

Fermentation of bamboo shoots using mature coconut water and its stability during storage at different conditions

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ABSTRACT

Fermented-bamboo-shoots are a common raw ingredient used in several Thai local dishes. However, contamination of harmful and undesirable microorganisms in the fermented bamboo shoots has been reported due to improper and long fermentation processes. Mature coconut water (MCW) could shorten the bamboo shoot fermentation process and improve the product quality. This study aimed to develop fermented bamboo shoots using mature coconut water and determine their stability during storage in different conditions. Bamboo shoots from Prachinburi province were mixed with 25 g/kg of Kosher salt and fermented with MCW to obtain fermented bamboo shoots with coconut water (FBSC) at room temperature. Traditional fermented bamboo shoots (TFBS), prepared using 100 g/kg of Kosher salt, were used as a control. After fermentation, the samples were packed in polypropylene (PP) or polyethylene terephthalate (PET) bags and stored at different temperatures for 60 days. The number of bacteria, pH, and total acidity (TA) were determined during fermentation and storage, as well as the color changes of the shoots. The results indicated that in the fermentation process, MCM with low salt concentration could effectively increase the number of lactic acid bacteria (LAB) greater than the traditional method. The number of LAB in FBSC was 9.78±0.43 CFU/mL of LAB, while TFBS had 8.08±0.02 log CFU/mL. Lower pH was significantly found in FBSC (3.89±0.01) than in TFBS (4.00±0.01), while TA of FBSC was significantly higher than that of TFBS, which were 11.25 ± 0.01 g/L and 6.00 ± 0.01 g/L, respectively. During storage, it was found that fermentation methods, types of packaging, and storage temperatures significantly affect the shoot colors but not pH and TA. PET bags could better delay the browning reaction of the fermented shoots than PP bags. The higher storage temperatures were, the faster color changes were observed. This study indicated that MCW could be used as fermentation media for bamboo shoots.

Keywords: Bamboo shoots, Coconut water, Fermentation, Stability

INTRODUCTION

Dendrocalamus asper, called in Thai Phai Tong, is one of the important bamboo species found in Thailand, especially in Prachinburi Province. They are commonly used for their edible shoots as they are sweet and have a unique taste. In addition, it was reported that the shoots contain certain nutritional values, including protein, carbohydrate, and fiber. They also have a good profile of minerals, consisting of potassium, calcium, manganese, and zinc [1, 2]. Since the shoots are very perishable and have a limited shelf life, They are generally preserved by boiling, drying, or fermenting [3, 4]. Fermentation of bamboo shoots is the most popular method to preserve the shoots as it is simple, requires less labor, and low cost. The fermented bamboo shoots could be prepared by peeling them and then slicing them 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 [4-6]. However, several reports mentioned the negative feedback of fermented bamboo shoots. Due to the long fermentation period, the fermented shoots could be contaminated with undesirable microorganisms such as *Escherichia coli, Staphylococcus aureus*, or *Clostridium botulinum* during fermentation and storage [7].

J Appl Res Sci Tech 2024;23(2):255918

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Mature coconut water (MCW) is a waste product from the coconut milk industry. In Thailand, MCW might produce nata de coco, while some are discharged directly into the drain in large quantities, roughly 200,000 tons/year [8]. MCW comprises several types of sugar, such as mainly sucrose, sorbitol, glucose, and fructose, followed by minor sugars, including galactose, xylose, and mannose [9]. It was used as a rich, nutritious media for lactic acid bacteria fermentation [8, 10] reported that MCM was used as media for probiotic Lactobacillus plantarum DW12. 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. Similarly, it could effectively grow Lactobacillus *casei* Shirota from 7.4 x 10⁸ CFU/mL to 2.5 x 10⁹ CUF/mL after 12 h of fermentation [11]. These indicated that MCM could be used as a medium to shorten the fermentation time and reduce contamination risks. This study aimed to investigate the effects of MCW as a media on bamboo shoot quality during fermentation and storage.

MATERIALS AND METHODS

Preparation of mature coconut water (MCW)

Freshly mature coconut water (MCW) was purchased from the 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 [12].

Bamboo shoot fermentation

Fresh bamboo shoots from Prachinburi Province were peeled, sliced, and soaked in tap water for 1 h to remove cyanogen [13]. The shoots were mixed with 25 g/kg of Kosher salt, less than the traditional method of 75%. The shoot was left at room temperature for 30 minutes before being 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 FBSC, were incubated at room temperature until the pH was below 4.6. Traditional fermented bamboo shoots (TFBS) were used as a control.

TFBS was prepared by following a traditional fermentation method from a local enterprise located in Prachinburi Province. Fresh bamboo shoots from Prachinburi Province, Thailand, were peeled, sliced, and soaked in tap water containing 10 g/kg of Kosher salt overnight. The bamboo shoots were then squeezed before mixed with 100 g/kg of Kosher salt and left for 30 min. After that, they were squeezed, packed in a plastic bucket, and added to drinking water until it covered the shoots for fermentation. The buckets were

then tightly closed with a cover. TFBS were incubated at room temperature until the pH was below 4.6.

During fermentation, both FBSC and TFBS were taken to determine pH by using a digital pH meter (pH 700, Eutech), total acidity. By titration, the number of total lactic acid bacteria (LAB) is determined by a pour plate method in Lactobacillus de Man, Rogosa, and Sharpe (MRS) agar.

Stability of bamboo shoots during storage

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 to the bags before being sealed. 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, and the number of bacteria, yeast, mold, and *E. coli*.

Determinations

1. Total sugar, total reducing sugar, and total acidity

Total sugars were determined colorimetrically using the phenol sulphuric acid and expressed as percentage sugar [14]. The absorbance was measured at 490 nm and expressed as glucose concentration (mg/mL). Similarly, the reducing sugars were determined colorimetrically using 3, 5-dinitro salicylic acid (DNS) reagent. The absorbance was measured at 540 nm and expressed as glucose concentration (mg/mL) [14]. Total acidity (TA) was determined by the titration [15]. Three mL of samples were titrated with 0.1 N NaOH solution until their pH reached 8.2. Titratable acidity was calculated by following eq. 1 and expressed as g/L of lactic acid.

$$\text{Fotal acidity} (g/L) = (V_o \times N \times MW)/(V_s)$$
(1)

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

2. Color

The color of fermented bamboo shoot samples was measured using a colorimeter (WR-10QC, FRU, China) and reported in CIELAB color scales L*, a*, and b* values [16].

Total color difference (TCD) was also calculated by following eq 2.

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

where 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.

Microbial determination

The microbial determination for fermented bamboo shoots was carried out. 25 g fermented bamboo shoots were blended in 225 mL of phosphate-buffered saline (PBS), an isotonic buffer solution used for bacterial cell dilution, by a stomacher (Bagmixer 400, Interscience, France) for 60 s [17]. Appropriate dilutions from samples were made using sterile PBS water (9 mL). The samples were placed on 3M petrifilm aerobic count plates to determine total plate counts and 3M petrifilm E. coli count plates, while pour plated method was performed using MRS agar to measure for total lactic acid bacteria and yeast and mold by using potato dextrose agar by a pour plate method. The samples were then incubated at 35 °C for 48 h. The experiment was performed in triplicate, and the average number of colony-forming units per gram (CFU/g).

Statistic analysis

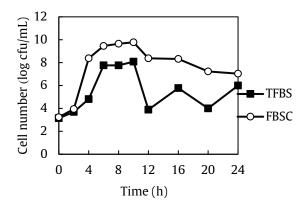
Each experiment was conducted three times. The influences of the various parameters were assessed by one-way analysis of variance (ANOVA) and the Duncan test for mean comparison. Differences were considered significant at a confidence level superior to 95%. The SPSS statistical program version 16.0 was used for the analyses.

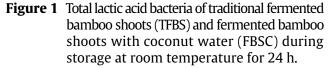
RESULTS AND DISCUSSION

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 g/L and 1.17±0.21 g/L, 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 coconut water (IMCW) and overly-mature coconut water (OMCW), which were 5.60±0.14 and 4.85±0.17 [°]Brix, respectively [18]. The pH and TA of coconut water depended on fruit maturity. The pH of coconut water increased with fruit maturity. Tan et al. (2014) revealed that the 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 [18]. Malic acid is the dominant organic acid in coconut water. In contrast with pH, coconut water's TA It was reported that TA of IMCW was 0.89 g/L, which was more significant than followed by those of MCW (0.76 g/L) and OMCW (0.61 g/L) [18]. The TSS and pH of the MCW could be useful in judging 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 [19]. 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 [20]. These could affect the taste and flavor that consumers accept [19, 20].

Sugars are the main fraction of soluble solids in coconut water. Coconut water contains sucrose, sorbitol, glucose, and fructose, followed by minor sugars, including galactose, xylose, and mannose [21]. These changes in 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) increased but decreased in reducing sugars (fructose and glucose) [22, 23]. MCW contained both reducing and non-reducing sugars. According to Rethinam and Krishnakumar (2022) [24], in the early stages of maturity, the sugars present were almost entirely reducing sugars, particularly glucose and fructose (>75%) [24], 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 $(2.49 \pm 0.11 \text{ g}/100 \text{ mL})$, while young malayan yellow dwarf showed the lowest of that 0.54 ± 0.11 g/100 mL) [25]. 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 [26].





Microbiology of fermented bamboo shoots

The number of LAB produced in the FBSC was significantly higher than in TFBS (Figure 1). After 24 h 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. These could be attributed to chemical compositions in MCW. It was revealed that MCW were composed of sugars, vitamins, amino acids and minerals. All of them were nutrient sources for microbial growth such as *Lactobacillus plantarum, Bacillus clausii*, or *Saccharomyces boulardii* [8, 26]. Moreover, pH of MCM was also suitable for

bacterial growth. It was reported that lactobacilli could grow in a ranging pH between 4.5 and 6.5, while the optimal pH was between 5.5 and 6.2 [27, 28].

Bamboo shoots could be fermented by a mixture of LAB such as Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus delbrueckii, Leuconostoc citreum, Leuconostoc fallax, Leuconostoc lactis, Leuconostoc mesenteroides, Streptococcus lactis, and Pediococcus pentosaceus [29]. In addition, salt concentrations also affected the number and diversity of the microorganisms during fermentation. Our results showed that FBSC, which contained 25 g/kg of salt, had a significantly greater number of microbes than TFBS, in which 100 g/kg of salt was used. Guan et al. (2022) [30] reported that salinity was a crucial factor shaping the variation in microbial community composition. Jing-Fang Shen et al. (2023) [31] found that the higher the salt concentration, the lower the microbes produced. Too high salt concentrations could reduce acid production, causing lactic acid bacteria to be less able to convert sugar, while promoting yeast growth [32]. This could indicate that the dual effects of MCM and low salt concentrations probably shorten fermentation time and reduce the risk of contamination.

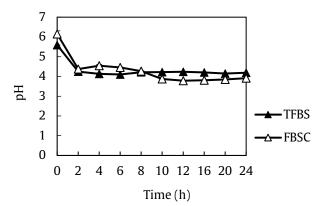


Figure 2 The pH of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) during fermentation at room temperature for 24 h.

During fermentation, dynamic changes in the physicochemical properties, including pH (Figure 2) and titratable acidity (g/L) (Figure 3) were observed. The pH value is an important parameter for fermentation. The decrease of pH and the increase of TA were mainly due to sugar consumption and the formation of organic acids, naturally occurring by lactic acid bacteria [31].

The pH of TFBS declined gradually from 6.00 ± 0.00 to 4.00 ± 0.01 , while that of FBSC decreased from 6.15 ± 0.16 to 3.89 ± 0.01 after fermentation for 24 h. In contrast, titratable acidity (g/L) of TFBS and FBSC 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 polysaccharides, oligosaccharides, and monosaccharides. During the

fermentation, LAB decomposed and utilized carbohydrates in bamboo shoots to produce organic acids, resulting in flavor formation. With the accumulation of organic acids, pH and TA were changed [33].

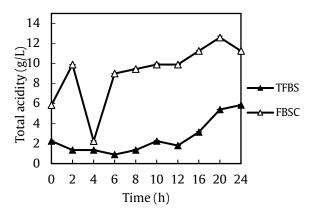


Figure 3 The total acidity of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) during fermentation at room temperature for 24 h.

Stability of fermented bamboo shoots during storage

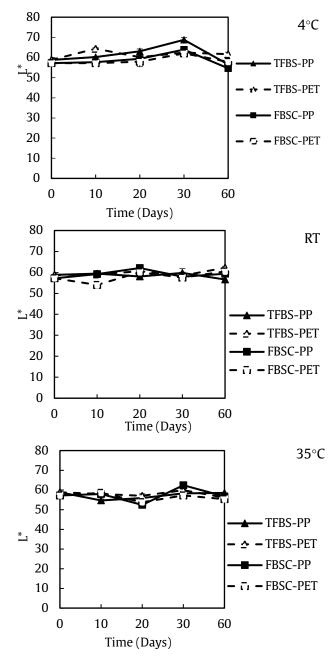
1. Colors

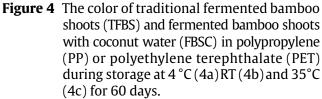
Color changes of TEBS and FBSC stored in different conditions were observed. At day 0, the L^* , the lightness, of all samples was not significantly different, as shown in Figure 4. They were ranked between 56.3-63.4. At 4 °C after 60-day storage, it was found that types of packaging bags influenced the bamboo shoot colors.

FBSC and TFBS in PET (FBSC-PET and TFBS-PET) were lighter than those 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) (Figure 4a). Similar results were shown in the samples stored at room temperature (RT). PET could protect the samples 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 4b). 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 4c).

The a^{*} could indicate a darker brown color of the bamboo shoots. The greater a^{*} was shown, the redder the samples would be observed. The results showed that the samples' redness increased with the storage temperature. After storage for 30 days at 4 °C, the a^{*} of FBSC-PP (8.03 ± 0.74) was significantly higher than TFBS-PP (5.93 ± 0.87), while FBSC-PET (4.10 ± 0.96) and TFBS-PET (3.70 ± 0.17) were not significantly different (Figure 5a).

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The a* of the samples stored at room temperature (RT) or 35 °C increased more noticeably than that at 4 °C. At RT after 60 days of storage at RT, a* of FBSC-PP (5.37 ± 0.49) and FBSC-PET (5.53 ± 0.49) were significantly lower than that of TFBS-PET (7.43 ± 0.25) and TFBS-PP (8.03 ± 0.40) (Figure 5b). Similarly, a* of the sample stored at 35 °C, regardless of packaging types, FBSC was significantly less red than TFBS. The highest a* was observed in TFBS-PP (9.73 ± 0.15), followed by TFBS-PET (6.13 ± 0.06), FBSC-PET (3.63 ± 0.15) and FBSC-PP (3.27 ± 0.31) (Figure 5c).

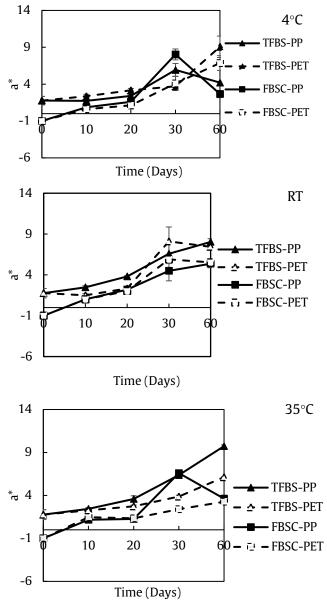


Figure 5 The greenness (a*) of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C (5a) RT (5b) and 35°C (5c) for 60 days.

The b* indicates yellow color. The greater b* was, the more yellowish the sample would be. The results were reported that after storage for 60 days at 4 °C, b* of all samples increased; TFBS-PET (6.13 ± 0.06) was higher than FBSC-PP (3.27 ± 0.31), but they were not significantly different. However, they were significantly greater than FBSC-PP (11.63 ± 1.02) and TFBS-PET (11.93 ± 0.93) (Figure 6a). When the storage temperatures were elevated, b* of all samples significantly increased (Figure 6b and 6c). Evidently, at 35 C, b* were ranked between 16.57-21.4. TFBS-PP (21.40 ± 0.30) and FBSC-PET (18.83 ± 0.67) significantly, followed by TFBS-PET (16.57 ± 0.49).

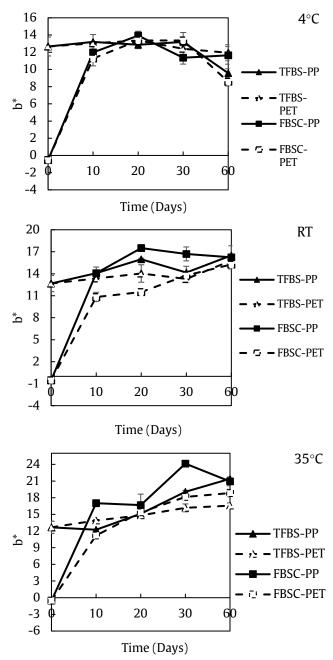


Figure 6 The color b* of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C (6a) RT (6b) and 35°C (6c) for 60 days.

TCD is an index of color change during storage. The color change greater than 2 is visible [34]. According to our study, several factors affected the color changes of the fermented bamboo shoots during storage, including fermentation methods, types of packaging, and storage temperatures. After storage for 60 days, the results showed that fermentation with MCM had higher changes than the traditional methods. Regardless of storage temperature and packaging types, TFBS showed less TCD than FBSC. Noticeably, TFBS-PET (7.54±1.23) and TEBS-PP (7.78±0.58) were significantly lower than FBSC-PET (17.43±0.18) and FBSC-PP (18.16 ± 1.58) when they were stored at RT. Similarly, at 35 °C, FBSC-PET (20.03 ± 0.67) and FBSC-PP (21.91 ± 0.77) were significantly higher than TFBS-PET (6.81 ± 1.43) and TFBS-PP (11.86 ± 1.05) (Figure 7).

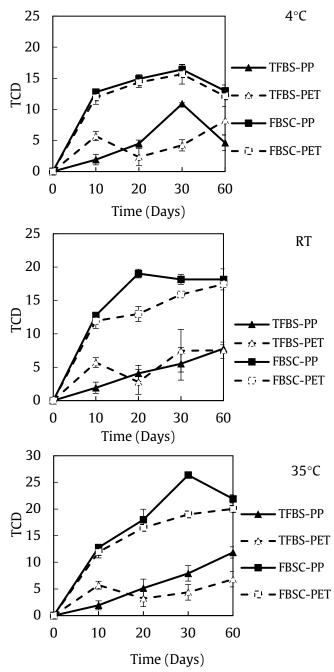
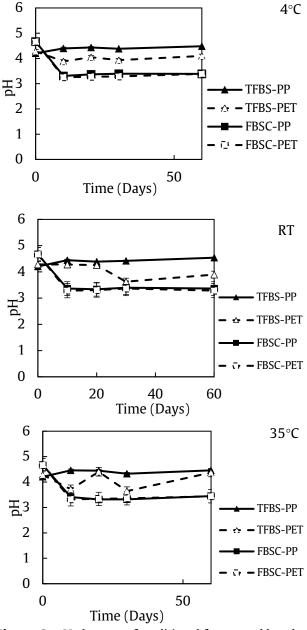


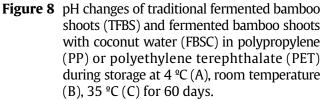
Figure 7 Total color difference of traditional fermented bamboo shoots (TFBS) and fermented bamboo shoots with coconut water (FBSC) in polypropylene (PP) or polyethylene terephthalate (PET) during storage at 4 °C, room temperature (28 °C), 35 °C, for 60 days.

This would contribute to the anti-browning activity of salt [35]. It was reported that salts could delay the browning reaction. Their efficiency was different depending upon their chemical compositions. Anti-browning activity of salts was greatest when

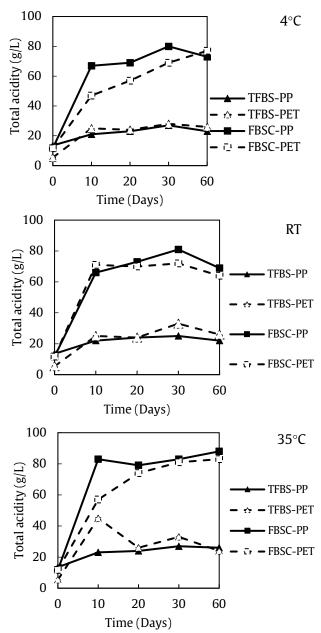
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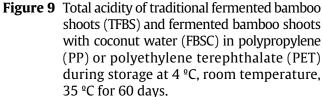
sodium, potassium, or calcium ions bind with chloride or phosphate, followed by sulfate and nitrate. Tissue extracted from chloride- and fluoride-treated slices showed polyphenol oxidase (PPO) activity, the same as that of control (non-treated slices). However, the PPO activity dropped when the slices were added to NaF and NaCl solutions. The level of polyphenols in treated slices was NaF > NaCl > control [36].





In addition, chemical reactions during fermentation, including non-enzymatic browning, enzymatic browning, and maillard reaction, could cause the color change in bamboo shoot. As bamboo shoot cells contain lutein and chlorophyll, they are generally yellowish green. These pigments could be degraded by acids produced during fermentation process. Moreover, the bamboo shoots could undergo maillard reaction, resulting in browning, and loss of original color [37, 38]. Ngadze et al. (2018) [39] reported that blanching bamboo shoots at 100°C for 10 min could inactivate endogenous enzymes, causing the shoots to become lighter. Moreover, the increase in temperature affected the total color difference and b^{*}. The shoots turned darker yellow at a higher temperature due to color degradation and non-enzymatic browning reaction [40, 41].





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According to the results, PET bags could delay the color changes of the fermented bamboo shoots during storage. Package materials can limit gas and water vapor or moisture penetration, affecting rates of oxidation and browning reactions [42, 43]. It was found that PET showed better barrier properties in prolonging the shelf-life of shelled bamboo shoots than PE and PVC, when they were stored at room temperature and 10 °C [44]. PET jars were reported to have greater ability to retain the quality of cookies supplemented with 6% and 8% bamboo shoot powder and dietary fiber than LDPE pouches when stored at ambient conditions for 90 days [45]. On the contrary, PP has a moderate barrier to moisture, gases and odors, compared to PET [46]. Types of packaging could influence the translucence and colors of samples. It was found that only PP packages failed to protect litchi fruit from browning reactions during storage at room temperature for 5 days [47].

pH and total acidity

The changes in pH of TFBS and FBSC during storage at different conditions are shown in Figure 8. The pH of FBSC and TFBS on 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 decreased (Figure 8a), 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 8b. At 35 °C, the maximum pH value was found in TFBS-PP, which was 4.46±0.01 and the lowest pH was 3.44±0.05 belonging to FBSC-PET (Figure 8c).

During storage, TA was significantly increased (Figure 9). Regardless of fermentation methods, nonsignificant differences were found between PP and PET bags. Niazmand et al. (2021) observed that titratable acidity contents of barberries packaged in different films increased by 38% after storage for six months [48].

Microorganisms

During storage, TFBS and FBSC were measured for microorganism analysis. The results showed that all samples had a total plate count of 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. Several studies showed the quality characteristics of fermented bamboo shot products during storage.

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 [49]. Cho and Song (2021) inactivated microorganisms in Korean vegetables "doenjang" by conventional heat treatments. Less than 10¹ CFU/g in doenjang were reduced when the product was heated at 75 - 85 °C for 60 min. The inactivation effect was partially improved when the heating temperature was 105 °C for 20 min. Mold, yeast, and vegetative cells with weak heat resistance could be inactivated at $100-105^{\circ}C$ [50]. Similarly, it was reported that one-log reduction of vegetative bacteria, such as *E. coli* and *Salmonella* spp could be achieved by heating samples at 70 °C for 1 min. 90% of *Aeromonas hydrophila* and yeast could be killed within a few seconds at 60 °C [51].

CONCLUSIONS

Mature coconut water could improve the fermentation process of bamboo shoots. The number of lactic acid bacteria was higher than the traditional method. During storage, fermentation methods, types of packaging, and storage temperatures significantly affect the shoot colors but not pH and TA. Mature coconut water had no positive effects on the color of the fermented shoot. PET bags could better delay the browning reaction of the fermented shoots than PP bags. This study indicated that MCW had the potential to be used as a low-cost fermentation media for bamboo shoots, which could help shorten the fermentation time and reduce risks of contamination for traditional fermentation.

ACKNOWLEDGEMENT

This work was supported by Program Management Unit for Area-Based Development (PMU-A), Office of National Higher Education Science Research and Innovation Policy Council (NXPO) (Grant number: RDG61A0023 and Project number: OTOPRMUTT61E04) and Rajamangala University of Technology Thanyaburi.

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