COMPARISON OF FERMENTED BAMBOO SHOOTS FROM PRACHIN BURI PROVINCE PREPARED BY USING TRADITIONAL METHOD AND COCONUT WATER FERMENTATION

YAOWALAK CHANGKIEW

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Name-Surname	Ms. Yaowalak Changkiew
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ABSTRACT

This study aimed to investigate effects of mature coconut water on characteristics of fermented bamboo shoots during fermentation and storage as well as effects of safflower oil emulsions on quality of the shoots during storage.

Fermented bamboo shoots with coconut water (FBSC) were prepared by mixing sliced bamboo shoots with salts and fermented with mature coconut water at room temperature for 36 h. The bamboo shoots fermented by the traditional method (TFBS) were used as a control sample. FBSC and TFBS were then packed in polypropylene (PP) or polyethylene terephthalate (PET) bags and stored at 4 °C, room temperature, and 35 °C for 60 days. In addition, effects of safflower oil emulsions as soaking solutions on FBSC quality were determined during storage in an accelerated condition. During fermentation and storage, FBSC and TFBS were determined for number of lactic acid bacteria, pH, and total acidity, while colors, pH, total acidity, and total microorganisms, antioxidant activity were evaluated during storage.

The results showed that during fermentation, FBSC had significantly greater total number of lactic acid bacteria, pH, and total acidity than that of TFBS. PET bags could significantly prevent FBSC and TFBS from browning reaction better than PP bags. The pH and total acidity of the samples were stable after storage for 10 days. Less than 30 colonies of bacteria were found in all samples. Safflower oil emulsions could increase phenolic acid and antioxidant activity of the shoots. This study indicated that the mature coconut water could be used to produce fermented bamboo shoots.

Keywords: fermented bamboo shoots, shelf life, coconut water, safflower oil, packaging

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Yaowalak Changkiew

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List of Abbreviations

C	Citric Acid
CWD	Coconut Water Drink
FBS	Fermented Bamboo Shoot
FBSC	Fermented Bamboo Shoot with Mature Coconut Water
MCW	Mature Coconut Water
PET	Polyethylene Terephthalate
PP	Polypropylene
PPO	Polyphenol Oxidase
S	Sodium Chloride
SC	Sodium Chloride in Citric Acid
SO	Safflower Oil
SW	Salt water
TFBS	Traditional Fermented Bamboo Shoot

CHAPTER 1
INTRODUCTION

1.1 Background and Statement of the Problems

Bamboos belong to the grass family Poaceae with 75 genera and have over 1,250 species worldwide. They encompass mainly the tropical, subtropical, and mild temperature zones of the world. Most of them are found in Asia such as China, Japan, India, Malaysia, and Thailand [1] [2]. In Thailand, more than 60 species have been found. They belong to 12 genera, while the main ones are *Thyrsostachys siamensis*, *Bambusa polymorpha*, *Bambusa mana*, *Bambusa tulda*, *Bambusa arundinacea*, *Dendrocalamus hamiltonii*, *Dendrocalamus giganteus*, and *Dendrocalamus asper* [3] [4]. *Dendrocalamus asper* are called in Thai as Phai Tong. They are one of the important bamboo specie, mainly found in Prachinburi Province. Phai Tong are commonly used for their edible shoots, which are sweet and have unique taste. Thai people like to consume the bamboo shoots because of not only good taste and flavour but also high nutritional values. The bamboo shoots contain high protein (3.59g/100g), carbohydrate (4.90 g/100g), and fiber (3.54 g/100g) but low fat (0.40 g/100g). Moreover, they also have a good profile of minerals, consisting potassium (K), calcium (Ca), manganese (Mn), zinc (Zn), chromium (Cr), copper (Cu), iron (Fe), plus lower amounts of phosphorus (P), and selenium. Antioxidant substances could be extracted from the shoots, which are mainly phenols, polyphenols, flavonoids, vitamin C, vitamin E, beta-carotene. These are known to have the potential to reduce disease risk. There were many studies reported that the shoots could reduce constipation, help digestion, and potentially lessen cardiovascular diseases (CVDs) and cancers [5] [6]. However, bamboo shoots are very perishable and have a limited shelf life. They are therefore generally boiled, dried, or fermented [7]. Fermentation of the bamboo shoots is the most popular method to preserve the shoots in Prachinburi province. It is because fermentation is simple, less labor, and low cost. The fermented bamboo shoots could be simply prepared by peeling the shoots and then slicing into small thin pieces. The thin pieces are then washed and mixed with salt and kept in closed containers for at least 30 days before being ready to eat. The fermentation time could be shortened by using mature coconut water, a waste product from the coconut milk

industry, containing enough nutrients and essential compounds to grow lactic acid bacteria [8] [9]. Several reports mentioned about the negative feedbacks of fermented bamboo shoots. Due to the long period of fermentation, the fermented shoots could be contaminated with undesirable microorganisms such as *Escherichia coli*, *Staphylococcus aureus* during fermentation and storage. Pasteurization or sterilization are required to ensure the safety [8] [9]. Besides, browning is usually occurred in naturally fermented bamboo shoots during storage, which is unacceptable from consumers. This results in price reducing. The whiter the fermented bamboo shoots are, the higher prices are required. Therefore, this study aims to develop biological and physical quality of fermented bamboo shoots by using coconut water fermentation and vacuum packaging techniques.

1.2 Purpose of the Study

The overall goal of this thesis is to develop quality of local fermented bamboo shoots. The studies were separated into three parts. The objectives of each part were as following:

1.2.1 To develop fermentation process of bamboo shoots by using mature coconut water

1.2.2 To evaluate effects of storage conditions on the fermented bamboo shoot's shelf life

1.2.3 To determine ability of safflower oil on inhibition of browning reaction in fermented bamboo shoots during storage

1.3 Theoretical Perspective

In this thesis, we have the scopes and the limitation of studying which are concerned to the pickled bamboo shoots and using coconut water fermentation research.

1.4 Delimitations and Limitations of the Study

For this thesis, we have the scopes and the limitations of studying which are concerned to the previous works which are:

1.4.1 To extend the concept develop the fermentation process of bamboo shoots by using mature coconut water

1.4.2 To extend the concept of storage conditions on the fermented bamboo shoot's shelf life

1.4.3 To extend the concept of effects of safflower oil in inhibition of browning reaction in fermented bamboo shoots during storage.

1.5 Significance of the study

The advantage of education and studying in this research could improve and develop the concepts and knowledge in applied biology and food sciences.

CHAPTER 2
LITERATURE REVIEWS

2.1 Bamboo shoots

Bamboos are giant perennial arborescent grass. They belong to the family of *Poaceae* and subfamily *Bambuseae*. Bamboos originate from China and widely distribute across continents, especially in tropical, subtropical and temperate regions with a mostly mesic to wet season. Up to date, more than 1642 species in 75 genera of bamboo have been evidenced to be distributed throughout the world, in which over 600 species were grown in China, 300 species were found in India, while Japan, Philippines, and Thailand had 237, 55, and 50 species, respectively [14] [10]. Bamboos mostly found in Thailand belong to the genus of *Dendrocalamus*. *Dendrocalamus asper* are one of the well-known bamboo species, which are known as Rough Giant Bamboo. It is a giant tropical and subtropical dense clumping specie native to Southeast Asia [2][11] such as China, Thailand, Vietnam, Malaysia, Indonesia and Philippines.[12]. *D. asper* have large woody culms between 15-20 m and 8-12 cm in diameter. The lower culms show aerial roots (rootlets) from the nodes. Culm internodes are 30-50 cm, pale green and covered with short brown hairs. Leaf sheaths initially sparsely hairy, becoming glabrous; ligule truncate, ca. 2 mm. Timbers of the bamboo are generally used as a building material for heavy construction, and shoots are consumed as a vegetable. The fresh bamboo shoots own crisp and crunchy texture as well as sweet taste, imparting a unique flavor [10] . Thai people like to consume the bamboo shoots because of not only good taste and flavour but also high nutritional values. Figure 2.1 demonstrated traditional production of bamboo shoot products.

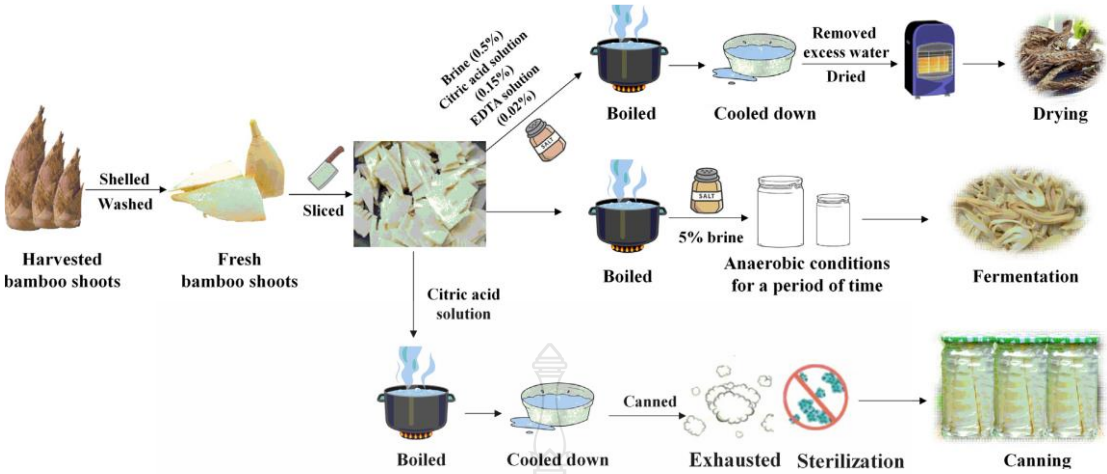


Figure 2.1 Processing workflows of bamboo shoot products via boiling, drying and fermentation

SOURCE: [13]

2.1.1 Nutritional value of bamboo shoots

The bamboo shoots contain high protein, carbohydrates, and fiber but low fat. They have a good profile of minerals, consisting potassium (K), calcium (Ca), manganese (Mn), zinc (Zn), chromium (Cr), copper (Cu), iron (Fe), plus lower amounts of phosphorus (P), and selenium (Se) (Table 2.1). Additionally, they are a rich source of natural antioxidants such as phenols, polyphenols, vitamin C, vitamin E, beta-carotene, flavonoids. Protocatechuic acid, p-Hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-Coumaric acid, and ferulic acid were phenolic compounds that could be extracted from the shoots [2] [6]. These compounds are known to have the potential to reduce disease risk. There were many studies reported that the shoots could reduce constipation, help digestion, and potentially lessen cardiovascular diseases (CVDs) and cancers [5] [6]. There are research reports of bamboo shoots that contain generally tyrosine, arginine, histidine, and leucine as amino acids. The presence of tyrosine facilitates biochemical metabolism of our body as it is a major constituent of adrenals which are precursors for adrenaline, necessary for active body metabolic activities [14].

Table 2.1 Nutrient content of freshly harvested shoots of *Dendrocalamus asper*

Nutrients	*D. asper*
Amino acids (g/100 g)	3.12
Protein (g/100 g)	3.59
Carbohydrate (g/100 g)	4.90
Fats (g/100 g)	0.40
Fibres (g/100 g)	3.54
Vitamin C (mg/100 g)	3.20
Vitamin E (mg/100 g)	0.91
Calcium (mg/100 g)	5.51
Phosphorus (mg/100 g)	40.95
Iron (mg/100 g)	3.37
Sodium (mg/100 g)	10.14
Potassium (mg/100 g)	46.4
Magnesium (mg/100 g)	10.14

SOURCE: Modified from [5] [6]

Although bamboo shoots offer nutritional value, there are many reports mentioned that fresh bamboo shoots contain potentially toxic compounds called cyanogenic glycosides, linamarin and taxiphillin, which break down upon disruption of the plant cells to form hydrogen cyanide (HCN). HCN is harmful to aerobic organisms. It inhibits electron transport, mitochondrial oxygen utilization and cellular respiration, adversely insulting functionality of heart, brain and liver and causing respiratory failure [15]. FAO (1991) [16] [17] [18] suggested that the acute lethal dose of cyanide for human is 0.5–3.5 mg/kg of body weight and the permissible limit of cyanogen content in food is 500 mg/kg. However, shoots of some bamboo species have cyanogen levels much higher than the permissible limit. It could be as high as 1951.49 mg/kg, depended on bamboo species as shown in table 2.1 [12]. According to its severe toxicity, cyanide in bamboo shoots must be detected at low concentrations prior to consumption. Therefore, appropriate processing methods to reduce or remove cyanogen toxicity in the shoots are needed

Table 2.2 Total cyanogenic glycoside content in fresh shoots of *Dendrocalamus asper*.

Species	Cyanogen content (mg/kg fresh weight)
Chimonobambusa callosa	31.68 ± 2.12
D. asper	766.66 ± 8.12
Dendrocalamus calostachys	636.77 ± 6.10
Dendrocalamus flagellifer	1893.67 ± 22.16
Dendrocalamus giganteus	988.17 ± 18.21
Dendrocalamus hamiltonii	733.92 ± 9.41
Dendrocalamus hookerii	1315.78 ± 8.40
Dendrocalamus longispathus	1951.49 ± 28.20
Dendrocalamus manipureanus	1347.98 ± 21.10
Dendrocalamus membranaceus	514.80 ± 3.15
Dendrocalamus sikkimensis	778.27 ± 6.17
Phyllostachys mannii	36.32 ± 2.18

SOURCE: [19]

Reduction in cyanogen level can be achieved by several processing methods such as slicing, peeling, soaking, cooking such as drying, boiling, soaking, and fermentation [16] [17] [7]. Moreover, those methods not only remove cyanogenic glycosides but also reduce acidity and bitterness from fresh bamboo shoots. Drying is effective in reducing water activity, inactivating enzymes and inhibiting microbial growth and thus could be preserve fermented bamboo shoots. Usually, the freshly-harvested bamboo shoots have a moisture content over 90 g/100 g. As completely dried, the moisture content of bamboo shoots decreased to an extent lower than 10 g/ 100 g. In drying of bamboo shoots, solar drying, hot air drying, microwave drying and combined drying were applied [18]. Drying of bamboo shoots by using high drying temperature reduced cyanide residue of fresh bamboo shoots. Fresh bamboo shoots were dried in a heated-air tray dryer at 40, 50, and 60 °C until the moisture contents of the shoot reached 16%. The resulted showed that cyanide residue in the fresh shoots were decreased with increased temperatures. After

drying the bamboo shoots at 60 °C, cyanide residue was reduced from 636.95 mg/kg to 6.60 mg/kg [20]. Besides, high drying techniques were also applied to the shoots to remove cyanide residue. Freeze and superheated steam was employed for the reduction of cyanogen in bamboo shoots. It was mentioned that around 80% of cyanogen glycoside was reduced after vacuum freeze drying for 24 h at –50 ºC. Moreover, superheated steam drying at 120-160 ºC could decompose the taxiphyllin which causes bitterness in the shoots [16]. Drying is also effective in reducing water activity, inactivating enzymes and inhibiting microbial growth [18].

Cooking and boiling greatly reduces the antinutrients from the vegetables and fruits. During boiling or cooking, cell walls rupture which permit leakage of cell content including antinutrients and toxic substances [19]. There was research reported that boiling bamboo shoot in an open vessel for three to four hours can reduce the toxicity through the nonenzymatic hydrolysis of taxiphyllin. Bamboo shoots cooked at 98–102 ˚C for 148–180 minutes showed the maximum removal of HCN (97% removal) and left a residue level of about 27 mg/kg HCN (1000 mg/kg in fresh shoots) [21] Similarly, 91% reduction of cyanide content was observed following slicing and cooking bamboo shoot in boiling water for 15 minutes. The content decreased from 40 mg/kg to 3.7 mg/kg when boiled for 15 minutes and it further reduced to 1.9 mg/kg when boiled for half an hour. The content was not detected in shoots boiled for 60 minutes. Cyanide levels for canned and packaged bamboo shoots samples ranged from non-detected to 5.3 mg/kg [21].

Soaking is a simple traditional practice which is followed in the processing of shoots for food in almost all the species of bamboo. Soaking of shoots is quite effective in eliminating cyanogens particularly in those species which have low contents. The soaking of shoots can be for few hours as in case of *Chimonobambusa callosa* and *Phyllostachys mannii* which have very low cyanogen content in fresh shoots to long term treatment in closed containers or in running water in rivers and streams in those species which have very high content of cyanogen in the fresh shoots [19]. The decrease in cyanogen also depends on some factors like temperature, time and soaking medium in which the material is soaked. Overnight soaking of bamboo shoot slices results in enzymatic hydrolysis of taxiphyllin by ß-glucosidase to glucose and 4- hydroxyl

mandelonitrile, which is further hydrolyzed to HCN and benzaldehyde by the activity of hydroxynitrilelyase enzyme [19].

2.2 Fermented bamboo shoots

Fermentation of bamboo shoots is one of the traditional methods to preserve the shoots in many countries, especially during offseason, as it is simple, less labor, and low cost. The processing methods and flavors of the fermented bamboo shoots of each country are different [13]. There were more than ten fermented products from bamboo shoots that were historically recorded in many countries as shown in Table 2.3 [13].

Table 2.3 Fermented products from bamboo shoots around the world

Regions	Fermented product
China	Ulanzi
Indonesia	Gulei rebung, Sayur ladeh lun-pia
India	Amil, Edung, Eeku, Eepe, Ekung, Eup Hendua, Godhak, Hiring, Hikhu, Hithyi, Ikung, Iromba, Khorisa-tenga, Kardi, Khorisa, Kupe, Lung-siej, Lungseij, Midukey, Rawtuai rep, Soibum, Soidon, Soijim, Syrwa, Tama, Ushoi
Meghalaya	Iromba
Philippines	Dinengdeng na Labong, Ginataang Labong, Labong,
Thailand	Naw-mai-dong, Nor-mai-dorng

SOURCE: [13]

In Thailand, the bamboo shoots could be traditionally fermented by these following steps. The shoots are peeled and sliced into small thin pieces. The thin pieces are then washed and mixed with salt and kept in closed containers. The fermentation is then occurred spontaneously by mostly lactic acid bacteria. The lactic acid fermentation converts fresh bamboo shoots to tasteful pickles. The fermentation could take at least 3 months before being ready to eat. The fermented bamboo shoots are often used as ingredients in many Thai dishes such as gang gai naw mai dong (sour bamboo curry),

sup nor-mai, stri-fried bamboo shoot with egg and kaeng nor mai ga tee. In order to produce fermented bamboo shoots, there are several factors that needed to be considered and controlled such as oxygen access, sodium chloride content, and proper acidity. They result in the quality of the final fermented bamboo shoot products and customer satisfactions. Moreover, there are several reports mentioned about the negative feedbacks of traditionally fermented bamboo shoots.

2.2.1 Lactic acid fermentation

Lactic acid bacteria (LAB) are a group of organisms that ferment sugar (i.e., glucose) predominantly to lactic acid. They are gram-positive, non-sporulating rods and cocci having low guanine-cytosine content. As mentioned, LAB convert the carbohydrate contents of the vegetables and fruits into lactic acid, which decreases the pH of the fermented products to around 4.0 ensuring stability. Lower pH value restricts the growth of spoilage flora and pathogenic bacteria [22]. Most of the LAB grow an-aerobically but they are also aero-tolerant. This group of bacteria is divided into two sub-groups including homo- fermentative and hetero-fermentative species. homo-fermentative bacteria produces a single fermentation product, i.e., lactic acid *via* the glycolytic pathway [23]. Members of the genera are *Pediococcus*, *Streptococcus* and *Lactococcus*. The fermentation of one mole of glucose yields two moles of lactic acid. For hetero-fermentative bacteria, they produce LA plus appreciable amount of ethanol, acetate and CO_2 via the phosphogluconate/phosphoketose pathway [23]. Bacteria involved in this group belong to genera *Leuconostoc* and *Lactobacillus*. In traditional fermentation of vegetable and fruits, LAB typically occur spontaneously [25]. The main species often found from raw or spontaneously fermented vegetables and fruits were *Leuconostoc*, *Lactobacillus*, *Weissella*, *Enterococcus*, *Pediococcus* and, especially, *Lactobacillus plantarum.* In fermented bamboo shoots, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus Corniformis*, *Lactobacillus fermentum*, *Lactococcus lactis*, *Lactobacillus mesenteroides*, *Enterococcus durans*, *Streptococcus lactis*, and *Lactobacillus casei* were usually found [24]. Not only is fermentation a process to preserve food, it also adds to the nutrient value, enhances flavor, and improves pharmacological values. The bacteria could posse functional probiotic properties as well as B-vitamin supplier to human body [25] [26]. Fermented bamboo shoot forms a rich ecological niche which harbours a plethora

of microorganisms [27]. The unique microflora in fermented food could increase the protein, vitamin, and fatty acid levels [9].

2.2.2 Factors affecting fermentation

There are several factors that influence the growth and activity of lactic acid bacteria in bamboo shoot fermentation, including oxygen concentration, pH, and salt concentrations.

Lactic acid bacteria are aero-tolerant anaerobes, which are not sensitive to oxygen but can grow in its presence as well as in absence [28]. However, oxygen could induce the growth of some spoilage microorganism causing product contamination. The fermentation process relies on the rapid colonization of the food by LA-producing bacteria, which lower the pH and make the environment unsuitable for the growth of spoilage organisms. Oxygen is also excluded as the lactobacilli favour an anaerobic atmosphere. Restriction of oxygen ensures that yeasts do not grow [22].

The pH is another critical factor in preservation and developing aroma and flavour of many fermented products [22]. Generally, lactic acid bacteria could effectively grow in a near neutral pH [29], while some bacteria such as *Lactobacillus* and *Streptococcus* can survive at lower pH (3.0–4.0) [30] [31]. During fermentation, the bacteria produced lactic acids causing reduction of pH. It was found that pH of 4.5 was an optimal pH for fermented vegetable products such as kimchi, sauer-kraut, pickled and cucumber, etc [32]. The overgrowth of lactic acid bacteria diminished product quality, resulting in unfavourable taste.

In order to prevent any growth of spoilage microorganisms, salts are normally added to create favorable conditions to lactic acid bacteria, which can range from 20 to 80 g/l. High salt concentration could inhibit growth of non-desirable organisms, while lactic acid bacteria are halophilic bacteria. This salt tolerance provides them an advantage over less tolerant species and allows lactic acid fermentation to be occurred [22]. Salt induces plasmolysis in plant cells which releases mineral salts and nutrients from the vacuole and creates anaerobic conditions for proper growth of LAB around the submerged product [22] [33]. Salt concentrations also play an important role in fermentation times of vegetables. The pH decreases rapidly during the first 3-5 days

from 6.5 to below 5.0, when 8% salt was added. In the other hand, fermentation took for 1-2 months to reach the pH below 5.0 when 3-4% salt was used [32] [34].

2.2.3 Microbial ecology in fermented bamboo shoots

Spontaneous fermentation is commonly used in traditional fermentation of fruits and vegetables. During fermentation, alteration of microbial populations was happened. Many reports have investigated the microbial changes. Jiang-sun, fermented bamboo shoots prepared by mixing the shoots with salt, sugar and fermented soybeans and layering in a bucket to allow fermentation for 1 month. The results showed that *Enterococcus faecium* and *Lactococcus lactis* were found as the major lactic acid bacteria in the initial fermentation. The strain was observed in the raw substrate of fermented soybeans. Then, *E. faecium* and *L. lactis* were completely replaced by *Lactobacillus plantarum* after 3 days of fermentation. The physiological analysis revealed that only *L. plantarum* was able to propagate in MRS broth with an initial pH of 4.2. On the other hand, *L. plantarum* and *Lac. lactis* subsp. *lactis* were the main LAB species found in the bamboo shoots. *L. plantarum* became the major LAB during the fermentation. The pH of 4.2 was observed in the 1-day fermented sample, and a pH of 3.5 was observed in the 30-days sample [35] [36] [37].

Sichuan pickle, a vegetable product prepared by traditional vegetable pickle fermentation method, were investigated for their spoilage and pathogenic bacteria during fermentation. It was found that at the beginning of spoilage stage, the pathogenic *Vibrio penaeicida* and the spoilage and pathogenic *Pseudomonas fluorescens* were detected, while, in middle spoilage stage they shifted to the pathogenic *V. penaeicida* (51.25%), the spoilage and pathogenic *Pse. fluorescens* (3.75%), the spoilage *Pse. Chlororaphis* (7.5%) and then to the pathogenic *V. penaeicida* (30.59%), *Halomonas variabilis* (10.59%) and *Arcobacter marinus* (5.88%). The spoilage *Lactobacillus alimentariu* (5.88%) were found in latter spoilage stage. Moreover, other undesired microorganism including *Pseudoalteromonas nigrifaciens*, *Psychrobacter alimentarius*, *Marinomonas*, *Cobetia marina*, *Celerinatantimonas* and *V. litoralis* were also detected in the spoilage process [38]. This information could indicate that control of fermentation process effectively would be needed in order to eliminate the microbial spoilage in fermented products. Microbial spoilage of food is a complex event, in which the combination of

microbial and biochemical activities may interact, and can be considered as any change which renders a product unacceptable for human consumption [39].

2.3 Mature coconut waters for fermentation

Coconut is an important fruit tree found in tropical regions. Its fruit can be made into a variety of foods and beverages. Global coconut-derived products are commonly tender coconut water and coconut milk. World trade of coconut-derived products have been growing. Technavio's market research [33] [34] forecasts that, during the period of 2016–2020, the global market of coconut-derived products would show a compound annual growth rate of around 27% and 15%, for tender coconut water and coconut milk, respectively. Consumption of coconut water has been increasing worldwide and represents one of the fastest growing beverage categories [42]. The market trends are being driven by increasing health-consciousness among consumers, as coconut water is natural hydrating qualities, and enhances taste. Besides, mature coconut milk is one of the main ingredients for cooking Thai and Indian foods. Generally, production of coconut milk generates high volume of mature coconut water, which is normally discharged, as only the coconut meat is used for producing coconut milk [43]. In Thailand, mature coconut water might be used to produce nata de coco while some are discharged directly into the drain in large quantities, roughly 200,000 tons/year.

Coconut water is low in fat and calories. Soluble sugars are the main components found in coconut water, but it also contains proteins, vitamins and minerals. Coconut water is considered as tender coconut water (TCW) and matured coconut water (MCW), based on maturity, cultivation areas and subsequently, storage and post-harvesting processing [44] [45]. TCW is commonly consumed as beverage in the tropics, while MCW is mostly discharged because only coconut meats are utilized for different culinary purposes as mentioned earlier [46]. However, it was found that MCW are composed of several types of sugar, such as mainly sucrose, sorbitol, glucose and fructose, followed by minor sugars including galactose, xylose and mannose [47] as well as containing unique chemical compositions of sodium, potassium, phosphorus, chloride and magnesium, vitamins, free amino acids and growth promoting factors [43]. The nutritional composition of MCW has been documented as shown in Table 2.4 [45]. This

therefore makes MCW important to be effectively used as a nutrient source for lactic acid fermentation [48].

Table 2.4 Nutrients in mature coconut water

Nutrients	content/ 100 mL
Na	23.71 mg
K	361.20 mg
Ca	15.88 mg
Mg	15.10 mg
Fe	417.19 µg
Se	0.94 µg
Cu	4.03 µg
Zn	13.65 µg
Mn	454.60 µg
Glucose	1.51 g
Total sugars	3.47 g

SOURCE: [45]

MCW could be used as a rich nutritious media for probiotic *Lactobacillus plantarum* DW12 to produce fermented MCW beverages. It was reported that fermentation of the probiotic in MCW showed a sharp increase of cell density from 7.01 log CFU/mL to 8.34 log CFU/mL after 24 h of incubation and a significant increase of the total acidity with a significant drop of pH from 6.00 to 3.37. It was also found that after 48 h incubation, fermented MCW contained 281 mg/100 mL of glutamic acid when 0.5% monosodium glutamate was added to the MCW, providing 12.8 mg GABA in 100 mL of fermented MCW [43]. MCW from *Cocos nucifera* and processed coconut water drink (CWD) were added with *Lactobacillus casei* shirota and incubated at 36 °C for 48 h. During fermentation, it was found that the probiotic in MCW grew more effectively than in CWD. After 6-h incubation, the number of viable cells in MCW was 7.4 x 10^8 CFU/mL and reached 2.5 x 10^9 CUF/mL within 12 h of incubation. On the contrary, the

cells slowly grew in CWD and did not reach the value of 10^9 CFU/mL until 48h of culture. This showed that the probiotics had better adaptation in MCW than in CWD, which might be due to preservatives in CWD, causing some inhibitory effect concerning the growth of lactic acid bacteria [49].

2.4 Browning reaction

An important factor causing quality deterioration of bamboo shoots is the development of browning on the shoot surface. Browning reactions are generally assumed to be a direct consequence of polyphenol oxidase action on polyphenols to form quinones, which ultimately polymerize to produce the browning appearance in fresh-cut fruit and vegetable products [50]. Browning is usually occurred in naturally fermented bamboo shoots during storage, which is unacceptable from consumers. This results in price reducing. The whiter the fermented bamboo shoots are, the higher prices are required.

Browning can be divided into 2 categories: enzymatic browning and non-enzymatic browning. Enzymatic browning reactions are primarily catalysed by polyphenol oxidase (PPO) (monophenol dihydroxyphenylalanine: oxygen oxidoreductases, EC 1.14.18.1) in the presence of oxygen and reducing substances [51]. Thus, its control is essential for preserving the quality of food products. The degree of browning depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of PPO. This copper-containing enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity) (Figure 2.4). To date, several methods have been used to assess the inhibition of PPO activity in fruits and vegetables because the phenolic composition is closely related to food properties, such colour [52]. Although sulfites have been successfully used as anti-browning agents, they have been associated with severe allergy-like reactions in certain populations and, as a result, the Food and Drug Administration (FDA) has limited their use to only a few applications [53].

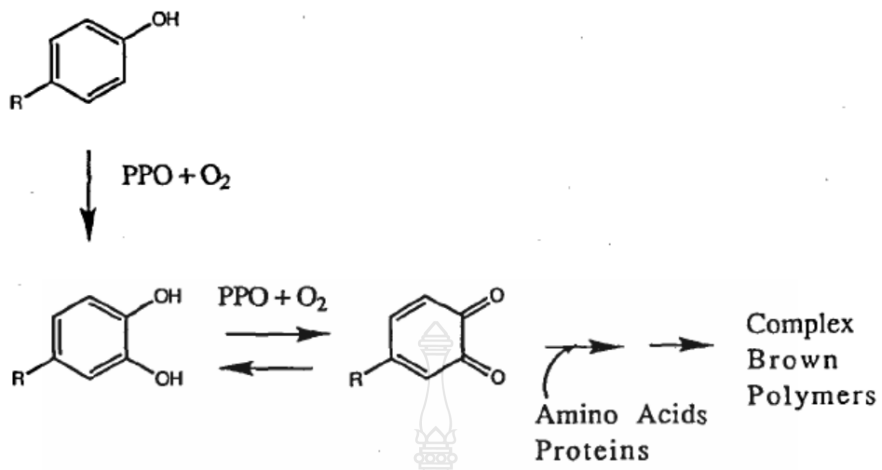


Figure 2.2 The simplified processes of enzymatic browning by polyphenol oxidase

SOURCE: [54]

Non-enzymatic browning is the result of the Maillard reaction. It includes condensation between reducing sugars and amino acids, caramelization, ascorbic acid degradation and pigment destruction [51]. Maillard reactions lead to changes in food colors, organoleptic properties, protein functionality, and protein digestibility. Maillard reactions are initiated by condensation of amino groups on protein, peptides, and amino acids with carbonyl groups on reducing sugars, resulting in Schiff base formation and rearrangement to Amadori or Heyns products (Figure 2.3). Maillard reactions affect multiple food quality parameters, including organoleptic properties, color, and protein functionality. Unique aroma profiles are developed dependent on temperature−time profiles used during food processing. In some cases, Maillard reactions contribute to desired changes such as the generation of delicate flavors, whereas in other cases undesired quality. changes are obtained, especially if the Maillard reactions are too pronounced, producing bitter and burnt flavors. Thus, being able to control Maillard reactions during food production and storage is important from a food quality perspective [55].

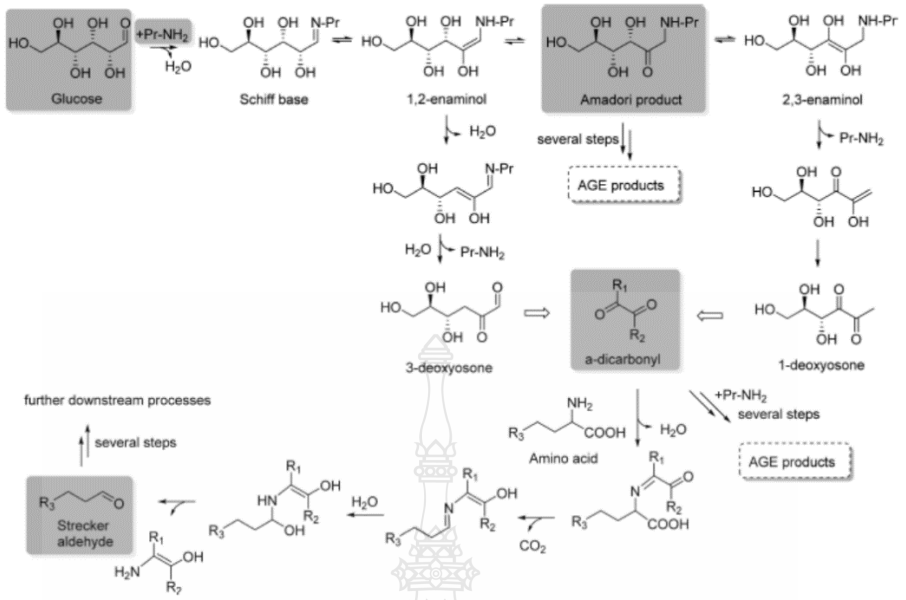


Figure 2.3 Simplified scheme of the Maillard reaction of a reducing sugar

SOURCE: [55]

It was reported that non-enzymatic browning in Golden Delicious and Amasya apple juice concentrates was investigated during storage at different temperatures for 4 months. The results showed that the browning changes were observed. Lightness values of the juices decreased by increasing storage time and temperature, which was more remarkable when the samples were stored at 50 and 60 °C. The researchers explained that Maillard reaction was a major reason causing the color changes. Type of sugars and amino acids were major factors affecting the Maillard reaction browning [56]. Reactions between sugars and amino acids in the Maillard reaction produce a multitude of compounds through interconnected chemical pathways. The course of the pathways changes depending on not only the nature of the amino acids and sugars but also the processing conditions such as temperature, water activity. Comparison of the data from the four amino acids studied showed a common pathway, which involved 73 Maillard reaction products (MRPs) where the differences were due only to the nature of the amino acid side chain [57].

Besides, there were studied that pigment degradation could cause non-enzymatic browning reactions. Many anthocyanin-containing foods are thermally processed to

ensure their safety, and stored for some time before being consumed. However, the combination of thermal processing and subsequent storage has a significant impact on anthocyanins. This study aimed to investigate the color, chemical stability, and antioxidant capacity of thermally treated anthocyanin aqueous solutions during storage at 4, 25, 45, and 65 °C, respectively. Anthocyanin aqueous solutions were thermally treated before storage. The results showed that the degradation rate of anthocyanins in aqueous solutions was much faster than those in real food. The color of the anthocyanin aqueous solutions changed dramatically during storage [58] [59].

2.5 Inhibition of browning reaction

Extensive research has focused on control of browning in foods. Several approaches to browning inhibition have been explored. Inhibitors of enzymatic browning can be done by reducing agents, chelating agents, and acidulants [60] [61].

2.5.1 Reducing agents/Antioxidants

Reducing agents or antioxidants such as ascorbic acid, thiols, or polyphenols could prevent browning reaction by chemically reducing the enzymatically formed or endogenous *o*-quinones to the colorless diphenols [62], or reacting irreversibly with the *o*-quinones to form stable colorless products analogous to the action of sulfites. Sulfiting agents are used in foods to control a number of detrimental processes including oxidation, enzymatic and nonenzymatic browning, and microbial growth. The use of sulfite treatment is common practice for the food industry. Unfortunately, the safety of sulfites in foods has been questioned because of alleged health hazards to asthmatics and because of the allergic reaction that sulfites cause in a certain segment of the population [63]. They have been associated with severe allergy-like reactions in certain populations and, as a result, the Food and Drug Administration (FDA) has limited their use to only a few applications [52]. These disadvantages have prompted the search for alternative analytical methods, and a number have been reported. Studies have shown that acidified sodium chlorite has a strong antimicrobial efficacy against various human pathogens on fresh and fresh-cut fruits and vegetables. Sodium chlorite probably has a dual role in browning inhibition and strong pathogen inactivation [64]. Moreover, ascorbic acid is a good antioxidant and reduction agent that eliminates oxygen in polyphenol oxidase reactions.

In action, approximately 0.1-0.5% of ascorbic acid may have a protective effect against enzymatic browning. 0.5% Ascorbic acid+ 1mg / liter of sodium chloride for 5 minutes was used to inactivate PPO; this was most effective treatment for delaying browning [65].

2.5.2 Chelating agents

The enzyme PPO contains copper in its active site. In the context of PPO-catalysed browning, chelating agents are believed to either bind to the copper in the active site or reduce the level of copper available for incorporation into the holoenzyme. EDTA (ethylenediaminetetraacetic acid) is a natural preservative appropriate for use in food processing. Both EDTA and its sodium salt (1 mg/L) are widely used as a metalchelating agent [61] [54] [65]. Metaphosphate is another chelating agent that has been used as antibrowning agents particularly for fresh-peeled fruits and vegetables. It is typically used at levels of 0.5 to 2% by pre-dissolving the compounds in water or by combining with other antibrowning agents [54].

2.5.3 Acidulants

Enzymes could lose their catalytic functions when they are in extremely low pH conditions. Acidulants such as citric, malic, tartaric and malonic acids, and inorganic acids such as phosphoric and hydrochloric acids can be used as anti-browning agent by lowering the pH of foods to below the pH necessary for optimal catalytic activity. The pH optimum of PPO activity s in the pH range 6-7 and the enzyme is inactive below pH 4 [61]. However, it could vary dependent to the source of the enzyme and the nature of the substrate. Citric acid in the concentration of 0.75% is an acidulant commonly used. It is readily available and cost-effective, while others are their limited availability, higher price and negative impact on taste [61] [65]. Moreover, it may have a dual inhibitory effect on PPO by both lowering of the pH and chelating the copper at the active site of the enzyme [54].

2.6 Safflower oil (SO)

The safflower is an ancient agricultural crop widely cultivated worldwide. It is a member of *Compositae* family which includes artichoke, chicory and sunflower. The parts of the plant can be used for different purposes. Its colorful petals could be prepared for food colorants, flavors, and dyes. Seeds could be produced as vegetable oils, bird

feeds, and foliage for cattle feeding [66]. The safflower oil is used as cooking oil. It has high linoleic acid contents and characteristics of nutty flavor with a distinct pale yellow to golden color. The oil is composed of 6–8 % palmitic, 2–3 % stearic, 16–20 % oleic and 71–75 % linoleic acids, and exhibits the highest linoleic acid content among all the commercial oils. As the main characteristics of the oil, specific gravity of 0.919–0.924, refractive index of 1.473–1.476, titer of 15–17 °C, flash point of 121–149 °C, free acidity of 0.15–0.60 %, saponification value of 186–194 mg KOH/g oil, iodine value of 141–147 g/100 g oil, unsaponifiable matter of 0.3–0.6 %, peroxide value of 0–1.0 mequiv O_2/kg oil (fresh oil), moisture and volatile matter content of 0.03–0.1 % have been reported [66] [67] [68]. Moreover, effects of safflower oil on human health have been reported. It could exhibit antibacterial activity, inhibition of platelet aggregation, anti-inflammatory action, inhibition of tumor promotion as well as hyperlipidemia, hypertension, and hyperglycemia [69].

Safflower seeds and safflower oil has been well-known for their antioxidant activities. It was found that *Carthamus tinctorius* L. seed extract contained 126.0± 2.4 mg GAE/g and 62.2±1.9 mg QE/g of total phenolic and flavonoid contents, respectively. The major phenolic compounds was epigallocatechin (109.62 mg/g), with a 4-hydroxy benzhydrazide derivative and gallocatechin presented at 18.28 mg/g and 17.02 mg/g, respectively. The extract exhibited remarkable radical scavenging activities, FRAP (ferric reducing antioxidant power) and reducing power in a dose-dependent manner. Moreover, the oxygen radical absorbance capacity (ORAC) value of CSE (0.1 mg/mL) was 62.9 ± 4.7 μM TE (trolox equivalent)/g [70]. Besides, safflower oil also showed antiaging activity. It was found that the inhibition of safflower oil extracts in the collagenase assay was between 47% and 72.1% compared to the positive control (83.1%), while inhibition in the elastase assay ranged from 32.2% to 70.3%, with the positive control being 75.8%. These results highlighted the interest in safflower oil as a source of phenols with valuable antioxidant and antiaging activity and use for cosmetics [71].

2.7 Food packaging

The packaging is a key food processing unit operation serving functions of containment, protection, preservation, storage and enhances the margin of food safety

[72]. Food packaging technologies involves retardation in oxidation, hindered the respiratory process, and prevention of microbial attack during storage. The impermeability of the packaging material to vapors and gases such as water, oxygen, carbon dioxide and aromas is an essential design consideration for the longevity of the packaged food product and hence the key to successful food packaging [73] [74]. High levels of oxygen present in food packages play an important role in quality of foods. Oxygen may facilitate microbial growth, off-flavors and off-odors development, color changes, and nutritional losses, causing significant reductions in the shelf life of foods [74]. Many technologies have been developed to eliminate or reduce the levels of oxygen inside packs. These include modified atmosphere packaging, oxygen scavenger sachet, and vacuum packaging. Typically, oxygen scavengers are used in packages that have airtight seals and are used in conjunction with other means of preservation, such as chemical preservatives, reduced water activity, reduced pH, and vacuum packaging. For instance, some type of polymer had been used as a reduced oxygen scavenger and incorporated in polymer packaging materials to limit the amount of oxygen in packaging [75]. Therefore, the control of oxygen levels in food packages is important to limit the rate of these deteriorative and spoilage reactions in foods.

The food products employ different package designs and purposes, including glass-bottled, aluminum, polypropylene, and polyethylene terephthalate. The glass, it has been well-known that the atoms and molecules in glass have an amorphous random distribution. Scientifically this means that it has failed to crystallize from the molten state, and maintains a liquid-type structure at all temperatures. In appearance, it is usually transparent but, by varying the components. It is hard and brittle, with a chonchoidal (shell-like) fracture [76]. It is a well-researched packaging material for food, but the disadvantages of glass include its fragility and heavyweight. If a little crack occurs, it affects the packaged food for storage and could be dangerous to consumers. Aluminum is commonly used to make cans and foil. It is a lightweight, silvery-white metal derived from bauxite ore, where it exists in combination with oxygen as alumina. Magnesium and manganese are often added to aluminum to improve its strength properties. Unlike many metals, aluminum is highly resistant to most forms of corrosion; it's a natural coating of aluminum oxide that provides a highly effective barrier to the effects of air, temperature,

moisture, and chemical attack [77]. For plastic materials, they have the advantages of being lightweight and durable. Polypropylene (PP) has the lowest density of plastics for commodities, while polyethylene terephthalate (PET) is more resistant to oxygen and fat permeability than other plastic materials [78] [77]. Both of them are typically used as food packages due to its control of oxygen levels in the packages. This is important to limit the rate of deteriorative and spoilage reactions in foods.

2.8 Related research

The diversity and dynamics of the dominant bacterial communities arising during the pickling process of bamboo shoots (*Dendrocalamus latiflorus*) were investigated by nested polymerase chain reaction denaturing gradient gel electrophoresis combined with quantitative real-time polymerase chain reaction. Several kinds of halophilic bacteria were detected during early sampling time (0 - 3 days). After pickling for 7 days, *Lactococcus lactis* significantly increased and became the first dominant bacterium. After pickling for 14 days, *Weissella* sp. bands appeared and quickly became dominant on the 21st day. As maturation progressed, *Lc. lactis* decreased in intensity whereas *Weissella* sp. increased in intensity [79].

Mature coconut water is a waste product from the coconut milk industry. It is sour and unpalatable, yet it contains sufficient nutrients for microbial growth. It could be used as a nutrient source for lactic acid bacteria to produce γ-aminobutyric acid or GABA [82]. It was shown that *Lactobacillus acidophilus* and *Lactobacillus plantarum* had great potentials to increase GABA content in mature coconut water. Fermented coconut water can be formulated as a healthy functional drink as GABA is known to have therapeutic value in alleviating stress [44]. Moreover, coconut water could be used to grow *Saccharomyces cerevisiae* LBCM 678. After fermentation, higher levels of desirable compounds, such as esters, alcohols, and ethanol as well as lower volatile acidity, acetaldehyde, and methanol were found when compared to *Saccharomyces cerevisiae* LBCM: 671 and 676 [81].

Safflower oil is a very valuable product for the body and human health. It is rich in macro- and microelements, vitamins and minerals, and also has antioxidant properties. The amount of unsaturated fatty acids such as oleic (18.31±0.874%) and cis-linoleic acid

CHAPTER 3
MATERIALS AND METHODS

3.1 Chemicals, apparatus, and By-product

3.1.1 Chemicals

Name	Brand/ borough /Country
1. Citric acid	KEMAUS/Cherry brook/Australia
2. Ascorbic acid	Sigma-aldrich/Germany
3. Sodium hydroxide	KEMAUS/Cherrybrook/Australia
4. Methanol	RCILabscanlimited/Bangkok/Thailand
5. Potassium metabisulphite	Univar/New Zealand
6. Ethanol absolute	VWR/France
7. Safflower oil	TCSmart/Thailand
6. Sodium carbonate	Univar/New Zealand
8. Folin–Ciocalteus Phenol	SRL/Maharashtra/India
9. 1,1-diphenyl-2-picryl-hydrazyl	SIGMA-ALDRICH/Germany
10. Sodium chloride	SRI/Maharashtra/India
11. Gallic acid monohydrate	SIGMA-ALDRICH

3.1.2 Apparatus

Name	Model/Brand/Country
1. High spread homogenization	D500/Wiggens homogenizer
2. Vortex	G560E/Scientific Industries/U.S.A

3. Ultrasonication	VCX 130/Vibra cell/USA
4. Vacuum packager	VM300TE/A/Brother/China
5. Precise color reader	WR-10QC/FRU/China
6. Incubator	JSGI-250T/JSR
7. Mixer grinder	MX-AC400/Panasonic/India
8. Refractometer	Master refractometer/ ATAGO/ Japan
9. *E. coli*/Coliform Count Plate	3M™ Petrifilm™ /USA
10. Aerobic count plate	3M™ Petrifilm™ / USA
11. Stomacher	BAGMIXER 400/interscience/ France
12. Counting scales 4-point	PA214/OHAUS/USA
13. Counting scales 2-point	JLGO2GE/Mettler/Switzerland
14. Thermo scientific centrifuge	2-16PK/sartorius/Germany
15. Micropipettes P1000, P200	WITOPET PREMIUM/witeg Lebortechnik GmbH/ Germany
16. Micropipettes P 5 ml	IKA PETTE
17. Droplet size	MAL1099267/Mastersizer3000
18. ELISA plate to measure OD	EZ Read 2000/biochrom/England
19. pH meter	pH700/Eutech

3.2 Microbiological and chemical determination of local fermented bamboo shoots

Twenty-five grams of fermented bamboo shoots obtained from a community enterprise in Prachinburi Province were stored in polypropylene bags and separately tighten with a rubber band. The sample was kept at 4 °C, room temperature (25±3 °C), and 35 °C for 28 days. During incubation, the samples were taken to determine for the number of total bacteria, yeast and mold in nutrient agar and potato dextrose agar, respectively as well as *Bacillus cereus* in Mannitol–egg yolk–phenol red–polymyxin agar and *Escherichia coli* by using the 3M petrifilm. pH and total acidity of the fermented bamboo shoots were measured by pH meter and titration and, respectively.

3.3 Fermentation of bamboo shoots and storage

3.3.1 Traditional fermentation of bamboo shoot

Fresh bamboo shoots from Prachinburi Province, Thailand were peeled, sliced, and soaked in tap water containing 10 g/kg of Kosher salt for one night to delay browning reaction during preparation. The bamboo shoots were then squeezed prior to be mixed with 100 g/kg of Kosher salt and left it for 30 min. After that, they were squeezed, packed in a plastic bucket, added with drinking water until it covered the shoots for fermentation. The buckets were then tightly closed with a cover. The samples, named as TFBS, were incubated at room temperature until the pH was below 4.6. After fermentation, TFBS were taken to determine for pH by using a digital pH meter (pH 700, Eutech), total acidity by titration, the number of total bacteria by using 3M petrifilm and total lactic acid bacteria by a pour plate method in Lactobacillus de Man, Rogosa and Sharpe (MRS) agar.

3.3.2 Fermentation of bamboo shoots with mature coconut water

Freshly mature coconut water (MCW) was purchased from Uthong market in Pathum Thani province, Thailand. It was pasteurized at 100 °C for 30 min to reduce the risk of microbial contamination before use. MCW was measured for pH, total acidity, total sugars, total soluble solids using an abbe refractometer (Master refractometer, ATAGO, Japan), and total reducing sugars [87]. Fresh bamboo shoots from Prachinburi Province were peeled, sliced, and soaked in tap water for 1 h to remove cyanogen [88]. The shoots were mixed with 25 g/kg of Kosher salt, which was less than the traditional method for 75%. The shoot was then left at room temperature for 30 min, prior to be squeezed and packed in a plastic bucket. The pasteurized MCW was added until it covered the shoots. The buckets were then tightly closed with a cover. The samples, named as FBSC, were incubated at room temperature until the pH was below 4.6. During fermentation, the sample was collected to determine for pH by using a digital pH meter (pH 700, Eutech), total acidity by titration, the number of total bacteria using 3M petrifilm and total lactic acid bacteria by a pour plate method in Lactobacillus MRS agar (MRS). The results of FBSC were then compared to TFBS.

3.3.3 Storage stability

After fermentation, TFBS and FBSC (40 g) were packed in polypropylene (PP) or polyethylene terephthalate (PET) bags. Twenty-five ml of saline water (4.85% NaCl) was then added into the bags prior to be sealed under vacuum condition. The samples in bags were then pasteurized in boiling water for 30 min and left at room temperature overnight. The samples were then stored at 4 °C, room temperature (25±3 °C), and 35 °C for 60 days. During storage, the shoots were measured for their colors (CIE Lab scale), pH, total acidity, the number of total bacteria, yeast and mold and *E. coli*.

3.4 Effects of soaking solutions on fermented bamboo shoots during storage

To prevent browning reaction occurred in FBSC, the effects of safflower oil was studied for its ability to reduce browning reaction. The safflower oil emulsion was prepared as a soaking solution to compare with the other three soaking solutions, namely 4.85 g/100 mL of sodium chloride solution (S), 0.2 g/100 mL of citric acid solution (C), and sodium chloride in citric acid (SC).

3.4.1 Safflower oil emulsion preparation

Different emulsions of safflower oil (O) in water were prepared. The oil at different concentrations (0.1g/100 mL and 0.2 g/100 mL) separately mixed with 4.85 g/100 mL of sodium chloride solution, 0.2 g/100 mL of citric acid solution, and citric acid (0.2 g/ 100 mL) containing 4.85 g/100 mL of sodium chloride solution. The samples were named as SO, CO, and SCO, respectively. The emulsions were homogenized by using high spread homogenization (14,000 rpm for 2-15 min) (D500, Wiggens homogenizer, Korea) or ultrasonication (80 Hz for 2-15 min) (VCX 130, Vibra cell, USA). The schematic chart of emulsion preparation was shown in Figure 3.1. The obtained emulsions were left at room temperature for 3 days to observe their separation. The sample having high stability was selected to be used as a soaking solution in the future experiment.

3.4.2 Droplet size

Droplet sizes of safflower oil emulsion were measured by laser diffraction in a Mastersizer3000 (MAL1099267). The refractive indices of essential oil and water were used as particle and dispersant, respectively. Results are given in volume mean diameter (D4,3). Measurements were made in duplicate [156].

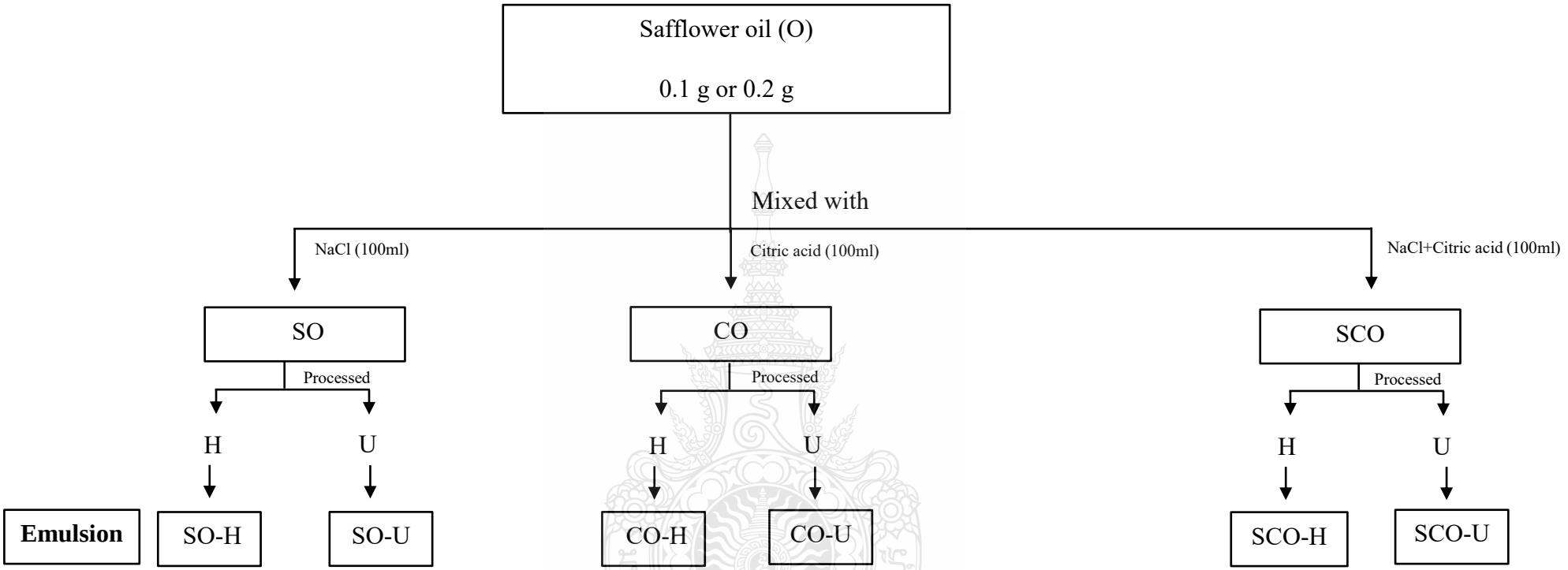


Figure 3.1 The schematic chart of emulsion preparation

NOTE: Sodium chlorine (S), citric acid (C), sodium chlorine + citric acid (SC), safflower oil (O), High speed homogenization (H), Sonicater at 80 Amp for 2 min (U)

3.4.2 Effects of soaking solutions

FBSC was prepared as the section of 3.3.2. After fermentation, the fermented bamboo shoots (15 g) were separately packed in polyethylene terephthalate (PET) bags. The soaking solutions including SC, SCO-H (0.1g/100 mL of safflower oil), and SCO-U (0.1g/100 mL of safflower oil) were added into the shoots at the ratio of the solution to the shoots at 1:1 (Figure 3.2). A commercial fermented bamboo shoot (140g of bamboo shoo, salt (4.85g/100 mL), citric acid (0.01g/100mL) was used as a control. The fermented bamboo shoots were then stored in an accelerated condition (55 °C for 7 days). During storage, the samples were taken to analyze for color changes, total phenolic contents, and antioxidant activity by DPPH.

3.4.3 Sensory evaluation

FBSC was prepared according to the section 3.3.2. SCO-H was used as a soaking solution. FBSC was kept at 4 °C for 7 days before using it to conduct sensory evaluation. The sample was prepared by cooking the shoots with eggs according to the recipe that was generally traditional. Add vegetable oil (15 cc) in a pan then the bamboo shoots 300 g. Then crack the egg (45 cc) and mix. After that season with soy sauce (5 cc), sugar (25 cc), oyster sauce (5 cc) and little water (60 cc). Turn up to high heat. Stir quickly until all ingredients are mixed well. Stir until the egg is almost cooked and the bamboo shoots are soft (for 10 min). Remove from heat. Commercial fermented bamboo shoots, freshly prepared TFBS, and freshly prepared FBSC were used to compare.

The samples were evaluated by 50 panelists. The test was accomplished based on Hedonic 9-point scale on the following factors: appearance, color, flavor, taste, texture, and overall acceptance by panelists and scaled from 1 to 9. The samples, each of which received three-digit code, served in plastic containers under normal light. The panelists received the random samples. They were asked to rinse their mouth with water between each simple test.

3.5 Determinations

3.5.1 Microbial determination

The microbial determination was determined by blending 25 g of samples with 225 mL of phosphate-buffered saline (PBS) by a stomacher (BAGMIXER 400, interscience, France) for 60 s. Appropriate dilutions from samples were made using sterile PBS water (9 mL). The samples were placed on 3M petrifilm to determine for total plate counts and *E. coli*, while potato dextrose agar was used for yeast and mold. Pour plate method was performed using MRS agar to measure for total lactic acid bacteria. The samples were then incubated at 37 °C for 48 h. The experiment was performed in triplicate and the average number of colony-forming units per gram (CFU/g).

3.5.2 pH and total acidity

The pH of samples were measured by using a pH meter. Total acidity (TA) of the fermented solution (section 3.3.1 and 3.3.2) and soaking solutions (section 3.3.3) were determined by the titration [152]. Three ml of samples were titrated with 0.1 N NaOH solution until their pH reached 8.2. Titratable acidity was calculated by following the eq. 1 and expressed as g/L of lactic acid.

$$\text{Total acidity (g/L)} = \frac{V\ of\ NaOH \times N \times MW}{V\ of\ sample} \quad (1)$$

where V was volume 0.1 N NaOH, N was Normality of NaOH and MW was Molecular Mass of lactic acid (90.08 g/mol).

3.5.3 Total sugars

Total sugars of MCW and fermented coconut water were determined colorimetrically using the phenol sulphuric acid method and expressed as percentage sugar [154]. The absorbance was measured at 490 nm and expressed as glucose concentration (mg/ml).

3.5.4 Reducing sugars

The reducing sugars of MCW and fermented coconut water were determined colorimetrically using 3, 5-dinitro salicylic acid (DNS) reagent and expressed as % [87] [154]. The absorbance was measured at 540 nm and expressed as glucose concentration (mg/ml).

3.5.5 Color of fermented bamboo shoots (FBS)

The color of FBS samples was measured using the chroma (WR-10QC, FRU, China) and reported in CIELAB color scales $L^* = (L_2\text{-}L_1)^2$, $a^* = (a_2\text{-}a_1)^2$, and $b^* = (b_2\text{-}b_1)^2$ values (L^* is the degree of lightness to darkness, a^* is the degree of redness to greenness, and b^* is the degree of yellowness to blueness) [155]. Total color difference (TCD) was calculated by following the eq. 2.

$$TCD = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (2)$$

3.5.6 Total phenolic contents

The fermented bamboo shoots was extracted by ethanol (99%). Five gram of the shoots were blended with 20 mL of ethanol for 1 min. The mixture was filtered. The supernatant was collected to determine for total phenolic contents and antioxidant activity. The total phenolic content was determined by modifications of the Folin–Ciocalteu method [144]. Briefly, 200 µL of the extract 1 mg/mL) were mixed thoroughly with 200 µL of Folin–Ciocalteu reagent (SRL, Maharashtra, India) for 3 min, followed by the addition of 685 µL of 0.7% (w/v) sodium carbonate (Univar, New Zealand). The mixture was allowed to stand for a further 60 min in the dark, and absorbance (EZ Read 2000, biochrom, England) was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

3.5.7 The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the shoot extract from 3,5 was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, with some modifications [144]. Briefly, 2 mL of each extract were mixed with 2 mL DPPH (SIGMA-aldrich, Germany) solutions and incubated in the dark at room temperature for 1 h. The absorbance of the mixture was then measured at 517 nm. Ascorbic acid (Sigma-aldrich, Germany) was used as a positive control. The ability of the sample to scavenge DPPH radical was determined.

The antioxidant activity was calculated by following the eq. 3 and expressed as (%).

$$\text{DPPH scavenging (\%)} = \frac{Control(A) - \text{Sample}(A)}{Control(A)} \times 100 \qquad (3)$$

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Microbiological and Chemical Determination of Traditional Fermentation Bamboo Shoots

Total aerobic bacteria (TPC), *B. cereus* (BC), *E. coli* (EC), yeasts and molds (YM) were found in the TFBS after storage at 4 °C, room temperature (RT), and 35 °C for 28 days. As shown in Figure 4.1, TPC, BC, and YM were found in the shoots after purchasing. The number of TPC, BC, and YM were 5.84±0.01, 6.06±0.12, and 5.81±0.01 log CFU/g, respectively. After 28 days of storage, *E. coil* were detected in all samples, which were 4.11±0.11, 6.03±0.01, and 5.28±0.01 log CFU/g for TFBS stored at 4 °C, RT, and 35 °C, respectively. Additionally, the number of TPC, BC, and EC of TFBS stored at high temperatures (RT and 35 °C) was increased more rapidly than that stored at 4 °C. These results indicated that TFBS of the community enterprise was unacceptable to be consumed.

According to the safety requirements for agricultural commodity and foods in Thailand, the maximum unacceptable of *E. coli* in foods is 3 log CFU/g, while *Staphylococcus aureus* and *B. cereus* in foods must be less than 2 log CFU/g (มผช.1166/2004) [89] [90]. Several studies reported that food poisoning in human was caused by the consumption of foods contaminated with these bacterium and its enterotoxin [91] [92] [93]. Ha, Shakur, and Do, (2019) mentioned that bamboo shoots could be the source of microbiological contamination. The contamination could be also related to hygienic conditions, including quality and material of container, use of hands, hands with gloves, and use of knives, etc. [94] [95]. The lack of hygienic precautions and bad cultural practices of indigenous fermentation, or uncontrolled fermentation increased the risk of microbiological contamination [96]. The risk could be diminished by training of producers and handlers to good practices, improving the hygienic quality of fermented products [8]. Moreover, the use of hurdle technologies like bio-preservation, food additives, or the use of efficient starters could also contribute to improve pathogenic control through sustainable solutions [97].

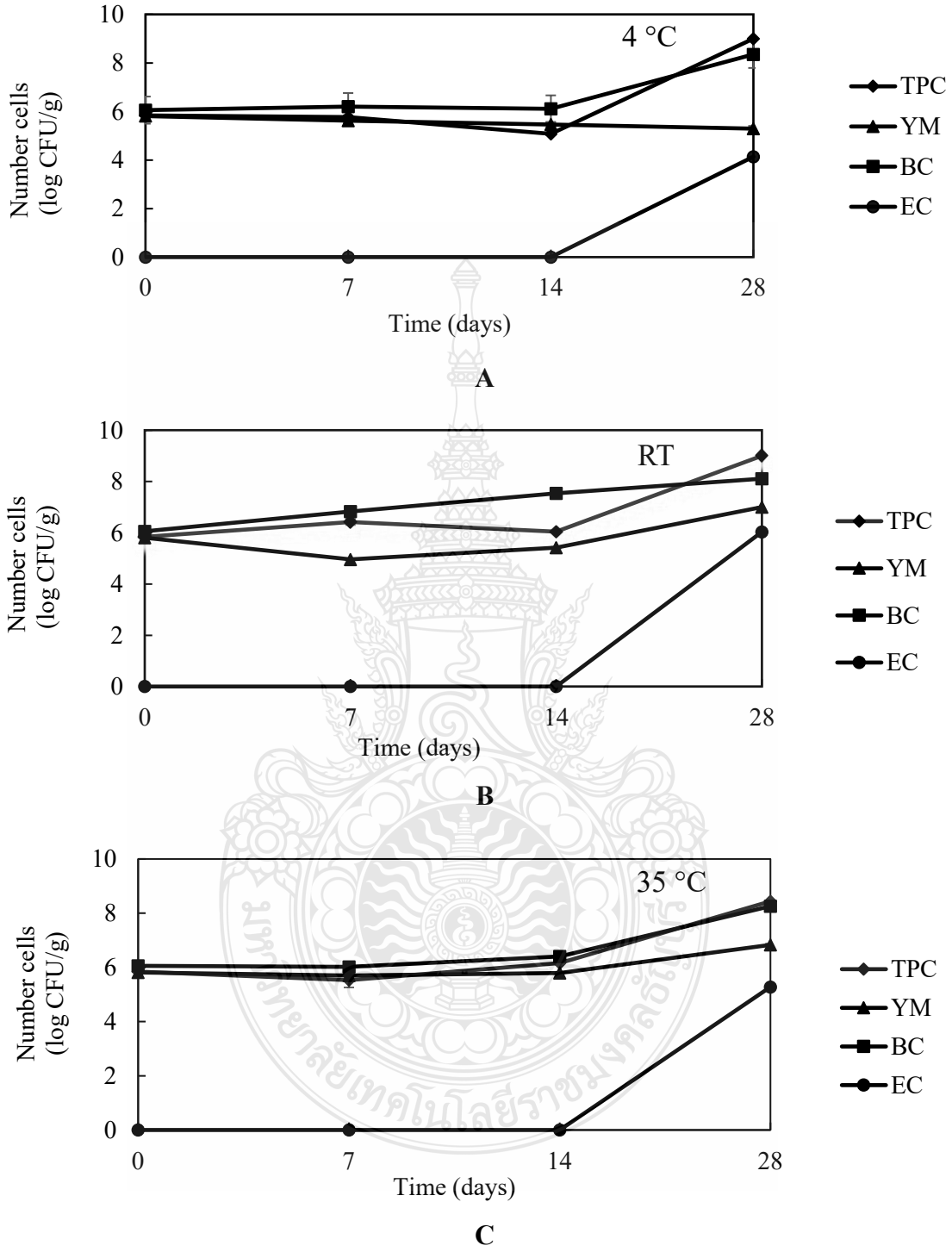


Figure 4.1 The microbial contamination of traditional fermented bamboo shoots (TFBS) during storage at 4°C (A), room temperature (B), and 35°C (C) for 28 days.
NOTE : TPC = total plate count, YM = yeasts and molds, BC = *B. cereus*, EC = *E. coli*

The pH of TFBS stored at 4 °C, RT, and 35 °C for 28 days were significantly increased from 3.16±0.23 at day 0 to 3.35±0.12, 3.55±0.02, and 3.44±0.03, respectively ($p<0.05$) (Figure 4.2a). Total acidity (g/L) of TFBS were continuously reduced during storage. The initial total acidity (g/L) of TFBS was 22.95±0.35 (g/L) and were not significantly reduced to 25.50±0.07 (g/L), 23.50±0.07 (g/L), and 24.00±0.28 (g/L) for that kept at 4 °C, RT, and 35 °C for 28 days, respectively (Figure 4.2b). The reduction of total acidity (g/L) could be possibly due to the microbial contamination of TFBS.

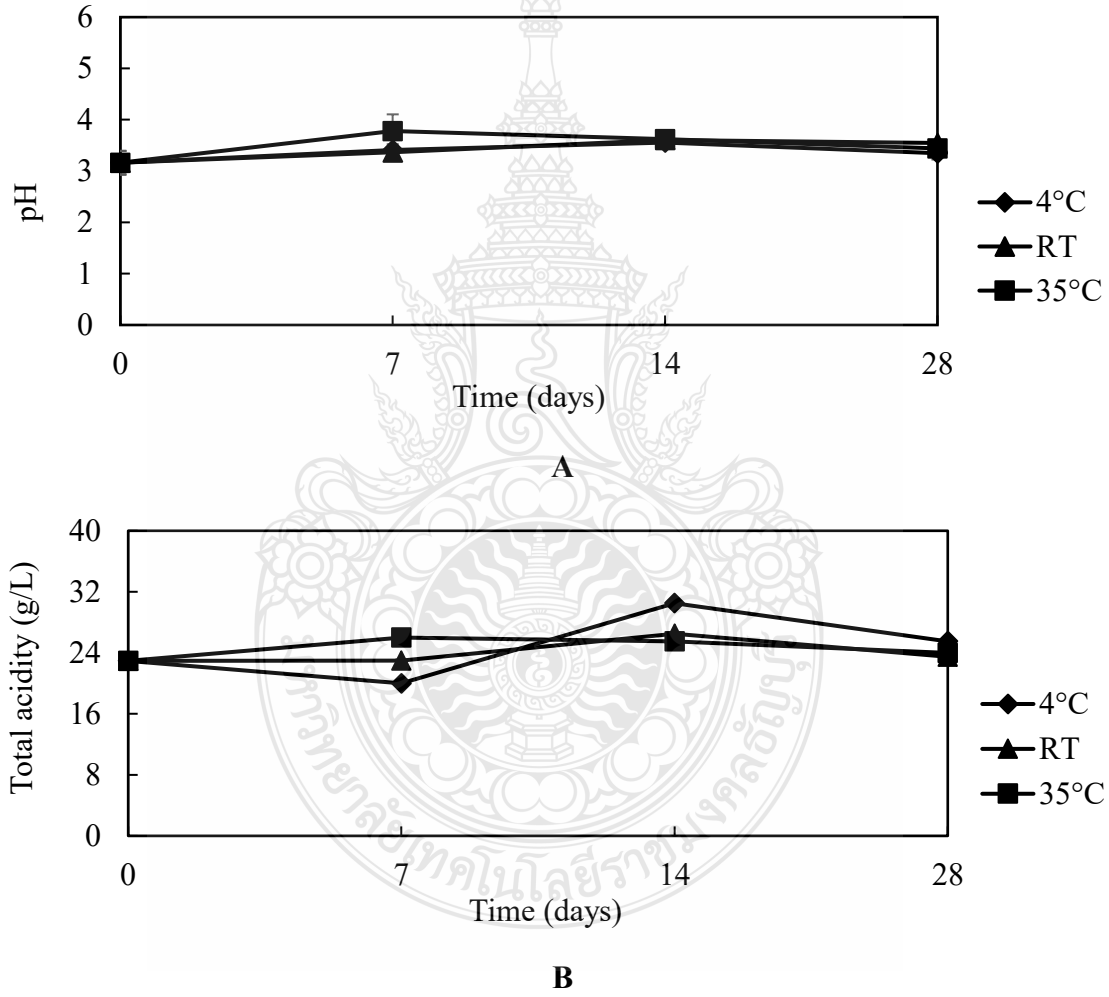


Figure 4.2 The pH (A) and total acidity (B) of traditional fermented bamboo shoots (TFBS) during storage at 4°C, room temperature , and 35°C for 28 days.

In this study, *B. cereus* and *E. coli* were detected at the beginning of the storage and after 14 days of storage, respectively. It was reported that the presence of *Staphylococcus* spp. and *B. cereus* in foods normally associated with individual hygiene of food handlers [98]. Similarly, it was reported that coliforms, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella oxytoca* and *Clostridium sporogenes* were spoilage and pathogenic microorganisms that could be detected in roots and tuber fermented foods [99]. *B. cereus*, *Enterobacter aerogenes*, *E. coli*, *Pseudomonas aeruginosa*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and *S. aureus* were also found in fufu, fermented cassava roots [100]. Similar pathogens such as *E. coli*, *B. cereus*, *S. aureus*, spp. and *Streptococcus faecalis* isolated from gari and pupuru, fermented cassava food products in Nigeria [101].

Kim and Breidt (2007) described that lactic acid could be degraded to propanol by spoilage microorganisms in reduced NaCl fermented cucumber slurries when its pH was between at 3.2-3.5 [102]. The prevention of anaerobic lactic acid degradation could be carried out by using 6% of NaCl in fermentation process and halting the fermentation at pH lower than 3.2 [101]. Spontaneous fermentation of lactic acid bacteria, especially in traditional food production, could be contaminated by pathogenic and spoilage microorganisms at any points of fermentation process, when poor hygiene practices and non-stile or open-system status of the entire fermentation were carried out [102] [103] [104]. Spontaneous vegetable fermented products usually encountered with difficulty of product quality and safety control [105].

4.2 Fermentation of bamboo shoots by using mature coconut water

4.2.1 Chemicals of mature coconut water (MCW)

MCW used in this study had 5 °Brix of total soluble solids, while pH and %TA were 5.59±0.10 and 1.17±0.21%, respectively. It also consisted of 26.07±0.13 g/L of total sugar and 23.54±0.15 g/L of reducing sugar. These were important factors affecting the growth of bacteria. It was reported that MCW showed higher TSS (6.15±0.21 °Brix) than immature coconuts water (IMCW) and overly-mature coconut water (OMCW), which were 5.60±0.14 and 4.85±0.17 °Brix, respectively [106]. The pH, and %TA of coconut water depended on the fruit maturity. The pH of coconut water

increased with the fruit maturity. Tan et al. (2014) revealed that pH of coconut water obtained from coconuts IMCW, MCW and OMC were recorded at 4.78±0.13, 5.34±0.12, and 5.71±0.10, respectively [106]. Malic acid is the dominant organic acid in coconut water. In contradict with pH, %TA of coconut water was decreased with the fruit maturity. It was reported that %TA of IMCW was 0.089±0.00%, which was greater than followed by those of MCW (0.076±0.01%) and OMCW (0.061±0.00%) [106]. The TSS and pH of the MCW could be useful to judge the consumers' acceptance and spoilage potential. TSS value could indicate the sweetness of the coconut water, while pH of coconut water could affect its flavor, consistency, and shelf life [107]. In order to ensure quality control, it was suggested that MCW should have pH values between 5.3 and 5.8 and Total soluble solids (TSS) values between 3.9 and 5.5 °Brix [108]. These could affect its taste and flavor that consumers accepted [107] [108].

In generally, sugars are the main fraction of soluble solids in coconut water. Coconut water contained sucrose, sorbitol, glucose and fructose, followed by minor sugars including galactose, xylose and mannose [109]. These changes of the sugar contents in coconut water could be due to the formation of sucrose at the expense of fructose and glucose. Researchers reported that during maturity, non-reducing sugar contents (sucrose) was increased but decreasing in reducing sugars (fructose and glucose) [110] [111]. MCW contained both reducing and non-reducing sugars. According to Rethinam and Krishnakumar (2022)[112], in the early stages of maturity, the sugars present were almost entirely reducing sugars, particularly glucose and fructose (>75%), but in the latter stages, the non-reducing sugar (sucrose) content increased. Similarly, it was found that the MCW of malayan yellow dwarf yielded the highest sucrose (2.49 ± 0.11 g/100 mL), while young malayan yellow dwarf showed the lowest of that (0.54 ± 0.11 g/100 mL) [45]. Thus, the composition and physicochemical properties of coconut water vary with maturity of the coconut fruit. Moreover, it was revealed that coconut water could be fermented by *Lactobacillus plantarum*, *Bacillus clausii*, or *Saccharomyces boulardii* to develop symbiotic drink [113].

4.2.2 Fermentation of bamboo shoots

The change in total LAB counts in the TFBS and FBSC during fermentation at room temperature for 36 h were shown in Figure 4.3. FBSC had greater number of LAB than FBSC since the 4th of fermentation. The number of LAB of TFBS was increased from 3.15±0.21 log CFU/ml to 8.08±0.02 log CFU/ml, while FBSC had 9.78±0.43 log CFU/ml, which was increased from 3.24±0.34 log CFU/ml. This indicated that LAB could more rapidly grow in FBSC than TFBS. During fermentation of FBSC, the cells started decreasing and reached to 8.32±0.01 log CFU/ml and 7.04±0.06 log CFU/ml for 16 h and 24 h, respectively. LAB in TFBS was not found after 36-h fermentation.

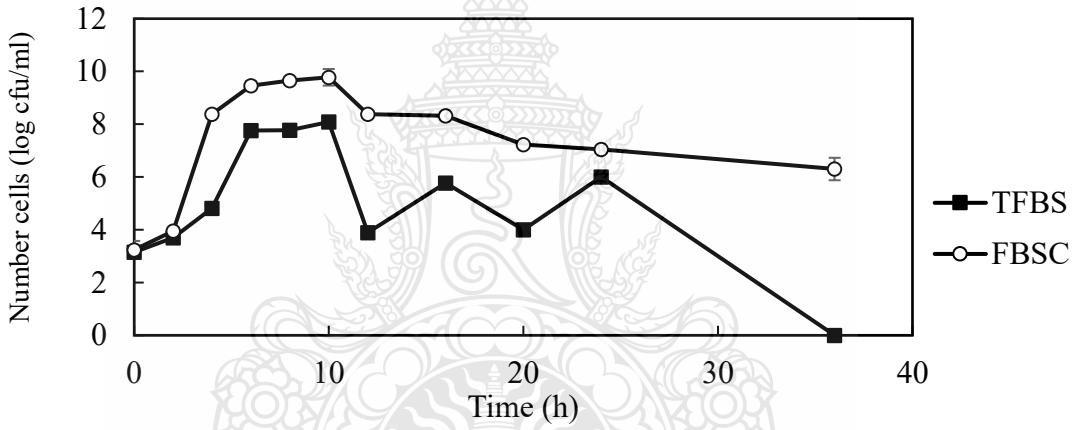


Figure 4.3 Total lactic acid bacteria of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) during storage at room temperature for 36 hours.

Salt concentrations affected the number and the diversity of the microorganisms during fermentation. From our results, it was evident that FBSC, which contained 2.5% of salt, had significantly greater number of microbes than TFBS, which 10% of salt was used. Similarly, Guan et al. (2022) [114] reported that salinity was one of the crucial factors in shaping the variation in the composition of microbial community. Jing-Fang Shen et al. (2023) [115] found that the higher the salt concentration, the lower the microbes produced. Too high salt concentrations reduced acid production, causing lactic acid bacteria to be less able to convert sugar, while promoting the growth of yeast

[116]. Several available reports indicated that naturally fermented bamboo shoot contains a mixture of microbial population viz. *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus corniformis*, *Lactobacillus delbrueckii*, *Leuconostoc citreum*, *Leuconostoc fallax*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Streptococcus lactis*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans* and *Pediococcus pentosaceus* [117].

During the fermentation process, dynamic changes in the physicochemical properties, including pH (Figure 4.4) and titratable acidity (g/L) (Figure 4.5) were observed. The pH of TFBS declined gradually from 6.00±0.00 to 4.00±0.01, while that of FBSC was decreased from 6.15±0.16 to 3.89±0.01 after fermentation for 36h. In contrast, titratable acidity (g/L) of TFBS and FBSC were increased steadily from 2.00±0.07 g/L to 6.00±0.01 g/L and 5.85±0.21 g/L to 11.25±0.21 g/L, respectively. Bamboo shoots contain high amounts of carbohydrates, mainly including polysaccharides, oligosaccharides and monosaccharides. During the fermentation, LAB decomposed and utilized carbohydrates in bamboo shoots to produce organic acids, resulting in the flavor formation. With the accumulation of organic acids, pH and TA were changed [118].

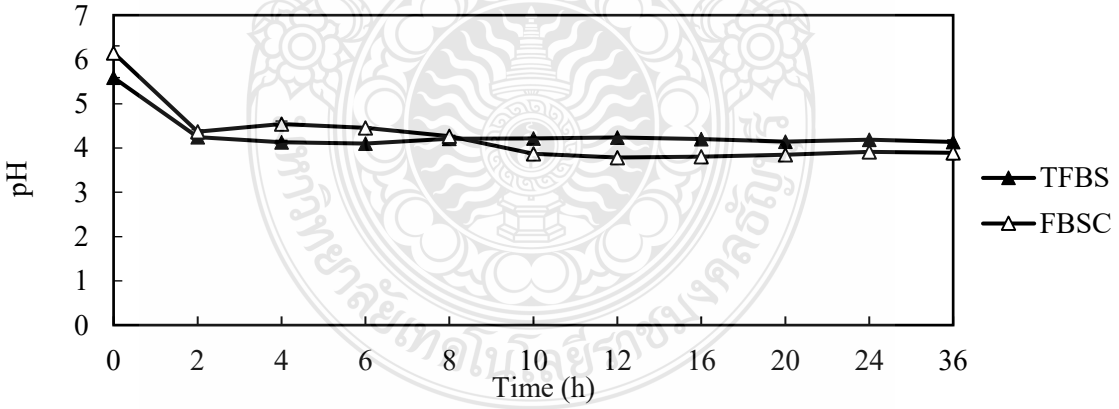


Figure 4.4 The pH on microbial growth of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) during fermentation at room temperature for 36 hours.

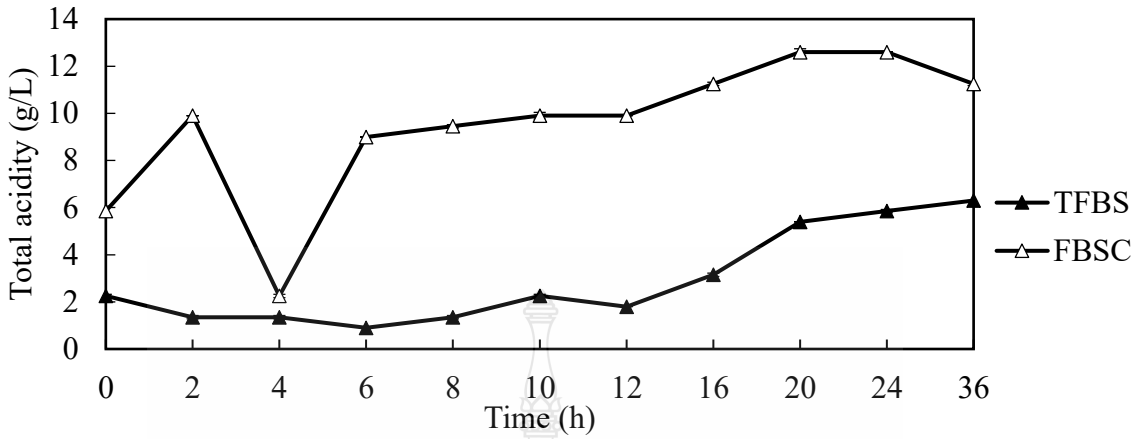


Figure 4.5 Total acidity of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) during fermentation at room temperature for 36 hours.

4.3 Stability of fermentation bamboo shoots during storage in different packaging types and temperatures

4.3.1 Color

Color changes of TEBS and FBSC during fermentation process were presented in Figure 4.6 - 4.8. At the day 0, the *L**, indicating the lightness, of all samples was not significantly different. L* was ranked between 56.3-63.4. At 4 °C after 60-day storage, the result was shown that types of plastic bags had an effect on bamboo shoot colors. Both FBSC and TFBS in PET were lighter than that were packed in PP. L* of TFBS-PET (61.63±1.00) was significantly higher than FBSC-PET (57.83±1.69), followed by TFBS-PP (56.57±0.25) and FBSC-PP (54.63±0.59) ($p \geq 0.05$) (Figure 4.6). Similar results were shown in the samples stored at room temperature. PET could protect the bamboo shoot better than PP. The highest lightness was found in TFBS-PET, which was 63.03±0.64 and significantly different when compared with FBSC-PET (60.47±1.53), followed by FBSC-PP (59.37±1.57) and TFBS-PP (56.63±0.58) (Figure 4.7). At 35 °C, L* of TFBS and FBSC were not significantly different, although different types of plastic bags were used. L* of FBSC-PET, TFBS-PET, and FBSC-PP were 55.33±0.93, 56.13±1.05 and 56.70±0.56, respectively. L* of TFBS-PP (58.53±0.21) was

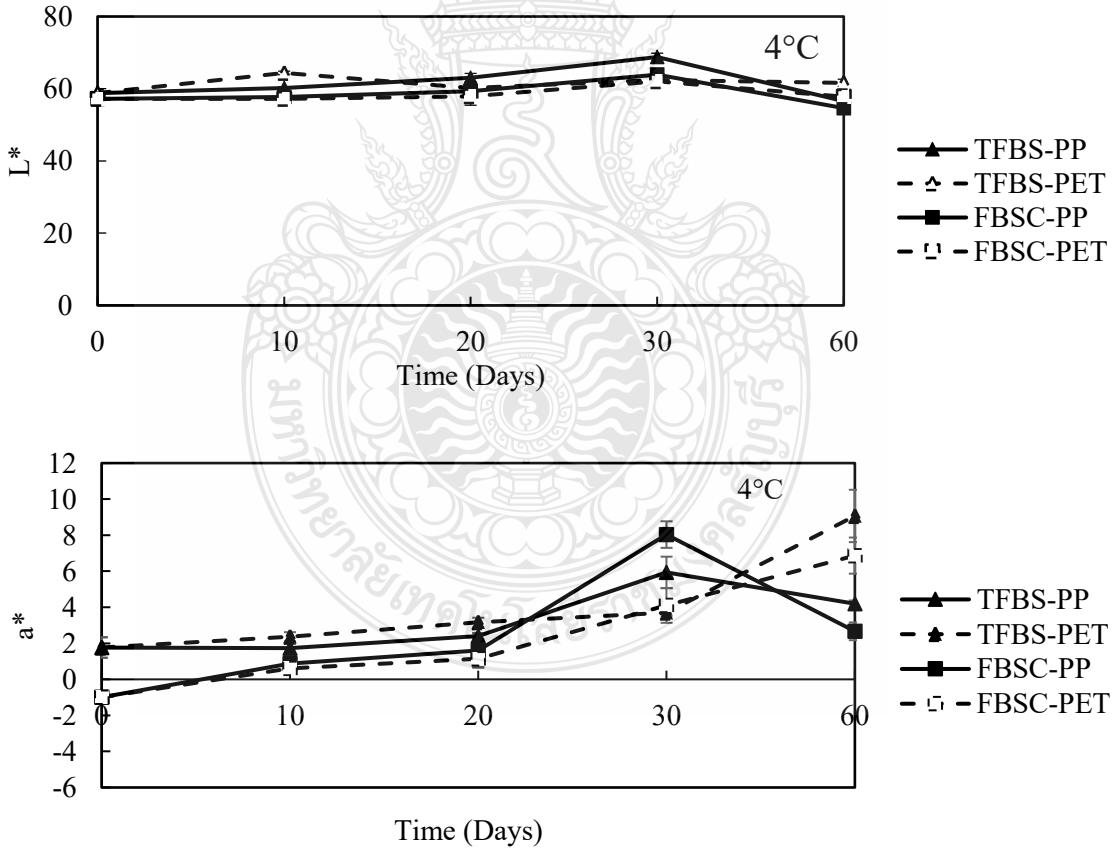
significantly greater than others but not significantly different when compared with FBSC-PP (Figure 4.8).

For the greenness (a*), TFBS-PET stored in 4°C for 60 days showed higher a* than other treatments (9.08±1.45), while FBSC-PP had the lowest a* (2.67. ±0.49). The a* could indicate a darker brown color of the pickled bamboo shoots. For 35°C, it was found that FBSC-PP (3.63±0.15) was significantly higher than FBSC-PET (3.27±0.31), followed by FBSC-PET (5.53±0.25) and FBSC-PP (5.37±0.59), which were not significantly different ($p \geq 0.05$) (Figure 4.7 and Figure 4.8). The results showed that storage at room temperature (RT) or 35 °C had effects on increase of redness. The total color difference (TCD) is an index of color change during pasteurized treatment. The color change of greater than 2 is visible. According to our study, TCD was found to increase during storage and reached to 12.99±0.94 for FBSC-PP) and 4.62±1.25 for TFBS-PP, when they were stored at 4 °C (Figure 4.9). This indicated that temperature affected the color changes during storage.

According to the results, color changes could be occurred due to instability of pigments in the bamboo shoots. It was found that unstable pigments (such as chlorophyll and anthocyanin) in bamboo shoot cells were degraded at different extents [119]. The total color difference obviously increased as the temperature increased as a result of the increase in b*. Therefore, the slices turned a darker shade of yellow at a higher temperature, which might be attributed to higher surface color degradation and non-enzymatic browning resulting from the air temperature increase [120] [121]. Moreover, the color change in bamboo shoot during fermentation might be due to the chemical reaction. The chemical reaction mainly includes non- enzymatic browning, enzymatic browning, and maillard reaction. Fresh bamboo shoots are generally yellowish green, because the bamboo shoot cells contain lutein and chlorophyll. During the fermentation process, the acidic environment degrade pigments such as lutein and chlorophyll, and the bamboo shoots can undergo maillard reaction during fermentation, resulting in browning, and loss of original color [122] [123] [124]**.** Ngadze et al. (2018) [125] reported that the enzyme bleaching by hot treatments would result in a lighter product through inactivation of the endogenous enzymes by the pre-incubation step (100°C for 10 min). During hot extractions other mechanisms such as non-enzymatic

browning would have occurred such as phenolic compounds (PCs) metal ion complexes (mainly Fe and Cu), ascorbic acid degradation and/or Maillard reactions, resulting in the mixed effects on color that occurred.

Moreover, it was reported that salts could delay browning reaction. Their efficiency was different deepened upon their chemical composition. Anti- browning activity of salts was greatest when sodium, potassium, or calcium ions bind with chloride or phosphate, followed by sulfate and nitrate. Polyphenol oxidase (PPO) activity of tissue extracted from chloride- and fluoride-treated slices was not different to control but when added into the assay solution, NaF > NaCl both showed lower PPO activity at pH 3–5 compared to control buffer. The level of polyphenols in treated slices was NaF > NaCl > control. Addition of chlorogenic acid to slices enhanced browning but NaCl and NaF counteracted this effect [126].



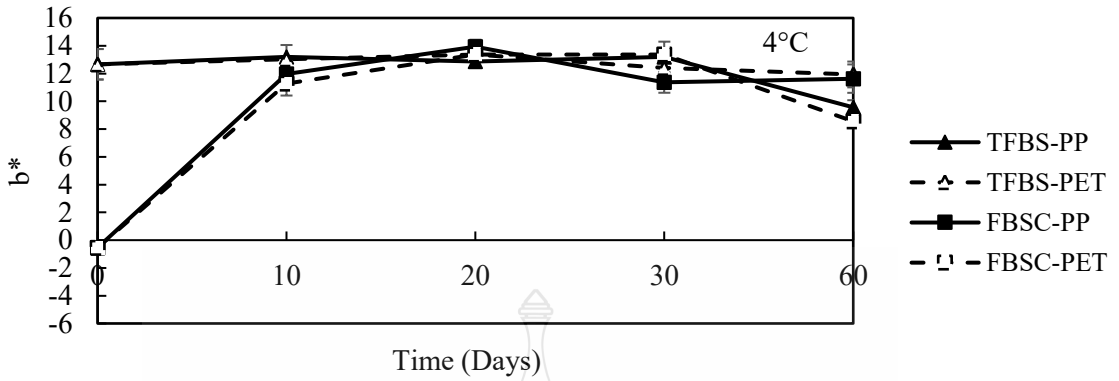
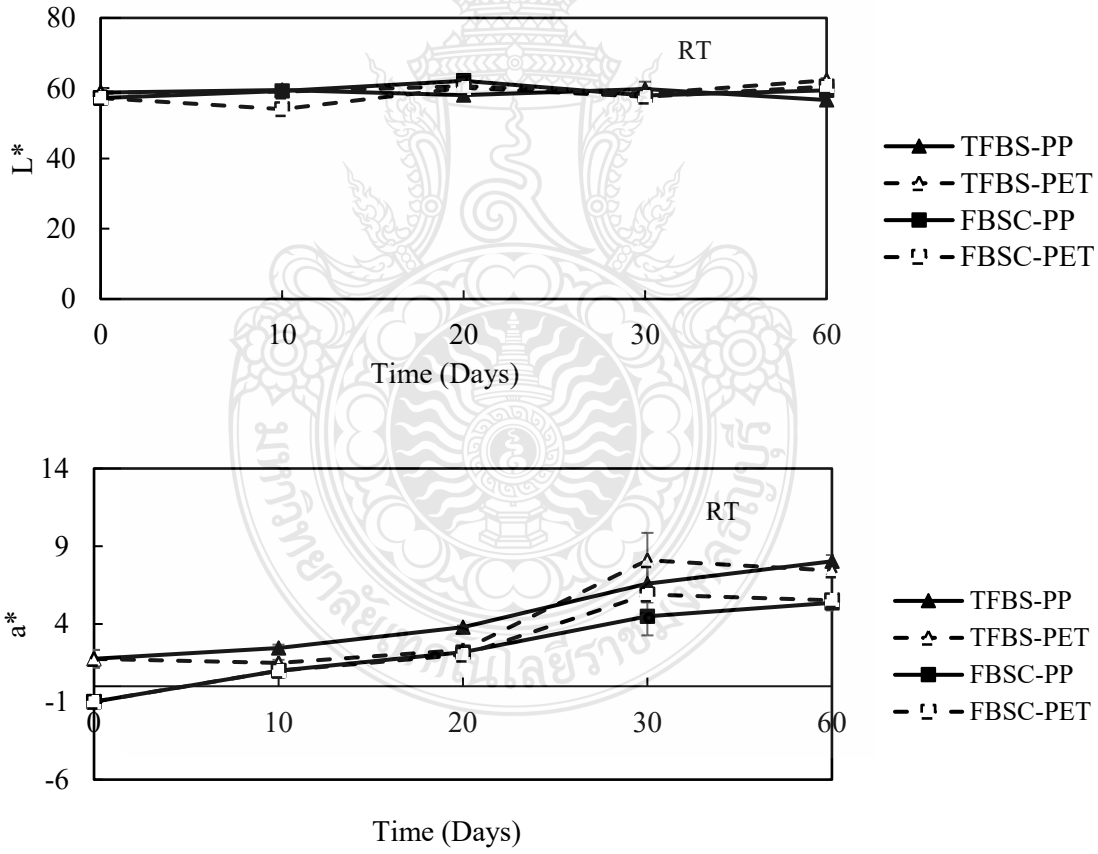


Figure 4.6 The color of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C for 60 days.



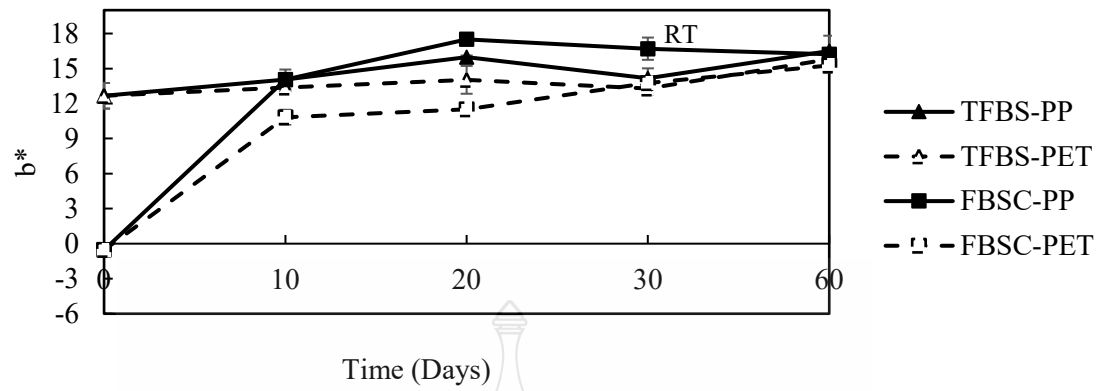
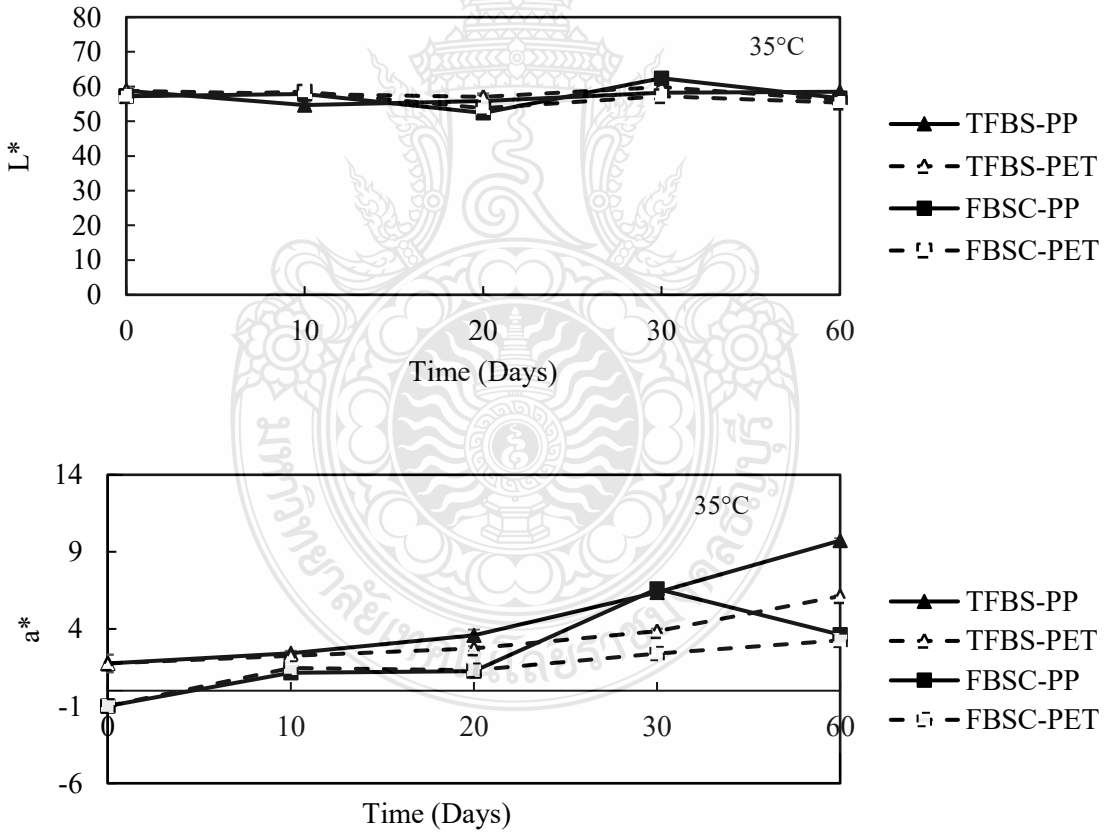


Figure 4.7 The color of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at room temperature (RT) for 60 days.



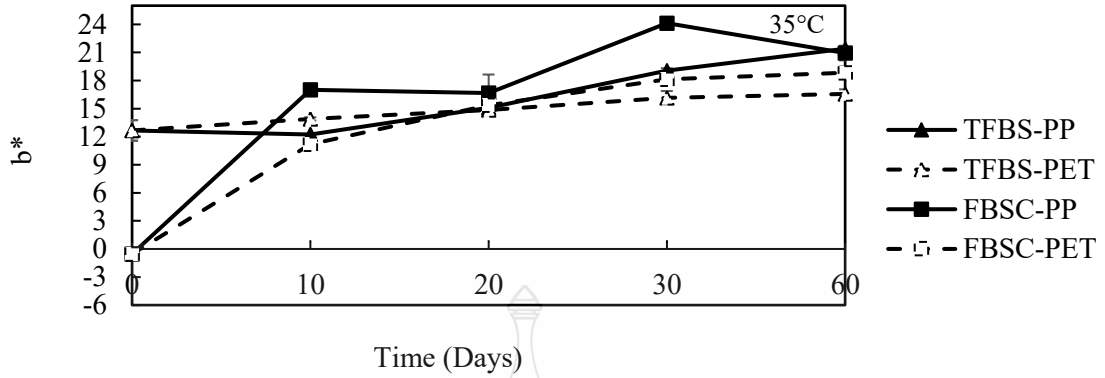
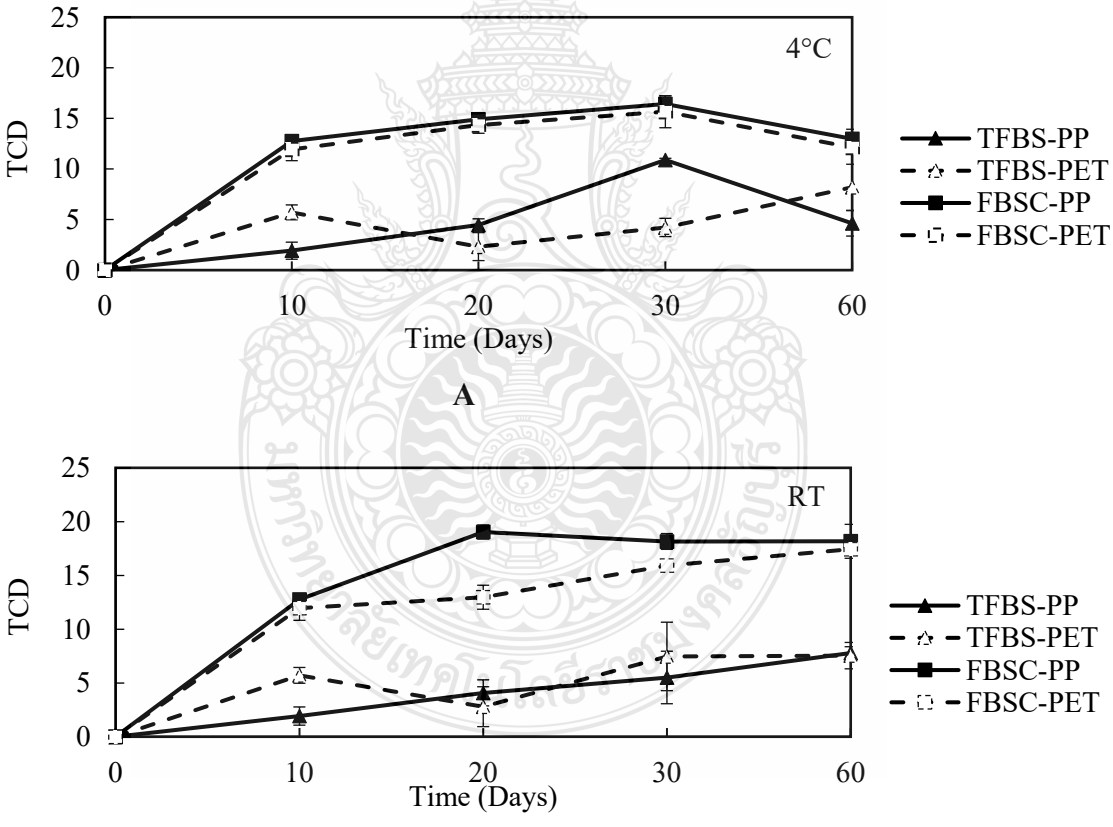
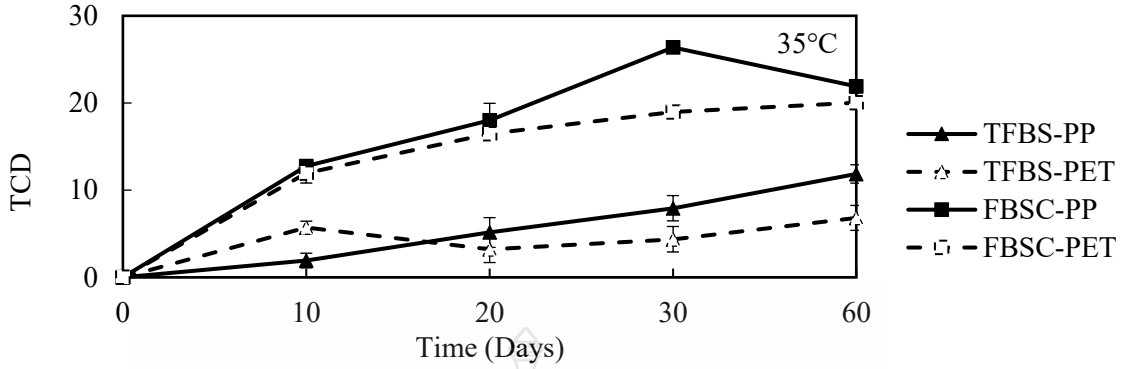


Figure 4.8 The color of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 35 °C for 60 days.



A

B



C

Figure 4.9 Total color difference of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C (A), room temperature (B), 35 °C (C), for 60 days.

4.3.2 pH and total acidity

The changes in pH of TFBS and FBSC during storage at different conditions were shown in Figure 4.10. The pH of FBSC and TFBS on the day 0 was 4.67±0.01 and 4.29±0.05, respectively. After storage for 60 days at 4 °C, pH of all samples were decreased (Figure 4.10A), however they were not significantly different. At RT, pH ranged between 3.29±0.04 (FBSC-PET) and 4.54±0.02 (TFBS-PP) shown in Figure 4.10B. At 35 °C, the maximum pH value was found in TFBS-PP, which was 4.46±0.01and the lowest pH was 3.44±0.05 belonging to FBSC-PET (Figure 4.10C). During storage, TA was significantly increased (Figure 4.11). Regardless fermentation methods, PP and PET bags were found that there were non-significant differences. Niazmand et al. (2021) observed that the changes in titratable acidity content of barberries packaged in different films followed an upward trend until the end of the third month. After that, until the end of the storage period, the changes were almost negligible. After 6 months, the average acidity of all samples was 2.4 g/100 g, which indicates a 38% increase compared with the initial value [131].

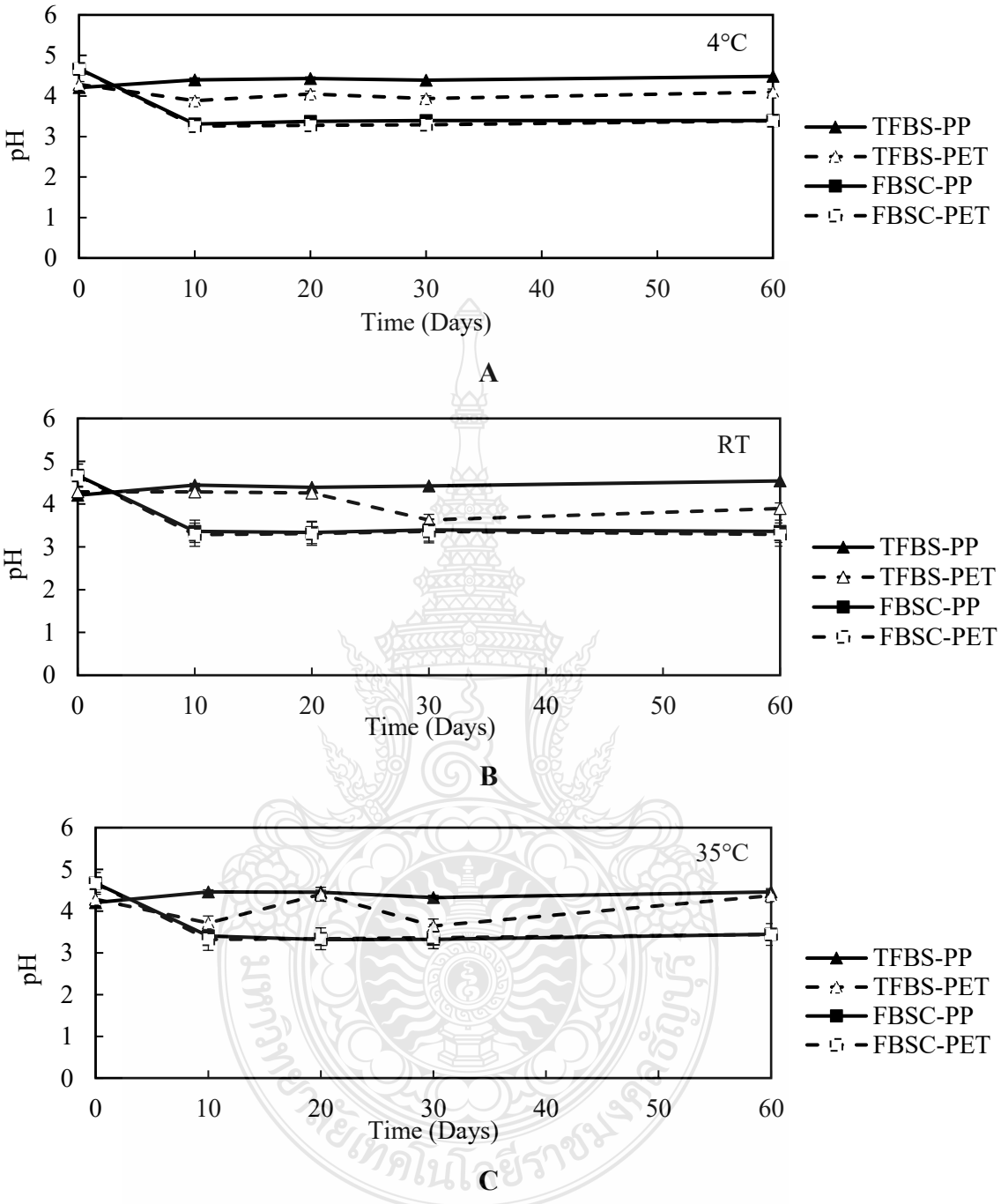


Figure 4.10 pH changes of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or poly polyethylene terephthalate (PET) during storage at 4 ºC (A), room temperature (B), 35 ºC (C) for 60 days.

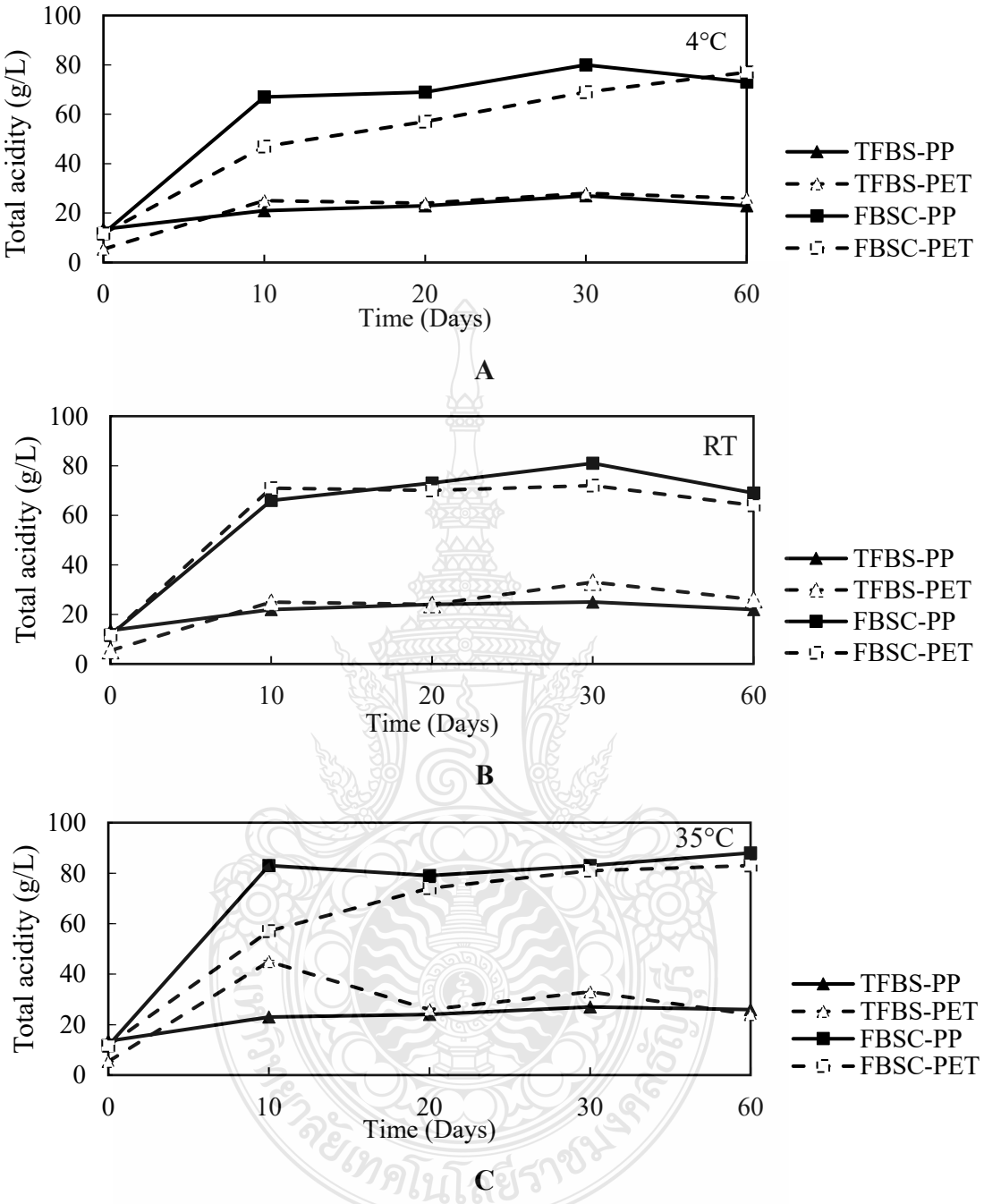


Figure 4.11 Total acidity of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or poly polyethylene terephthalate (PET) during storage at 4 ºC (A), room temperature (B), 35 ºC (C) for 60 days.

4.3.3 Microorganisms

During storage, TFBS and FBSC were measured for microorganism analysis. The results showed that all of the sample had of total plate count bacteria, coliforms, yeast and mold less than 30 CFU/g. Storage conditions have an important effect on the quality of fermented foods through the activity of various microbes. Numerous studies have been conducted to evaluate the quality characteristics of fermented bamboo shot products during storage.

Cho and Song (2021) investigated the inactivation effect against the various microorganisms in korean vegetables “doenjang” by conventional heat treatment, total bacterial counts were examined with a change in heating temperature. The total count of bacteria was reduced by less than 10^1 CFU/g when heated for 60 min at around 75 – 85 °C, which is the condition for pasteurization in the industrial product of doenjang. At the heating temperature of 105 °C for 20 min, the inactivation effect was partially improved, showing a reduction of 10^1 – 10^2 CFU/g of the total count of bacteria. An amount of 10^1 – 10^2 CFU/g of the mold, yeast, and vegetative cells with weak heat resistance are killed at (100–105) °C heating [132]. Traditional pasteurization refers to heat treatment of food (usually below 100 °C) to destroy micro-organisms of public health significance. Pasteurization processes used in the industry do kill microorganisms in foods; they only target pertinent pathogens and lower levels of spoilage organisms that may grow during storage and distribution [133]. For 1 min at 70 °C was sufficient to achieve a one-log reduction of all the other vegetative bacteria, such as *E. coli* and *Salmonella* spp. Conversely a few seconds at 60 °C is enough to have 90% of *Aeromonas hydrophila* and yeast, which have the lowest heat resistance, inactivated [134].

4.4 Improvement of fermented bamboo shoot stability during storage

4.4.1 Characteristics of safflower oil (SO) emulsion for soaking solution

The stability of SO emulsion prepared by different conditions was determined and shown in Table 4.1. There were no emulsions separating into an opaque white layer at the top and a turbid layer and/or a clear layer at the bottom at 0 day. However, after 3 days of storage, a thin layer of oil was found at the top of all treatments of SO homogenized or sonicated with sodium chloride (S) and that was homogenized

with citric acid (C) for 2 min. Preparation of SO emulsion in citric acid contained sodium chloride (SC) could maintain stability of SO emulsion. The particle size distribution of the SO emulsions was measured and shown in Table 4.2. The esterification can offer the emulsion with smaller size by increasing the interaction between oil and water phase [135]. Citric acid had a notable effect on the oil droplet size distributions of the emulsions. The carboxyl groups of citric acid could form new hydrophobic surfactant [136].

Table 4.1 Stabiltiy of safflower oil (SO) emulsion in different solutions

Methods	SO (g/100 mL)	Solutions	Retention time (min)			
			2	5	10	15
Homogenization	0.1	C	+	-	-	-
		S	+	+	+	+
		SC	-	-	-	-
	0.2	C	+	-	-	-
		S	+	+	+	+
		SC	-	-	-	-
Ultrasonication	0.1	C	-	-	-	-
		S	+	+	+	+
		SC	-	-	-	-
	0.2	C	-	-	-	-
		S	+	+	+	+
		SC	-	-	-	-

NOTE: (–) not separated (+) separated SO = safflower oil, C = citric acid, S = sodium chloride, and SC = mixture of sodium chloride and citric acid

Table 4.2 The change emulsion of soaking solutions curve of droplets

Treatments	D [4,3] (μm)
0.1SCO-H	37
0.2SCO-H	69.6
0.1SCO-U	1.31
0.2SCO-U	1.47

NOTE: 0.1SCO-H High spread homogenization 14,000 rpm Time 2min, 0.1% Safflower Essential Oil (SO), (0.2SCO-H) High spread homogenization, 0.2% (SO), (0.1SCO-U) Ultrasonication Amp 80% Time 2 min, 0.1% SO (0.2SCO-U) Ultrasonication Amp 80% Time 2 min, 0.2% SO.

The total phenolic contents of control at day 0 was 34.41±0.79 mg/g. In the present study, the phenolic content in the fermented bamboo shoot sample was found to be 49.03±1.70 mg/g (control), 68.65±0.39 mg/g (SC), 67.42±2.23 mg/g (SCO-H) and 69.41±0.92 mg/g (SCO-U) for 7 days. The phenolic content of the control was less than that of the SCO-H, SC and SCO-U samples, respectively. Browning of fruit and vegetables was related to increasing phenylalanine ammonia-lyase activity and increased levels of phenolic compounds [137]. Changes in colour were correlated with total phenolic content. The phenolic contents of fermented bamboo shoot in laminate (V) and polypropylene bags were similar and higher than those stored in low density polyethylene (LDPE) packaging. The quinone and lignin of fermented bamboo shoot stored in V bags were lower than those stored in PP and LDPE bags [139]. The variation in the total phenolic content was attributed to many factors including genotype, agronomic practices, maturity level at harvest, postharvest storage, climatic and geographical locations. Phenolic acids and their derivatives and flavanoids are dominant phenolic compounds in seeds rich oil. The antioxidant capacities of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule. Several studies indicated that phenolic acids esterified with a group such as sugar enhance antioxidant potency of the molecule [140] [141]. However, tocol content together with unsaturation degree of oil have dramatic impact on its oxidative stability. Antioxidant activity of tocol depends on their chemical nature and concentration [142]. Safflower oil had almost 2/3 times more tocol level than

that of poppy oil; however, its oxidative stability value was half of poppy oil induction period. This might be resulted by ever small amount polyunsaturates and long-chain fatty acids present in safflower oil negatively impacted the oxidative stability [143]. The total phenolic content of the methanolic root extract, calculated from the calibration curve (R^2 = 0.998), was 45.17± 1.70 gallic acid equivalents/g. Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity [144].

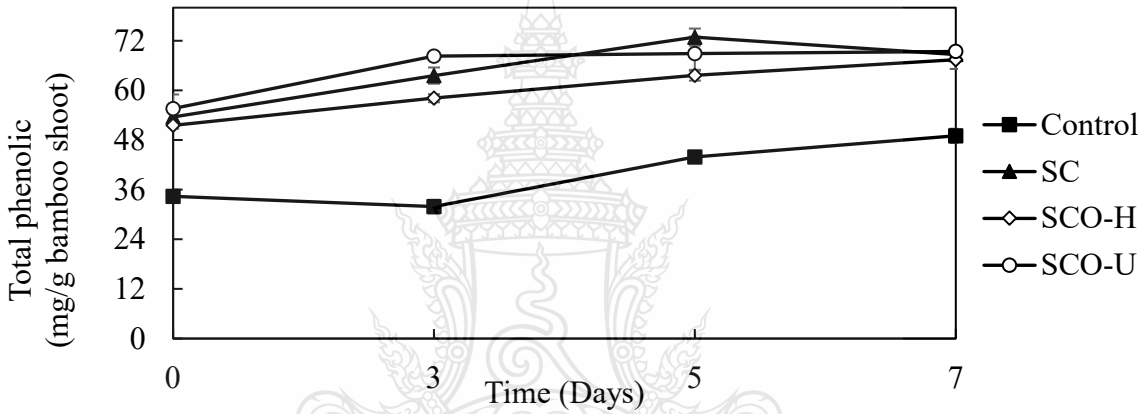


Figure 4.12 Total phenol content of fermented bamboo shoots with coconut water (FBSC) in commercial product (control), sodium chloride and citric acid (SC), sodium chloride and citric acid by high spread homogenization and sodium chloride and citric acid by ultrasonication Amp 80% Time 2 min during storage at 55 °C for 7 days.

Plants rich in secondary metabolites, including phenolics and antioxidant activity due to their redox properties and chemical structures. The bamboo shoot extract had strong antioxidant activity. Similar results were reported by Sonar et al. (2015) [146]. The methanolic extracts of different fermented bamboo shoot samples exhibited significant free radical scavenging activity ranging between 70.84 and 95.37%. Badwaik et al. (2014) [27] reported that antioxidant activity increased from 26.67% to 49.20% and 55.35% after 8 days of bamboo shoot natural anaerobic fermentation and that mixed with

Garcinia pedunculata Roxb., respectively. Safflower seed oil could exhibit high scavenging effects against DPPH free radicals [147]. Antioxidants can react with oxygen to suppress the initiation of browning. They were also able to react with the intermediate products, thereby breaking the chain reaction and inhibiting melanin formation [148].

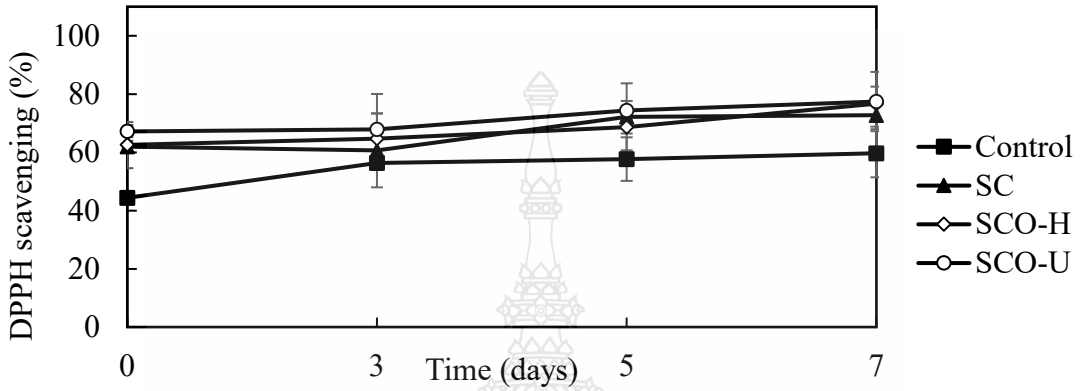


Figure 4.13 Antioxidation activity by DPPH assays of fermented bamboo shoots with coconut water (FBSC) in commercial product (control), sodium chloride and citric acid (SC), sodium chloride and citric acid by high spread homogenization and sodium chloride and citric acid by ultrasonication Amp 80% Time 2 min during storage at 55 °C for 7 days.

4.4.2 Color

Color of fermented bamboo shoots showed noticeable differences compared to the control (Figure 4.14 and Table 4.3). The colour changes of the Fermented bamboo shoot solution were observed to occur more rapidly throughout storage with increasing the storage temperature. The results are in accordance with the findings of various researchers [149]. The slightly higher yellowness (b*) values of treated products may be attributed to the oil base and antioxidants present in bamboo essential oil. Moreover, colour shades that these oils present could justify the increase in b*, since oils have a yellowish coloration [150]. TCD was found to increase with accelerated temperature and reached a value of 6.93±6.94 (Control), 9.62±7.15 (SC), 18.95±4.38 (SCO-H) and 19.83±7.75 (SCO-U) shown in Figure 4.14.

Table 4.3 Chang color fermented of bamboo shoot at 0 to 7 days for 55 °C.

Treatment	Time (Days)			
	0	3	5	7
Control				
SC				
SCO-H				
SCO-U				

NOTE: (Control) Commercial, (SC) Sodium chloride + Citric acid, (SCO-H) Sodium chloride + Citric acid + oil (High spread homogenization), (SCO-U) Sodium chloride + Citric acid + oil (Ultrasonication)

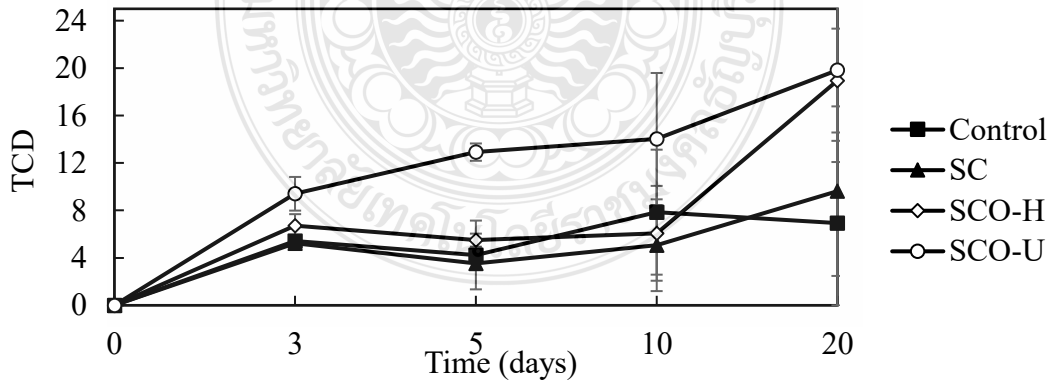


Figure 4.14 Total color difference of fermented bamboo shoots with coconut water (FBSC) in commercial product (control), sodium chloride and citric acid (SC), sodium chloride and citric acid by high spread homogenization and sodium chloride and citric acid by ultrasonication Amp 80% Time 2 min during storage at 55 °C for 20 days.

4.4.3 Sensory evaluation

FBS with the following recipe: control, TFBS, FBSC, and FBSC-SCO cooked with egg with the same ratio of control seasonings in all samples.

The results showed that the sensory attributes of taste in the FBSC (SCO) samples were similar to those for the untreated ones. FBSC-SCO processed by homogenization received a higher score for the attributes of flavor appearance and overall probably because a better brightness (L* value) and less salty taste were found they are shown in Table 4.4. However, commercial samples or control samples that were soaked in a solution with sulfide became a white color of fermented bamboo shoots. It was found that consumers did not accept control because they were more aware of the negative effects of chemicals in food, samples without chemicals are therefore overall acceptability.

Table 4.4 Sensory evaluation of soaking solutions on fermented bamboo shoots during storage.

Treatment	Color	Flavor	Taste	Texture	Appearance	Overall Acceptability
Control	6.50 ± 1.89^{a}	4.83 ± 2.05^{b}	3.33 ± 2.09^{b}	4.65 ± 2.08^{c}	6.60 ± 1.85^{a}	3.93 ± 2.00^{b}
TFBS	5.35 ± 1.59^{b}	4.73 ± 2.29^{b}	4.08 ± 2.35^{b}	5.60 ± 1.93^{b}	5.23 ± 1.73^{c}	4.73 ± 2.26^{b}
FBSC	6.15 ± 1.66^{a}	5.55 ± 1.91^{ab}	5.88 ± 1.92^{a}	6.90 ± 1.24^{a}	5.78 ± 1.79^{bc}	6.25 ± 1.82^{a}
FBSC + SCO	6.63 ± 1.19^{a}	6.33 ± 1.62^{a}	5.43 ± 1.96^{a}	6.68 ± 1.53^{a}	6.50 ± 1.34^{ab}	6.30 ± 1.74^{a}

All data were the mean ± S.D, Values with different letters within one column are significantly different ($p < 0.05$).

***** Control: commercial product

CHAPTER 5
CONCLUSION

Mature coconut water could improve fermentation process of bamboo shoots. The number of lactic acid bacteria was higher than the traditional method. PET bags and salt played important roles in delaying browning reaction of fermented bamboo shoots when they were stored at 4 °C and room temperature. However, if the storage temperature was high as 35 °C, L* indicating lightness of the shoots was not depended on types of plastic bags, PP or PET. Safflower oil emulsion used as soaking solution could be prepared by both homogenization and sonication at the oil concentration of 0.1% and 0.2% without any emulsifiers. Sonication provided smaller oil droplets affecting quality of the fermented bamboo shoots during storage. Addition of safflower oil emulsion prepared in citric acid containing sodium chloride into fermented bamboo shoots helped prevent the bamboo shoots from color changes better than the emulsion with only sodium chloride, or only citric acid. According to sensory evaluation, overall scores of consumer acceptance of the fermented bamboo shoots with coconut water was over the traditional one and addition of safflower oil emulsion did not affect the consumer acceptance.

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APPENDICES

Appendix A

Determinations

Table 4.5 The pH and total acidity of traditional fermented bamboo shoots (TFBS) during storage at 4°C, room temperature, and 35°C for 28 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH0W	Between Groups	.000	2	.000	.000	1.000
	Within Groups	.004	3	.001		
	Total	.004	5			
pH28D	Between Groups	.029	2	.015	29.033	.011
	Within Groups	.001	3	.000		
	Total	.031	5			

pH0W

	pH	N	Subset for alpha = 0.05
			1
Duncan[a]	4C	2	3.1850
	25C	2	3.1850
	35C	2	3.1850
	Sig.		1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

pH28D

	pH	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	4C	2	3.3550		
	35C	2		3.4500	
	25C	2			3.5250
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TA0D	Between Groups	.000	2	.000	.000	1.000
	Within Groups	.004	3	.001		
	Total	.004	5			
TA28D	Between Groups	.029	2	.015	29.033	.011
	Within Groups	.001	3	.000		
	Total	.031	5			

TA0D

	TA	N	Subset for alpha = 0.05
			1
Duncan[a]	4C	2	3.1850
	25C	2	3.1850
	35C	2	3.1850
	Sig.		1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

TA28D

	TA	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	4C	2	3.3550		
	35C	2		3.4500	
	25C	2			3.5250
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 4.6 The color of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C, room temperature, and 35°C for 60 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TFBSpp	Between Groups	7.482	2	3.741	25.508	.001
	Within Groups	.880	6	.147		
	Total	8.362	8			
TFBSpet	Between Groups	79.820	2	39.910	21.304	.002
	Within Groups	11.240	6	1.873		
	Total	91.060	8			
FBSCpp	Between Groups	33.787	2	16.893	16.261	.004
	Within Groups	6.233	6	1.039		
	Total	40.020	8			
FBSCpet	Between Groups	39.536	2	19.768	9.818	.013
	Within Groups	12.080	6	2.013		
	Total	51.616	8			

TFBSpp

			Subset for alpha = 0.05	
	TmL	N	1	2
Duncan[a]	4C	3	56.5667	
	RT	3	56.6333	
	35C	3		58.5333
	Sig.		.838	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TFBSpet

	TmL	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	35C	3	56.1333	
	4C	3		61.6333
	RT	3		63.0333
	Sig.		1.000	.257

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

FBSCpp

	TmL	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	4C	3	54.6333		
	35C	3		56.7000	
	RT	3			59.3667
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

FBSCpet

	TmL	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	35C	3	55.3333	
	4C	3	57.8333	57.8333
	RT	3		60.4667
	Sig.		.074	.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 4.7 Total color different of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C, RT and 35 °C for 60 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
T4	Between Groups	133.510	3	44.503	23.268	.000
	Within Groups	15.301	8	1.913		
	Total	148.811	11			
TRT	Between Groups	309.306	3	103.102	94.274	.000
	Within Groups	8.749	8	1.094		
	Total	318.056	11			
T35	Between Groups	449.448	3	149.816	142.991	.000
	Within Groups	8.382	8	1.048		
	Total	457.829	11			

T4

	TCD	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	TFBS-PP	3	4.6207		
	TFBS-PET	3		8.1898	
	FBSC-PET	3			12.1115
	FBSC-PP	3			12.9873
	Sig.		1.000	1.000	.460

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TRT

	TCD	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	TFBS-PET	3	7.5387	
	TFBS-PP	3	7.7818	
	FBSC-PET	3		17.4335
	FBSC-PP	3		18.1656
	Sig.		.783	.416

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

T35

	TCD	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	TFBS-PET	3	6.8144		
	TFBS-PP	3		11.8575	
	FBSC-PET	3			20.0300
	FBSC-PP	3			21.9085
	Sig.		1.000	1.000	.055

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 4.8 pH changes of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or poly polyethylene terephthalate (PET) during storage at 4 ºC, room temperature, 35 ºC for 60 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
P4	Between Groups	2.668	3	.889	762.419	.000
	Within Groups	.009	8	.001		
	Total	2.678	11			
PRT	Between Groups	3.021	3	1.007	1.492E3	.000
	Within Groups	.005	8	.001		
	Total	3.027	11			
P35	Between Groups	2.843	3	.948	1.223E3	.000
	Within Groups	.006	8	.001		
	Total	2.849	11			

P4

	pH	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	FBSC-PET	3	3.3867		
	FBSC-PP	3	3.3900		
	TFBS-PET	3		4.1000	
	TFBS-PP	3			4.4833
	Sig.		.908	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PRT

	pH	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	FBSC-PET	3	3.2867			
	FBSC-PP	3		3.3633		
	TFBS-PET	3			3.8933	
	TFBS-PP	3				4.5400
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

P35

	pH	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	FBSC-PET	3	3.4400		
	FBSC-PP	3	3.4467		
	TFBS-PET	3		4.3733	
	TFBS-PP	3			4.4567
	Sig.		.777	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 4.9 Total acidity of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or poly polyethylene terephthalate (PET) during storage at 4 ºC, room temperature, 35 ºC for 60 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
T4	Between Groups	8.542	3	2.847	341.700	.000
	Within Groups	.067	8	.008		
	Total	8.609	11			
TRT	Between Groups	6.089	3	2.030	135.315	.000
	Within Groups	.120	8	.015		
	Total	6.209	11			
T35	Between Groups	12.249	3	4.083	544.407	.000
	Within Groups	.060	8	.007		
	Total	12.309	11			

T4

			Subset for alpha = 0.05	
	TA	N	1	2
Duncan[a]	TFBS-PP	3	.7667	
	TFBS-PET	3	.8667	
	FBSC-PP	3		2.4333
	FBSC-PET	3		2.5667
	Sig.		.217	.111

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TRT

	TA	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	TFBS-PP	3	.7333	
	TFBS-PET	3	.8667	
	FBSC-PET	3		2.1333
	FBSC-PP	3		2.3000
	Sig.		.219	.134

Means for groups in homogeneous subsets are displayed.

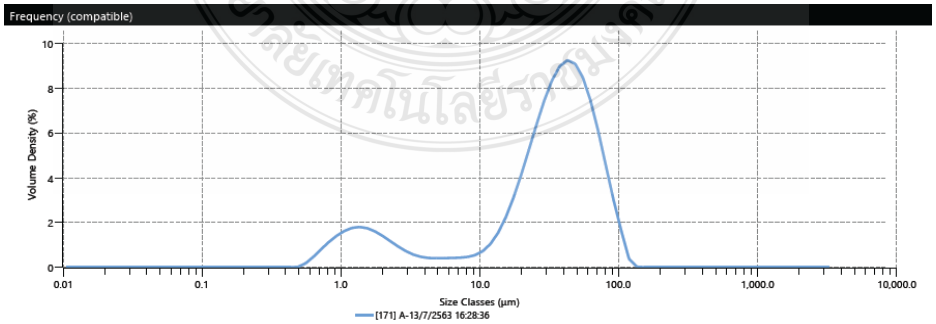
a. Uses Harmonic Mean Sample Size = 3.000.

T35

	TA	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	TFBS-PET	3	.8000		
	TFBS-PP	3	.8667		
	FBSC-PET	3		2.7667	
	FBSC-PP	3			2.9333
	Sig.		.373	1.000	1.000

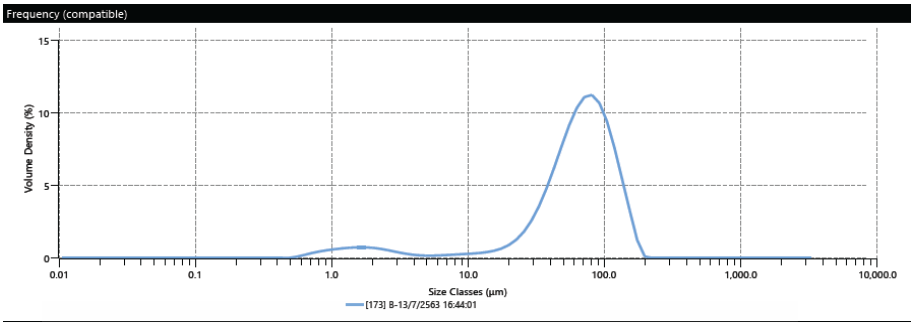
Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.



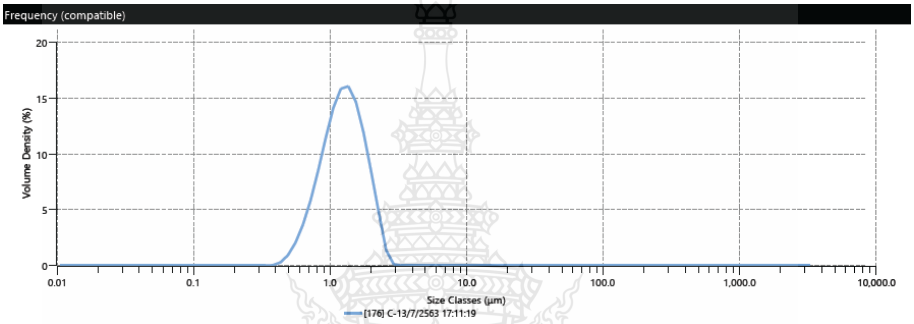
A

D [4,3] 37µm



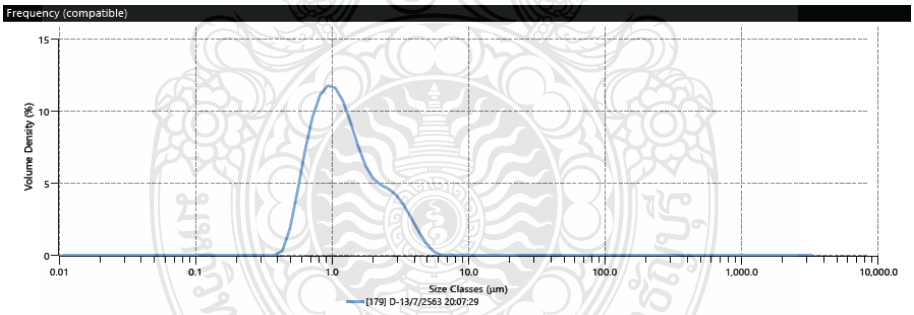
B

D [4,3] 69.6 µm



C

D [4,3] 1.31µm



D

D [4,3] 1.47µm

Figure 4.15 The change emulsion of soaking solutions curve of droplets (A) High spread homogenization 14,000 rpm Time 2min, 0.1%Safflower Essential Oil (SO), (B) High spread homogenization, 0.2%(SO), (C) Ultrasonication Amp 80% Time 2 min, 0.1% SO (D) Ultrasonication Amp 80% Time 2 min, 0.2% SO.

Table 4.10 Total phenol content of fermented bamboo shoots with coconut water (FBSC) in commercial product (control), sodium chloride and citric acid (SC), sodium chloride and citric acid by high spread homogenization and sodium chloride and citric acid by ultrasonication Amp 80% Time 2 min during storage at 55 °C for 7 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
day0	Between Groups	23989.982	3	7996.661	134.148	.000
	Within Groups	238.443	4	59.611		
	Total	24228.425	7			
day7	Between Groups	11533.657	3	3844.552	108.638	.000
	Within Groups	141.555	4	35.389		
	Total	11675.211	7			

day0

			Subset for alpha = 0.05		
	Phenolic	N	1	2	3
Duncan[a]	control	2	1.3763E2		
	SC	2		2.5430E2	
	SCOH	2		2.5466E2	
	SCOU	2			2.7763E2
	Sig.		1.000	.964	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

day7

	Phenolic	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	control	2	127.6250	
	SCOH	2		206.1450
	SC	2		214.3000
	SCOU	2		222.4450
	Sig.		1.000	.055

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 4.11 Antioxidation activity by DPPH assays of fermented bamboo shoots with coconut water (FBSC) in commercial product (control), sodium chloride and citric acid (SC), sodium chloride and citric acid by high spread homogenization and sodium chloride and citric acid by ultrasonication Amp 80% Time 2 min during storage at 55 °C for 7 days.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
d0	Between Groups	23.262	3	7.754	1.710	.302
	Within Groups	18.137	4	4.534		
	Total	41.399	7			
d7	Between Groups	272.691	3	90.897	6.574	.050
	Within Groups	55.310	4	13.827		
	Total	328.001	7			

d0

	DPPH	N	Subset for alpha = 0.05
			1
Duncan[a]	SCOU	2	86.6129
	SCOH	2	89.8589
	control	2	90.5629
	SC	2	90.9274
	Sig.		.118

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

d7

	DPPH	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	control	2	79.0366	
	SCOU	2	82.7001	
	SCOH	2	85.2442	85.2442
	SC	2		94.8100
	Sig.		.176	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Biography

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