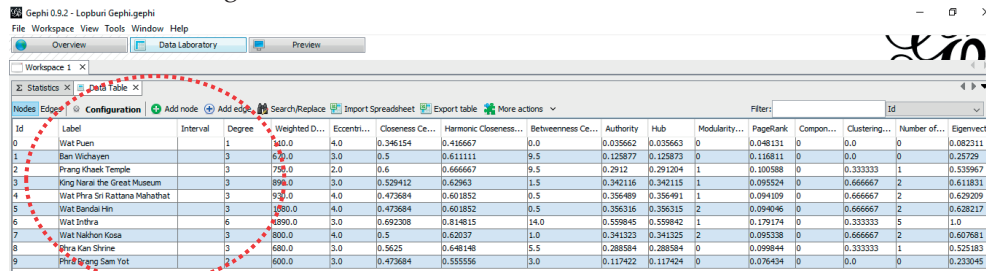


Figure 6. Graph showing walking routes for tourism in Lopburi by Verification using Gephi

Checking the graph obtained from the Gephi has a similar pattern to the graph obtained by drawing the graph with the actual site survey data and the data from the google.co.th/ maps.

4.4.3.2 Checking the data



Id	Label	Interval	Degree	Weighted D...	Eccentr...	Closeness Ce...	Harmonic Closeness...	Betweenness Ce...	Authority	Hub	Modularity...	PageRank	Compon...	Clustering...	Number of...	Eigenvect...
0	Wat Puen	1	60.0	4.0	0.346154	0.416667	0.0	0.035662	0.035663	0	0.048131	0	0.0	0	0	0.082311
1	Ban Wichayen	3	20.0	3.0	0.5	0.611111	8.5	0.125877	0.125873	0	0.116811	0	0.0	0	0	0.25729
2	Prang Khaek Temple	3	750.0	2.0	0.6	0.666667	9.5	0.2912	0.291204	1	0.100588	0	0.333333	1	0	0.535967
3	King Narai the Great Museum	3	890.0	3.0	0.529412	0.62963	1.5	0.342116	0.342115	1	0.095524	0	0.666667	2	0	0.611831
4	Wat Phra Sri Rattana Mahathat	3	930.0	4.0	0.473684	0.601852	0.5	0.356489	0.356491	1	0.094109	0	0.666667	2	0	0.629209
5	Wat Bandai Hin	3	1880.0	4.0	0.473684	0.601852	0.5	0.356316	0.356315	2	0.094046	0	0.666667	2	0	0.628217
6	Wat Inthra	6	8690.0	3.0	0.692308	0.814815	14.0	0.559845	0.559842	1	0.179174	0	0.333333	5	1.0	0
7	Wat Nakhon Kosa	3	800.0	4.0	0.5	0.62037	1.0	0.341323	0.341325	2	0.095338	0	0.666667	2	0	0.607681
8	Phra Kan Shrine	3	680.0	3.0	0.5625	0.648148	5.5	0.288584	0.288584	0	0.099844	0	0.333333	1	0	0.525183
9	Phra Prang Sam Yot	600.0	3.0	0.473684	0.555556	3.0	0.117422	0.117424	0	0.076434	0	0.0	0.0	0	0	0.233045

Figure 7. Graph showing distance, degrees, and weights by verification using Gephi

Distance data entered into Gephi, degrees, and weights of each graph corresponding to the graph obtained using graph theory analysis.

5. Results

The walking route for tourism in Lopburi has a walking distance of 2,090 meters and takes 10 places in 25 minutes to reach 10 places

When analyzing the data obtained from the basic concepts of graph theory and matrix concepts, with satellite maps and schematics from google.co.th/maps Along with exploring the real place, the suitable walking routes for traveling in Lopburi are as follows:

1. If arriving by private car, bus (where parking is available), or train, it is best to start from *Phra Prang Sam Yot* → *Phra Kan Shrine* → *Wat Nakhon Kosa* → *Wat Inthra* → *Wat Bandai Hin* → *Wat Phra Sri Rattana Mahathat* → *King Narai the Great Museum* → *Prang Khaek Temple* → *Ban Wichayen* → *Wat Puen*
2. If traveling by motorcycle may start from no. 1 above or start from *Wat Puen* → *Ban Wichayen* → *Prang Khaek Temple* → *King Narai the Great Museum* → *Wat Phra Sri Rattana Mahathat* → *Wat Bandai Hin* → *Wat Inthra* → *Wat Nakhon Kosa* → *Phra Kan Shrine* → *Phra Prang Sam Yot*

Both routes have a total walking distance of 2,090 meters and a total walking time of 25 minutes to each location (does not count the distance and visit time at each location).

6. Discussion and Conclusion

6.1 Discussion

Research trends in graph theory applied to multiple dimensions such as architecture, Tourism, medical, transportation, etc [13,14]. Similarly, this research presents the application of graph theory in the form of a combination of computer programming technology to more than examine the reliability of mathematical proofs. The Lopburi walking route graph is a path graph with 10 walking points and 9 routes corresponding to the definition of a path graph, that is, a graph with order n and size $n - 1$

If set P_n as a path graph Which $n \geq 1$ by (distance) between u and G in graph v is the shortest length of $u - v$ comparing with path $u - v$ all in graph G substituted by $d_G(u, v)$

Because the walking route graph for tourism in the city of Lopburi contains a path. *Wat Puen* → *Ban Wichayen* → *Prang Khaek Temple* → *King Narai the Great Museum* → *Wat Phra Sri Rattana Mahathat* → *Wat Bandai Hin* → *Wat Inthra* → *Wat Nakhon Kosa* → *Phra Kan Shrine* → *Phra Prang Sam Yot* which is the shortest path, The graph must not be longer than 9.

6.2 Conclusion

6.2.1 In this research, the resulting graph is not a cycle graph. The starting point and the ending point, if it is the same point, must repeat the same route at the location of *Wat Puen* ↔ *Ban Wichayen*.

6.2.2 If the resulting graph is a cycle graph, It can compare to the algorithm of the traveling salesman problem [15].

6.2.3 This research uses an adjacency matrix to find paths that walk to adjacent locations.

6.2.4 In this research, the resulting graph has 10 points. Using the path, nine walking routes are obtained.

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Efficiency Enhancement with Rule-Based Method for Credit Classification

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Abstract: The efficiency enhancement with the Rule-based Method is a data mining technique to study the relationship between credit borrowers and deciding credit approval and reduce the risk of bad debt in the future. This research aims to efficiency enhancement with the Rule-based method for credit classification, which is the credit types data, numeric, and nominal used for the category from the cooperative savings database by using the Gain Ratio as a measurement unit of the sampling (Entropy) and filter to select important variables. Therefore, the researcher uses the K-fold cross-validation method by dividing the data to perform the test into equal K-part numbers into training and testing data sets. Then Rule-based approach of data mining techniques in WEKA software version 3.9.4viz Decision Table, RIPPER (JRip), OneR, and Partial Rule (PART) to efficiency enhancement of the model for credit classification to get more accurate and reliable by measuring the efficiency of the model with Recall, Precision, and F-measure. The results of the research can be found that both the Gain Ratio and the outlier data filter can make the efficiency of the model with the Rule-based method using the Partial Rule to get the highest Recall value of 4.1%, the highest Precision value of 4.0%, and highest F-measure value by 5.4%. Besides, the Partial Rule can make the model's efficiency for credit classification get a Recall of 86.1%, Precision of 85.9%, and F-measure of 85.6%. Thus, all values were more efficient than the Decision Table, JRip, and OneR.

Keywords: Gain Ratio; Rule-Based; Efficiency; Model

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1. Introduction

Currently, financial institutions lending to incur income from interest and aims to expand the size of the credit amount regardless of the quality of the released loans will cause bad debt problems to affect financial institutions lacking liquidity in the system. Generating the amount of more Non-performing Loan must set aside reserves money for Non-performing loan that does not incur the additional income that financial institutions are unable to expand lending as a result of the problem of making financial institutions must set aside debt reserves money a lot of money. When the bad debt or risk assets are high, that is necessary to build financial stability and trust among members, depositors, shareholders, and stakeholders [1]. To improve consideration and credit approval by the rule-based methods, it is a data mining technique to assist in

credit analysis in predicting credit borrowers from the model. It is a search for patterns or rules to build new knowledge with large amounts of hidden data to make credit analysis more efficient. Therefore, this research presents learning with rule-based methods, namely Decision Table, RIPPER (JRip), OneR, and Partial Rules (PART) and uses the principle of the K-fold cross-validation to separate data sets into a learning data set and testing data set, including the Gain Ratio is a measurement unit of the sampling (Entropy) and filter to select important variables in comparing the model efficiency with the value of Recall, Precision, and F-measure respectively. It was used to enhance the efficiency of the credit classification model obtained from the credit database of the savings cooperatives.

2. Materials and Methods

The purpose is the efficiency enhancement of the model using the Gain Ratio for selecting variables' importance and filtering out the outliers.

3. Theory and Literature Reviews

3.1 Theory

3.1.1 Data classification with Rule-based [2] is a model that shows a set of rules to have pattern characteristics 'IF-THEN' by one of the rules shown in the form below.

IF condition THEN conclusion

Such as R1: IF age = youth AND student = yes THEN buys_computer = yes

For example, the R1 consists of two parts: the condition of 'IF' called 'rule antecedent' or 'precondition', which includes a set of attributes that comprise the various conditions. The R 1 rule consists of attributes that indicate characteristics of people of both attributes: age = youth and student (student = yes) [3]. The condition of the rule will be linked to various attributes together with an AND symbol to indicate that the data must satisfy the specified conditions. In the second part, it is called 'rule consequence', and in the example of a rule, 1R can be written as a rule.

$$R1: (age = youth) \wedge (student = yes) \Rightarrow (buys_computer = yes)$$

If the record data x one according to the condition of the rule antecedent can tell that the R 1rule covers x record data or x record is covered by the R 1rule. Therefore, when the data set is composed of multiple records data, it will be able to find the percentage of all the records in the data set covered by the R 1rule, which it will call the coverage percentage as coverage, which can be calculated as follows.

$$\text{coverage (R)} = n_{\text{covers}} / |D|$$

Where n_{covers} is the number of records in the data set covered by the R rule, and $|D|$ is the number of records of all data in the dataset.

The rule's coverage value is used to evaluate the rule's data classification efficiency and the measurement of accuracy in the data classification of rules can be calculated as follows.

$$\text{Accuracy (R)} = n_{\text{correct}} / n_{\text{covers}}$$

Where n_{correct} is the number of records was classified correctly under the rules R

3.1.2 The Rule-based classification techniques in data mining used in research are as follows.

3.1.2.1 Decision Table Technique: [4]it is managing the group in a decision table style by taking the results of learning from the input data to have similar data characteristics to be in the same group by the last attribute of the table is used for determining the group to want to divide also.

3.1.2.2 PART Technique: [5] it is managing the group by the results in the form of rules to take data of each attribute as a condition in deciding the characteristics of the similar data to be in the same group by the data value of the attribute has the continuous value to use the signs "<", "<=", ">=", ">". In considering the discontinuous data part will use the sign "=" and use the condition "AND" for linking between attributes will link till the last attribute to divide the groups.

3.1.2.3 RIPPER Technique: [6] It is a rule that results from dividing the data classification rules into groups according to the target class and then using the pruning principle to select the rule more effectively than the specified error rate to use the data prediction.

3.1.2.4 OneR Technique: [7] It is an algorithm that provides an easy and intuitive way by Rule-based principles of creation for the classification by choosing the minor error value attribute for just one attribute to be the class predictor of the data which will be got the data classification rule of a smaller number.

3.1.3 Gain Ratio

GainRatio value is a measurement unit of the sampling (Entropy) to be important in predicting and the usability of the outlier data filter. When using knowledge gain to split the dataset, will be made inclination by the considered attribute has a considerable occurrence value to make the higher knowledge gain: $\text{Gain}(A) = \text{Info}(D) - \text{Info}_A(D) = \text{Info}(D) - 0 = \text{Info}(D)$ from the inclination may arise from the knowledge gain and $\text{Info}(D)$, known as entropy of D is the mean of the amount of data required to identify a category of records in data set which is dependent on the ratio of the number of the records corresponding to each category. [8] The researcher tries to reduce the inclination by developing new data division indicators with normalization for the knowledge gain and value calculation 'split information'.

$$\text{SplitInfo}_A(D) = - \sum_{j=1}^v \frac{|D_j|}{|D|} \times \log_2 \left(\frac{|D_j|}{|D|} \right)$$

By $\text{SplitInfo}_A(D)$ shows the amount of data to consider in the data set D into v subsets according to the value in attribute A . From $\text{SplitInfo}_A(D)$ value, the calculation can find the gain ratio below.

$$\text{GainRatio}(A) = \frac{\text{Gain}(A)}{\text{SplitInfo}(A)}$$

3.2 Literature Reviews

Almost every technique in data mining can create effective rule-based prediction models. Still, they cannot make effective rule-based prediction models from the real data and many researchers were applied to select more efficient variables as follows.

Phadung and Jaree [9] propose to develop an intrusion detection model and use four classification techniques, including Decision table, Naive Bayes, RIPPER, and PART decision list in data mining. The statistics used the percentage, Precision, Recall, and F-Measure. The research findings showed that RIPPER has the highest Precision, up to 99.00%. Then, the PART decision list has a precision up to 98.20%. Follow by Decision Table has the highest Precision, which is up to 97.50%. The lowest Precision is Naive Bayes 49.40%. Therefore, the RIPPER model has the highest average significantly.

Nongluk et al. [10] compare the efficiency of data mining classification techniques between K-nearest Neighbor (K-NN), Rule-based, and Decision trees by using the various parameters. The variant standard data sets such as Spambase, Zoo, and Nursery from the UCI Machine Learning Repository were used by selecting different parameters and characteristics. The accuracy and efficiency of each technique were analyzed and compared by specifying various parameters. Moreover, the data set was tested in varied 12 models per test set. The most accurate approach has more effective and appropriate for the characteristics of that data set. Finally, the analysis results have shown that K-NN and Decision Tree techniques are suitable for the slight attribute data set. At the same time, the Rule-based methods are appropriate for the massive attribute data set.

Kittisak [11] proposes a relationship between health conditions and diseases. The partial Rules algorithm outperformed the Decision tree. The Precision and recall are 88.60% and 89.20%, respectively, and the F-measure is 88.80%. A group of experts was invited to examine the obtained rules. Some inappropriate

attributes and rules were eliminated. The outcome was 44 rules and these rules were embedded into the automatic health screening system that allows people to examine their health condition by themselves.

Fadi et al. (2012) [12] propose investigating the Arabic text classification problem. In particular, four different Rule-based classification approaches: Decision trees (C4.5), Rule Induction (RIPPER), Hybrid (PART), and Simple Rule (One Rule), are evaluated against the published Corpus of Contemporary Arabic text collection. Analyzing the experiment results, we determine the most suitable classification algorithms for classifying Arabic texts.

Shafiullah et al. [13] propose improving the existing approach's performance. A rule-based learning method using statistical analysis has been presented in this paper to select a unique classifier for the same application. This study has been conducted using six classifiers, namely REP Tree, J48, Decision Stump, IBK, PART, and OneR, with twenty-five datasets. WEKA tool has been used in this study to develop the prediction model.

Rajalakshmi et al. [14] propose to help improve the quality of data. Discretization is dividing a continuous attribute into a finite set of intervals to generate an attribute with a few distinct values. This paper handle continuous values of the iris data set taken from the UCI machine learning repository. Data set used in various classification algorithms, namely J48, Random Forest, REP Tree, Naive Bayes, RBF network, OneR, BF Tree, and Decision Table. The performance measures are accuracy and error rate noted before and after discretization.

Shathian [15] proposes using data mining techniques to apply knowledge from data mining as the guidelines decision support credit approval. To study this experiment with harmonized data group classification group 3 technical result rule is Decision Tree: C 4.5, Decision Tree: Partial Rules and Decision Tables trial results found that classification groups Decision Tree: Partial Rules, the result is maximum is 72.7 % by data classification.

4. Research Methodology

In this research, the researcher divided the research methods as follows.

4.1 Data Collection: All the data are collected in 14 attributes, especially age, sex, status, revenue, occupation, province etc. Also, there is a data set to use in the study to be a number of 2,404 records from the savings and credit cooperative limited database. Afterwards, the data is converted into the CSV file format to use in the credit consideration, as shown in Table 1 and Figure 1.

Table 1. Attributes for the research

Attributes	Attributes Type	Meaning
Age	Numeric	Age
Sex	Nominal	Male,Female
Status	Nominal	Family, Single
Member Period	Nominal	Year
Salary	Numeric	Income
Capital stock	Numeric	Number of capital stock
Collateral	Nominal	Estate, Capital stock, Bank account
Approval	Numeric	Credit Limit Current
Instalment	Numeric	Payment
All instalments	Numeric	Term
Objectives	Nominal	Consumption,House,Car,Business
Occupation	Nominal	Governmental, Private ,Freelance
Address	Nominal	Rural ,Suburb , Town
Class	Nominal	Normal, Risk

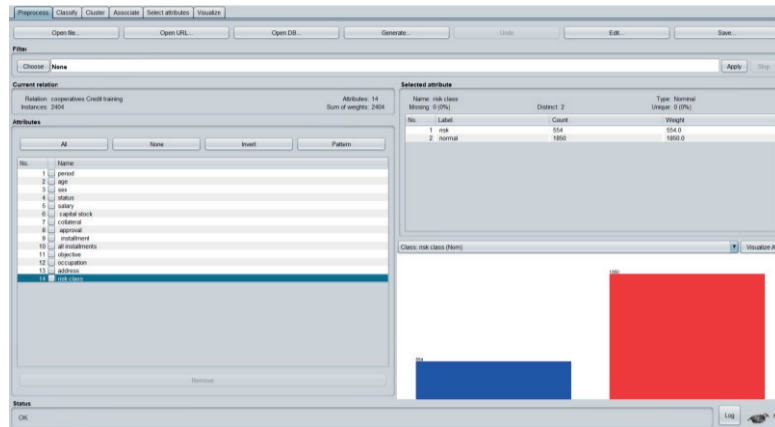


Figure 1. The number of credit data set before preprocessing

4.2 Data preprocessing: [16] Data transformations of the attributes analysis or the selection of variables are important for forecasting with Gain Ratio. Outlier data filters with the Gain Ratio will be used for creating trees. If any data is not classified with C4.5, the outlier data will be removed from the above preparation dataset, decreasing the data by only 1.33,% as shown in Table 2.

Table 2. The number of data before Rule-based methods

Class	Raw data	GainRatio	Outlier filter
Normal	1,850	1,850	1,823
Risk	554	554	549
Total	2,404	2,404	2,372

As for filtering out the outliers, it is an outlier by detecting an error value in the data set that is higher than or lower than normal. It cannot be classified in any group and falls outside the group. For example, no outlier is the value at 2,372 and yes outlier is the value at 32. Thereby, it should be discarded by an outlier of 32 values as in Figure 2 and Figure 3 . Besides, one raw data set was not filtered out of the attributes. In contrast, the same raw data set was filtered out the attributes by Gain Ratio. Afterwards, both groups were predicted by Rule-based methods. Therefore, it was found that the results of the testing are different from Table3.

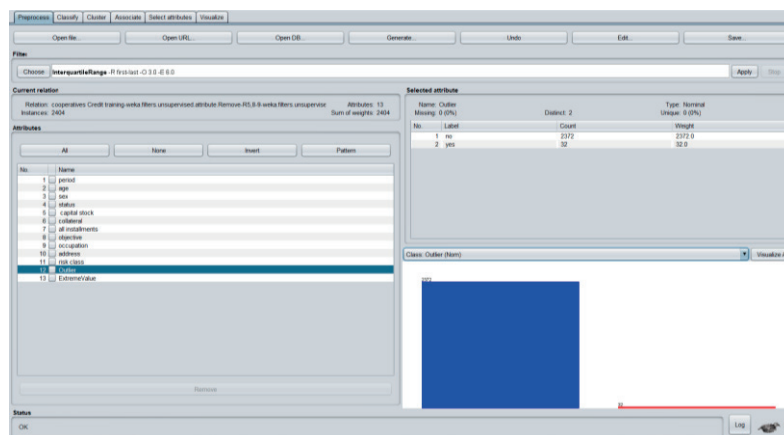


Figure 2. Before filtering out the outliers of the credit data set

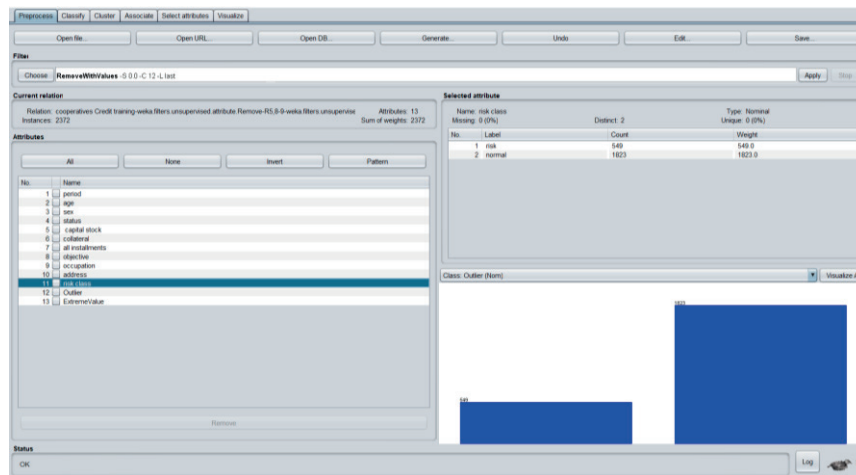


Figure 3. The number of credit data set after filtering outlier

4.3 Modeling: A prediction modelling by Rule-based can choose four methods, namely Decision Table, PART, RIPPER, and OneR. Therefore, the efficiency measurement of the model experiments to divide 2 data sets into a training data set and a testing data set using the -10fold cross-validation principle by dividing the data into several parts (K-fold cross-validation) for example, 10-fold cross-validation. It is to divide the data into 10 parts. Each of the parts has a number of the data equal parts. After that, [17] the data of one part is used to test the model's efficiency to cycle until the complete number of splitting and use Recall, Precision, and F-measure value demonstrates the model's efficiency below.

4.3.1 Recall is a measurement of correct of the model by considering the separation of each class: $\text{Recall} = \text{TP} / \text{TP} + \text{FN}$

4.3.2 Precision is a measurement of the model accuracy by considering the separation of each class: $\text{Precision} = \text{TP} / \text{TP} + \text{FP}$

4.3.3 F-measure is a simultaneous measurement of Precision and recall of the model by considering the separation of each class: $\text{F-measure} = 2 * (\text{Precision} * \text{Recall}) / (\text{Precision} + \text{Recall})$

Where accuracy is a measure of the model's accuracy by considering including all classes.

5. Results

Classification by Decision Table, JRip, OneR, and PART with each method and the efficiency measurement of the model by Recall, Precision, and F-measure. The test results of Table 3, Figure 3, Figure 4, and Figure 5 are as follows.

Table 3. Recall, Precision, and F-measure of Rule-based methods

Rule-based methods (10-fold)	Recall			Precision			F-measure		
	Raw data	GainRatio	Outlier filter	Raw data	GainRatio	Outlier filter	Raw data	GainRatio	Outlier filter
DecisionTable	0.639	0.700	0.683	0.426	0.514	0.490	0.511	0.593	0.571
JRip	0.689	0.750	0.743	0.789	0.814	0.812	0.590	0.668	0.664
OneR	0.721	0.730	0.725	0.738	0.694	0.700	0.672	0.666	0.665
PART	0.762	0.820	0.861	0.766	0.819	0.859	0.740	0.802	0.856

Recall values were derived after selecting the variable which the method of Decision Table and JRip get higher values at 6.10%. As for the technique of OneR, and PART is more value at 0.9% and 5.8%. Afterwards,

the variable selection and filtering of the outlier decreased efficiency by 1.7%, 0.7%, and 0.5%, except that PART increased by 4.1%. Therefore, it was found that the results of the method were shown the recall of the model viz PART was the highest value at 86.10%, OneR was the value at 73.0%, JRip was the value of 75.0%, and Decision Table was the value at 70% as Figure 4.

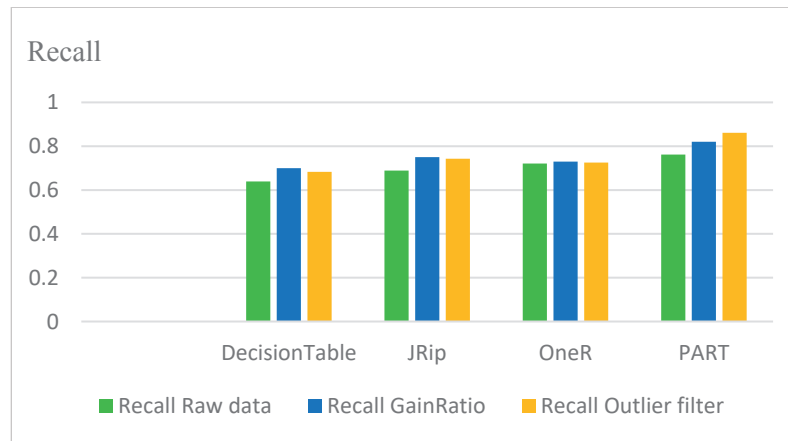


Figure 4. Recall values of the prediction rule

Precision values were derived after selecting the variable which the method of Decision Table, JRip and PART get higher values at 8.8%, 2.5%, and 5.3%. The method of OneR decreased by 4.4%. Afterwards, the variable selection, and filtering of the outlier, the efficiency is reduced by 2.4% and 0.2% except for OneR, and PART is increased by 0.6% and 4.0%. Therefore, it was found that the results of the method were shown Precision of the model viz PART was the highest value at 85.9%, OneR was the value at 73.8%, the JRip was the value at 81.4%, and Decision Table was the value at 51.4% as Figure 5.

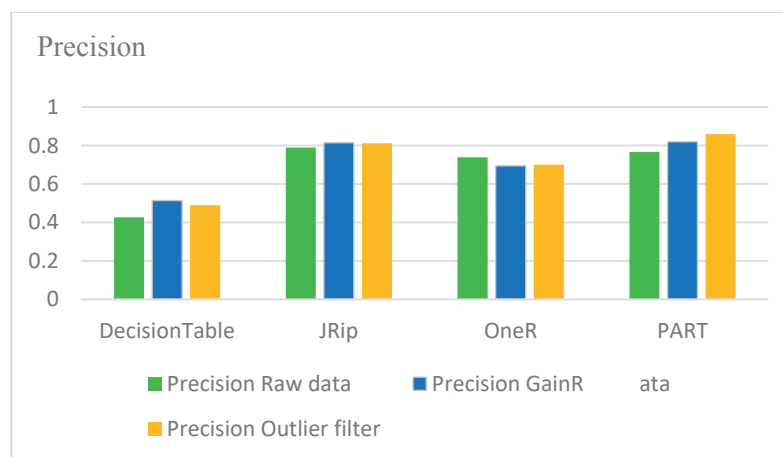


Figure 5. Precision values of the prediction rule

F-measure values were derived after selecting the variable in which the Decision Table, JRip, and PART get higher values at 8.2%, 7.8%, and 6.2%. As the method of OneR is decreased by 0.6%. Afterwards, the variable selection, and filtering of the outlier, the efficiency is reduced by 2.2%, 0.4%, and 0.1%, except for PART is increased by 5.4%. Therefore, it was found that the results of the method were shown the F-measure of the model viz PART was the highest value at 85.6%, OneR was the value of 67.2%, JRip was the value of 66.8%, and Decision Table was the value at 59.3% as Figure 6.

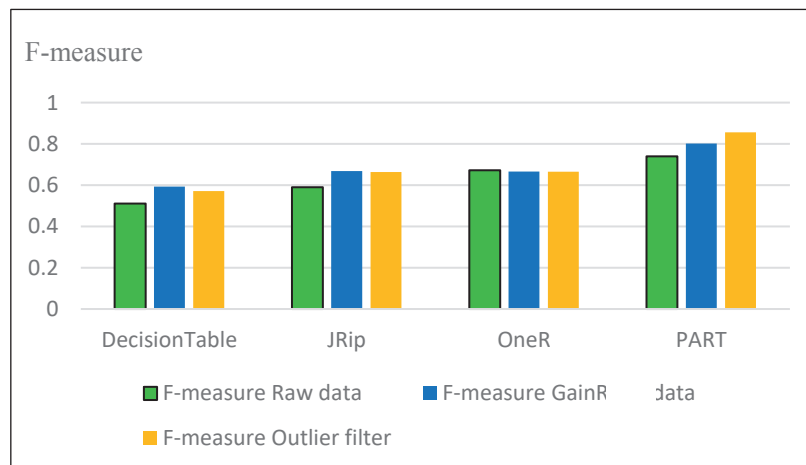


Figure 6. F-measure values of the prediction rule

6. Conclusions

The efficiency of the predictive rule model (Rule-based) uses the Gain Ratio to select the attributes and choose the data with the outlier to remove them. The model was used to compare four methods: Decision Table, JRip, OneR, and PART. Consequently, the results were found that both the Gain Ratio and the outlier filter to make the model's efficiency get PART method by the higher recall at 4.1%, higher Precision at 4.0%, and higher F- measure at 5.4%. Therefore, the PART method created the model with a Recall of 86.1%, Precision of 85.9%, and F-measure of 85.6%. Hence, all values were higher than the method of Decision Table, JRip, and OneR, respectively. Furthermore, the researcher studied the data structure, data cleaning, and data reduction to choose an appropriate PART model to apply as a guideline for future credit approval of savings cooperatives.

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Effect of Extracted Galangal Rhizome and Gelling Agents on Quality Attributes of Galanga Jelly Product

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Abstract: This research aimed to study the effects of extracted galangal rhizome and gelling agents on the quality attributes of the jelly product. The ratios between extracted galangal rhizome and water used in the study were 0:100, 25:75, and 50:50 (w/w). Gelling agents used in the study were only gelatin combined with potato starch. The products were tested by physical characteristics, chemical, and sensory acceptability. The sample product with extracted galangal rhizome was yellower than the product without extracted galangal rhizome. In addition, the higher the amount of extracted galangal rhizome, the greater the yellowness of the product. Increasing the levels of extracted galangal rhizome showed a tendency to increase the L^* , a^* , and b^* values. Results obtained from a texture analyzer showed that using 20g of gelatin, 2.5g of potato starch, and 25% of extracted galangal rhizome had the highest values of hardness, springiness, and chewiness ($P \leq 0.05$) of 7.63 N, 10.21 mm and 76.76 (N•m), respectively. The water activity was in a range of 0.86-0.88 and the moisture content was 18.30-18.74%. Total phenolics content and IC₅₀ were 13.52-19.99 mgGAE/g of sample and 0.20-0.32mg/ml of jelly extract, respectively. From sensory evaluation, using 25% of extracted galangal rhizome and 2.5g of potato starch, and 20g of gelatin as gelling agents resulted in the highest overall acceptability.

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1. Introduction

Galanga (*Alpinia galanga* (L.) Willd.) is a plant in the Zingiberaceae family. Examples of plants in this family are Fingerroot, *Amomum testaceum*, True Cardamom, Ginger, Turmeric, and *Curcuma aromatica*. Galanga is called differently depending on its origin such as Se-oe-khoei (Mae Hong Son), Kha yuak (Northern Thailand), Kha luang (Northern and Northeast Thailand), and Katuk karohini (Central Thailand). It is reported that 100 grams of its baby rhizome provide 20 kilocalories [1]. The same amount of galangal rhizome (100 g) also provides 14 g, 9 g, 49 g, 19 g, 9 g, 3g, 16g, and 0.2-1.5% of moisture, total ash, matter soluble in 80% ethanol, matter soluble in water, total sugar, total nitrogen,

total protein, and essential oil content (dry wt.), respectively [2]. Galanga has numerous benefits, and its rhizome can be an ingredient in many recipes, spices, and medicines. Moreover, galanga also has medicinal properties. Its rhizome can treat stomachache, colic, and flatulence [3]. Its flower can be put over the skin to heal dermatophytosis [4]. Researchers reported that the dry root and rhizome had been used for their antioxidant, antidiabetic, antiulcer, antidiarrhea, anti-emetic, analgesic, anti-inflammatory, and anticoagulation effects [5-7]. However, galanga is a pungent and spicy herb that needs to be diluted before processing into other products.

Jelly is a trendy dessert nowadays, especially for children and teenagers, because they enjoy its chewy texture. Jelly has a gooey and flexible texture and comes in attractive shapes and colors. Jelly is made of the gelling agent [8]. Carrageenan, gelatin, starch, and pectin are widely used gelling agents [9]. Gelatin is a natural hydrocolloid protein found in animal bones, skin, and connective tissue [10]. This research used gelatin and potato starch as the gel-forming agents. Gelatin is most widely used in the gummy confection or confectionery gel industry as it quickly produces a stable gel texture and plays a role as an emulsifier [11]. Meanwhile, potato starch is more appropriate for gummy-type candies because of its clarity and elasticity over other starches after cooking [12].

After being solubilized, the softness of the jelly can range from soft to hard. This type of gelling agent solubilizes at 40 °C and has pH stability. It is common to use gelatin in desserts to give them a melt-in-your-mouth texture, a good taste, and a good smell [13]. Starch is a food additive used in desserts to help improve the surface of the jelly by thickening and stabilizing it. It prevents the crystallization of sugar, a sweetener that helps form the jelly's shape and prolongs the product's shelf life [14]. According to the Thai Industrial Standard Institute, sweeteners approved for desserts are sucrose, invert sugar, invert syrup, fructose syrup, glucose syrup, etc. The acids commonly used in desserts are citric acid, malic acid, lactic acid, and fumaric acid. Adding an acid is important for the taste and helps stabilize the jelly. However, too much acid would ruin its stabilization of it. Usually, the pH of jelly should be around 2.8-3.5 [15] (p. 2).

This study aimed to study the appropriate ratio of extracted galangal rhizome and gelling agents to make the jelly, galanga jelly's physical and chemical properties, and consumers' acceptance of galanga jelly in terms of senses.

2. Materials and Methods

2.1. Ingredient preparation

Galangal rhizomes were purchased at the local market in Nakhon Ratchasima province, Thailand. The rhizomes had to be fresh, not too immature, or too mature. There were short rhizomes around the roots. The outer skin of the roots was orange-brown. The roots' inside were yellow and in good condition without a foul smell. The rhizomes were cleaned, and unwanted parts such as pseudostems, some root parts went wrong, or soil were cut out. Then, the root was left to dry. After that, the rhizomes were sliced into little pieces and mixed with clean water using a blender (the ratios between galanga rhizome and water were 0:100, 25:75, and 50:50 (w/w), respectively). A cheesecloth was used to strain the extracted galangal rhizome. Then the extracted solution was used for the jelly production process. The galangal rhizome ratios at different levels were determined by the solubility of sample preparation between the rhizome and water. Then, the galangal rhizome was blended with clean water and filtered to be the primary raw material to produce the jelly.

2.2. Jelly production process

According to the study of ingredients in jelly production, there was a direct variation of formulas used. The formulas for the jelly production had different amounts of extracted galangal rhizome. Each formula had ingredients as follows. 100 mL of the extract was mixed with 36.5 g of sucrose, 18.25 g of glucose syrup, and 0.55 g of citric acid. At the same time, the study of results of gelling agents was conducted on gelatin bloom 250 and potato starch. The study used 6 jelly formulas as follows:

0:100+G = Water: Galangal rhizome 0:100 and 20g of gelatin

25:75+G = Water: Galangal rhizome 25:75 and 20g of gelatin

50:50+G = Water: Galangal rhizome 50:50 and 20g of gelatin

0:100+GS = Water: Galangal rhizome 0:100 and 20g of gelatin, 2.5g of potato starch

25:75+GS = Water: Galangal rhizome 25:75 and 20g of gelatin, 2.5g of potato starch

50:50+GS = Water: Galangal rhizome 50:50 and 20g of gelatin, 2.5g of potato starch

The ingredients and process were divided into two parts. The first part was letting the gelatin sit in room temperature water (30 ml) until it became more significant. The second part was mixing extracted galangal rhizome, sucrose, potato starch, and glucose syrup. The mixture had to be simultaneously stirred until the temperature was raised to 110 °C. Then, two parts were mixed and let sit until the final total soluble solids (TSS) content was 70°Brix and pH 3.5. Afterwards, pour the mixture into silicone molds, and put them in the fridge for 24 hours (at a temperature between 4-8 °C and relative humidity between 85-95%). Then, take the jellies out of the silicone molds (size of 2.4 x 2.4 x 1.9 cm³) and store them in containers to study their physical and chemical properties and test consumers' acceptance.

2.3. Physical analysis

2.3.1. Color measurement

The surface color of the jelly was measured with a chroma meter (CR-400, Minolta Co., Osaka, Japan). The measurement was repeated six times. The results were expressed as L* (lightness), a* (redness-greenness), and b* (yellowness-blueness).

2.3.2. Texture analysis

Texture profile analysis (TPA) was carried out using a texture analyzer (TA General, Brookfield engineering Labs Inc., USA) with a 5 kg-load cell. Compression tests evaluated Experiments, which generated a plot of force (g) versus time (s). A 25.4 mm diameter TA 11/1000 probe was used to measure the textural profiles of samples.

2.4. Chemical analysis

2.4.1. Water activity (*a_w*) and Moisture content

Water activity (*a_w*) and Moisture content were measured at room temperature (25 °C), Aqualab equipment (water activity analyzer, aw-SPRINT-TH500, Novasina, Switzerland) and moisture equipment (moisture analyzer, MA37, Sartorius, Germany), respectively.

2.4.2. Total phenolic content and antioxidant activity

Jellies extracts were prepared by mixing one gram of sample with hot water (90-95°C) for 15 min. After centrifugation, the obtained extracts were kept at 4°C until further analysis (applied from [17] (p. 2)).

Extracted galangal rhizomes at different concentrations (galanga rhizome: water at 0:100, 25:75, and 50:50) and galanga jellies (extracted galangal rhizome: water at 0:100+G, 25:75+G, 50:50+G, 0:100+GS, 25:75+GS, and 50:50+GS) were determined for the total phenolic content and antioxidant activity.

The total phenolic content (TPC) measurement process was done by using the method in [18] (p. 360). The extracted sample (100uL) and 100 uL of Folin-Ciocalteu's phenol reagent were mixed for 3 minutes. Then, 1 ml of Na₂CO₃ solution (20%) was added to the mixture. The mixture was then incubated for 30 minutes in a room with no lights. The absorbance was measured at 750 nm to the blank. Gallic acid was used to obtain the standard curve. TPC unit was described in mg, and gallic acid was equivalent to the extracted galangal rhizome per gram (mg GAE/ml sample) and per 100g of galangal jelly (mg GAE/g).

DPPH method was used to determine the antioxidant measurement. The DPPH assay measured the electron-donating ability of the extracts and commercial antioxidants according to the bleaching of purple-coloured methanol solution. The assay added 5 ml of 0.004% DPPH in methanol to 50 µl of samples at different concentrations. After 30 minutes of incubation at room temperature, the absorbance reaches 517 nm. The bleaching of the purple color indicated the positive antioxidant activity. The percentage of free radical inhibition by DPPH was calculated by equation (1) [18] (pp. 360-361).

$$\%I = \left[\frac{(AB-AA)}{AB} \right] \times 100 \quad (1)$$

Where: AB = the absorbance of a blank after 30 min
AA = the absorbance of the sample after 30 min

The results were reported as IC_{50} , calculated from the percentage of free radical inhibition (%) versus concentrations of the respective sample curve.

2.5. Sensory evaluation

The testers evaluated product acceptance on the nine-point hedonic scale. One point was the least liked and the 9 point was the most appreciated. The sample group was 30 trained panellists, and each person tasted 6 samples simultaneously. The researcher assessed the likings in appearance, colour, flavour, taste, texture, and overall acceptability.

2.6. Statistical Analysis

This study used a Completely Randomized Design (CRD) to analyze the physical and chemical qualities and the consumers' acceptance in terms of senses. The study used Randomized Complete Block Design (RCBD) to assess the rates in terms of senses 3 times. Then, I used the data from the assessment to do the Analysis of Variance (ANOVA) and compared the averages' difference at the confidence level of 95% using SPSS software.

3. Results and Discussion

3.1. Physical analysis

Different amounts of extracted galangal rhizome and gelling agents influenced the physical properties of the jelly product. According to the study, the different amounts of extracted galangal rhizome and gelling agents influenced the product's properties in each formula in terms of color and texture.

3.1.1 Appearance

The appearance of the product from each formula is shown in Figure 1.

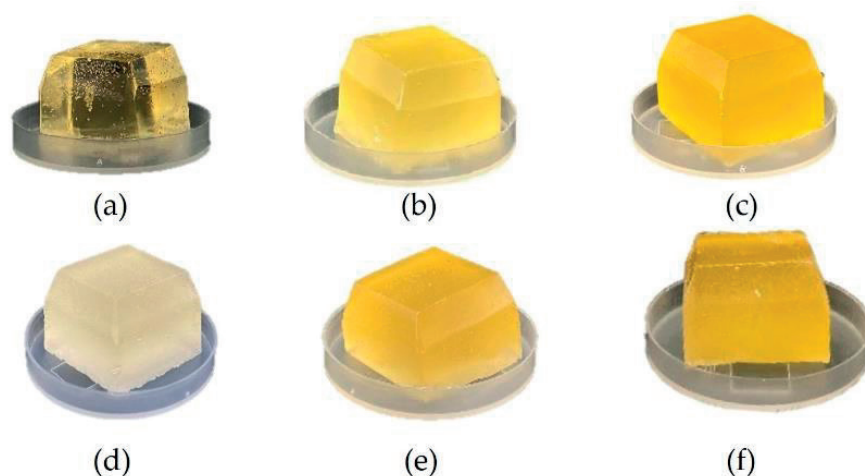


Figure 1. Samples of galanga jelly product with a ratio of extracted galangal rhizome and water at 0:100+G (a), 25:75+G (b), 50:50+G (c), 0:100+GS (d), 25:75+GS (e), and 50:50+GS (f).

Note: G = Gelatin and S = Potato starch

The sample product from every formula ranged from transparent to yellowish transparent. From the figures, the samples products with extracted galangal rhizome (Figures 1b, 1c, 1e, and 1f) were yellower than those without extracted galangal rhizome (Figures 1a and 1d). In addition, it was found that a higher amount of extracted galangal rhizome could influence the sample product to be more yellow. However, the different amounts of extracted galangal rhizome and gelling agents could barely affect the shape of the sample product.

3.1.2 Color parameters

The result from color evaluation by the CIE system, which consisted of L^* (lightness), a^* (redness), and b^* (yellowness), as described in Table 1, showed that the different amounts of extracted galangal rhizome had a statistically significant influence on a^* (redness) and b^* (yellowness) at ($P \leq 0.05$) but did not influence L^* (lightness) at ($P > 0.05$).

Table 1. Effects of galanga and water ratio and type of gelling agents on color parameters of the jelly product

Treatments	Color parameters		
	L^* (ns)	a^*	b^*
0:100 + G	53.14 ± 0.44	2.27 ± 0.14 ^b	6.00 ± 0.15 ^c
25:75 + G	53.34 ± 0.57	2.49 ± 0.20 ^b	15.53 ± 0.73 ^b
50:50 + G	53.62 ± 0.50	5.19 ± 0.28 ^a	20.92 ± 0.20 ^a
0:100 + GS	52.89 ± 0.14	2.45 ± 0.38 ^b	6.05 ± 0.07 ^c
25:75 + GS	53.28 ± 0.44	2.56 ± 0.12 ^b	15.85 ± 0.26 ^b
50:50 + GS	53.50 ± 0.47	4.74 ± 0.34 ^a	20.93 ± 0.17 ^a

Note: Values are the means of triplicate ± standard deviation. Those numbers with the different superscript letters in a block differ significantly ($P \leq 0.05$). ns, not significant ($P > 0.05$).

G = Gelatin and S = Potato starch

Using different gelling agents did not significantly influence L^* , a^* , and b^* at ($P > 0.05$). L^* , a^* , and b^* ranged between 52.89-53.62, 2.27-5.19, and 6.00-20.93, respectively. Higher amounts of extracted galangal rhizome influenced a^* and b^* to be higher. The sample from the formulas 50:50+G showed the highest L^* , which was 53.62, while the formulas 50:50+G and 50:50+GS showed the highest a^* and b^* , which were 5.19 and 20.93, respectively.

From studying the different amounts of extracted galangal rhizome and gelling agents that influenced the chemical properties of the jelly product. The ratios between extracted galangal rhizome and water used in the study were 0:100, 25:75, and 50:50 (w/w). Gelling agents used in the study were only gelatin combined with potato starch. This study aimed to study physical and chemical properties in appearance, color, texture, moisture, and water activity, including consumers' acceptance in terms of senses. The results showed that the jelly product's properties differed in each formula. The sample product that used extracted galangal rhizome as an ingredient would be yellower than the sample product without extracted galangal rhizome.

Moreover, with the human eye, using a different amount of extracted galangal rhizome and gelling agents could barely affect the shape of the jelly product. From studying the physical and chemical properties of the jelly product, it was found that higher amounts of extracted galangal rhizome would increase the redness and yellowness of the jelly product because galanga has flavonoids [19] (pp. 121-127). Flavonoid is part of polyphonic compounds commonly found in plant-based food. It produces red, yellow, purple, and blue pigmentation [19] (pp. 121-127). Therefore, increasing extracted galangal rhizome would increase flavonoids in the jelly product. The jelly product would have more redness and yellowness.

3.1.3 Texture profiles

According to the study about the different amounts of extracted galangal rhizome and gelling agents that influenced the texture by using texture profile analysis (TPA), it was found that the different amounts of extracted galangal rhizome and gelling agents had a statistically significant influence on hardness (N), springiness (mm), and chewiness (N•m) as described in Table 2.

Hardness (N) is the force from compressing to reduce the amount of the sample but ruin the shape simultaneously. It was found that hardness ranged between 3.33-7.63 N. The different amounts of extracted galangal rhizome and gelling agents had a statistically significant influence on hardness at ($P \leq 0.05$). While the sample from the formula 25:75+GS, which was the formula that contained 25% of extracted galangal rhizome and used gelatin and potato starch as gelling agents, had the lowest hardness at 3.33 N, the sample from the formula 0:100+G had the highest hardness at 7.63 N. The sample that used gelatin and potato starch would give higher hardness when compared to gelatin alone ($P \leq 0.05$).

Table 2. Effects of galanga and water ratio and type of gelling agents on texture profile of jelly product

Treatments	Hardness (N)	Springiness (mm)	Chewiness (N•m)
0:100 + G	6.61 ± 0.01 ^b	9.55 ± 0.48 ^{ab}	54.46 ± 2.41 ^b
25:75 + G	3.33 ± 0.00 ^e	9.61 ± 0.36 ^{ab}	26.70 ± 2.28 ^e
50:50 + G	5.59 ± 0.01 ^c	9.03 ± 0.36 ^{bc}	50.60 ± 3.20 ^b
0:100 + GS	7.63 ± 0.01 ^a	10.21 ± 0.64 ^a	76.76 ± 4.58 ^a
25:75 + GS	4.91 ± 0.00 ^d	8.85 ± 0.29 ^{bc}	38.43 ± 1.26 ^d
50:50 + GS	6.43 ± 0.01 ^b	8.70 ± 0.38 ^c	44.63 ± 3.28 ^c

Note: Values are the means of triplicate ± standard deviation. Those numbers with a different superscript letter in a block differ significantly ($P \leq 0.05$).

G = Gelatin and S = Potato starch

In addition, using extracted galangal rhizome as an ingredient in the formula could lower the hardness compared to the formula that did not contain extracted galangal rhizome. Springiness (mm) describes whether the product would return to its original shape after being compressed. It was found that the springiness ranged between 8.70-10.21 mm. The sample from the formula 50:50+G had the lowest springiness at 8.70 mm, while the highest springiness, 10.21 mm, came from the sample from the formula 0:100+G. The sample that used gelatin and potato starch would give higher springiness when compared to gelatin alone. Moreover, using extracted galangal rhizome as an ingredient in the formula tended to lower the springiness. Chewiness (N•m) describes elastic resistance from the food when chewed. It was found that the chewiness ranged between 26.70-76.76 N•m. Using different amounts of extracted galangal rhizome and gelling agents had a statistically significant influence on chewiness at ($P \leq 0.05$). The sample from the formula 25:75+GS had the lowest chewiness at 26.70 N•m ($P \leq 0.05$), while the highest chewiness, which was 76.76 N•m, came from the formula 0:100+GS. Using galanga as an ingredient in the formula could lower the chewiness ($P \leq 0.05$).

Hardness was the force from compressing to ruin the shape of the sample. The chewiness was elastic resistance from the sample when chewing or crushing until the shape was ruined. Springiness was the ability of the sample to return to its original shape after it had been compressed. When combining gelatin and potato starch as gelling agents, the jelly product would have a more robust structure, causing it to be more complex, thicker, and stretchier. It would need more energy to crush. Therefore, the hardness, the chewiness, and the gumminess would be increased because potato starch had macromolecules and consisted of polymer with 2 types of α -D-glucose units: Amylose and Amylopectin. Amylose was a linear polymer of glucose with an α 1,4-linked D-glucose bond [21-22] (pp. 1151-1152) (p. 149). Apart from being a gelling agent, the functional properties of potato starch were being a thickening agent [21] (p. 1552). The gel which contained potato starch would be thick. Therefore, mixing potato starch and gelatin would make the structure of the gel harder. The hardness would be higher than gel made from gelatin alone. When the hardness was higher, the chewiness and the springiness would also be higher. In addition, there was a report that mixing corn potato starch with gelatin would make the hardness higher than using gelatin alone because the structure of gel made from only gelatin had porosity. The resulting gel would be soft and velvety. Adding corn potato starch would decrease the porosity and produce a more compact appearance. Hence the hardness would be higher [23] (pp. 240-241). Using 25% of extracted galangal rhizome, potato starch, and gelatin as gelling agents resulted in the highest overall acceptability because of the appropriate galanga smell. Mixing potato starch and gelatin could improve the texture of the jelly; hence it was preferred by the customers.

3.2. Chemical properties

3.2.1. Moisture content and water activity (a_w)

According to the study about moisture content and a_w of jelly products that used different amounts of extracted galangal rhizome and gelling agents, results from the experiments were 18.30-18.74% and 0.86-0.88, respectively. The difference between the amount of extracted galangal rhizome and gelling agents had a statistically significant influence on the jelly product's moisture content ($P \leq 0.05$). The jelly product with extracted galangal rhizome had a higher moisture content than the jelly product without extracted galangal rhizome ($P \leq 0.05$), and the moisture content tended to decrease when the amount of extracted galangal rhizome was increased. The sample from the formula 0:100+GS had the highest moisture content at 18.74%. Using different gelling agents did not have a statistically significant influence on the moisture content of the jelly product ($P > 0.05$), as described in Table 3.

Table 3. Effects of extracted galangal rhizome and water ratio and type of gelling agents on water activity and moisture content of the jelly product

Treatments (extracted galangal rhizome: water)	Water activity (a_w) (ns)	Moisture content (%)
0:100 + G	0.87 ± 0.17	18.63 ± 0.10^a
25:75 + G	0.86 ± 0.74	18.58 ± 0.03^a
50:50 + G	0.86 ± 0.75	18.30 ± 0.04^b
0:100 + GS	0.87 ± 0.38	18.74 ± 0.11^a
25:75 + GS	0.88 ± 0.18	18.43 ± 0.39^{ab}
50:50 + GS	0.87 ± 0.01	18.36 ± 0.14^b

Note: Values are the means of triplicate $\pm$ standard deviation. Those numbers with a different superscript letter in a block differ significantly ($P \leq 0.05$). ns, not significant ($P > 0.05$).

G = Gelatin and S = Potato starch

According to the a_w of the jelly product in table 3, using different amounts of extracted galangal rhizome and gelling agents did not significantly influence the jelly product ($P > 0.05$). The formulas with the lowest a_w were 50:50+G and 25:75+G. Both formulas had the same value, which was 0.86.

From the experiments, a_w and moisture content were 18.30-18.74% and 0.86-0.88, respectively, consistent with the study mentioned above. Increasing extracted galangal rhizome would decrease moisture content. The jelly product that used 50% of extracted galangal rhizome had the lowest moisture content because increasing extracted galangal rhizome would reduce the amount of water, which was a component of jelly, which caused moisture content to decrease. Moreover, In the gelled gummy systems, water acts as a plasticizer to aid gelation of gelatin formation [25] (p. 181). Gelatin has a high affinity for water (the value of bound water 0.44 g/g dry material) in the sucrose/potato starch/gelatin system. The higher the solid content in gelatin solution, the faster the gel formation is affected by gel concentration [8].

Nonetheless, the experiment's water activity and moisture content were consistent with a study indicating that general jelly products had a moisture content of less than 20%, and water activity ranged between 0.7-0.8 [20] (pp. 37-41).

3.2.2. Phenolic compounds and antioxidant activity

The amounts of phenolic compounds and antioxidant activity (IC_{50}) of extracted galangal rhizome in various concentrations (galanga rhizome: water at 0:100, 25:75, and 50:50) and galanga jellies (extracted galangal rhizome: water at 0:100+G, 25:75+G, 50:50+G, 0:100+GS, 25:75+GS, and 50:50+GS) are shown in Figure 2. Considering the amounts of phenolic compounds and IC_{50} , it was found that galangal extract with the highest concentration (0:100) had the most phenolic compounds and IC_{50} ($P \leq 0.05$), which equalled 28.73

± 0.25 mg GAE/g of the sample (extracted solution) and 0.83 ± 0.03 mg/ml of jelly extract, respectively. Considering the amounts of phenolic compounds and IC_{50} of galanga jellies, it was found that the sample of 0:100 extracted galangal rhizome, including the 0:100+G and 0:100+GS jelly samples, which were the control sample, indicated the low amounts of phenolic compounds and IC_{50} while using gelatin or gelatin mixed with potato starch to produce jelly in the same ratios of extracted galangal rhizome which were 50:50+G and 50:50+GS did not cause any difference to the phenolic compounds of the samples, plus the 25:75+G and 25:75+GS jelly samples had statistically different amounts of phenolic compounds ($P \leq 0.05$). However, the amounts of phenolic compounds in both samples were not hugely different, that was 13.52 ± 0.20 mg GAE/g of the sample (e) and 14.96 ± 0.23 mg GAE/g of sample (d), respectively. Similarly, IC_{50} of the 50:50+G and 50:50+GS samples were statistically different but not hugely different, that was 0.32 ± 0.01 mg/ml of jelly extract (C) and 0.28 ± 0.01 mg/ml of jelly extract (D), respectively.

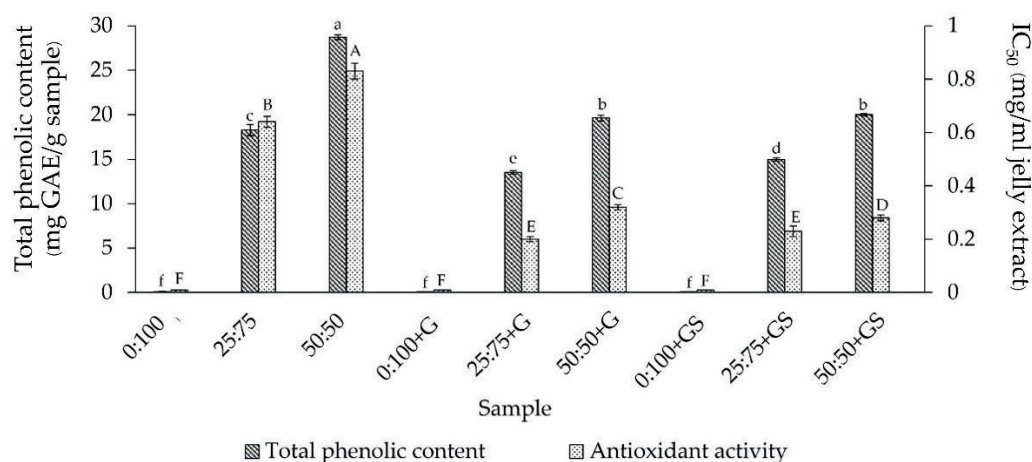


Figure 2. Total phenolic content and IC_{50} of extracted galangal rhizome and galangal jellies.

Note : G = Gelatin and S = Potato starch

The increase and decrease in the amount of extracted galangal rhizome usage caused the amounts of phenolic compounds and IC_{50} of extracted galangal rhizome and galangal jellies to differ. These properties are directly varied according to the quantities of extracted galangal rhizome used as an ingredient to produce the jelly.

According to the review of total phenolic content and IC_{50} of the galangal rhizome extract from water extraction in many research, it was reported that the amount of total phenolic content in galangal rhizome extract derived from water extraction (10% fresh galangal) was 8.25 ± 0.78 mg GAE/g of extraction while the amount of total phenolics in galangal rhizome extract derived from 50% ethanol extraction (10% fresh galangal) was 31.49 ± 4.09 mg GAE/g of extraction and fresh galangal extract derived from water extraction had $IC_{50} = 54.39$ mg/ml, plus fresh galangal extract derived from ethanol extraction had $IC_{50} = 10.50$ mg/ml [18] (pp. 358-365) and [24] (pp. 44-46). The total phenolic content of galangal extract from 5% of root-derived from water extraction at the temperatures of 40°C, 50°C, 60°C, and 70°C were approximately 0.0125, 0.0135, 0.014, and 0.0185 mg GAE/mg DW, respectively. The IC_{50} was approximately 0.45, 0.60, 0.74 and 0.50 mg/ml, respectively. In addition, 4% galangal extract had a total phenolic of 442.6 ± 69.1 mg GAE/100g, and the IC_{50} was 3554.9 ± 55.6 ppb [25] (pp. 24-25). Nonetheless, the total phenolic content and IC_{50} were different due to the extraction methods and the concentration of galangal rhizome. For example, [18] (p. 362) extracted galangal by distilling galangal powder at 80°C for 3 hours, while [24] (p. 41) used hot water at different temperatures for 1 hour to extract galangal. [25] (p. 23) extracted galangal by boiling galangal before mixing it with distilled water. The ratio of boiled galangal and distilled water was 1:25.

Many phytochemicals are found in galangals, such as flavonoids, terpenoids, saponins, and phenolic acids, including essential oils [26] (p. 3). Flavonoids are the prominent phytochemicals found in galangal [4] (p.1). Flavonoids can prevent oxidative stress by eliminating free radicals (antioxidant) and

antimicrobial properties [26] (p. 4) because flavonoids are sensitive to their environmental conditions (temperature, light, oxygen, and pH) [27] (p.1). Therefore, galangal jelly production used heat to process the product, and it caused IC₅₀ of the jelly to be lower when compared to start extract (extracted galangal rhizome). This was similar to the study of [28] that developed the production process of jelly from berry extracts by using the microwave to reduce the heating process from the normal process. Moreover, using gelatin as a gelling agent to produce jelly might cause IC₅₀ to be lower. This may be due to the aggregation of gelatin molecules among each other, and the active amino acids along the polypeptide chains were bonded to other reactive groups [29]. The antioxidant activity varied with the types of gelling agent used. The results indicated that different gelling agents contained other antioxidants, which could scavenge radicals at varying degrees [29].

Moreover, using potato starch mixed with gelatin as gelling agents in jelly products, potato starch also affects phenolic compounds when phenolic compounds or purified phenolic compounds (e.g., phenolic acids, flavonoids, coumarins, stilbenes, and tannins) stay into potato starch in terms of gelatinization and retrogradation due to the reaction altering various functional properties of potato starch such as rheological properties, gelatinization, retrogradation, and gelling. While plant extracts addition provides a practical method to modify the potato starch, employing pure phenolic compound gives insight into the mechanism such as phenolic structure-potato starch property relationships [30] due to the reaction of the solubilized polyphenols and potato starch. The hydroxyl and carboxyl groups of the solubilized phenolic compounds may interact with water and indirectly with the hydroxyl groups of potato starch through hydrogen bonding, altering the water and potato starch properties [31-32].

3.3. Sensory evaluation

To evaluate the sensory attributes, including appearance, color, flavour, taste, texture, and overall acceptability of different samples were served to the trained panellists (30 panellists) who were asked to evaluate them on a 1-9 point hedonic scale, and the mean scores obtained by various samples for different sensory attributes are presented in Table 4.

Table 4. Evaluation of sensory attributes of the jelly product

Treatments	Sensory attributes					
	Appearance	Color	Flavour	Taste	Texture	Overall acceptability
0:100 + G	6.33 ± 1.72 ^c	5.13 ± 0.50 ^c	4.73 ± 1.28 ^d	5.33 ± 1.42 ^{bc}	5.10 ± 1.86 ^b	5.46 ± 1.33 ^b
25:75 + G	7.23 ± 1.45 ^{ab}	7.06 ± 0.86 ^{ab}	7.13 ± 1.73 ^{ab}	6.20 ± 1.32 ^a	6.53 ± 1.22 ^a	6.80 ± 1.27 ^b
50:50 + G	7.46 ± 1.40 ^a	6.56 ± 1.04 ^b	6.33 ± 0.80 ^c	4.90 ± 1.47 ^c	6.10 ± 1.51 ^a	5.60 ± 1.71 ^b
0:100 + GS	6.50 ± 0.27 ^{bc}	5.53 ± 1.38 ^c	4.40 ± 2.07 ^d	6.00 ± 1.25 ^{ab}	6.30 ± 1.23 ^a	5.83 ± 1.11 ^b
25:75 + GS	7.23 ± 1.00 ^{ab}	7.46 ± 0.57 ^a	7.56 ± 0.67 ^a	6.36 ± 1.03 ^a	6.86 ± 0.37 ^a	7.23 ± 1.07 ^a
50:50 + GS	6.80 ± 1.03 ^{abc}	6.66 ± 0.99 ^b	6.60 ± 1.03 ^{bc}	5.66 ± 1.97 ^{abc}	6.20 ± 1.18 ^a	6.03 ± 1.47 ^b

Note: Values are the means of triplicate ± standard deviation. Those numbers with a different superscript letter in a block differ significantly ($P \leq 0.05$). The scale is arranged such that; 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like lightly, 5=Neither like nor a dislike, 4 = Dislike slightly, 3= Dislike somewhat, 2 = Dislike very much, and 1 = Dislike extremely.

G = Gelatin and S = Potato starch

The average sensory attributes scores of the jelly product were statistically analyzed for variance among the jelly prepared by different samples. The result of sensory attributes on appearance, color, flavour, taste, texture, and overall acceptability gave the average score in the ranges of 6.33-7.46, 5.13-7.46, 4.40-7.56, 5.33-6.36, 5.10-6.86 and 5.83-7.23, respectively. In the case of appearance attribute, sample 50:50+G presented the highest appearance attribute score (7.46), and sample 0:100+G presented the lowest score (6.33). The sample

with the highest color, flavour, taste, and texture score was 25:75+GS which were 7.46, 7.56, 6.36, and 6.86, respectively. In the case of color jelly prepared from the sample, 0:100+GS gave the lowest score (4.40). Moreover, sample 0:100+G represented the lowest score on color, taste, and texture, which were 5.13, 5.33 and 5.10, respectively.

In terms of overall acceptability, there was a highly significant difference among the prepared jelly. Table 4 shows that jelly prepared from sample 25:75+GS showed the highest score of other samples (7.23), and sample 0:100+GS got the lowest score (5.83).

Accordingly, using 25% of extracted galangal rhizome, potato starch, and gelatin as gelling agents in galangal rhizome jelly products resulted in the best physical and chemical properties, including the best customer acceptance in terms of senses. That could lead to the selection of suitable gelling agents to use commercially to increase the value and ways to consume galanga, which is one of the Thai herbs.

The reason that the sample 25:75+GS received the highest consumer acceptance rating might be probably the lowest hardness and chewiness. They were then related to the texture profile in Table 2. The texture is critical feature consumers expect from gummy that consistency impacts the chewability of gummy [33] (p. 2). Chewiness measures the level to which the gummy's elasticity texture is chewable and describes the chewing sensation [11] (p. 7). In addition, [33] (pp. 6-7) reported that the gummy, which had low hardness and chewiness values, received a high score of overall acceptability than the high hardness and chewiness values.

4. Conclusions

The amounts of galangal juice and different gelling agents used in the production process affected the quality of the galangal jelly. Using 25% of galangal juice and potato starch mixed with gelatin as gelling agents in galangal jelly received the highest acceptance in terms of overall acceptability (7.23). Due to the galangal has many benefits as nutraceuticals then, galangal has been developed in the jelly form. The jelly product form is a medium for delivering nutrients to consumers more effortless than the tablet form, causing consumers to swallow or chew quickly. Using galangal rhizome as the main substance to develop confectionaries that nutritional value is recommended in the range of 25% to not more than 50%. Galangal rhizomes jelly is the development of health food products that add value to Thai herbs and guidelines for selecting the suitable gelling agent for the commercial jelly product

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The Impact on Properties of Block Rubber of Using Adulterated Coagulants in Cup Lumps of Natural Rubber

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Abstract: Cup lumps are raw materials used in block rubber production and commonly contain adulterants among the chemicals used for rubber coagulation. This study aimed to identify the composition and concentration of components in commercial coagulants for producing cup lumps in the Northeastern and Northern regions of Thailand. A further goal was to study the impact of using these cup lumps to make block rubber. Detection of coagulant concentration by ion chromatography found that 25% of the samples were composed of formate ions and 50% of the samples were composed of sulfate ions ranging from 17.89 to 93.46% and 36.17 to 97.74% by weight, respectively. Among the adulterated coagulants, they were mixed with formate ions and chloride ions in 18.75% of the samples and sulfate ions mixed with chloride ions in 6.25% of the samples. Additionally, block rubber containing adulterants had Po, PRI, and Mooney viscosity values that meet the lower grade STR 20 standard. Block rubber made from cup lumps coagulated using formic acid showed physical properties that met the STR 5 standard.

Keywords: Adulterated coagulant; Block rubber; Cup lumps

1. Introduction

Cup lump natural rubber is the starting raw material in block and crepe rubber production. It has a production volume of 1.49 metric million or 32% of Thailand total production of raw rubber materials [1]. Most of this is produced in the northeastern region accounting for 80% of the country's output. Cup lumps of rubber are obtained from latex coagulated in a cup. The shape of the cup lumps is, therefore, that of its container. They have many sizes, both small and large. The volume and weight of cup lumps are unspecified, ranging from 100-500g [2]. Cup lumps are produced using acid as a coagulant or allow natural coagulation with no acid. Non-acidic cup lumps of natural rubber do not settle properly. Over an extended period, this will cause spoilage, air bubbles and porosity in the cup lumps. The result will be an initial weakness and low viscosity in addition to having poorer physical properties than if acid was used to coagulate latex [3].

Additionally, malodors will be generated due to the volatile organic compounds in non-rubber, including ketones, aldehydes, aromatic compounds, esters, acids, alkanes, alcohols and cyclic compounds [4-5]. Decomposition results in the foul 'rotten egg' smell of hydrogen sulfide and mercaptan, while

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acid coagulation of rubber effectively separates the non-rubber from the rubber. More protein is in the serum fraction than in the rubber, resulting in a less foul odour. Only good quality cup lumps can produce good quality block rubber [6].

The Rubber Research Institute of Thailand recommends using formic acid as a coagulating agent as it is a readily degradable organic acid with no residue in the product [3, 7]. However, some manufacturers of rubber coagulating agents mixed various adulterants into their products and some producers added other materials to the coagulants. In 2021, the ROAT received a letter from the Thai Rubber Association (TRA) complaining about using adulterated latex coagulants to make cup lumps, especially in Thailand's North and Northeast regions. In particular, there is a problem of degrading cup lumps with ammonia when the rubber is cut into small squares inside the cups. This is done regardless of the damaged product. The practise is widespread in the upper northeastern provinces of Bueng Kan, Nong Khai and Udon Thani. Additionally, formalin was added to latex to maintain a new condition before delivery to latex processors who make concentrated latex or rubber sheets. Other contaminants included vulcanized rubber and remnants of material from manufacturing foam rubber. Additionally, soil, sand, cement, molasses and inappropriate binders such as sulfuric acid and chloride salts were found in the cup lumps. In 2013, the Rubber Research Institute of Thailand also received complaints from the TRA that cup lumps produced in the northeastern region were not of good quality. As a result, tire manufacturers did not accept rubber shipments from the northeast region due to residual sulfuric acid in the products. Sulfuric acid causes the rubber to become sticky and dark in colour. This affects adhesion between the wire and the rubber of tires. There is also a problem with the joints of the tire valves. They become disconnected from the rubber during tire assembly. Moreover, exposure to sulfuric acid can irritate the skin, eyes, and nose. The formation of gaseous sulfur compounds such as SO_2 and H_2S can also poison the environment. In light of these problems, Tassanakul [3] prepared a manual for producing good quality cup lumps of natural rubber for farmers to implement. This manual also covers good manufacturing practices (GMP) for making crepe rubber from high-quality cup lumps. Such information has been developed as a good manufacturing practice for crepe rubber production [8-9], a good agricultural practice for rubber to produce fresh latex [10], and a good agricultural practice for rubber to make cup lumps [11]. These are optional rubber standards that promote and support the dissemination of quality control of agricultural products.

The objective of the current study was to determine the composition of each commercial adulterated coagulant in the northeastern and northern regions of Thailand. We studied the chemical and physical properties of block rubber that contain adulterants used in making cup lumps of natural rubber. Additionally, procedures are proposed to prevent the use of adulterated coagulants in producing cup lumps. This very serious problem causes great damage to Thailand and the world rubber industry. Overall, the impact derived from the scientific finding of using commercial adulterated coagulants must be solved by the relevant organization.

2. Materials and Methods

This research was a quantitative study. It examined 60 rubber thickening agents found in the northeastern and northern regions. The experiments were divided into three phases as follows. In the first step, 50 of 60 coagulating agents were selected by random sampling. This was done with a quick test kit that detected the presence of organic acids using Gentian violet [12]. The samples were checked for sulfate adulteration using a 10% barium chloride solution to adopt the ISO 2480:1972 [13]. Then the samples were checked for chloride salts adulteration with a 10% silver sulphate solution in a process for adopting the ISO 2479:1972 [14]. The pH values of the samples were checked using litmus paper.

In the second step, 16 of 60 samples were selected for analyzing the concentrations of formate (HCOO^-), sulfate (SO_4^{2-}) and chloride (Cl^-) ion by using ion chromatography.

For the third step, natural rubber latex was subjected to five (5) types of rubber coagulants. They were (1) formic acid, (2) sulfuric acid, (3) calcium chloride salts, (4) formic acid with calcium chloride salts, and (5) sulfuric acid with calcium chloride salts. All of them are commercial grade. Label directions were followed for each coagulant to form cup lumps. This produced crepe rubber that was sent to a shredder and then to a drier

in the block rubber factory at the Factory Management Division 4, Rubber Authority of Thailand, Khun Han District, Sisaket Province. To study the chemical and physical properties of these materials, including the amounts of impurities (dirt), the ash content (ash), volatile matter content (V.M.), nitrogen content as well as the initial plasticity (Po), plasticity retention index (PRI) and Mooney viscosity (Vr) were compared to Standards STR 5, STR 10 and STR 20 [15]. This study was conducted in 2020-2021 at the Factory Management Division 4, Khun Han District, Sisaket Province. Additionally, a small group meeting was held to determine measures to prevent the use of adulterate coagulants in the production of cup lumps and improve the quality of rubber.

3. Results and Discussion

3.1 Determination of the presence of formic acid, sulfuric acid, chloride salts of latex coagulants using a test kit and pH using litmus paper

A total of 50 coagulants were sampled. They were solutions packaged in plastic and glass bottles as well as large plastic containers. Package volumes ranged from 750 mL to 5 L. It was found that some brands of latex coagulants had the same logo, but their colours were different. Some were colourless and transparent, while other solutions were light yellow, dark yellow and black. Some solutions of the same brand had different colours. There were coagulants of the same brand for making cup lumps and rubber sheets. Some labels specified the methods of use, while others did not. Some could be used directly, while others required dilution. None of the latex coagulants specified its type and concentration. The date of manufacture was not determined, nor was the expiry date, except for one sample that contained formic acid, identified as "Formic". That one sample had detailed information clearly stated on its label. It accounted for 2% of all samples tested. Gentian violet solution was used to determine the type of organic acids present, for the organic solution will appear blue-violet. If the solution is inorganic, it will be changed from blue-violet to green or yellow, depending on the acidity of the solution. The yellow indicates the pH of 1–3, while the green colour has a pH range of > 3-5.

It was found that only 11 samples (22%) contained only organic acid as formic acid. A test for sulfuric acid was done using a barium chloride solution. It was observed that 17 samples (34%) caused the solution to change from clear to opaque as barium sulfate precipitation. A test for chloride salts was done using a silver sulphate solution. Silver chloride was precipitated as a discolouration of the clear solution to one that was milky-white. Five (5) samples (10%) were found to contain organic acids (formic) mixed with chloride salts. Mixtures of sulfuric acid and chloride salts were detected in 17 samples (34%), as shown in Table 1.

Table 1. The results of formic acid, sulfuric acid and chloride salts presence in various coagulants

Type of Coagulants	Number of samples	%
1. Formic acid	11	22
2. Sulfuric acid	17	34
3. Chloride salts mixed with formic acid	5	10
4. Chloride salts mixed with sulfuric acid	17	34
Total	50	100

Most (88%) of the coagulants had a pH of 1, which is strongly acidic. It was found that most (78%) of the coagulating agents were adulterated using both inorganic acids along with sulfate or chloride salts. This is consistent with the study of Tassanakul [16], who found that commercially available coagulants were more likely to be adulterated with inorganic acids. This is because many coagulants are produced by entrepreneurs who do not regard the adverse effects on rubber producers and the environment. People have reported a burning pain on their skin after handling these products caused by vapour behaviour from sulfuric acid. Additionally, the cambium of rubber trees is damaged and darkened by a strong acid as sulfate changes to sulfide. This is consistent with other studies by Srihamongkul and Kasikanan [17]. They found that the pH of

the aqueous serum obtained from coagulation is harmful to the skin and rubber surfaces in the long term. Products that contain adulterants are a fraction of the cost of those made with formic acid. There is a faster coagulating time, but the dry cup lumps are dark and sticky, while the resulting rubber is hard, lacks flexibility and has a lower viscosity.

3.2 Quantitative determination of formate, sulfate and chloride ions using ion chromatography

Sixteen (16) specific rubber coagulant samples were taken for the quantitative determination of formate (HCOO^-), sulfate (SO_4^{2-}) and chloride (Cl^-) ion concentrations for analysis using ion chromatography. Four (4) samples (25%) contained only formic acid. Of these, only one formic acid brand, BASF, with a formate ion concentration of 93.46%, was correctly labelled (94%). In other samples, the concentrations of ionic formate were 17.89, 31.99 and 54.35% by weight, respectively, for Dao-D, Dee-F and Reua-B brands. Eight (8) samples (50%) contained sulfuric acid as a rubber coagulating agent and the sulfate concentration was between 36.17 and 97.74% by weight. Additionally, three (3) samples (18.75%) samples contained rubber coagulating agents comprising a mixture of formate and chloride ions. The remaining sample (6.25%) was adulterated with a combination of sulfate and chloride ions, marketed as the "good quality brand, C.H.". Among the samples, two had chloride salts concentrations ranging from 30.78 and 36.78% by weight. The Rod-K brand contained formic acid, while the Hi-S brand contained only chloride salts (Table 2). A coagulant producer should show the product composition and concentration of acid on the label. This is so that farmers can use such information to make purchasing decisions. However, producers use similar brand names, which confuses the market. For example, Su-S-A and Su-S-B have similar names but differ only in colour. Those producing latex coagulants should consider the impacts on the quality of rubber, the people who produce rubber and the environment.

Table 2. Concentrations of formate (HCOO^-) sulfate (SO_4^{2-}) and chloride ions (Cl^-) in coagulants were determined by using ion chromatography

No.	Trade name	Concentration (% by weight)		
		HCOO^-	SO_4^{2-}	Cl^-
1	Rod-K	11.54	n.d	30.78
2	Ruea-B	54.35	n.d	n.d
3	BASF	93.46	n.d	n.d
4	Su-S-A	n.d	43.13	n.d
5	Su-S-B	n.d	47.88	n.d
6	Ton-Y	n.d	36.17	n.d
7	CH	n.d	40.96	0.95
8	Nak-k-l	0.68	n.d	2.50
9	Nak-k-S	0.63	n.d	2.38
10	Sing-MT	n.d	92.43	n.d
11	Phae-D	n.d	97.74	n.d
12	Suea-KC	n.d	37.64	n.d
13	Suea	n.d	53.53	n.d
14	Dao-D	17.89	n.d	n.d
15	Dee-F	31.99	n.d	n.d
16	Hi-S	n.d	n.d	36.78

3.3 Chemical and physical properties of block rubber made from cup lumps using various coagulating agents

The chemical and physical properties of block rubber made from cup lumps was studied using five (5) coagulants. They consisted of 1) formic acid, 2) sulfuric acid, 3) calcium chloride salts, 4) formic acid combined with calcium chloride salts, and 5) sulfuric acid combined with chloride salts. The formic acid concentration in sample 1 was 0.6% on a dry rubber weight basis. Samples 2-5 were used according to information on their package labels. It was found that using formic acid alone when production was controlled

according to good agricultural practice (GAP) standard [11], block rubber properties showed higher Po and PRI values than from samples of cup lump rubber coagulated with sulfuric acid and chloride salts. The rubber coagulated with the latter two agents had a higher moisture content than those made with formic acid. This is because sulfates and salts absorb moisture from the air. Rubber coagulated with calcium chloride salts has a Po value of less than 30, which does not meet the STR 20 standard. This is a criterion for the lowest quality of block rubber. Its PRI value is lower than that using a formic acid coagulant. This agrees with the work of Pimrat, Poonsawat and Sahagaro [18]. It was found that using sulfuric acid above the specified level resulted in decreased PRI values and lower viscosity.

Using chloride salts causes 50% more rubber deterioration than using formic acid alone (Table 3). This is in agreement with the study of Tassanakul [19] that the use of calcium chloride alone was found to have a severe effect on cup lump quality. It makes the resulting cup lumps harder and increases its viscosity. Products made from such rubber deteriorate more quickly. The cup lump surfaces are dark and sticky. Many block rubber manufacturers have encountered problems with poor quality rubber and non-uniform properties. This affects the use of block rubber and limits the products it can use, especially in vehicle tires. Additionally, a foul smell arises from the serum water at the collection points. This affects the community and the environment in the long run [20]. When cup lumps are produced without quality control, their properties are inconsistent, especially the Mooney viscosity. This is because the natural rubber's structure comes from forming protein chains with lipids at the end of the chains, making them longer. The result increases with time, causing higher viscosity and storage hardening [21]. Xu Chen et al. [22] found different coagulation methods could also affect the storage behaviours of natural rubber since coagulation methods could affect the content of non-rubber components.

Table 3. The chemical and physical properties of block rubber using various coagulants

Type of coagulants/Standard	Dirt, max (%)	Ash, max (%)	Nitrogen, max (%)	VM, max (%)	Po, min	PRI, min	Vr ML(1+4 100 °C)
STR* 20	0.16	0.80	0.60	0.80	30.0	40.0	NS
STR 10	0.08	0.60	0.60	0.80	30.0	50.0	NS
STR 5	0.04	0.60	0.60	0.80	30.0	60.0	NS
Formic	0.011	0.32	0.34	0.40	44.7	82.0	80.7
Sulfuric	0.023	0.34	0.39	0.50	39.0	72.6	77.9
Calcium chloride	0.013	0.87	0.36	1.03	23.5	41.1	55.6
Formic + Calcium chloride	0.025	0.89	0.35	0.66	20.5	24.4	49.2
Sulfuric + Calcium chloride	0.028	0.37	0.45	0.49	29.5	69.5	61.5

Remark: STR means Standard of Thai Rubber

3.4 Recommendations for measures to control the use of non-formic acid coagulation agents in the production of cup lumps

According to the Rubber Authority of Thailand, formic acid is recommended as a latex coagulant in the production of cup lumps because it is an organic acid that readily decomposes and does not affect the environment. The cup lumps from this method have good physical properties, are safe for operators, and do not injure the cambium of the rubber trees. The surface of rubber trees does not show dryness syndrome. Manufacturers and distributors of rubber coagulation agents often mix sulfuric acid and chloride salts into their products. This affects the quality of the block rubber used in many rubber products, especially vehicle tires, which use the highest volume of block rubber. One way to control this is to enable farmers to produce

good quality cup lumps. Therefore, we propose seven (7) measures to prevent rubber coagulation agents that are not formic acid, as follows:

1. Make the use of non-formic acid, or inorganic acid should be subject to regulation. Short-, medium-and long-term plans are urgently needed to develop the Rubber Authority of Thailand's policy to prohibit non-formic acid coagulants.

2. It is proposed that the Rubber Authority of Thailand confer with their legal department to formulate a Letter of Consumer Protection that will be sent to the Consumer Protection Board, which regulates advertising, labelling and contracting. The letter should ask that latex coagulants become controlled products under Section 30 of the Consumer Protection Act of 1979. If the Consumer Protection Committee on Advertising, Labeling and Contracts determines that regulation should exercise control, it will be issued under Section 31. It will regulate the production of all rubber coagulants. Such products must have a quality certificate indicating the name, place of manufacture, composition, coagulant concentration, quantity, method of use, dates of manufacture and expiry. These should be clear and attached to the container.

3. It is proposed that the Rubber Authority of Thailand begin discussions with the Department of Agriculture on this matter to address the problem of offences committed under the Consumer Protection Act of 1979 or other regulations that fall under the authority and responsibility of the Department of Agriculture.

4. It is proposed that the Rubber Authority of Thailand work with the Thai Rubber Association and request cooperation from STR 10 and STR 20 block rubber manufacturers to purchase quality rubber materials that meet the standard qualifications. Farmers should be trained to use coagulants that contain formic acid or an organic acid that produces rubber with comparable properties. There should be no adulterants such as bark, vulcanized rubber, calcium salt, bio-fermented fluids, wood vinegar, sulfuric acid or acids that claim the trade names of "organic acids", "biological acids" etc.

5. The Rubber Authority of Thailand should expedite public relations, release information on electronic media, radio, television, and newspapers, and mail promotional materials to inform governmental and non-governmental agencies concerned with this matter. The private sector, farmer groups, and rubber tree farmers should be included. The effects of non-formic acid latex coagulants should be urgently and continuously presented. This is to prevent damage to the quality of Thai rubber.

6. It is proposed that the Rubber Authority of Thailand and related agencies promote, support and motivate farmers and farmer groups to produce good quality cup lumps that meet GAP standards. This is to produce quality rubber, increase productivity and improve safety. It is a worthwhile investment to produce rubber that can be made into premium-grade crepe rubber. This will upgrade production throughout the manufacturing chain and move the industry toward sustainability and internationalization.

7. It is proposed that the Rubber Authority of Thailand modify its post-harvest and harvest management policies for rubber production according to GAP standards. The policy must be implemented to drive improved production of quality rubber to achieve maximal effectiveness.

4. Conclusions

Thailand is the world's largest producer of block rubber. This is the reason for the promotion of producing rubber in the northeastern and northern regions to meet the demands of the world market. Cup lumps are products that are popular among farmers. So, the producers of coagulants see this as an opportunity to make adulterated coagulants with sulfuric acid and chloride salts. This is widespread in the industry and it is hard to control. Studies have shown that coagulants are sold that contain inorganic rather than organic acids. In the production of block rubber, manufacturers cannot distinguish which coagulants have been used in the cup lumps. Additionally, rapid detection of sulfate adulteration with a barium chloride solution and checking for adulteration with chloride salts using a silver sulphate solution can be done. If it is desirable to determine the concentration of various adulterants, ion chromatography can determine the concentrations of these substances. The results showed that the number of samples containing sulfuric acid was more than those with

formic acid. Coagulants were also adulterated with formic acid mixed with chloride salts and sulfuric acid mixed with chloride salts. According to the GAP, using formic acid in cup lumps to produce block rubber showed that the chemical and physical properties pass the STR 5 standard for block rubber. Other block rubbers were adulterated with sulfate and chloride salts. Their flexibility deteriorated with viscosity so that it was lower than the STR 20 standard, which was Thailand's lowest grade of block rubber. Therefore, the Rubber Authority of Thailand, as a central organization and management of the entire system, should be strictly implemented for using adulterated coagulants not to have a broad impact on the properties of block rubber.

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Effect of Probiotic Used on Phytoplankton Community Structure in White Shrimp (*Litopenaeus vannamei*) Pond

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Abstract: Probiotic use is one major approach to eliminating waste accumulation in shrimp farming. This study investigated the effects of probiotic use on the abundance and diversity of phytoplankton at the farm level. The results revealed 4 divisions for 12 genera of phytoplankton, consisting of Cyanophyta (*Oscillatoria* sp., *Pseudanabaena* sp., *Planktolyngbya* sp. and *Merismopedia* sp.), Chlorophyta (*Scenedesmus* sp. and *Monorapidium* sp.), Bacillariophyceae (*Nitzschia* sp., *Pleurosigma*/Gyrosigma sp. and *Navicula* sp.) and Pyrrophyta (*Protoperidinium* sp., *Ceratium* sp. and *Prorocentrum* sp.). Pyrrophyta exhibited the highest density, followed by Cyanophyta, Bacillariophyta and Chlorophyta. The cell density of 4 divisions was high at the start rearing e.g., Pyrrophyta 47,991 cells/L (85%), Cyanophyta 3,141 cells/L (5%), Bacillariophyta 3,530 cells/L (6%), and Chlorophyta 1,965 cells/L (3%). However, the density drastically changed at the end of the study in 19 days, especially in Pyrrophyta, for which cell density dropped to 4,705 cells/L (48%). The density of Cyanophyta increased towards day 7 and decreased at the end of the study (2,126 cells/L, 22%). The density of Bacillariophyceae and Chlorophyta were reduced in the initial cultivation from day 1 to day 10 but increased again before the end of the study for 1,176 cells/L (12%) and 1,698 cells/L (18%). The research indicates that adding probiotics to shrimp ponds can affect the abundance of the phytoplankton community. Probiotics can reduce an abundance of Pyrrophyta and Cyanophyta (biotoxin producing group.), while they show a trend to increase an abundance of Chlorophyta and Bacillariophyta.

Keywords: Probiotic; Shrimp; Phytoplankton; Chlorophyta; Bacillariophyta

1. Introduction

The shrimp aquaculture industry is a significant agricultural activity driving the economy in several coastal countries [1]. Economic competition results from shrimp production with the highest benefit. Huge amounts of high-protein feeds, chemicals and antibiotics are used, neglecting the maintenance of the environment both inside and outside ponds [2]. Indeed, water effluent from shrimp aquaculture should be treated before re-circulating to new crops or discarding into surrounding environments. Probiotics, which contain the most beneficial bacteria, inhibit pathogenic microorganisms, enhance and promote shrimp's immunity and growth, and improve water quality in ponds [3-5]. Water quality control by probiotics is carried out by utilizing excess nutrients in shrimp ponds. However, using probiotics by directly adding them to culturing water may influence the community structure of microbiota, such as phytoplankton. The phytoplankton plays an important role in aquatic photosynthesis, leading to oxygen production and nutrient control such as nitrate and phosphate [6]. Moreover, phytoplankton is a natural feed, providing water shade to reduce shrimp cannibalism [7]. Nutrients are necessary for both phytoplankton and probiotics. Therefore, competition between microorganisms occurred. Probiotics can use nutrients better than phytoplankton. They can control the abundance of undesired phytoplankton. Therefore, this research aimed to study the effect of probiotic use on the phytoplankton community in shrimp farms.

2. Materials and Methods

2.1 Shrimp farm

The experimental shrimp farm was in Songkhla province, southern Thailand. Farmers performed the study investigating shrimp ponds and the culture system. Shrimp ponds were prepared by clearing bottom waste and mud, followed by drying for 2–3 weeks. Lime and dolomite (1 ton each) were added to the floor of the ponds to increase pH and alkalinity. Seawater (about 48,000 m³) was added to water depth 1.5 m in 2 rai (0.32 hectares) of the pond. Post-larva stage (PL15) of white shrimp (*Litopenaeus vannamei*) (approximately 200,000 individual/rai) was seeded in the pond. An automatic feeding machine was in operation every 5 minutes from 7 am to 6 pm. Oxygen was added by utilizing 3 mixing turbines with 50% and 100% efficiency during both day and night, respectively.

2.2 Probiotic application

Commercial probiotic product was composed of 3 significant *Bacillus* species, including *B. subtilis*, *B. megaterium* and *B. licheniformis*. The use of probiotics in shrimp ponds was carried on by farmers, which commenced on day 20 continuing until day 74 of culture. One hundred grams of probiotic powder were inoculated in a 200 L plastic tank containing shrimp feed of 0.5 kg, molasses of 0.5 kilograms, and tap water of 200 L. Oxygen was supplied by an air pump for 24-36 hours before use. Two hundred litres of about 10⁶ CFU/ml probiotics were scattered over the pond every 3 days.

2.3 Phytoplankton collection

The collection of phytoplankton was started on day 55 of shrimp culture. Based on probiotic use, phytoplankton was collected every 3 days from day 20 until day 74 of shrimp culture. Sample collection was performed from 9–10 am before adding a new batch of probiotics to avoid the mistaken re-sampling of newly added probiotics. Phytoplankton cells from five locations in the pond were collected by passing a 20 L water sample through a 20 µm plankton net mesh. Then, cells were fixed with 10% formalin before further study in the laboratory [8].

2.4 Water parameters

Several water parameters were monitored during cultivation. Temperature, dissolved oxygen, pH and salinity parameters were measured at the time of phytoplankton collection (9–10 am) [6].

2.5 Phytoplankton study

One millilitre of phytoplankton sample was dropped into Sedgewick Rafter Cell. The morphological characteristics of phytoplankton were then studied under a light microscope (CHS, Olympus). Phytoplankton species and characteristics were identified by manual method and recorded before the cell abundance of phytoplankton was calculated with three replicates [8].

3. Results and Discussion

3.1 Phytoplankton community

The results of the phytoplankton study showed that 12 genera within 4 divisions of phytoplankton were found. The identification study exhibited 4 genera of division Cyanophyta (*Oscillatoria*, *Pseudanabaena*, *Planktolyngbya* and *Merismopedia*), 2 genera of division Chlorophyta (*Scenedesmus* and *Monorapidium*), 3 genera of division Bacillariophyceae (*Nitzschia*, *Pleurosigma* or *Gyrosigma* and *Navicula*) and 3 genera of division Pyrrophyta (*Protoperidinium*, *Ceratium* and *Prorocentrum*) (Figure 1). The total phytoplankton cells for 7 studies of 19 days were 186,967 cells/L. Highest cell abundance were the following divisions: Pyrrophyta (133,938 cells/L or 71.6%), Cyanophyta (36,991 cells/L or 19.7%), Bacillariophyta (8,984 cells/L or 4.8%) and Chlorophyta (7,053 cells/L or 3.7%), respectively (Figure 2).

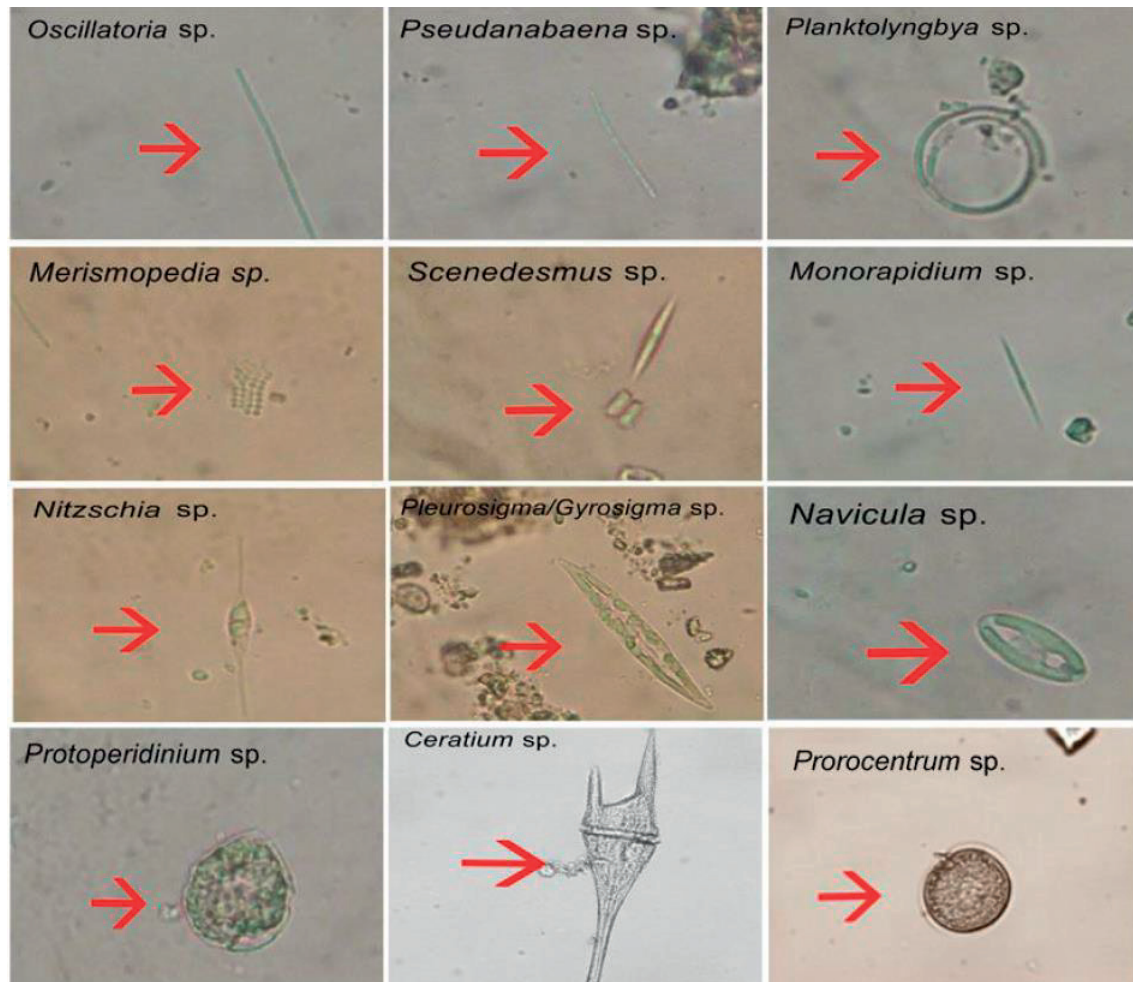


Figure 1. The morphology of phytoplankton was founded in a shrimp pond.

The most abundant genus of phytoplankton was *Protoperidinium* (133,517 cells/L), followed by *Planktolyngbya* (31,726 cells/L) and *Ceratium* sp., which were found at the lowest density (2 cells/L) (Table 1). These 4 divisions of phytoplankton commonly found in shrimp farms were previously reported. Meanwhile, this study found Pyrrophyta division had the highest concentration. In contrast, de Paiva-maia et al. [9] suggested that Cyanophyta had the highest concentration in shrimp farms (*Litopenaeus vannamei*), followed by Chlorophyta, Bacillariophyta and Pyrrophyta, respectively. [9] proposed that less concentration of Pyrrophyta resulted from the influence of probiotics.

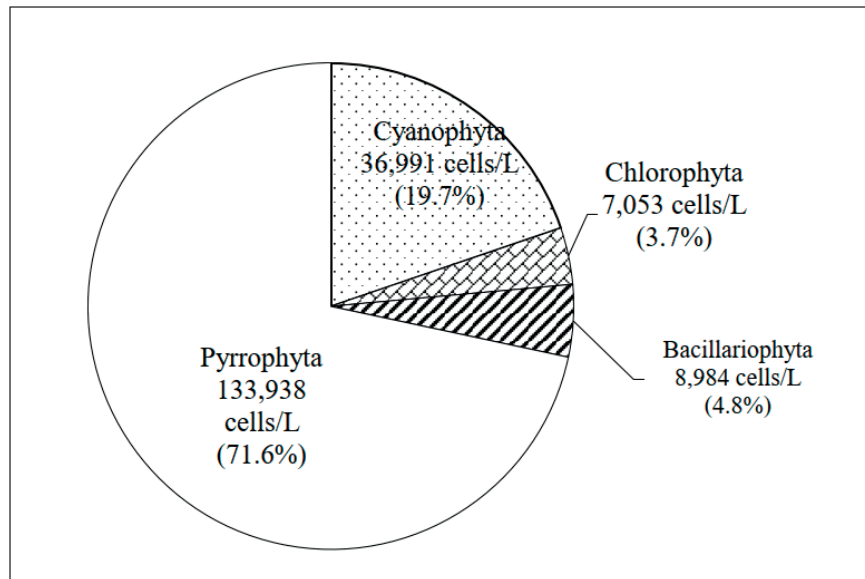


Figure 2. Phytoplankton communities in shrimp pond.

Like [9], Yusoff et al. [10] found that Cyanophyta was the highest community in treated ponds but lower than in controlled ponds as they operate with commercial bacteria. High organic substances in water are growth requirements of Pyrrophyta and Cyanophyta, which in these divisions can generally be found in eutrophic aquaculture ponds, as mentioned in previous studies [11, 12]. However, [9-10] have suggested that probiotics can be controlled to pyrrophyta and Cyanophyta. The controlling mechanisms may be nutrient use competition and antagonisms (antibiotics, enzymes, bacteriocin, etc.).

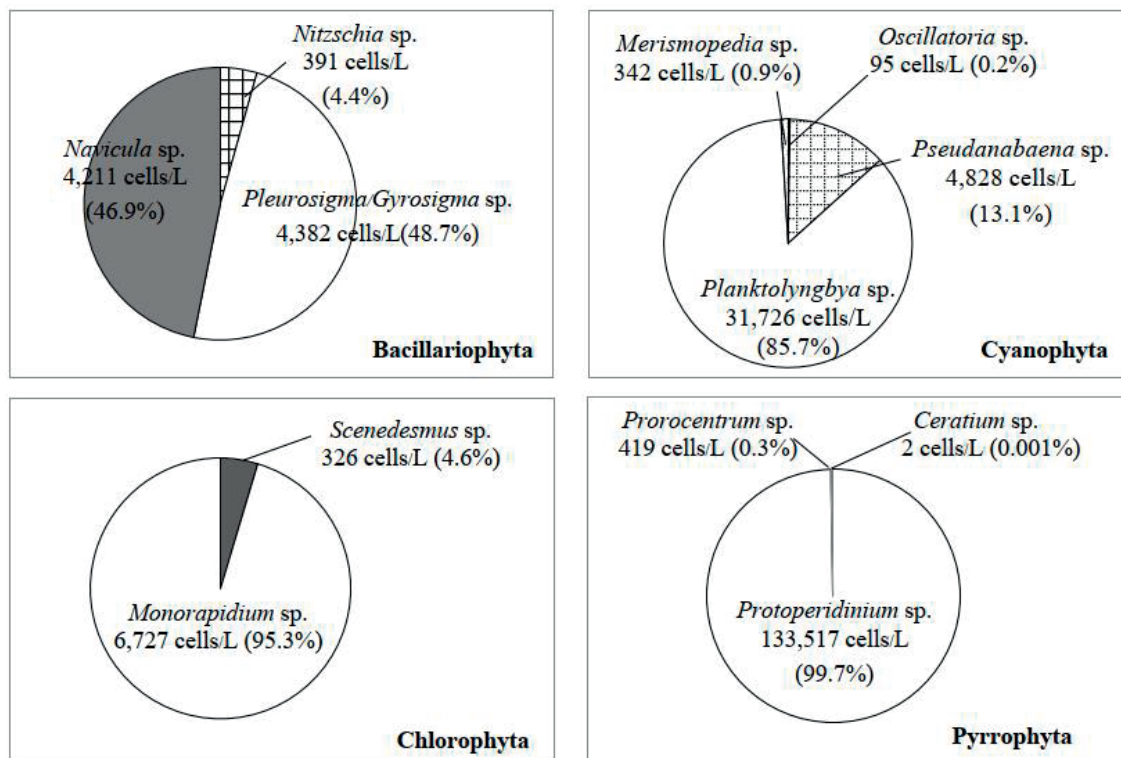


Figure 3. The abundance of phytoplankton cells is based on species.

Figure 3 showed the richness of species of pyrophyta was *Protoperidinium* sp. (99.7%), followed by *Prorocentrum* sp. (0.3%) and *Ceratium* sp. (0.001%), respectively. The richness of species of Cyanophyta was *Planktolyngbya* sp. (85.7%), followed by *Pseudanabaena* sp. (13.1%), *Merismopedia* sp. (0.9%) and *Oscillatoria* sp. (0.2%), respectively. In contrast, the highest population species of Bacillariophyta was *Pleurosigma* sp. or *Gyrosigma* sp. (48.7%), followed by *Navicula* sp. (46.9%) and *Nitzschia* sp. (4.4%), respectively. The most abundant species of Chlorophyta were *Monorapidium* sp. (95.3%), followed by *Scenedesmus* sp. (4.6%). This study was in contrast with the report of Lukwambe et al. [13], which investigated the effects of commercial microbial agents (probiotics) on phytoplankton community structure in intensive white shrimp (*Litopenaeus vannamei*) aquaculture ponds. Their results showed that Bacillariophyta (*Navicula* sp., *Coscinodiscus* sp. and *Skeletonema* sp.) and Cyanophyta (*Oscillatoria* sp. and *Spirulina* sp.) were the highest populations. However, most *Protoperidinium* sp. occurring in shrimp ponds may negatively affect other species because it has been proposed as a biotoxin producer [14].

Table 1. The abundance of phytoplankton cells (every 3-days) from a 19-day study.

Phytoplankton	Abundance (cells/L)							
	D1	D4	D7	D10	D13	D16	D19	Total
Cyanophyta	3141	8600	10778	5067	5564	1715	2126	36991
- <i>Oscillatoria</i> sp.	0	7	19	15	18	15	21	95
- <i>Pseudanabaena</i> sp.	3100	95	189	363	431	249	401	4828
- <i>Planktolyngbya</i> sp.	35	8485	10531	4620	4994	1360	1701	31726
- <i>Merismopedia</i> sp.	6	13	40	68	121	91	3	342
Chlorophyta	1965	378	417	344	1553	698	1698	7053
- <i>Scenedesmus</i> sp.	50	18	18	47	103	57	33	326
- <i>Monorapidium</i> sp.	1915	360	399	297	1450	641	1665	6727
Bacillariophyta	3530	1562	323	493	1402	498	1176	8984
- <i>Nitzschia</i> sp.	176	104	97	10	4	0	0	391
- <i>Pleurosigma</i>								
<i>Gyrosigma</i> sp.	2214	992	23	120	467	221	345	4382
- <i>Navicula</i> sp.	1140	465	203	363	931	278	831	4211
Pyrrophyta	47991	9615	25976	16712	22614	6325	4705	133938
- <i>Protoperidinium</i> sp.	47829	9509	25874	16697	22607	6309	4692	133517
- <i>Ceratium</i> sp.	2	0	0	0	0	0	0	2
- <i>Prorocentrum</i> sp.	161	105	102	15	7	16	13	419
Grand total	56628	20155	37494	22616	31133	9236	9705	186967

3.2 Daily abundance of phytoplankton

The result showed that division Pyrrophyta was the most abundant in all experiments. They dominated the study period in the 48-85% (Figure. 4). Pyrrophyta had the highest cell abundance ($P < 0.05$)

significantly on day 1 with $47.99 \pm 9.41 \times 10^3$ cells/L (85%). While Cyanophyta, Bacillariophyta and Chlorophyta had a cell abundance on day 1 of 6%, 6% and 3%, respectively.

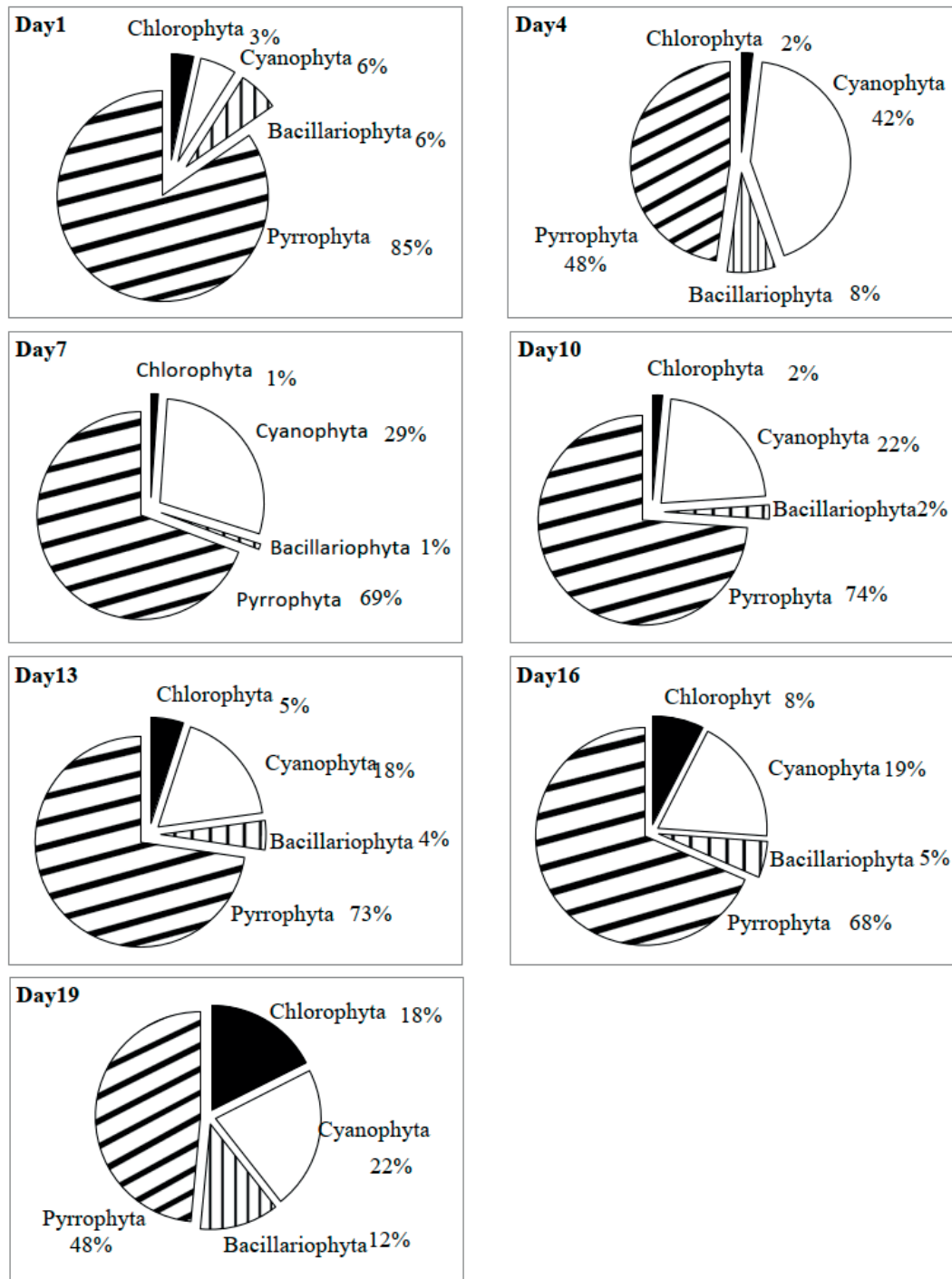


Figure 4. Abundance percentage of phytoplankton divisions every 3 days.

However, the total cell abundance of all phytoplankton on the first day of probiotic use was highest at $56.63 \pm 4.51 \times 10^3$ cells/L (Table 2). When the probiotic was used until day 4, the Pyrrophyta had declined to 48% ($9.61 \pm 1.08 \times 10^3$ cells/L), whereas Cyanophyta had rapidly increased to 42% ($8.60 \pm 0.97 \times 10^3$ cells/L). After that, Pyrrophyta increased again with a 68-74% range on days 7, 10 and 13, then consistently decreased from day 16 to day 19. As Pyrrophyta, division Cyanophyta was the second group of abundance since days 7,

10, 13, and 16 with a range of 18-29%. On day 19, the cell abundances of division Chlorophyta and Bacillariophyta had significantly increased compared to previous times. Unfortunately, shrimps were harvested on day 19 from the first study day, and the author could not continue monitoring the phytoplankton population's dynamic. However, the result showed that using probiotics over periods (at least 19 days) will change the abundance of the phytoplankton population.

Moreover, the profile of the total abundance of all 4 divisions per day dropped from day 1 to day 19. This was revealed in each division's concentration profile, which declined since using the probiotic. The result suggested that probiotics might control the phytoplankton population. Probiotics compete with phytoplankton in shrimp ponds as they can utilize nutrients faster than phytoplankton. This is in addition to their excellent cell regeneration and faster growth [9, 15].

Moreover, probiotics may produce antagonistic and antibiotic compounds to inhibit other phytoplanktons. In our study, green algae (Chlorophyta) were found in small amounts because it is usually distributed in fresh water. There were few green algae which have resistant to saline water. According to the results, probiotics can control the abundance of harmful phytoplankton like Cyanophyta and Pyrrophyta. In contrast, probiotics have enhanced phytoplankton populations like Bacillariophyta and Chlorophyta. These two divisions are suggested as desired phytoplankton because they are important as food for higher consumers [15, 16]. Figure 4 shows the decrease of pyrrophyta and the increases of Bacillariophyceae and Chlorophyta on day 19. On the contrary, at the end of their experiment, de Paiva-maia et al. [9] reported only 2 divisions with the highest abundances in ponds, Cyanophyta and Bacillariophyta.

Table 2. The abundance of phytoplankton is based on the studied day.

Day	Abundance ($\times 10^3$ cells/L $\pm$ SD)				
	Chlorophyta	Cyanophyta	Bacillariophyta	Pyrrophyta	Total
D1	1.96 $\pm$ 0.17 ^a	3.14 $\pm$ 0.99 ^a	3.53 $\pm$ 0.13 ^a	47.99 $\pm$ 9.41 ^b	56.63 $\pm$ 4.51
D4	0.37 $\pm$ 0.08 ^a	8.60 $\pm$ 0.97 ^b	1.56 $\pm$ 0.11 ^a	9.61 $\pm$ 1.08 ^b	20.16 $\pm$ 0.54
D7	0.41 $\pm$ 0.04 ^a	10.77 $\pm$ 3.12 ^b	0.32 $\pm$ 0.07 ^a	25.97 $\pm$ 1.63 ^c	37.47 $\pm$ 1.47
D10	0.34 $\pm$ 0.06 ^a	5.06 $\pm$ 1.58 ^b	0.49 $\pm$ 0.10 ^a	16.71 $\pm$ 1.56 ^c	22.62 $\pm$ 0.86
D13	1.55 $\pm$ 0.61 ^a	5.56 $\pm$ 1.41 ^b	1.40 $\pm$ 0.16 ^a	22.61 $\pm$ 2.64 ^c	31.13 $\pm$ 1.08
D16	0.69 $\pm$ 0.06 ^a	1.71 $\pm$ 0.79 ^b	0.49 $\pm$ 0.03 ^a	6.32 $\pm$ 0.62 ^b	9.24 $\pm$ 0.39
D19	1.69 $\pm$ 0.51 ^{ab}	2.12 $\pm$ 0.05 ^b	1.17 $\pm$ 0.09 ^a	4.70 $\pm$ 0.32 ^c	9.71 $\pm$ 0.21
Total	7.05 $\pm$ 0.24	36.99 $\pm$ 0.95	8.98 $\pm$ 0.04	133.94 $\pm$ 3.15	186.97 $\pm$ 1.48

*Mean of the different alphabet labels within the same row indicate significant differences ($P < 0.05$).

*Mean of the same alphabet label within the same row indicate no significance ($P > 0.05$).

3.3 Population dynamics of phytoplankton

According to the experiment, this study found that the Cyanophyta community continuously increased from day 1 (3,141 cells/L) and showed the highest community on day 7 (10,778 cells/L), then slowly declined until day 19 (2,126 cells/L). Chlorophyta showed the most elevated community on day 1 (1,965 cells/L) and decreased to the lowest on day 10 (344 cells/L), afterwards continuously increased to 1,698 cells/L on day 19. Bacillariophyta had its highest population on day 1 (3,530 cells/L) and gradually declined to the lowest on day 7 (323 cells/L) before slowly increasing to 1,176 cells/L on day 19. The community of Pyrrophyta was at its highest on day 1 (47,991 cells/L) before fluctuating moderately until falling to a low on day 19 (4,705 cells/L). In Figure 5, all 4 divisions showed downward trends, especially Pyrrophyta. Chlorophyta, Bacillariophyceae

and pyrrophyta declined on day 4, whereas Cyanophyta increased. This might be due to high nutrient loading during the initial days of the experiment, which was beneficial for their growth. Paerl and Tucker [17] mentioned that Cyanophyta prefers a low ratio of N:P as [fix N₂ for their expansion and to effectively combat other phytoplankton, which probably contributed to its highest population early in the study, before slightly declining to day 19 with lower nutrient concentration. The decrease of Cyanophyta and Pyrrophyta was reported to be beneficial to shrimp culture due to their toxin and eutrophication being dangerous to shrimp [9, 10, 17].

Furthermore, at the end of the experiment, Chlorophyta and Bacillariophyceae had higher populations, which was profitable to shrimp growth as natural food, and for producing oxygen and being non-toxic [10]. However, the use of probiotics affected the population dynamic of phytoplankton. As the results showed, trends of Cyanophyta and pyrrophyta decreased, while Chlorophyta and Bacillariophyceae increased by the end of the experiment, with all divisions having smaller populations than initially. De Paiva-Maia *et al.* [9] mentioned that phytoplankton communities increased in probiotic treatments but less than in control. Nevertheless, they also confirmed that probiotics influenced dynamic phytoplankton changes.

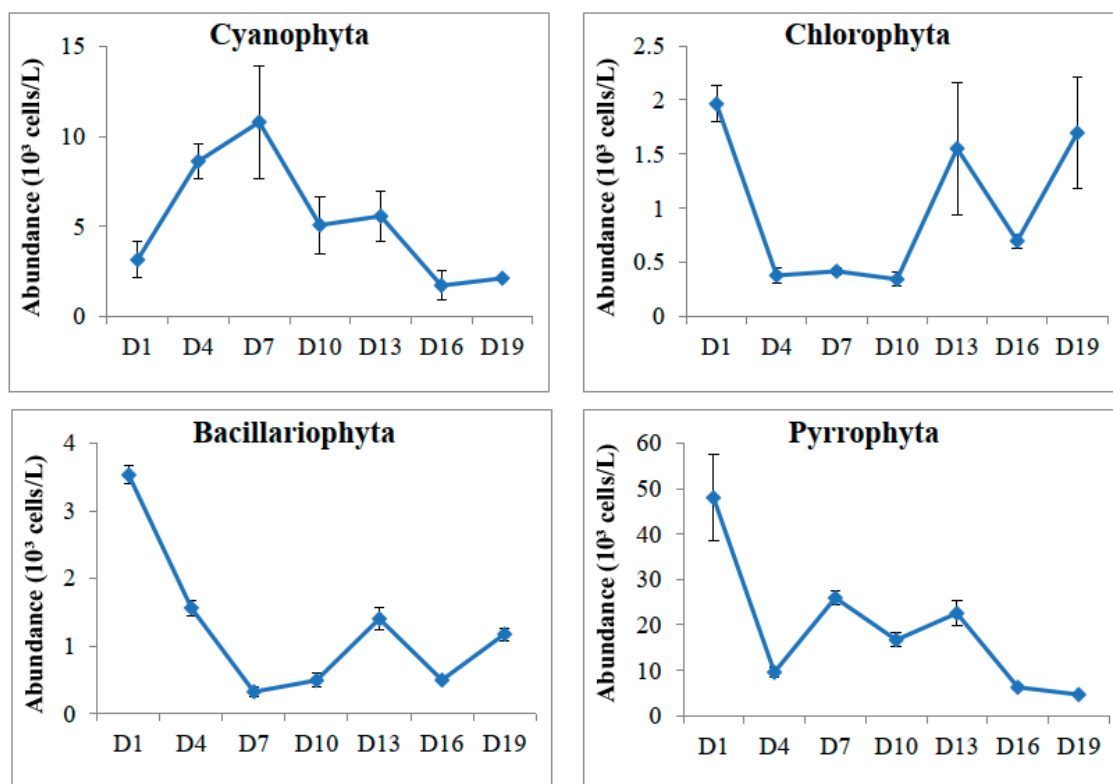


Figure 5. The abundance of phytoplankton divisions every 3 days.

3.4 Daily proportion of phytoplankton abundance

Phytoplankton abundance proportion was analyzed daily in 7 collection periods. It was found that pyrrophyta showed the highest balance and productiveness every day, followed by Cyanophyta, Bacillariophyceae and Chlorophyta, respectively (Figure 6). Bacillariophyta and Chlorophyta were useful as live feed but had low existence. In contrast, the populations of Pyrrophyta and Cyanophyta were high, as they may produce biotoxin and can be harmful to shrimp health. This study showed low populations of Pyrrophyta and Cyanophyta when probiotic was applied. Their blooming and biotoxin production can mitigate that. However, there was no control experiment without probiotics used in this study, which was the weakness of real culturing in the farm system. The study only monitored the evidence influencing probiotics on other phytoplankton in real culturing. So, it could not be assumed which phytoplankton group was the most dominant in the pond without a probiotic system. This experiment tried to compare with de Paiva-Maia *et al.*

[9], which had 2 treatments applied in shrimp ponds, with and without probiotic addition. They reported that Bacillariophyceae and Chlorophyta were the highest populations initially (~47% and 43%, respectively).

In contrast, Cyanophyta and Pyrrophyta concentrations were lower than 7%. After the probiotic was applied for 10-16 weeks, Cyanophyta and pyrrophyta increased to 32-48% and 15-39%, respectively. On the other hand, Bacillariophyceae and Chlorophyta populations fell at the study's end to 7-15% and 19-20%, respectively. Indeed, the phytoplankton abundance profile with and without probiotics was similar. However, the concentration of Cyanophyta with probiotic use was higher than the concentration without probiotic use.

In contrast, the concentration of pyrrophyta with probiotic use was lower than without probiotic use. Furthermore, Lukwambe *et al.* [13] also found higher Cyanophyta density than Bacillariophyceae after probiotic addition. From the experiment, when probiotics were applied it could be observed that Cyanophyta and pyrrophyta initially had higher populations than the other two, which was confirmed by the study of de Paiva-Maia *et al.* [9]. Despite high amounts of Cyanophyta and Pyrrophyta in this study, there was no blooming to reach the level that produces any toxicity, and the shrimp culturing cycle proceeded until harvest [10]. To develop into a bloom of Cyanophyta, typically, the high community of one or two rapid expansion species occupying for an extended period should exist [17]. Usually, Bacillariophyceae and Chlorophyta should have more population than Cyanophyta and pyrrophyta. The use of probiotics in shrimp farms can eliminate waste, improve water quality, and support shrimp health. Moreover, they also help by controlling and inhibiting the fast growth of phytoplankton belonging to a group of biotoxin producers by utilizing nutrients in the water column or producing some inhibitor agents to limit that phytoplankton. However, the intensive shrimp aquaculture system has neglected to manage desirable phytoplankton. Farmers are concerned about harmful phytoplankton because they may cause massive shrimp death. This point suggests that the decline of phytoplankton by the influence of probiotic use is not a critical problem, while probiotics' efficiency in water improvement is crucial.

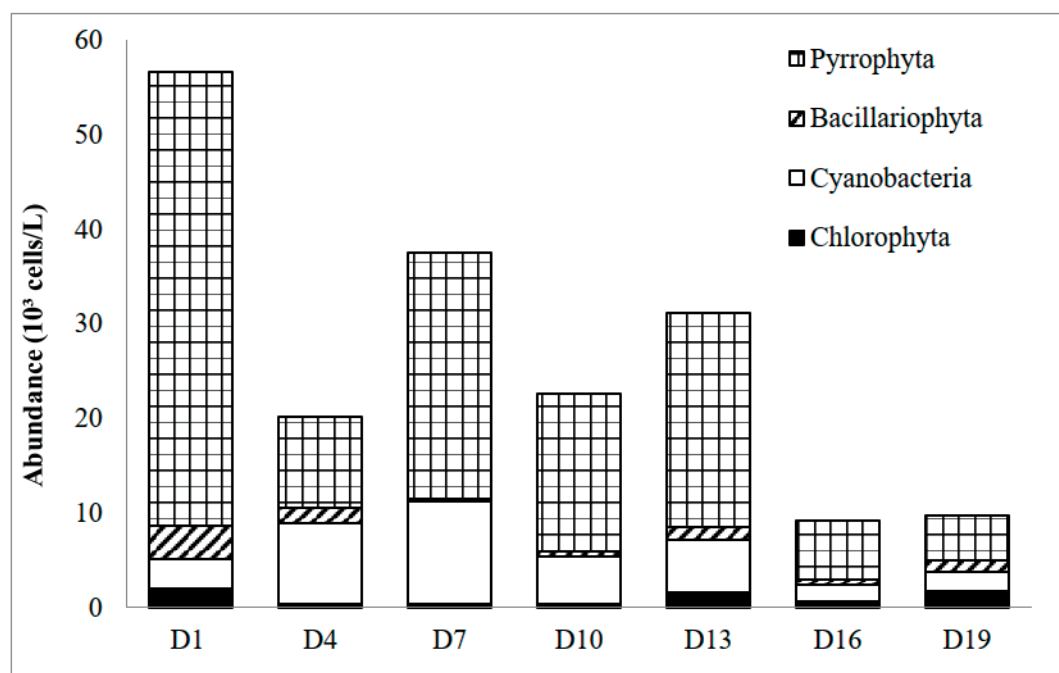


Figure 6. The daily abundance of phytoplankton.

3.5 Water Quality

Water temperature from day 1 to day 19 showed an average of 29.5 °C. In contrast, the averages of dissolved oxygen, pH and salinity of all 19 days were 6.2 mg/L, 8.0 and 11.4 ppt, respectively. The fluctuation was very low, with a minimal standard variation. None of the water quality parameters was significantly different between the sampling days ($P > 0.05$) (Table 3). The result corresponded with the study of Rajinikanth

et al. [18] that reported all water parameters (pH, water temperature and dissolved oxygen) were stable or only slightly changed during their study.

Table 3. Water parameter.

Parameter	Day								SD
	D1	D4	D7	D10	D13	D16	D19	Average	
Temperature (°C)	29.0 ^a	30.0 ^a	30.5 ^a	29.5 ^a	29.5 ^a	28.0 ^a	30.0 ^a	29.5	0.8165
DO (mg/L)	6.14 ^a	6.50 ^a	6.34 ^a	6.05 ^a	7.51 ^a	5.15 ^a	6.00 ^a	6.2	0.7050
pH	8.00 ^a	8.02 ^a	8.04 ^a	8.00 ^a	8.18 ^a	8.01 ^a	8.04 ^a	8.0	0.0634
Salinity (ppt)	13.0 ^a	10.5 ^a	10.0 ^a	12.0 ^a	13.0 ^a	10.0 ^a	11.0 ^a	11.4	1.3138

*Mean of the same alphabet label within the same row indicate no significance ($P > 0.05$).

4. Conclusions

Using probiotics can control an abundance of phytoplankton in shrimp ponds. Phytoplankton found in ponds of white shrimp (*Litopenaeus vannamei*) in the 19 days of the experiment (every 3 days sampling) was 12 genera from 4 divisions. This study found species from Cyanophyta (blue-green algae) were *Oscillatoria* sp., *Pseudanabaena* sp., *Planktolyngbya* sp., and *Merismopedia* sp. Chlorophyta (green algae) species found were *Scenedesmus* sp. and *Monorapidium* sp., while *Nitzschia* sp., *Pleurosigma/Gyrosigma* sp., and *Navicula* sp. represented Bacillariophyceae (diatom). Members of Pyrrophyta (dinoflagellate) were *Protopteridinium* sp., *Ceratium* sp., and *Prorocentrum* sp., where the highest cell abundance was also found for this division. The abundance profile of all divisions reflected the effects of probiotics on the phytoplankton community. Probiotics can control an abundance of division Pyrrophyta and Cyanophyta. In contrast, probiotics can enhance an abundance of division Bacillariophyceae and Chlorophyta at the end of the study. Water parameters in this study, e.g., temperature, dissolved oxygen, pH, and salinity, were highly stable and showed no significant difference. Probiotics solely influenced changes in the phytoplankton population for improving water quality and phytoplankton density control.

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Effect of Nickel Concentration on Ozone Production using Nickel-Antimony Doped Tin Oxide for Wastewater Treatment

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Abstract: Nickel-antimony doped tin oxide (NATO) catalyst has greatly considered its capability to treat high organic matter and eliminate colour in wastewater as palm oil mill wastewater with electrochemical ozone generation. This study presents the optimal Ni content of NATO and its properties to enhance ozone generation. The NATO anodes were fabricated by dip-coating, varying the Ni content between 0.5% and 5%. The key findings show that all anodes showed a single phase of rutile structure with the cracked mud on the surface. The binding energies of the $\text{Sb}3\text{d}_{3/2}$ peak at 540.48 eV and 541.58 eV agree with Sb^{3+} and Sb^{5+} , respectively. The optimal ozone current efficiency of 37% at the current density of ca. 0.22 A cm^{-2} in $0.5 \text{ M H}_2\text{SO}_4$ at 2.7V was obtained on the NATO anode with 2% Ni content calcined at 700°C . The palm oil mill wastewater treatment had the efficiency of decolourisation TOC and COD removal increased with increasing electrolysis time. The colour removal efficiency achieved was more than 85% for a reaction time of 15 min. The efficiency of TOC and COD removal was reached ca. 80% in 20 min. The overall results appeared that NATO with electrochemical ozone generation could be employed to treat palm oil mill wastewater with high efficiency due to $\bullet\text{OH}$ and O_3 .

Keywords: Palm Oil Mill Wastewater; Ni-Sb/SnO₂; Ozone; Electrochemical

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1. Introduction

Advanced oxidation processes (AOPs) have been extensively applied to water and wastewater treatment to eliminate organic substances and hazardous and toxic pollutants before treatment with conventional methods [1]. The mechanism of AOPs is based on hydroxyl radicals with high potential oxidation (2.87eV) [2]. AOPs technology consists of ozonation, UV/H₂O₂, photocatalytic oxidation, electro-oxidation and Fenton's oxidation [3]. Ozone is a powerful oxidizing agent. The most common method of generating ozone on the industrial scale is using Cold Corona Discharge (CCD) and the electrochemical generating of ozone. The latter has many potential advantages, including low voltage operation and the possibility of generating high concentrations of ozone in the gas and liquid phases with high current efficiency [4]. The water electrolysis can produce through a 6-electron process at an anode ($E^\circ = 1.51 \text{ V}$).

Several electrode materials have been studied concerning ozone activity and selectivity over many years, including graphite, glassy-carbon, platinum, boron-doped diamond (BDD) and Ni/Sb-SnO₂ (NATO) [5]. Recently, it has been reported that the applicability of the NATO electrodes was employed for the degradation of refractory pollutants, decolourisation, microorganisms removal and decomposition of organic compounds [6] [7]. Wang and co-workers [7] reported that the 4-chlorophenol (4-CP) was degraded using a NATO anode via an electrochemical process. It has been suggested that Ni co-doped Sb-SnO₂ could produce •OH radicals that can form ozone without organic molecules. Zakaria and Christensen (2014) [6] and Maneelok et al. (2018) [8] have reported complete decolourisation of Reactive Blue 50 (RB50) Dye solution due to the destruction of the anthraquinone moiety using NATO anode at 2.7V. Furthermore, Sun and co-workers (2015) [9] reported that co-doping of Ce-Ni into Sb-SnO₂ can enhance phenol degradation and TOC removal as well as electrocatalytic activity and service life due to the changes in the concentration of oxygen vacancies in the lattice of SnO₂ and the generation of reactive oxygen species (ROS) in the solution for indirect oxidation. With respect to previous studies, a novel material as NATO could enhanced the degradation of toxic organic compounds. However, most of studies above were investigated on synthetic wastewater, of which the composition was simple. While the real wastewater always contained organics, complex inorganics, solid and microorganisms [10]. Thus, the applicability of NATO anodes in real wastewater as Palm oil mill wastewater (POW) was investigated.

Palm oil mill wastewater treatment is crucial. In terms of composition, POW is a complex liquid consisting of macromolecules, such as polysaccharides, lipids, proteins, and many monocyclic and polymeric aromatic molecules with typically the chemical oxygen demand (COD) of 15,000-100,000 mg/L, biochemical oxygen demand (BOD) of 10,000-43,750 mg/L, pH of 3.4-5.2, oil and grease of 8 g/l, suspended solids of 20 g/l, and total solids of 40 g/l [11]. Several treatment methods for POW have been proposed, such as adsorption, electrocoagulation, photocatalytic oxidation, ultrafiltration and conventional method as aerobic and anaerobic treatment [12]. Alternatively, the electrochemical process was considered to overcome the drawbacks of traditional and wastewater treatment technologies due to its simplicity in operation, no chemical addition required, and less sludge production [13]. Thus, this research focused on the palm oil mill wastewater treatment by using electrochemical ozone generation at NATO electrodes while investigating the effect of Ni content on the performance of the NATO anodes concerning electrocatalytic activity and the active site for ozone generation. The surface properties of NATO anodes were carried out, and the ozone generation by the electrochemical process for POW treatment was evaluated.

2. Materials and Methods

2.1 Synthesis method

The electrode preparation and dip-coating synthesis to fabricate Nickel-Antimony doped Tin Oxide anodes (NATO) were adapted from Christensen et al. (2014) [6] and Maneelok et al. (2018)[8]. The precursor solutions contained SnCl₄.5H₂O, SbCl₃ and NiCl₂.6H₂O in the mole ratio Sn:Sb:Ni of 500: 8: x, where x represents the Ni content with varying doping levels at 0.5, 1, 2, 3, 4, and 5. All chemicals were dissolved in ethanol. The titanium substrates were dipped in the catalyst solution and were then calcined in a furnace at 700 °C for 1 hour. The electrodes were allowed to cool in the air, after which they were weighed.

2.2 Analytical method

The NATO anodes were characterised by structure, morphology and chemical composition. The determination of structure was carried out by X-ray diffraction (XRD). Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX) and X-ray photoelectron spectroscopy (XPS) were employed to observe the morphology and chemical composition, respectively. The electrochemical oxidation process measured the generation of ozone. 3 cm³ of 0.5M H₂SO₄ was utilised as an electrolyte. A 0.64 cm² Pt-Ti mesh was employed as a cathode. Both electrodes were comprised of the cuvette cell and placed in the cuvette holder in a UV spectrophotometer. A reference spectrum was collected with the potential at 2.7V for the 30s. The steady-state absorbance at 258 nm was used to calculate the current efficiency, assuming an extinction coefficient of 3000 M⁻¹ cm⁻¹ [5]. The current efficiency of ozone (%CE) was calculated from:

$$\eta = 58.A_{O_3} / Q$$

Where A is the ozone absorbance and Q is the charge passed

2.3 Wastewater collection and characterisation

The Palm oil mill wastewater was collected from the final anaerobic tank of Palm Oil Company, Nakhon Si Thammarat, Thailand. The samples were preserved in the fridge with a temperature of less than 4 °C. Decolourisation experiments were carried out using the NATO anodes at 2.7V as a function of electrolysis time (0.5, 5, 10, 15 and 30 min). M/NATO anode was employed with a 0.64 cm² Pt/Ti mesh as a cathode in a quartz cuvette cell placed in the spectrophotometer. During the experiment, the current and absorbance was recorded. Decolourization was determined from the absorbance of the 383 nm band [14] in the ratio A/A₀. Palm oil mill wastewater was analysed for COD using the Closed Reflux, Titrimetric Method (5220C) [15]. The high-temperature combustion method (5310B) was used to measure TOC. The Palm oil mill wastewater remediation was evaluated from the measurements of COD and TOC as follows:

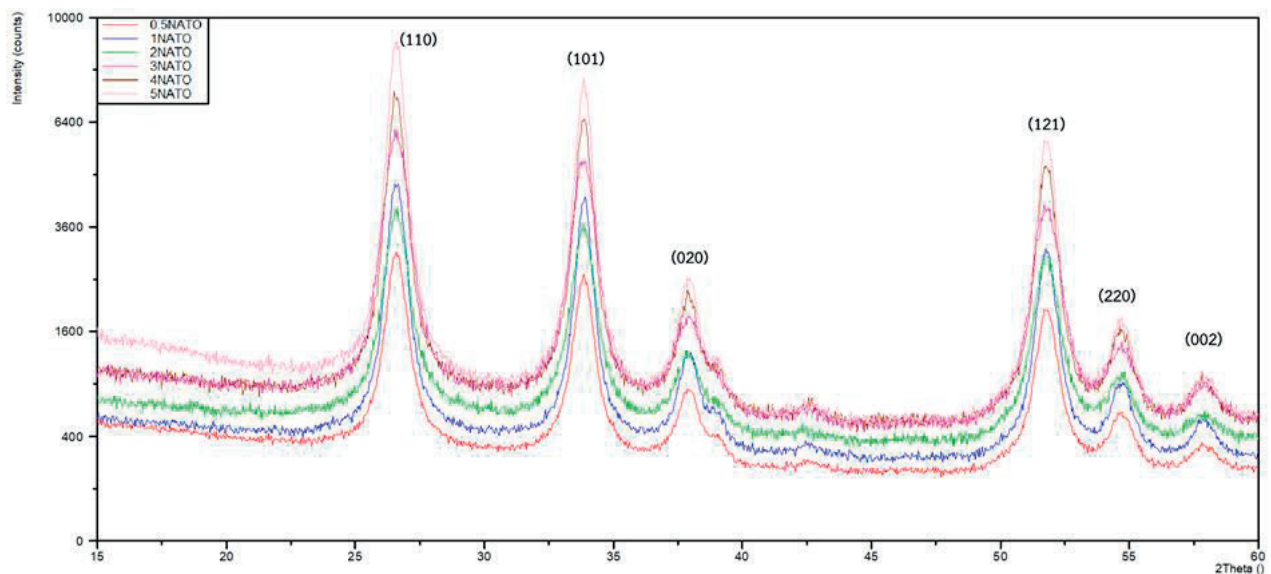
$$COD, TOC = \frac{100 \times (C_i - C_t)}{C_i}$$

Where C_i is the concentration of COD and TOC before treatment and C_t the COD and TOC after a time t [10]

3. Results and Discussion

3.1 The electrode surface characterisation

The prepared NATO electrodes calcined at 700 °C with different Ni content of Sn:Sb:Ni in a mole ratio of 500:8:x where x is 0.5-5 presents the Ni content. Figure 1 shows the XRD pattern of NATO with different Ni loading. It can be seen that the result of XRD patterns in all samples shows the single crystalline phase of the rutile structure. The crystal structures is the space group (SG): P4₂/mm and the unit cell parameter are the values of: a = b = 0.4738 nm and c = 0.3187 nm [4]. Regarding the peak position of all the XRD patterns, they (2θ = 26.6, 33.8, 37.9, 51.8, 54.7 and 57.9°) were attributed to 110, 101, 020, 121, 220, 002 planes of SnO₂. Hence, the patterns of all products agreed well with the reflection of a single phase of "SnO₂; Tin Oxide; Cassiterite, syn; Q: S; 00-041-1445" obtained from the crystallographic database ICSD 39173. No characteristic impurity was detected. When comparing the SnO₂ pattern with other research [8, 9]. It was found that the diffraction peaks of all products were quite the same as the position peaks of those. Regarding the effect of Ni content, there is no effect of Ni content up to 5%Ni on the structure. This suggests that the addition of nickel and antimony to tin oxide did not change the crystal structure, suggesting that Ni and Sb replaced Sn ion in the lattice of SnO₂. It can be explained that the ionic radius of Sb³⁺ and Sb⁵⁺ is 0.76 Å and 0.60 Å, respectively. Ni²⁺ and Ni³⁺ ionic is ca. 0.69 Å and 0.72 Å, respectively whilst the ionic radius of Sn⁴⁺ is 0.69 Å [9]. Hence, the Sb⁵⁺ and Ni²⁺ ions could substitute Sn⁴⁺ ions in the bulk of the lattice. The Sb³⁺ and Ni³⁺ ions might be placed at the surface [16].



Figures 1. The XRD patterns of the NATO samples were calcined at 700 °C with various Ni content from 0.5%Ni to 5% Ni.

The typical SEM and EDX images of the 5NATO electrode calcined at 700°C, as represented by the cross on the electrode, are shown in Figure 2. It can be seen from Figure 2(a) that the electrode morphology showed the smooth, cracked mud structure resulting from mechanical tension and stress inside the coating. These could occur from the dopant, the plasticity of the coating, and the difference between the thermal expansion coefficients of the Ti substrate and the coating [17]. Despite the change in nickel concentration, it is clear that the morphology of all anodes is quite the same. However, It has been reported that the Sb and Ni content can affect morphology. Wang et al.(2013) [18] reported that a NATO catalyst with a high nickel content and low antimony concentration appeared to display a smooth surface. The EDX spectra of 5NATO electrodes calcined at 700 °C at the cross are shown in Fig. 2(b). It can be seen from the figures that Sn, Sb and O were detected but no Ni due to the limitations of the instrument. However, the element Sn, Sb, Ni, O and Ti in term of weight percentage show the percentage of nickel were observed.

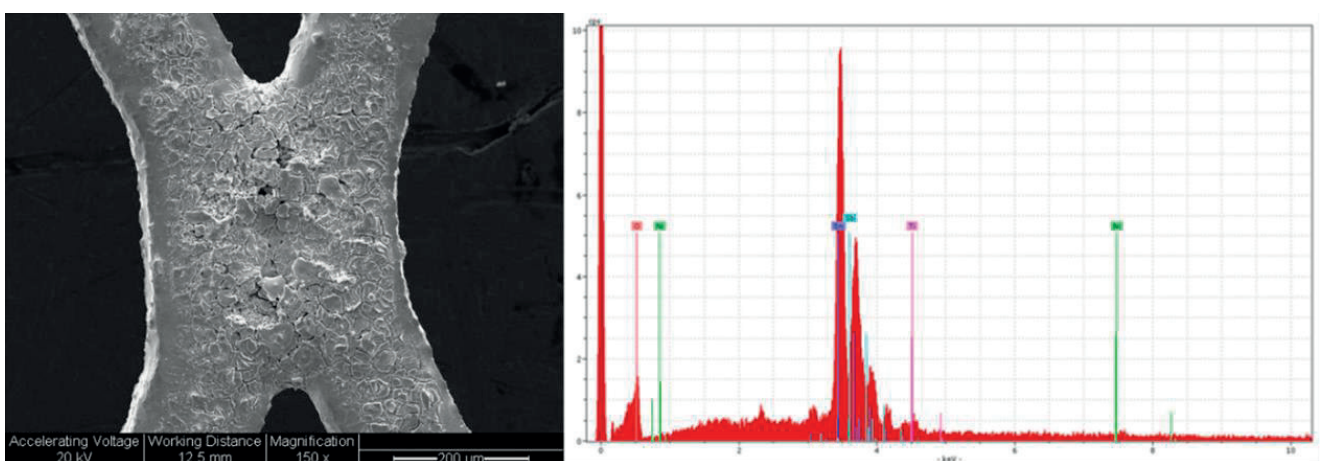


Figure 2.(a) A typical SEM image and **(b)** EDX spectra of 5NATO anode calcined at 700 °C: magnification x 150 and scale bar = 200 µm

The broad scan XPS spectrum clearly shows photoelectric peaks that correspond to Sn, Sb and O; however, no Ni peak was observed at the surface of the NATO. Figures 3(a) and (b) show the spectrum of

Sn3d and Sb3d states for 0.5NATO anode calcined at 700 °C, respectively. As can be seen from the figures, the spin-orbital of the Sn3d peak of all the samples split into Sn3d_{3/2} and Sn3d_{5/2}, with a symmetric peak shape component that was fitted at a binding energy of 495.5 and 487.9 eV, respectively. A good fit was obtained between the experimental and calculated data, which leads to assigning the state of Sn⁴⁺ within Sb-SnO₂ in agreement with the literature [16]. No chemical shift of the Sn3d state was observed after doping with Sb and Ni, and the separation distance between Sn3d_{3/2} and Sn3d_{5/2} peaks of both samples is equal to 8.4 eV, which agrees with Babar et al.(2011) [19]. They had detected a gap of ca.8.5 eV between the Sn3d_{3/2} and Sn3d_{5/2} peaks.

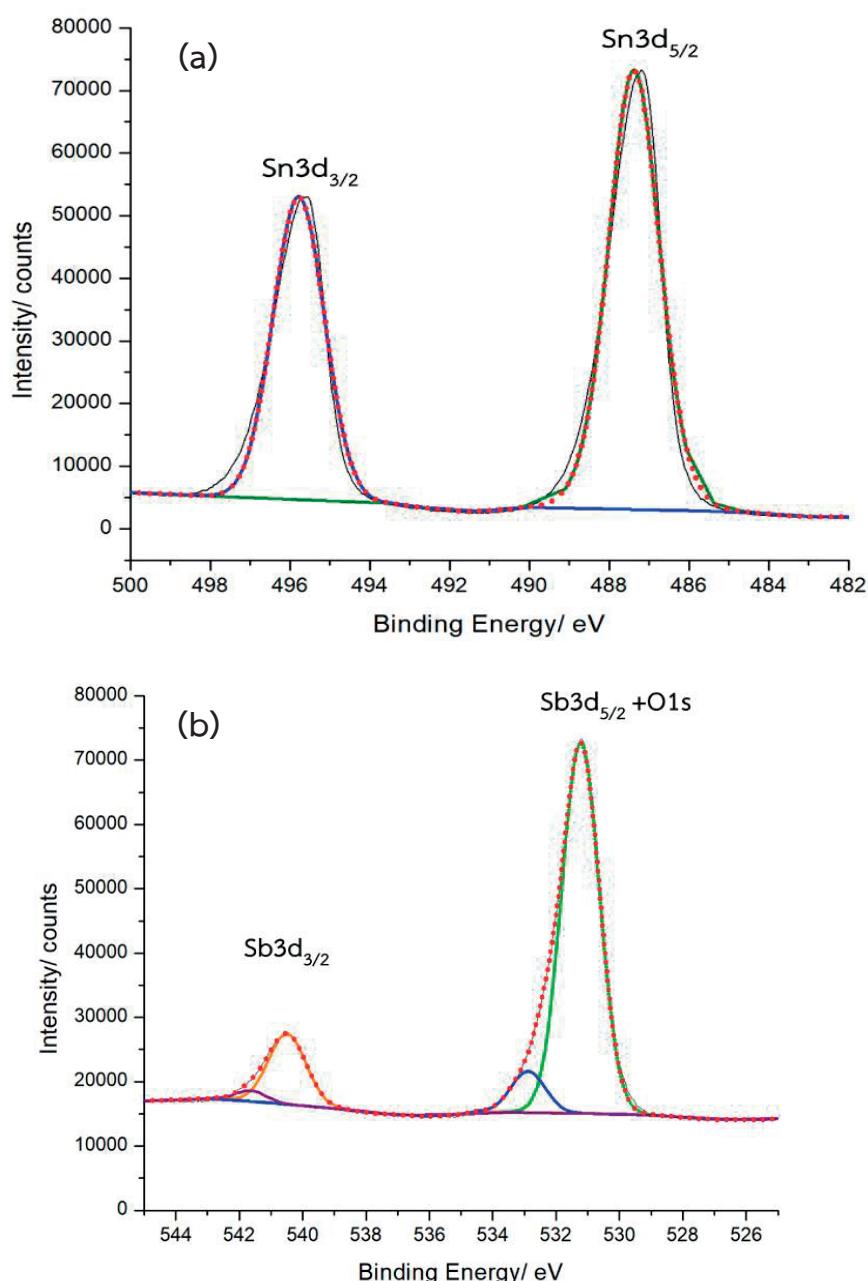


Figure 3. The fitting of the XPS spectra of the (a) Sn3d and (b) Sb3d peak of 0.5NATO electrode calcined at 700 °C: the dotted line is the raw data and the solid line is the synthesised curve and components.

As can be seen in figure 3(b), the binding energy of the Sb3d_{3/2} was at 540.48 eV and 541.58 eV corresponding to Sb³⁺ and Sb⁵⁺, respectively. The Sb3d_{5/2} peak shows that the Sb3d_{5/2} and O 1s region are dominated by the O 1s peak, which is broad and asymmetric, suggesting the other oxygen species contributions.

Peaks with binding energies in the range 530-531.5 eV in the spectra of metal oxides are commonly attributed to lattice oxygen species (MOx) [20]. Moreover, oxygen directly bonded to metals (M-O) gives increases to peaks in the range 530.0-532.0 eV, and metal hydroxides (M-OH) to peaks with binding energies of 531-533.5 eV [20]. Babar and co-workers (2011) [19] observed the XPS spectra of ATO thin film with binding energies between 530.45 and 530.57 eV were attributed to chemisorbed oxygen, along with a peak at 532 eV indicating M-OH.

3.2. Effect of Ni content on ozone generation

Figure 4 shows the current efficiencies and the current densities of the NATO anodes series as a function of the nickel content in the precursor solutions. As seen from the figure, the current efficiency of all NATO anodes showed the same behaviour: the current efficiencies increased steadily with increasing Ni content up to 2%Ni before decreasing Ni by doping Ni up to 5%. This might be due to an increase resistant to doping Ni. This result is in agreement with the work of Wang et al. (2006) [7] reported that the resistivities of Ni/Sb-SnO₂ electrodes effectively increased by a factor of ca.100 on doping with 1.0 at%Ni (from 0.2 Ωcm^{-1} for undoped Ni to 20 Ωcm^{-1} for 1 at.%Ni). Moreover, the current efficiency was decreased because the ratio of O₃ and O₂ active sites decreased with increasing Ni content. The optimum electrode was 2NATO calcined at 700 °C. The anode showed the highest current efficiency and density at ca. 37% and 0.22 A cm⁻², respectively. Wang et al.(2006) [7] and Shekarchizade and Amini (2011) [17] found that current efficiency decreased with Ni doping, with the optimum Ni concentration being ca. 0.2 at.%.

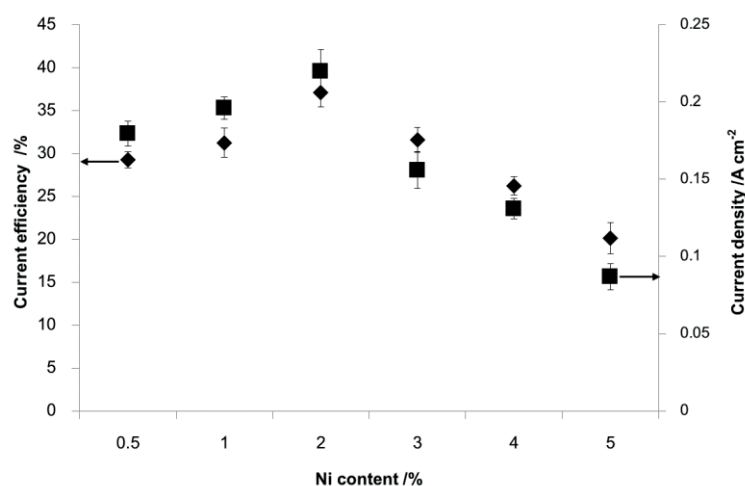


Figure 4. Plots of current efficiency and current density vs the nickel concentration in the precursor solution for the NATO series anodes of 0.5M H₂SO₄ at 2.7V.

3.3. Effect of ozone on degradation and decolourisation of POW

The properties of POW before and after the electrochemical ozone generation process by the 2NATO anode are summarized in Table 1. As can be observed from table 1, the pH increased after electrolysis due to hydroxide formation, and hence COD and TOC also decreased as a function of time.

Table 1. Characteristics of Palm oil mill wastewater after electrolysis for 30 minutes with electrochemical ozone generation process.

Parameter	Before ozonation	After ozonation
pH	5.14	5.87
COD (mg L ⁻¹)	8,540	1,238
TOC (mg L ⁻¹)	178.6	30.79

The effect of current density and ozone current efficiency on the removal of COD, TOC and colour from the POW was investigated on the 2NATO anode corresponding to the highest current efficiency. The experiment operated under the same conditions as in the previous section. Figure 5 shows removal efficiency for decolourisation, COD and TOC as a function of time. As may be seen, the absorbance decreased by ca. 80% in 10 min and ca. 90% in 25 min. COD and TOC removal in POW were achieved more than 80% after electrolysis for 15 min and 20 min, respectively. Regarding electrochemical ozone generation, it is generally assumed that water oxidation on NATO anodes involves the formation of OH radicals, which then react with dye molecules and toxic substance [21]:



A more detailed mechanism has been prepared by [21]. Firstly, water oxidation yields the adsorbed hydroxyl radical ($\text{OH}\cdot$) according to equation (2). This radical interacts with lattice oxygen at the surface yielding "active oxygen" MO_{x+1} , see reaction (3). Finally, the MO_{x+1} reacts with the organic pollutant (R) according to reaction (4):

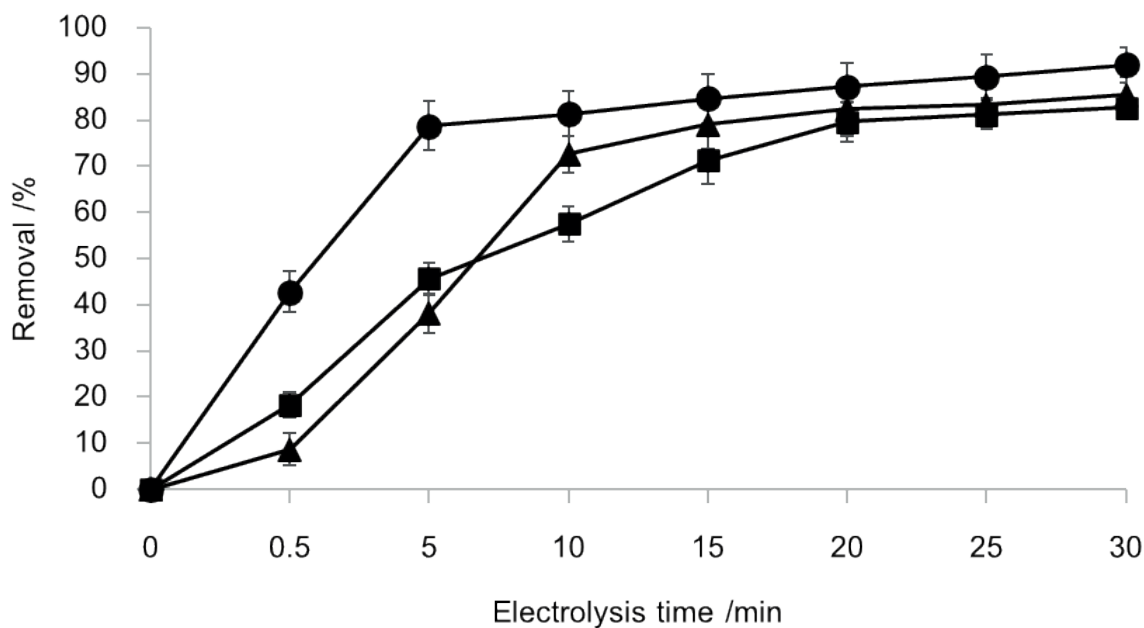
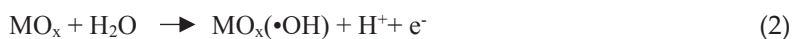


Figure 5. Plots of the removal of (●) colour, (▲) COD and (■) TOC removal of POW as a function of time at 2.7 V using 2NATO anode.

4. Conclusions

NATO anodes were prepared by dip-coating method with various Ni doping levels to investigate electrochemical ozone generation and their surface properties. Furthermore, the anodes were employed to examine the electrocatalytic activities for decolourisation and degradation of organic pollutants in POW. The XRD characterisation shows a single phase of rutile structure. Sb and Ni are substituted into the SnO_2 lattice, suggesting that Sb^{3+} ions at the surface of the crystallites provide the active sites for oxygen adsorption. The morphology of all NATO showed the cracked mud structure due to mechanical tension and stress inside the

coating. The element composition of NATO anodes shows Sn, Sb, Ni, O and Ti. For the chemical oxidation state, it was found that the peaks at 495.5 eV and 487.7 eV of the 0.5NATO are attributed to the Sn^{4+} state. The binding energy of the $\text{Sb}3d_{3/2}$ was at 540.48 eV and 541.58 eV, corresponding to Sb^{3+} and Sb^{5+} , respectively. The current efficiency and density increased with doping Ni up to 2%, then decreased gradually with increasing Ni content up to 5% due to its resistance. The optimum current efficiency of ca. 37% and current density of 0.22 A cm^{-2} were observed for the 2NATO. Decolourisation and degradation of POW, the colour removal was achieved ca. 80% in 10 min. The performance of COD and TOC reduction increased with increasing ozonation time. The efficiency of COD removal increased linearly up to > 80% after electrolysis for 15 min and 20 min for TOC. Hence, a higher electrochemical oxidation rate is observed at higher current densities due to more elevated ozone and hydroxyl radicals production.

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Preparation of Lead-Free X-Ray Shielding Materials Based on Natural Rubber/Barium Sulfate Composites

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Abstract: This work prepared the lead-free X-ray shielding materials based on natural rubber (NR) mixed with BaSO₄ particles at different BaSO₄ contents (0–100 phr) and different material thicknesses (0.5–2.5 cm). The X-ray shielding properties of NR/BaSO₄ composites were tested using a 100-kV X-ray generator. The results suggested that the increases in the BaSO₄ contents improved the x-ray shielding properties of the NR/BaSO₄ composites, such as linear attenuation coefficient (μ_l), mass attenuation coefficient (μ_m), and half-value layer (HVL). The highest X-ray attenuation was 94 % in 100 phr BaSO₄. In terms of mechanical properties, it was found that the density and hardness (Shore A) increased with increasing BaSO₄ contents. In contrast, the increases in BaSO₄ resulted in decreased tensile strength, elongation at break and tear strength. In summary, the overall properties suggest that these NR/ BaSO₄ composites could be developed as an effective X-ray shielding material instead of lead.

Keywords: Natural rubber; Barium sulfate; X-ray shielding; Composite materials

1. Introduction

X-ray is a relatively high-energy electromagnetic wave that plays important roles in various applications such as agriculture, food preservation, industry, and especially in medical fields for diagnosis and therapy [1]. However, exposure to X-rays could potentially lead to fatal health hazards in radiation workers and users and cause symptoms such as skin burns and vomiting within hours. It can also result in long-term health effects such as memory loss, cancer, and cardiovascular disease [2]. Therefore, effective shielding materials were important to prevent excessive X-ray exposure. It is known that the materials composed of high-atomic-number (Z) and high-density elements are commonly used as shielding materials, such as lead (Pb) [3-5].

However, lead is considered a hazardous chemical [6]. Recently, the radiation shielding materials with the addition of fillers such as tungsten oxide (W_2O_3) [7-8], bismuth oxide (Bi_2O_3) [8-10], and barium sulfate ($BaSO_4$) [7,11-12] were studied for replacement of lead (Pb). Among these materials, $BaSO_4$ is an exciting filler since it is environmentally friendly, safe, and highly effective in X-ray attenuation [12]. Previous investigations reported that it could be improved the radiation shielding properties of concrete [13] and it is also found that a shielding film prepared from $BaSO_4$ and polymeric silicon has higher tensile strength and X-ray shielding performance [7]. In addition, a polymeric composition consisting of ethylene propylene diene monomer (EPDM), industrial oil (IO), and alkyl phenol-formaldehyde resin (PF) with the addition of $BaSO_4$ can be applied to manufactured material for protection from radiation in offices of radiation therapy and diagnostics [12].

In this work, we aimed to develop flexible and lead-free X-ray-shielding materials that are safe and environmental-friendly based on NR/ $BaSO_4$ composites. Dunlop method is used for NR/ $BaSO_4$ composites foam production. This method is widely used because it is simple, convenient, reliable, cost-effective, and energy-efficient [14-15]. The X-Ray shielding properties are tested based on the thicknesses and $BaSO_4$ contents of NR/ $BaSO_4$ composites, such as X-ray transmission ratio, X-ray attenuation, linear attenuation coefficient (μ_l), mass attenuation coefficient (μ_m), and half-value layer (HVL). Furthermore, the mechanical properties of the composites were also investigated.

2. Materials and Methods

1. Materials

NR latex with 60 wt% dry rubber was acquired from the Center of rubber technology for community, Faculty of engineering, Thaksin University, Phatthalung, Thailand. Barium Sulfate was purchased from Scitrader Co, Ltd. (Thailand). Sulfur (S), zinc diethyldithiocarbamate (ZDEC), zinc 2-mercaptobenzothiazole (ZMBT), Wingstay®-L, zinc oxide (ZnO), Diphenylguanidine (DPG), and Sodium silicofluoride (SSF) was purchased from VPK Supply Co, Ltd. (Thailand). All chemicals were used in an aqueous dispersion form, prepared in the laboratory (Table 1).

2. Preparation of NR/ $BaSO_4$ composites

NR/ $BaSO_4$ composites were fabricated using the chemical proportions given in Table 1. First, NR latex was filtered and agitated by a whisk and bowl mixer for 3 min to liberate dissolved ammonia before foam preparation. After that, $BaSO_4$, S, ZDEC, ZMBT, Wingstay® L, ZnO, DPG and SSF were slowly added to the mixture and stirred for about 1 min. Then, the NR/ $BaSO_4$ foam was poured into a mold ($25 \times 25 \times 0.5$ cm³) to begin the gelling process. Finally, NR/ $BaSO_4$ was vulcanized at 100 °C for 1 h in an oven (ED 56, BINDER). The non-reactive reagents were removed by washing the solid foam with distilled water. After washing, the cured NR/ $BaSO_4$ was evenly dried in a hot air oven at 80°C for 24 h.

Table 1. Material formulations for Natural Rubber/Barium Sulfate Composites preparation

Ingredients	Total solid content (%)	Dry weight (phr*)	Wet weight (g)
HA latex	60	100	167
S	50	2	4
ZDEC	50	2.5	5
ZMBT	50	2	4
Wingstay® L	50	1	2
ZnO	50	5	10
DPG	40	2	5
SSF	20	0.5	2.5
$BaSO_4$ (phr)	0, 50, 75, 100		

* phr is the ratio unit of weight part of chemicals to 100 wt parts of rubber

3. Characterizations

3.1. Density and morphology studies

For all NR/BaSO₄ composites, the density was measured by comparative measurement of each sample mass in air and water using a digital scale (BSA Series, Sartorius). The experimental setup showed in Figure 1. The samples with dimensions of 20 × 20 × 5 mm³ were placed in the balance pan and recorded their dry weight. Next, the samples were placed under the submersed basket. Finally, the specific gravity was calculated by a digital scale. The specific gravity of the sample was multiplied by water density to obtain the density of the sample, as shown in Eq (1).

$$\text{specific gravity} = \frac{\text{density of the object}}{\text{density of the water}} \quad (1)$$

For the study of morphology, the samples were investigated using a scanning electron microscope (SEM; FEI / FEG Quanta 450), operating at 5 kV. Dried foams were vacuum-coated with gold before SEM investigation.

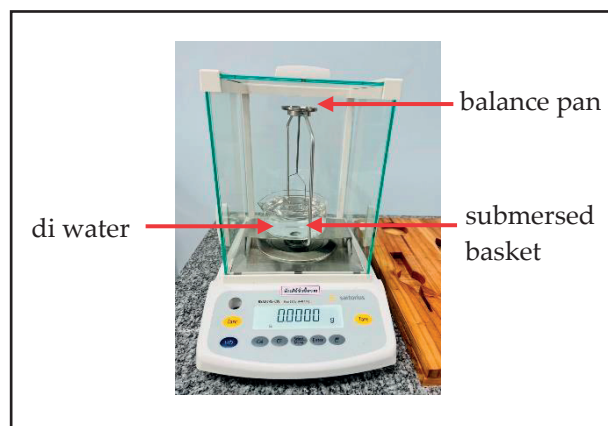


Figure 1. Setup for Density measurement.

2.2. Mechanical properties

Tensile and tear tests were conducted following ISO37-1 and ISO30-1, respectively, to characterize the mechanical properties of the NR/BaSO₄ composites. The tests were performed on a universal testing machine (Purchem Co., Ltd.) with a crosshead speed of 50 cm/min. Ultimate tensile strength, elongation at break and tear strength were determined. The hardness for all NR/BaSO₄ was tested using an automatic hardness tester (GX-02, Teclock).

2.3. X-Ray shielding properties

The X-Ray shielding properties of NR/BaSO₄ composites were performed at the Chaiya Hospital, Surat Thani Province, using the General X-ray Machine (MRAD-A32S, CANON) at 100 kV and the X-ray test equipment (X2, RAYSAFE) was used as the detector. All samples used in the experiment were 2 cm × 2 cm. The X-Ray shielding properties test was carried out on the different material thicknesses (0.5-2.5 cm). The narrow beam X-ray source was located 1 m from the surface of NR/BaSO₄ composites. The independent 0.05 s tests were performed and were recorded for each sample. The experimental setup is shown in Figure 2. From the experiment, the X-Ray shielding properties included X-ray attenuation [2], linear attenuation coefficient (μ_l), mass attenuation coefficient (μ_m), and half-value layer (HVL) [7] could be calculated using equation (2) – (5)

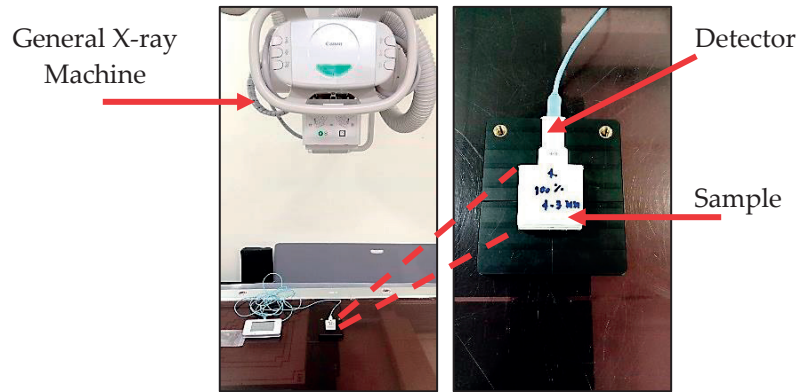


Figure 2. Setup for X-ray shielding measurement.

$$X - ray \text{ attenuation} = \frac{I_0 - I}{I_0} \times 100 \quad (2)$$

$$I = I_0 e^{-\mu_l x} \quad (3)$$

$$\mu_m = \frac{\mu_l}{\rho} \quad (4)$$

$$HVL = \frac{\ln 2}{\mu_l} \quad (5)$$

Where I , I_0 , μ_l , μ_m , ρ , HVL, and x was the intensity of transmitted X-rays, the intensity of incident X-rays, linear attenuation coefficient, mass attenuation coefficient, density, half-value layer, and thickness of the samples, respectively.

3. Results and Discussion

3.1 Density and morphology studies

The density of NR/BaSO₄ composites is shown in Figure 3. It was found that the densities of NR/BaSO₄ composites increased with BaSO₄ content mainly due to the much higher density of BaSO₄ particles. It was known that increased material density could improve the mass attenuation coefficient according to equation 4.

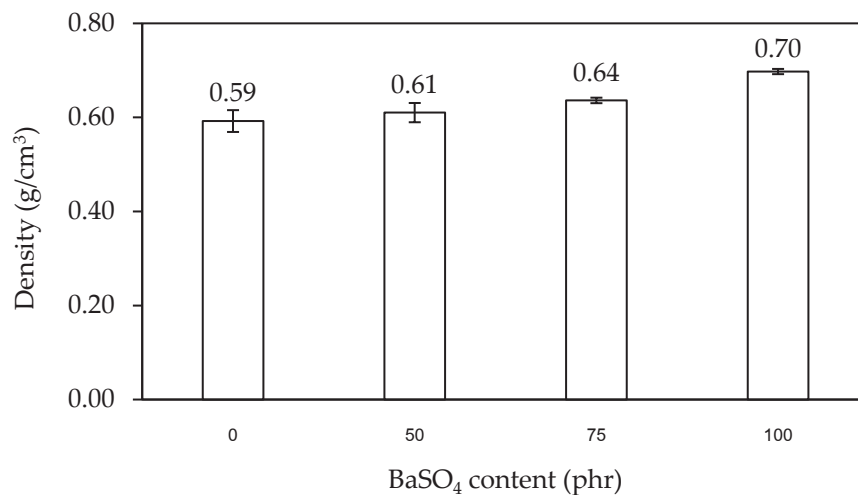


Figure 3. The densities of NR/BaSO₄ composites with varying contents of BaSO₄.

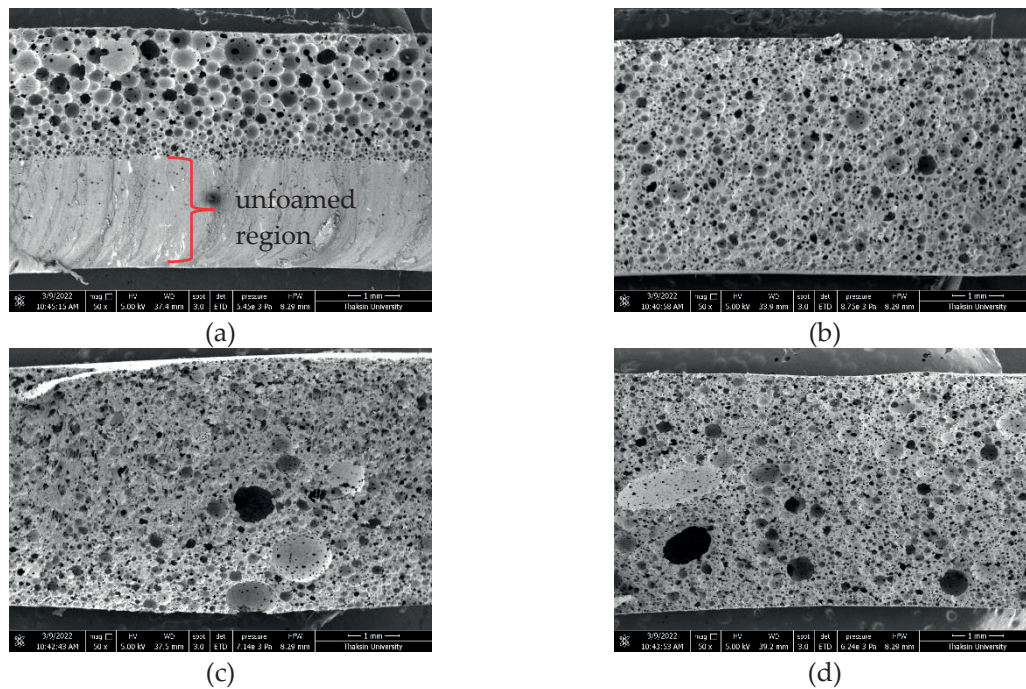


Figure 4. Cross-sectional microstructures of NR/BaSO₄ composites with varying contents of BaSO₄ (a), 0 phr, (b) 50 phr, (c) 75 phr, and (d) 100 phr.

Figure 4. represents the influence of BaSO₄ on morphologies of NR/BaSO₄ composites. From cross-sectional microstructures, All samples showed small open-cell structures inside NR/BaSO₄ composites from cross-sectional microstructures. However, NR/BaSO₄ composites had non-uniform cell structures. The composites without BaSO₄ consisted of large foam cells on the top, the tiny foam cells in the middle, and the non-foamed region on the bottom (Figure 3 (a)). Due to foams transforming into solid-state foams slowly, it is easier to diffuse gas into the top of the sample, which leads to foam collapse and increased density on the bottom [14]. On the other hand, samples with increasing BaSO₄ contents showed increasing large voids since foams transformed to solid-state foams rapidly. Gas phases decrease tensile strength, elongation at break, and tear strength [3].

3.2 Mechanical properties

As shown in Figure 5, the tensile strength, elongation at break, and tear strength decreased with increasing BaSO₄ content. These were due to more BaSO₄ content being added to the composites, leading to an agglomeration of BaSO₄ particles and worse particle dispersion[16]. On the other hand, hardness (Shore A) increased with increasing BaSO₄ contents. The increases in hardness could have been due to the high rigidity of the BaSO₄ particles. This result was consistent with Lim-aroon et al. (2019) [16], which prepared X-ray and gamma-ray shielding from NR/Bi₂O₃ composites.

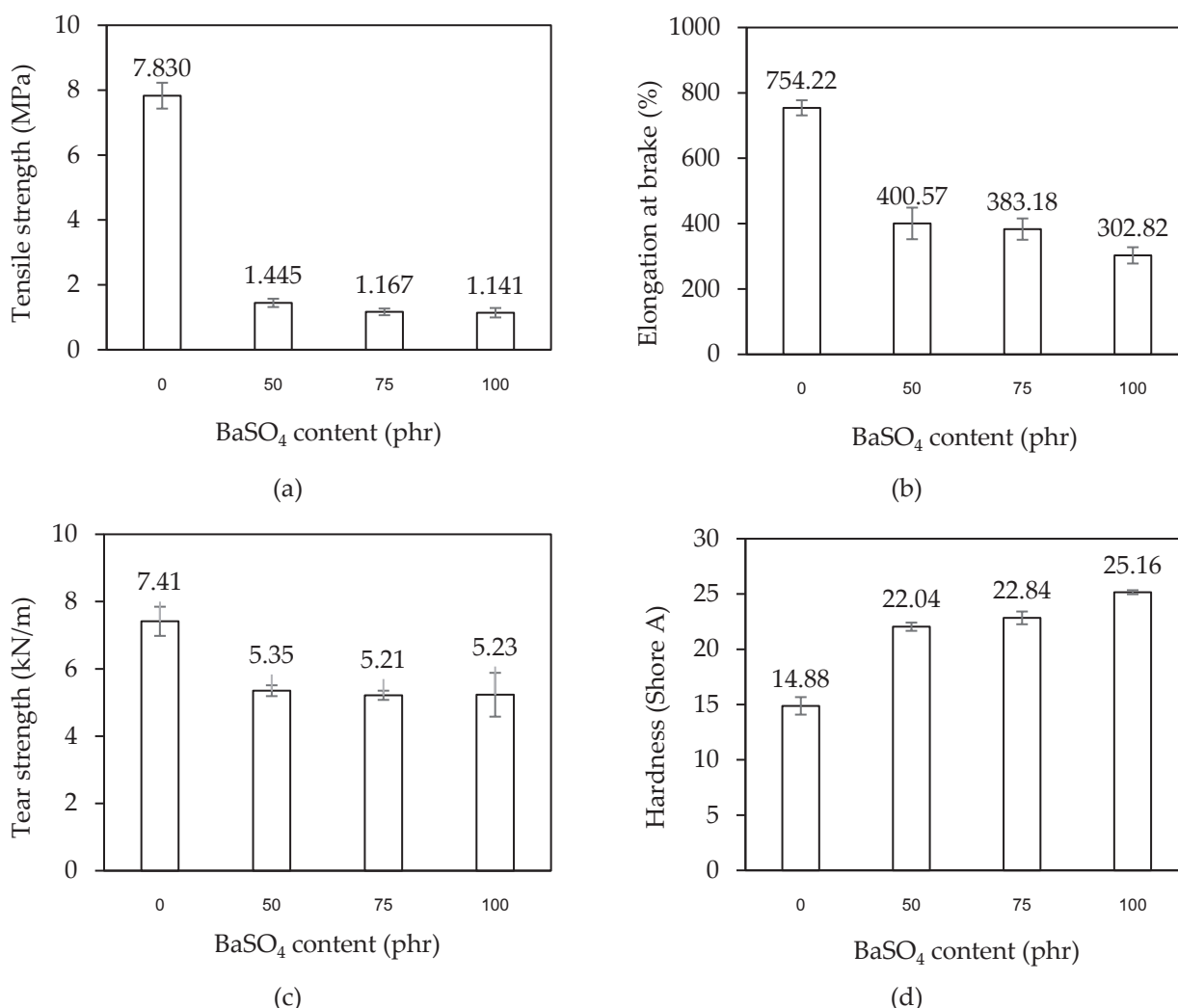


Figure 5. The mechanical properties of NR/BaSO₄ composites with varying contents of BaSO₄; (a) tensile strength, (b) elongation at break, (c) tear strength, and (d) hardness.

3.3 X-Ray shielding properties

Figure 6 shows the X-ray attenuation as a function of thickness for NR/BaSO₄ composites. It can be seen that the X-ray attenuation is higher compared to a pure NR. In addition, the X-ray attenuation increases with BaSO₄ content and thickness of samples due to the more remarkable ability of X-ray interaction and the impact of the photoelectric effect or Compton scattering with bismuth atoms in the material to attenuate and reduce X-ray energies and intensities [2]. For 2 cm thickness, the X-ray attenuation is highest at about 94% in 100 BaSO₄. From this result, it was possible to develop for actual use.

X-ray shielding properties of NR/BaSO₄ composites are shown in Table 2. The results showed that μ_l and μ_m increased, while HVL decreased with increasing BaSO₄ content. Causes may include the high Z number material (BaSO₄) [11], periodically distributed in the Na/BaSO₄ composites, which can act as an X-ray moderator resulting in μ_l improved HVL. At 100 phr of BaSO₄ content, the highest μ_l , μ_m , and the lowest HVL were 1.342 cm⁻¹, 1.923 cm²/g, and 0.517 cm, respectively. These results confirm that increasing the contents of BaSO₄ could improve the radiation shielding properties of NR/BaSO₄ composites.

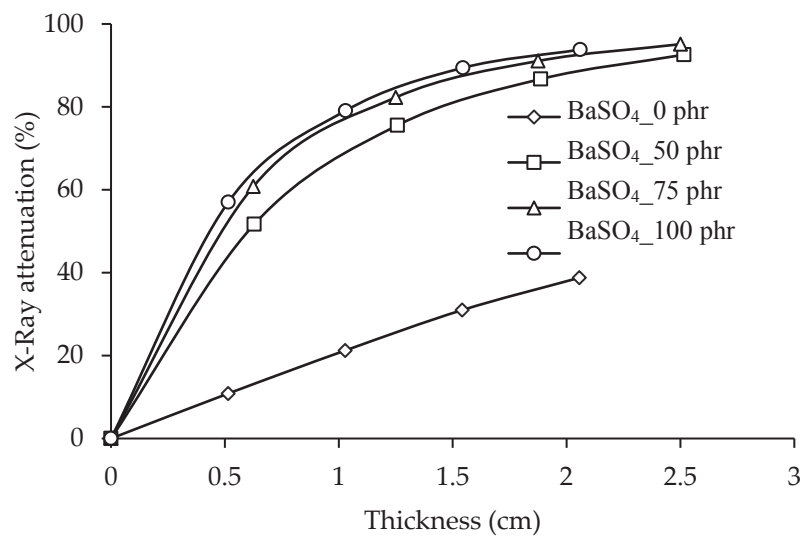


Figure 6. X-ray attenuation (%) of NR/BaSO₄ composites with varying contents of BaSO₄.

Table 2. The linear attenuation coefficient, mass attenuation coefficient and half-value layer of NR/BaSO₄ composites.

BaSO ₄ content (phr)	Linear attenuation coefficient; μ_l (cm ⁻¹)	Mass attenuation coefficient; μ_m (cm ² /g)	Half value layer; HVL (cm)
0	0.241	0.406	2.880
50	1.092	1.79	0.635
75	1.203	1.891	0.576
100	1.342	1.923	0.517

4. Conclusions

This work developed flexibility, lead-free, X-ray shielding materials based on NR/BaSO₄ composites with the contents varied BaSO₄ from 0 to 50, 75, and 100 phr. The results showed that BaSO₄ worked effectively as active fillers to shield X-rays. The NR/BaSO₄ composites had a higher linear attenuation coefficient (μ_l) and mass attenuation coefficient (μ_m) with increasing BaSO₄, while the HVL was decreased. Furthermore, it was found that the X-ray attenuation property improved with increasing BaSO₄ content and material thickness. The results also indicated that the increasing BaSO₄ increased the density and hardness of NR/BaSO₄ composites.

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Effects of Diet Supplemented with *Morinda citrifolia* Fermented Extract on the Growth Performance and Feed Conversion Ratio of *Oreochromis niloticus*

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Abstract: The use of additives in feeds to promote the growth of farmed fish has been rapidly expanding; among these are supplements derived from indigenous plants. Here, we analyzed the effects of satiation feeding of diet supplemented with noni (*Morinda citrifolia*) fermented extract on the feeding rate, growth performance and feed conversion ratio of Tilapia, *Oreochromis niloticus*. Fish with an initial average weight of 2.83 ± 0.01 g were fed with diets supplemented with noni fermented extract at 0 (control), 100-, 200- and 300-ml kg^{-1} for 12 weeks. Subsequent results showed that growth rate was correlated with or dependent on the concentration of noni fermented extract in the diet. The best result for growth performance, such as weight gain, average daily growth rate and specific growth rate, was found in a fish-fed diet supplemented with noni fermented extract at 200 ml kg^{-1} . However, the feed conversion ratio (FCR) is still higher than the control. The high FCR compared to control may be due to the anthraquinone in noni fermented extract, which decreases nutrient absorption. Fish fed with a 300 ml kg^{-1} noni fermented extract supplemented diet was the lowest growth performance and highest in FCR, suggesting that concentrations over 200 ml kg^{-1} might be detrimental to fish. Taken together, noni fermented extract can be a natural source of feed supplement to improve fish growth performance and may act as an appetite stimulant. However, due to its laxative effect, noni fermented extract supplementation should be given at appropriate levels and effective feeding management.

Keywords: *Morinda citrifolia*; *Oreochromis niloticus*; growth performance; satiate feeding

1. Introduction

Over the years, the aquaculture of tilapia (*Oreochromis niloticus*) has rapidly expanded to more than 130 countries worldwide [1]. Global production of tilapia farms is up 3.3% in 2020 and peaked at 6 million tons for the first time despite the impact of COVID-19. The global expansion of tilapia is because tilapia is a fish that can be grown in a diverse aquatic environment and can potentially replace marine fish products [2]. Currently, there are various methods used to increase the productivity of tilapia farming. Such approaches include using additives in feed formulas such as antimicrobials, antioxidants and enzymes to improve feed quality [3] and or supplementing with probiotics, prebiotics, immunostimulants and phytobiotics to increase the growth rate and health of tilapia [3-5].

Noni (*Morinda citrifolia*), a fruit-bearing tree native to Southeast Asia, is reported to exhibit phytobiotic properties, meaning compounds derived from plants that, when added to feed, affect the growth of animals [6]. Heinicke [7] and Widjastuti et al. [8] revealed that noni contains the precursor xeronine, which is important for metabolic processes that influence growth. Furthermore, earlier studies on noni suggest that the plant has antimicrobial properties [9, 10], antioxidant [11] and an appetite stimulant [12]. Based on the properties mentioned above, experimental application of noni as a component of feed formula for raising animals was reported. Examples include using noni, in fresh fruit form, for raising cattle [13] and noni fruit extract for raising red tilapia [14]. Their results suggest that, by feeding according to the percentage of body weight, noni in both the fresh fruit form and fruit extracts increased the growth rate of experimental animals and did not affect the efficiency of food use. However, in practice, most farmed tilapia are fed several times a day depending on the size and age of the cultured fish and each time, the fish are fed until they are full or until satiation. Therefore, this study's objective was to determine the effects of a fish diet supplemented with noni fruit extract on tilapia's feeding rate, growth, and feed conversion ratio. The supplemented diets were fed until satiation to simulate farm rearing conditions.

2. Materials and Methods

2.1 Experimental design

The experiment was designed completely randomized with four treatments and four replications. Noni fermented extract was supplemented into the diets at 0, 100, 200 and 300 ml kg⁻¹ feed. Nile tilapia was purchased from the Aquaculture Genetics Research and Development Center, Department of Fisheries, Chumphon Province, Thailand. A total of 800 Nile tilapia with an average weight of 2.83 ± 0.01 g and an average length of 5.27 ± 0.03 cm were used in this study. Fish were reared in hapa nets or hapas 1.5 m x 1.5 m x 1.5 m in size; each hapa holds 50 fish, and 16 hapas were used in total. The hapas were placed in an earthen pond 25 x 40 x 2.5 m in size. The tilapia was trained to feed on sinker pellet feed using a control diet for 7 days before the start of the experiment.

2.2 Preparation of Noni fruit extract and experimental diet

Noni fruit was cleaned with tap water and dried. After that, the fruit was cut into pieces about 1 cm thick. Three kilograms of noni fruit were mixed with 1 kg of sugar and kept in containers for 3 months. The extract was collected by squeezing the fermented fruit and filtering to separate the extract from the waste. The filtered extract was mixed with other ingredients to make the experimental diets following the methods described earlier with some modifications [15]. The contents of each diet are shown in Table 1. The ingredients of each diet were equivalently mixed using a Hobart mincer (model HL200) for 15 min and pelleted to make a pelleted diet of 1.5 mm in size. All diets were dried in a hot air oven at 60 °C for 12 hr. The experimental diets were stored in plastic bags and refrigerated at 4 °C until use.

2.3 pH measure of experimental diet

Experimental diets were ground with a medicinal mortar. One gram of the experimental diet was mixed thoroughly with 20 ml of distilled water. The pH of experimental diets was measured using a pH meter (model C835)

Table 1. Ingredients of experimental diets

Ingredient	Noni fruit extract in diets (ml)			
	0	100	200	300
Fish meal	220.00	220.00	220.00	220.00
Soybean meal	90.00	90.00	90.00	90.00
Broken rice	330.00	330.00	330.00	330.00
Rice bran	350.00	350.00	350.00	350.00
Vitamins and minerals mix ¹	10.00	10.00	10.00	10.00
Total (g)	1000.00	1000.00	1000.00	1000.00
Noni (ml)	0.00	100.00	200.00	300.00
Water (ml)	300.00	200.00	100.00	0.00
Proximate composition				
Moisture (%)	9.98	9.98	9.98	9.98
Crude protein (%)	25.03	25.03	25.03	25.03
Crude lipid (%)	6.19	6.19	6.19	6.19
Crude fiber (%)	4.66	4.66	4.66	4.66
Ash (%)	9.16	9.16	9.16	9.16
Gross energy (Kcal/100g)	387.30	387.30	387.30	387.30
pH of Experimental feed	5.66 ± 0.02	5.60 ± 0.01	5.16 ± 0.01	5.06 ± 0.03
pH of Noni fermented extract	3.08 ± 0.00			

¹ Vitamins and minerals mix/kg: vitamin A 1.20 MIU; vitamin D3 0.24 MIU; vitamin E 8,000 IU; vitamin B1 2.00 g; vitamin B2 2.00 g; vitamin B6 2.00 g; vitamin B12 0.001 g; vitamin C 22.00 g; pantothenic acid 5.00 g; nicotinic acid 5.00 g; folic acid 0.20 g; biotin 0.002 g; choline 2.00 g; iron (Fe) 2.00 g; copper (Cu) 2.00 g; zinc (Zn) 5.00 g; calcium 5.00 g; manganese (Mn) 0.8 g; selenium (Se) 0.01 g; lysine 8.50 g and methionine 3.00 g

2.4 Feeding management

Tilapia were fed with experimental diets twice daily, at 7:00 a.m. and 4:00 p.m., by satiation feeding for 12 weeks. The weight of the experimental diet of each hapa was recorded before feeding. The experimental diets were half sinking baskets, half floating in the water. The experimental diets were given to fish little by little. When the fish start to feel full, the fish will not come up to eat at the basket. The weight of the experimental diet for each hapa was re-recorded after feeding. Water quality, including temperature, dissolved oxygen, pH, ammonia and nitrites, was checked every 2 weeks. Water exchange (30 %) was done every 15 days.

2.5 Data collection and analysis

The weight, length, feed intake, and survival rate were noted at the end of the experiment. All the data collected were used to calculate growth efficiency, including weight gain, average daily gain, increase in length, specific growth rate, feed conversion ratio, feed intake and survival rate. The statistical results were analyzed using the obtained values to analyze variance (ANOVA) and comparing the mean difference in each experiment by Duncan's new multiple range test with a statistical program at 95% confidence level.

3. Results and Discussion

Feeding Nile tilapia with diet supplemented with noni fermented extract at 0 (control), 100, 200 and 300 ml kg⁻¹ by satiation feeding for 12 weeks showed that fish fed diet with supplemented noni fermented extract at 200 ml kg⁻¹ had the best growth performance in terms of final weight (34.31 ± 0.52 g), weight gain (31.49 ± 0.52 g), average daily gain (0.37 ± 0.01 g/fish/day), final length (12.38 ± 0.10 cm), length gain (7.13 ± 0.05 cm) and specific growth rate (2.97 ± 0.02 %/day). These parameters were significantly different ($P < 0.05$) from a fish-fed supplemented with noni fermented extract at 0 (control), 100 and 300 ml kg⁻¹. For the survival rate, fish-fed supplemented with noni fermented extract at all concentrations, including the control, had a survival rate of more than 90% (Table 2). The results indicated that noni fermented extract supplementation could enhance the growth of Nile tilapia. This is consistent with the results conducted earlier in cattle [13], in which they were fed 0, 0.09% and 0.18% noni supplementation in the diets. The cattle were fed diets at 2.5 % of their initial body weight for 28 days. Noni supplementation improved the average daily gain weight of the cattle as the percentage of noni increased in the diet. Moh et al. [16] reported that noni fruit extract supplementation could improve the growth of whiteleg shrimp in terms of body weight, total length, average daily growth and feed conversion ratio. They mentioned that their study's improvement of whiteleg shrimp's growth performance might be associated with the improved digestive enzymes induced by noni fruit extract. Their result showed increasing activities of amylase in whiteleg shrimp with increasing concentration of noni fruit extract. This affects the efficiency of using carbohydrates for growth. The fermentation of noni fruit for 3 months resulted in a noni fermented extract with a pH of 3.08 caused by the microbial product. The pH of experimental diets were also measured, as shown in Table 1. The pH of experimental diets decreased following increased noni fermented extract. The noni fermented extract, therefore, will make the environment in the digestive tract of fish slightly more acidic, which is suitable for the functioning of the enzyme pepsin and resulting in the efficiency of protein digestion and better absorption [17]. Our result showed that a fish-fed diet supplemented with noni fermented extract at 300 ml kg⁻¹, which has the lowest pH, fared the worst in terms of growth performance. This can be attributed to alcohol compounds in the fermented fruit, which might be toxic to the fish. In addition, the fermentation of noni has been shown to promote the survival of probiotic strains such as *Gluconobacter*, *Acetobacter*, *Lactobacillus*, and *Bifidobacterium* spp. [18, 19]. It is well known that probiotics could improve digestion by reducing the growth of harmful bacteria in the intestines so that good bacteria in the intestine increases. Moreover, the increased growth may be due to the proxeronine in noni that is converted to xeronine by the proxeroninase enzyme in the body. Xeronine is important for biochemical processes in the body, including protein synthesis, glucose absorption and other metabolic processes that affect growth [8, 20, 21].

The efficacy of noni fermented extract, in addition to enhancing growth, is that it can also stimulate appetite [12, 22]. Here, we employed feeding until satiation to simulate the current and most popular feeding practice for farmed tilapia. Our subsequent results showed that noni fermented extract could stimulate fish's appetite. The feeding rate of fish increased with the concentration of noni fermented extract supplemented in the diet. The feed conversion ratio analysis revealed that fish fed with a diet supplemented with noni fermented extract at 100 ml kg⁻¹ did not differ significantly from the control group ($P > 0.05$). Still, those fed with noni fermented extract at 200 and 300 ml kg⁻¹ had feed conversion ratios significantly higher ($P < 0.05$) than the control (Table 2). The results indicated that the amount of noni fermented extract in the diet affected the feed utilization of fish. It is to be noted that If the fish receives an excessive amount of noni fermented extract, the feed utilization of the fish will decrease. The decrease in feed utilization may be due to the anthraquinone found in noni [23]. Van Gorkom et al. [24] and Mueller et al. [25] reported that anthraquinone has laxative properties by causing damage to the intestinal lining cells, which may result in a reduction in the efficiency of nutrient absorption, enzyme secretion and intestine movements. In addition, excessive exposure to noni fruit extract carries a risk of hepatotoxicity. The liver is a vital organ involved in the digestive process. Shalan et al. [26] evaluated chronic toxicity of noni fruit in mice. The mice drank water mixed with noni fruit extract at 1 and 2 mg ml⁻¹ for 6 months. The result showed that the mice who drank water mixed with noni fruit extract at 2 mg ml⁻¹ had symptoms of hepatotoxicity with the destruction of liver cells and a 40% mortality rate after just 3 months of drinking water mixed with noni fruit extract. Moreover, Millonig et al. [27] and Stadlbauer et al. [28] also reported that noni fruit extract could induce hepatitis symptoms in humans.

The results of this experiment showed that noni fermented extract could stimulate appetite and increase growth as well as can be a laxative. Therefore, the use of noni fermented extract supplemented diets to enhance the development of fish must be applied at an appropriate rate as too high concentrations of noni fermented extract in the diet may negatively affect fish growth. This was evident in fish fed with a diet supplemented with noni fermented extract at 300 ml kg⁻¹. This experimental group has the worst feed conversion ratio, which is significantly different ($P < 0.05$) compared to other experimental diets. Our results indicate that satiation feeding with noni fermented extract supplemented diets should not be done as this will increase the level of noni fermented extract and should be given based on the percentage of fish body weight. The experiment of Kristiana *et al.* [14] investigated the effect of noni fruit extract supplementation on growth rates in red tilapia. The fish were fed a diet supplemented with noni fruit extract at 0, 100, 300 and 500 ml kg⁻¹ of feed and provided at a rate of 3% of the body weight for 30 days. Noni fruit extract at 500 ml kg⁻¹ of the meal was most effective in terms of specific growth rate and fish feed conversion ratio, which were the best and significantly different compared to the control ($P < 0.05$). The growth of tilapia in this study was lower than the normal growth rate of tilapia in commercial farms and lower than data reports in other publications, such as in Figueiredo-Silva *et al.* [29], wherein red tilapia cultured in the laboratory increased their body weight from 1.7 g to 67-83 g within 10 weeks. In this study, fish grew from 2 g to 34 g in 12 weeks. The growth performance of aquatic animals depends on several factors such as proximate diet composition, especially the percentage of protein, number of feeding per day, rate of feeding, etc. In our experiment, fish were fed diets containing 25% crude protein and 6% crude lipid with providing twice a day. Still, in the investigation of Figueiredo-Silva *et al.* [29], fish were fed with diets containing 35% crude protein and 12% crude lipid and fed three times a day, which suggests that the percentage of crude protein and crude lipid, as well as feeding frequency, were higher than in our study. In typical commercial farms, on the other hand, fish are fed with commercial diets containing at least 18% protein, but the feeding frequency is at least three times a day. These factors contributed to why growth performance was different in those settings.

The average water quality during the experiment was within the range suitable for aquaculture as specified by Boyd and Lichkoppler [30]. Specifically, the dissolved oxygen was at 5.60 - 5.90 mg l⁻¹; pH 7.20 - 7.70; water temperature 26.60 - 28.70 °C; ammonia 0.13 - 0.28 mg l⁻¹; and nitrites 0.01 - 0.03 mg l⁻¹.

Table 2. Growth performance of Nile tilapia fed with a diet supplemented with noni fermented extract at different concentrations for 12 weeks. Feed was given until satiation twice a day.

Growth performance	Noni fermented extract in diets (ml kg ⁻¹)				p-values
	0	100	200	300	
Initial weight (g/fish)	2.82 ± 0.02	2.84 ± 0.04	2.83 ± 0.02	2.83 ± 0.03	0.9041
Final weight (g/fish)	30.51 ± 0.94 ^b	30.64 ± 2.04 ^b	34.31 ± 0.52 ^a	25.79 ± 1.72 ^c	0.0001
Weight gain (g/fish)	27.69 ± 0.95 ^b	27.80 ± 2.05 ^b	31.49 ± 0.52 ^a	22.95 ± 1.72 ^c	0.0001
ADG (g/fish/day) ²	0.33 ± 0.01 ^b	0.33 ± 0.02 ^b	0.37 ± 0.01 ^a	0.27 ± 0.02 ^c	0.0001
Initial length (cm)	5.30 ± 0.08	5.27 ± 0.06	5.27 ± 0.05	5.26 ± 0.02	0.9383
Final length (cm)	11.83 ± 0.26 ^b	11.85 ± 0.37 ^b	12.38 ± 0.10 ^a	11.38 ± 0.22 ^c	0.0013
Length gain (cm)	6.55 ± 0.31 ^b	6.58 ± 0.41 ^b	7.13 ± 0.05 ^a	6.13 ± 0.19 ^c	0.0023
SGR (%/day) ³	2.83 ± 0.04 ^b	2.83 ± 0.09 ^b	2.97 ± 0.02 ^a	2.63 ± 0.08 ^c	0.0001
Feed conversion ratio	1.76 ± 0.13 ^c	1.90 ± 0.19 ^c	2.50 ± 0.04 ^b	3.78 ± 0.17 ^a	0.0001
Feed intake (g/fish)	48.75 ± 2.97 ^c	52.63 ± 4.75 ^c	78.61 ± 1.55 ^b	86.66 ± 6.02 ^a	0.0001
Survival rate (%)	91.00 ± 2.00 ^b	95.50 ± 3.00 ^a	93.50 ± 2.52 ^{ab}	97.50 ± 3.00 ^a	0.0269

¹ Different letter in the same row indicates a statistically significant difference at $P < 0.05$.

² ADG = Average daily gain

³ SGR = Specific growth rate

4. Conclusions

The effect of diet supplementation with noni fermented extract in tilapia is concentration-dependent. Specifically, our results revealed that fish fed with a diet supplemented with noni fermented extract at 200 ml kg⁻¹ by satiation feeding for 12 weeks showed the best growth rates. However, the feed conversion ratio was still higher than the control. The high feed conversion ratio may be attributed to the anthraquinone in noni fermented extract, which has a laxative effect and results in feed being pushed along faster in the digestive tract. This would then cause the efficiency of nutrient absorption to decrease. Our results revealed that feeding until satiation with diets supplemented with noni extract is not suitable. Therefore, to increase the efficiency of noni extract supplementation on tilapia growth rates, the amount of noni extract that the fish should receive must be controlled appropriately. Taken together, we have shown that noni fermented extract can be a potential natural growth enhancer for fish when given at appropriate levels coupled with sound feeding management. Further studies on the most suitable feeding program using noni fermented extract are currently being undertaken.

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