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Mansoor Abdul Hamid
Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia; Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia., chot@ums.edu.my

See Chee Hian
Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia., chot@ums.edu.my

Oslida Martony
Medan Health Polytechnic, Jl. Jamin Ginting, Km 13.5, Kel. Lau Cih Medan, Tuntungan 20136 Medan, Sumatera, Indonesia, chot@ums.edu.my

Mazarina Devi
Department of Industrial Technology, Faculty of Engineering, Universitas Negeri Malang, Jalan Semarang 5, 65145 Malang, Indonesia, chot@ums.edu.my

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Effect Of Steam and Microwave Blanching Against Enzymatic Browning of Chilled Saba Banana (Musa Spp., Bbb)

Mansoor Abdul Hamid[a,b*], See Chee Hian[b], Oslida Martony[c], Mazarina Devi[d]

[a]*Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.*
[b]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.*
[c]*Medan Health Polytechnic, Jl. Jamin Ginting, Km 13.5, Kel. Lau Cih Medan, Tuntungan 20136 Medan, Sumatera, Indonesia.*
[d]*Department of Industrial Technology, Faculty of Engineering, Universitas Negeri Malang, Jalan Semarang 5, 65145 Malang, Indonesia*
* *corresponding author, email: chot@ums.edu.my*

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ABSTRACT

The browning process, either enzymatic or non-enzymatic, is commonly used in food processing. Most browning process is avoided to maintain the food products' quality. This study was carried out to investigate the effect of steam blanching and microwave blanching against the enzymatic browning of chilled Saba bananas (Musa spp., BBB). The steam and microwave blanching in a range of 3 to 11 minutes and 30 to 90 seconds, respectively, were carried out to investigate the efficiency of deactivating the activity of the enzyme polyphenol oxidase (PPO). The results indicated that steam blanching for 7 minutes (SB7) and microwave blanching for 60 seconds (MB60) are the best treatments, reducing 92.07% and 82.63% activity of enzyme PPO, respectively. Bananas treated with SB7 and MB60 were then stored in chillers and showed no significant difference ($p>0.05$) in the activity of enzyme PPO for the 4 weeks of storage. Besides, the phenol content of both treated bananas demonstrated no significant difference ($p<0.05$) for the first 3 weeks. Both treated bananas also showed no significant difference ($p<0.05$) in the total color difference for the whole 4 weeks of storage. Moreover, the firmness of bananas treated with steam blanching is significantly ($p<0.05$) higher than that of bananas treated with microwave blanching, which started in week 1. The water activity of bananas treated with microwave blanching is significantly ($p<0.05$) lower than that of other samples. For the microbiological test, the numbers of bacteria, yeast, and mold in bananas from both treatments were still lower than the recommended standard. In conclusion, microwave blanching for 60 seconds is found to be the best treatment as compared to steam blanching, which have a sufficient reduction in PPO enzyme activity but still maintains the color, retains the lower water activity, and does not affect the texture of the treated banana.

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I. Introduction

Bananas is one of the most renowned fruits in the global marketplace, with a 4% share of total sales profits in 2014. Banana Saba is a cooking banana that is currently famous and has been used as an ingredient in banana fritters in Malaysia. The export of bananas (cooking bananas, raw or dried) from Sabah to the West Peninsula is gradually increasing, especially banana Saba, from 1,057.84 metric tons in 2012 to 8,674.74 metric tons in 2015. Banana is usually kept at a low temperature, around 13 oC, to slow down the production of ethylene and the ripening process, allowing the fruit to be kept for a longer time (Zhu et al., 2015). However, enzymatic browning represents another issue that will destroy the value of the banana. Enzymatic browning is a phenomenon usually found in fruits and vegetables that is caused by polyphenol oxidase (PPO, EC 1.10.3.1). This enzyme facilitates the hydrolysis of monophenol to ortho-diphenol (activity cresolase; EC 1.14.18.1), followed by the oxidation of ortho-diphenol to ortho-quinone (catecholase activity; EC 1.10.3.1) (Summervir & Monika, 2015). Quinone is a very active compound that will be modified into melanin through the polymerization process, which causes the browning of fruit and vegetables. Aside from, affecting

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appearance, the browning process also affects sensory properties, nutritional value, and commercial value (Hui, 2008).

To overcome this problem, anti-browning has become a necessary process in the food industry. Of all the anti-browning treatments available, blanching is the most frequently used method in the food industry due to its effectiveness and lower cost. Water blanching can control the browning of bananas, but it will cause the leakage of water-soluble vitamins and reduce their nutritional content. Thus, other methods, such as steam blanching and microwave blanching, should be considered. According to Cano et al. (1997), steam blanching banana slices for 5 minutes is effective in reducing the activity of enzyme PPO by as much as 96%. Although steam blanching involves a higher cost compared to water blanching, it is able to maintain nutrients and reduce water waste. For microwave blanching, Luis & Francisco (2013) found that microwave blanching is more effective in anti-browning compared to water blanching. It minimizes the operation time and is hence able to maintain the nutrients, taste, and color of the product (Puligundla et al., 2013). In this work, an investigation was carried out into the effects of steam blanching and microwave blanching on the activity of the enzyme PPO, phenol, texture, color, water activity, and shelf life of chilled banana Saba. Besides, the best treatment with a specific time factor will be explored in this investigation.

II. Method

A. Materials.

Raw banana Saba (Musa spp., BBB) with a maturity index of 4 and an average size of 6 x 1.5 inches2 was purchased from Big Market, Kota Kinabalu. The bananas were cleaned with pipe water and dried at room temperature. Each banana was carefully selected based on its visual appearance, shape, and size. After peeling and halving, the flesh was immediately immersed in an ice water bath.

B. Steam Blanching Treatment

Samples of the peeled banana fruits were steam blanched for (3, 4, 5, 6, 7, 8, 9, 10, and 11 minutes) with an electrical steamer as a preliminary study. The best time for steam blanching treatment was used for further investigation. The treated fruits were chilled by immersing them in an ice water bath. Then, the treated samples were dried, packed into a polyethylene bag, and sealed.

C. Microwave Blanching Treatment

Samples of the peeled banana fruits were microwave-blanched for (30, 4, 50, 60, 70, 80, and 90 seconds) with a conventional microwave (700W) as a preliminary study. The best time for steam blanching treatment was used for further investigation. The treated fruits were chilled by immersing them in the ice water bath. Subsequently, treated samples were dried, packed into polyethylene bags, and sealed.

D. Control

The peeled samples were packed into a polyethylene bag and sealed. All treated and control samples were then stored in a chiller at 2±1°C for a 4-week period.

E. Extraction of Enzyme Polyphenol Oxidase (PPO)

2.0 ± 0.1 g of banana was homogenized with 10 mL (0.05M) of sodium phosphate buffer (pH 7.0) containing 0.2 g of polyvinyl pyrrolidone (PVPP) for 2 minutes. Then, the homogenized mixture was centrifuged at 12,000 x g for 20 minutes at 42 ± 1 °C. The supernatant obtained was used to estimate the activity of the enzyme PPO (Huang et al., 2013).

F. Analysis of Enzyme Polyphenol Oxidase (PPO)

0.1 mL of crude enzyme was added to 0.1 mL of catechol substrate (10 mM) and dissolved in 2.9 mL of 0.05 mM sodium phosphate buffer (pH 7.0). The activity of PPO was expressed as enzyme units per mg protein. One unit (U) of activity PPO was equivalent to an increment of 0.001/min absorption at 398 nm (Huang et al., 2016).

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G. Phenol Analysis

Phenol content was determined using the method modified by Folin-Ciocalteu. 3.0 ± 0.1 g of banana flesh was immersed in 30 mL of methanol and extracted with the help of an ultrasonic at 50°C for 30 minutes to optimize the extraction. After that, it was centrifuged at 20°C and 10,000 x g for 20 minutes. The sediment was again immersed in 30 mL of methanol two more times. The reaction mixture contained 0.5 mL methanol extract, 5 mL distilled water, and 0.5 mL Folin-Ciocalteu. After 8 minutes, 1.5 mL of 20% sodium carbonate was added. The mixture was then stirred and left aside for 30 minutes. The absorbents were measured at 760 nm. Gallic acid solution (0 and 100 mg/L) was used to plot the standard curve. The result was expressed as gallic acid (mg) equivalent (GAEs) per 100 g of fresh weight (GAEs, mg/100 g of fresh weight) (Huang et al., 2016).

H. Texture Analysis

Before measurement, the chilled bananas were subjected to a thawing process for 1 hour to reach room temperature. After that, a texture analyzer (TA-XTPLUS, United States of America) was used to measure the texture. A convex probe with a 4 mm diameter and a 5 kg load cell was used. The flat surface of the banana was put on the surface of the platform, and the penetration was placed right in the center. A probe with a speed of 1 mm s-1 was used, and a compressive force of 5.0 mm penetration was used by Cecelia et al. (2013).

I. Analysis of Total Color Difference

A colorimeter (Konilta Minolta, Japan) was used to measure the color of the banana's external surface. The color measured was in coordination with CIE L*, a*, and b*. Value L* showed the brightness, while a* represented the degree of green and red, and b* signified the degree of blue to yellow. Value range L* (0–100); value range of a* and b* (-128–127). The value of the total color difference was calculated using the formula (ΔE= [(L1 – L2)2 + (a1 – a2)2 + (b1 – b2)2]1/2). Three readings were taken from three random places on the banana surface (Cano et al., 1997).

J. Analysis of Water Activity (Aw)

The water activity of the samples was measured using Hydrolab each week from weeks 0, 1, 2, 3, and 4. The banana sample was filled into the cup one-third full and closed with a probe. The value of water activity was recorded.

K. Microbiological test

Total Plate Count. 22.5 g of PCA was dissolved in 1000 mL of distilled water to produce the PCA agar. The mixture was stirred to make sure it was well mixed. Then, the solution was sterilized using an autoclave (Hirayama, HVE-50) at 121°C for 15 minutes. The PCA agar was then set aside to cool, and the temperature was maintained at 50°C by putting it in a water bath. A pour plate was used, and 1 mL of inoculum was transferred from a universal bottle of (10-2, 10-3, and 10-4). Each process was done in triplicate. The cooled agar medium was poured into a petri dish and homogenized by making an eight-shaped turn. The agar was turned upside down and kept in an incubator at 27°C for a day, while colonies were counted in the range of 30 to 300.

Yeast and Mold Count. 39.0 g of PDA was dissolved in 1000 mL of distilled water to produce the PCA agar. The mixture was stirred to make sure it was well mixed. Then, the solution was sterilized using an autoclave (Hirayama, HVE-50) at 121°C for 15 minutes. The PCA agar was then set aside to cool, and the temperature was maintained at 50°C by putting it in a water bath. A pour plate was used, and 1 mL of inoculum was transferred from a universal bottle of (10-2, 10-3, and 10-4). Each with duplication. The cooled agar medium was poured into a petri dish and homogenized by making an eight-shaped turn. The agar was turned upside down and kept in an incubator at 27°C for a day. Colonies ranged from 30 to 300.

L. Statistical Analysis

All the data obtained were analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 21. All data obtained from the analysis of the activity of enzyme PPO, phenol content, texture, total color difference, and water activity were analyzed using a variant one-way ANOVA and a post hoc Turkey test with a 95% confidence level showing a significant difference of ($p < 0.05$) for all the attributes tested (Cano et al., 1997).

III. Results and Discussion

A. Best Steam and Microwave Blanching Treatment of Saba Banana

The best blanching treatments were based on the reduction of enzyme PPO activity (%) as shown in Table 1 and Table 2.

Table 1. Reduction in the activity of enzyme PPO and percentage of reduction on the activity of enzyme PPO for steam and microwave blanching.

Steam			Microwave		
Time (min)	Activity of PPO enzyme ($min^{-1}mg^{-1}$protein)[1]	Reduction in PPO activity (%)	Time (Sec.)	Activity of PPO enzyme ($min^{-1}mg^{-1}$protein)[1]	Reduction in PPO activity (%)
control	73.15 ± 1.20^{d}	0	control	73.17 ± 1.18^{d}	0
3	36.00 ± 2.83^{c}	50.75	30	33.60 ± 3.25^{b}	54.04
4	37.75 ± 3.04^{c}	52.39	40	46.40 ± 4.53^{c}	36.53
5	33.05 ± 3.04^{c}	54.72	50	$41.95 \pm 2.33^{b,c}$	42.54
6	18.95 ± 2.90^{b}	74.01	60	12.70 ± 2.26^{a}	82.63
7	5.75 ± 0.78^{a}	92.07	70	12.20 ± 0.42^{a}	83.32
8	5.50 ± 0.35^{a}	92.48	80	11.75 ± 1.20^{a}	83.94
9	4.30 ± 0.13^{a}	94.12	90	10.20 ± 0.99^{a}	86.06
10	3.38 ± 0.19^{a}	95.38			
11	2.46 ± 0.16^{a}	96.64			

[1] (^{a} - ^{d}) Means within column not followed by the same letter differ ($p < 0.05$)

Both steam and microwave blanching treatments showed a positive impact on reducing the browning effect of the bananas. The blanched at a minimum of 7 minutes is able to reduce enzyme PPO activity significantly ($p<0.05$) compared to the blanching treatment of less than 7 minutes or lower by 92.07% enzyme activity reduction. There is no significant difference ($p > 0.05$) with a blanching time greater than 7 minutes, but in the longer blanched time, the texture becomes softer and decreases its overall quality (David, 2004). The microwave blanching treatment for 60 seconds is able to reduce the enzyme PPO activity up to 82.63%, which is significantly ($p<0.05$) lower than the blanching time of 60 seconds. 60 seconds is chosen as the best time for microwave blanching with no significant difference ($p > 0.05$) compared to the longer blanching time; moreover, the longer time has a negative impact as it affects the texture of the banana by disturbing the cell membrane (Luis & Francisco, 2013).

Both treatments showed no significant difference ($p <0.05$) in the reduction of activity of enzyme PPO (%) throughout the 4 weeks of storage. Besides, the enzyme PPO activity for both treatments on week 4 shows no significant difference ($p <0.05$) compared to week 0. SB7 records an 82.02% reduction in enzyme PPO activity on week 0 and 84.10% on week 4, while MB60 recorded 79.51% on week 0 and 80.00% on week 4 (Table 2). These results indicated that the pretreatment is effective in maintaining the browning compounds in the treated banana. The results align with Zhoa *et al.* (2021) finding that samples stored showed that the browning effect of PPO is increased by time at higher temperatures when exposed to light.

Table 2. Percentage of reduction in activity of enzyme PPO for chilled treated bananas and controlled bananas during storage period.

Treatment	Storage time (week)				
	0	1	2	3	4
	Reduction in PPO enzyme activity (%)				
SB7	82.02^{Aa}	82.86^{Aa}	81.27^{Aa}	83.05^{Aa}	84.10^{Aa}
MB60	79.51^{Aa}	75.80^{Aa}	76.61^{Aa}	76.98^{Aa}	80.00^{Aa}

[1] (^{a}) Means within column not followed by the same letter differ ($p < 0.05$)
[2] (^{A}) Means within a row not followed by the same letter differ ($p < 0.05$)
*Reduction on the activity of enzyme PPO, (%) calculated based on the controlled banana on week – 0
*Enzyme PPO activity expressed as $\Delta A_{398} = min^{-1} mg^{-1}$ protein for each 0.001 absorption
* All data are the average of three independent experiments

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B. Physicochemical and Microbiological Profiles of Steam and Microwave Blanched Sample During Storage

Several characteristics were measured in the two best blanching treatments to study its physicochemical properties, such as phenol content, color, texture, and water activity changes, as well as the microbiological community.

1) Phenol Content

The phenol content of bananas treated with steam blanching and microwave blanching on weeks 0 and 1 is significantly lower than that of control bananas (Table 3). This is due to the fact that phenol compounds are destroyed by heat generated during treatment. However, the phenol content of controlled bananas keeps decreasing significantly every week from week 0 to week 3, and it remains at week 4. On the contrary, the phenol content of SB7 and MB60-treated bananas is decreasing very slowly, and a significant difference only exists during week 4. The decreasing of phenol showed a similar trend, as found by Deng *et al.* (2018), in Litchi pericarp kept at ambient temperature with significantly higher loss percentages ($p<0.05$) as compared to samples stored at chill conditions.

Table 3. Phenol content for chilled treated bananas and controlled bananas during the storage period.

Treatment	Storage time (week)				
	0	1	2	3	4
	Phenol Content (GA mg/100g sample)				
Control	11.21 ± 1.08^{Aa}	9.29 ± 0.69^{Bb}	5.10 ± 0.58^{Cb}	2.29 ± 0.42^{Db}	1.38 ± 0.10^{Da}
SB7	7.17 ± 1.22^{Ab}	6.46 ± 0.85^{ABb}	5.26 ± 1.31^{ABb}	4.22 ± 1.37^{ABb}	3.50 ± 0.71^{Bb}
MB60	7.03 ± 0.83^{Ab}	6.22 ± 2.56^{ABb}	4.91 ± 0.88^{ABb}	4.26 ± 0.25^{ABb}	3.41 ± 0.25^{Bb}

[1] ($^{a-b}$) Means within column not followed by the same letter differ ($p < 0.05$)
[2] ($^{A-D}$) Means within a row not followed by the same letter differ ($p < 0.05$)
*Reduction on the activity of enzyme PPO, (%) calculated based on the controlled banana on week – 0
*Phenol content expressed as mg of Gallic acid / 100 grams of fresh weight
* All data are the average of three independent experiments

2) Color Changes

The total color difference for treated SB7 and MB60 showed no significant difference with controlled bananas during week 1, as summarized in Table 4. However, significant differences begin to occur from week 2 onward. The total color difference of controlled bananas increased significantly on week 2 and then it did not change much. In comparison between both treatments, the total color difference of the SB7-treated banana showed no significant difference compared to the MB60 throughout the 4-week storage period. Moon *et al.* (2020) indicated that color changes due to the enzymatic browning reaction affect the samples of fruits and vegetables, leading to a darker color, but this can be controlled by several synthetic chemical compounds commonly used as PPO inhibitors.

Table 4. Total color difference for chilled treated bananas and controlled bananas during the storage period.

Treatment	Storage time (week)				
	0	1	2	3	4
	Total Color Difference (%)				
Control	0	2.92 ± 2.18^{Aa}	13.04 ± 2.74^{Ba}	17.21 ± 3.45^{Ba}	15.92 ± 2.58^{Ba}
SB7	7.71 ± 2.76^{Aa}	7.38 ± 2.44^{Aa}	7.09 ± 1.02^{Ab}	8.60 ± 0.81^{Ab}	9.25 ± 1.48^{Ab}
MB60	5.06 ± 1.99^{Aa}	5.58 ± 0.64^{Aa}	6.60 ± 1.87^{Ab}	8.69 ± 2.17^{Ab}	9.15 ± 2.18^{Ab}

[1] ($^{a-b}$) Means within column not followed by the same letter differ ($p < 0.05$)
[2] ($^{A-B}$) Means within a row not followed by the same letter differ ($p < 0.05$)
*Reduction on the activity of enzyme PPO, (%) calculated based on the controlled banana on week – 0
*Total color difference calculated with formula ($\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$
* All data are the average of three independent experiments

3) Texture Profile.

The texture of SB7-treated bananas is significantly higher ($p<0.05$) than that of control bananas throughout the 4-week storage period (Table 5). This is due to the heat treatment involved during steam blanching, which causes the cross-link between the carboxyl group and pectin (Guillermo *et al.,* 2014). This increases the straightening of the cell wall, thereby increasing the texture. For MB60-treated bananas, the texture does not show any significant difference compared to the control banana, although the heat treatment does increase the texture. This is because microwave blanching will disturb the cell membrane and change the polymer structure of the cell wall. Thus, it decreases the texture of the MB60-treated bananas. Ahmed *et al.* (2023) found that the storage quality of dates can be prolonged by artificial neural networks (ANN) and modified atmosphere packing (MAP) kept at chill conditions, indicating minimal changes in their texture quality.

Table 5. Texture for chilled treated bananas and controlled bananas during storage period

Treatment	Storage time (week)				
	0	1	2	3	4
	Texture (kg^{-1})				
Control	$0.51{\pm}0.26^{Aab}$	$0.44{\pm}0.24^{Aa}$	$0.36{\pm}0.16^{Aa}$	$0.37{\pm}0.12^{Aa}$	$0.36{\pm}0.08^{Aa}$
SB7	$0.92{\pm}0.26^{Aa}$	$1.03{\pm}0.28^{Ab}$	$0.83{\pm}0.25^{Ab}$	$1.05{\pm}0.40^{Ab}$	$0.84{\pm}0.39^{Ab}$
MB60	$0.25{\pm}0.20^{Ab}$	$0.30{\pm}0.24^{Aa}$	$0.19{\pm}0.15^{Aa}$	$0.34{\pm}0.26^{Aa}$	$0.35{\pm}0.26^{Aa}$

[1] ($^{a-b}$) Means within column not followed by the same letter differ ($p < 0.05$)
[2] ($^{A-B}$) Means within a row not followed by the same letter differ ($p < 0.05$)
* All data are the average of three independent experiments

4) Water Activity

Throughout the 4 weeks of storage, the water activity of controlled bananas and treated bananas showed no significant difference ($p > 0.05$), respectively, as shown in Table 6. As compared among treated bananas and controlled bananas, the water activity of the controlled banana showed no significant difference compared to SB7-treated bananas, but it has a significant difference compared to MB60-treated bananas throughout 4 weeks of storage. This is due to the fact that the mechanism of the microwave is heated by the inner structure of the banana, which creates vapor pressure inside the banana. This pressure will push the water toward the banana's surface and evaporate it. Zambrano *et al.* (2019) reported that moisture content is a significant factor contributing to food spoilage, where the moisture content increased significantly with storage time.

Table 6. Water activity for chilled treated bananas and controlled bananas during storage period.

Treatment	Storage (Week)				
	0	1	2	3	4
	Water Activity (A_w)				
Control	$0.94{\pm}0.01^{Aa}$	$0.94{\pm}0.01^{Aa}$	$0.94{\pm}0.01^{Aa}$	$0.93{\pm}0.02^{Aa}$	$0.94{\pm}0.02^{Aa}$
SB7	$0.95{\pm}0.01^{Aa}$	$0.92{\pm}0.01^{Aa}$	$0.93{\pm}0.01^{Aa}$	$0.93{\pm}0.01^{Aa}$	$0.94{\pm}0.03^{Aa}$
MB60	$0.84{\pm}0.02^{Ab}$	$0.85{\pm}0.01^{Ab}$	$0.86{\pm}0.03^{Ab}$	$0.86{\pm}0.01^{Ab}$	$0.87{\pm}0.01^{Ab}$

[1] ($^{a-b}$) Means within column not followed by the same letter differ ($p < 0.05$)
[2] (^{B}) Means within row not followed by the same letter differ ($p < 0.05$)
* All data are the average of three independent experiments

5) Microbiological Status.

The bacteria count is increasing significantly ($p<0.05$) throughout the 4 weeks of storage, but they are still in the satisfaction category during week 4. According to the Microbiological Guidelines for Ready-to-Eat Food by the Centre for Food Safety, Food, and Environmental Hygiene Department of Hong Kong, a colony count of $<10^6$ cfu/g for extended shelf-life food products requiring refrigeration is included in the satisfaction count. The bacteria count is growing slowly due to the chilling storage and the heat-based blanching treatment that can kill most of the bacteria. Wang *et al.* (2023) indicated that the α-diversity of the microbiological colony in the sample stored at 4°C is much higher than that in the sample stored at 25°C due to the surface sample treatment that influenced the bacteria to cause spoilage.

Table 7. Total plate count (cfu/g) for chilled treated bananas and controlled bananas during the storage period.

Storage (Week)	Treatment		
	Control	SB7	MB60
0	<25	<25	<25
1	7.6×10^2	<25	<25
2	3.9×10^3	9.3×10^3	6.7×10^2
3	4.2×10^4	6.7×10^3	0.9×10^3

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Storage (Week)	Treatment		
	Control	SB7	MB60
4	2.3 x 10^5	4.8 x 10^4	1.2 x 10^4

The yeast and mold count for treated bananas and controlled bananas is increasing gradually, but it is still in the satisfactory category during week 4. According to the International Commission on Microbiological Specification for Food, the yeast population for fruit is usually in the range of 3.8 x10^4 to 6.8 x 10^5 cfu/g. The yeast and mold count are also growing slowly due to the heat-based blanching treatment and being packed into a polyethylene bag. Wang *et al.* (2023) found less fungal growth in the chilled sample, and the most abundant is the phyla group in the bacterial community.

Table 8. Total yeast and mold count (cfu/g) for chilled treated bananas and controlled bananas during storage period

Storage (Week)	Treatment		
	Control	SB7	MB60
0	<25	<25	<25
1	1.8 x 10^2	<25	<25
2	2.6 x 10^2	2.0 x 10^1	1.1 x 10^1
3	6.0 x 10^2	6.3 x 10^1	1.9 x 10^1
4	3.5 x 10^3	9.6 x 10^1	3.2 x 10^1

IV. Conclusion

Both steaming and microwave blanching showed a positive impact on reducing PPO enzymatic browning, with steaming at 7 minutes and microwave blanching at 60 seconds being chosen as the best for each treatment. Although SB7 showed the highest PPO reduction, MB60 is observed as the best treatment because it is not only significantly able to reduce the enzyme PPO activity, but it can also maintain the color, does not affect the texture of bananas, and reduces water activity throughout 4 weeks of storage.

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