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Influence of Antimicrobial Coating and Low Temperature Storage on Quality Changes of Queen Pineapple Fruit

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***Abstract* – The incorporation of low temperature, high relative humidity condition and coating for prolong postharvest storage life for 'Queen' pineapple cv. Ninh Binh in Vietnam was studied. Three types of antimicrobial coating solutions were used in this study including chitosan/methylcellulose (C/MC) solution, C/MC solution incorporated with vanillin (C/MC-vanillin) and carbendazim (C/MC-carbendazim). The pineapples, uncoated and coated with different film solutions, were stored at 12 ± 1°C, 85 ± 2% RH for 30 days and were examined for physical properties, physical-chemical properties and sensory values. The coating delayed the changes in total soluble solids, flesh firmness, flesh color and ethanol content. Specifically, pineapples coated with C/MC-carbendazim showed the lowest value on ethanol content and the highest value on TSS, firmness, flesh color and sensory. However, no significant change in weight loss was found for pineapples uncoated and coated with C/MC-carbendazim at low temperature and high relative humidity condition. These results showed that pineapples coated with C/MC-carbendazim and maintained at 12±1°C, 85±2% RH show the best fruit quality.**

***Keywords* – Carbendazim, Coating, Ethanol Content, Physical-Chemical Properties, Microbial Growth, 'Queen' Pineapple.**

I. Introduction

The shelf life of fruits, vegetable and foods in general is easily reduced by many factors. The main cause of food spoilage is the growth of microorganisms on the surface of the products. In order to extend the postharvest life for fruit products, chitosan, an antimicrobial film, has been used. Recently, the antimicrobial activity of chitosan/methylcellulose (C/MC) film has been developed. Adding benzoate and sorbate into C/MC films to improve antimicrobial activity has also been reported [6]. Essential plant oils, including anise, basil, coriander, oregano, garlic oil, potassium sorbate and bacteriocin (nisin) were incorporated into chitosan films and C/MC films as supplemental antimicrobial activity agents [19, 23, 24]. Carbendazim (methyl-2-benzimidazole carbamate - $C_9H_9N_3O_2$) is a widely used broad-spectrum benzimidazole fungicide that plays a very important role in plant disease control. Carbendazim solutions have been used directly to control of fungi and diseases of plants and fruits in postharvest food storage, in seed pre-planting treatment, and as a fungicide in paint, paper and wood. The fungicidal activity of benomyl is due to the presence of carbendazim [7, 16]. Blending carbendazim into C/MC film to create an antimicrobial environment on the surface material is a new trend in fruits and food packaging. There are no reports on the effect of carbendazim concentration on the properties of C/MC based films.

In order to maintain fresh quality of fruits, postharvest treatment needs to be applied, including coating. Several studies have been done and indicated that chitosan coating had the potential to improve the quality maintenance, prolong storage life of cucumbers [8], strawberries [9, 12], apricot [11], longan [15], litchi [14], tomatoes [18] and mango fruit [1]. Application of chitosan-based coating incorporated with citric acid and potassium sorbate to delay the decay of fresh longan fruit was reported [2]. Maintaining and extending the shelf-life of fresh-cut pineapple by wrapping with chitosan/methylcellulose and vanillin films was examined [21].

The influence of storage temperature on ethanol content, microbial growth and other properties of Vietnamese pineapple fruit cv. Ninh Binh was studied [20]. The results showed that 12 ± 1°C and 85% RH were the optimal conditions needed to preserve the pineapples while maintaining a good quality level without chilling injury symptoms. As far as we know, there is little available scientific literature about the use of C/MC incorporated antimicrobial agent for maintaining and extending the shelf life of fresh pineapple. Therefore, the aim of this study was to determine the effect of chitosan/methylcellulose film incorporated with carbendazim and vanillin coating on the physical properties, physical - chemical properties and sensory values of 'Queen' pineapple during storage at 12 ± 1°C and 85% RH.

II. Materials and Methods

A. Film-solution preparation

Chitosan with a degree of deacetylation of 90% and purity >99.75% (Bannawach Bio-line Co. Ltd., Thailand) was prepared by dissolving 1.5 g of chitosan in 100 mL of 1% acetic acid solution. One gram of methylcellulose (viscosity 10-25 mP.s, Methocel # 64605) was dissolved in 100 mL of ethanol:water (1:3). Solutions of chitosan and methylcellulose were then mixed in a beaker with a stirrer. One gram of polyethylene glycol (PEG) 400 was used as a plasticizer. Vanillin, 0.9 g (Sigma, St. Louis, USA) was incorporated after the temperature of the solution reached its melting point (83°C) (Sangsuwan et al., 2008b). In another process, carbendazim (active ingredient, BaSF Co., Thailand), 1.6 g/100 g solid of C/MC, was blended into the C/MC coating solution. These solutions were then filtered, degassed and conditioned at 25±2°C.

B. Raw Materials

Queen pineapples (*Ananas comosus L.*) cv. Ninh Binh, planted at a private farm in Tam Diep district, Ninh Binh province in Vietnam were used for this research. In the main season (April & May), 140-145 days after full bloom, pineapple fruit cv. Ninh Binh were selected for uniformity in shape, size and similarly in maturity stage 2 (10-25% yellow color of fruit skin). The pineapple crown leaves were cut with the crown length about 1cm from the top of fruit. After that, the pineapples were washed by water and drained for 10 to 15 minutes.

Fruits were dipped in different coating solutions (C/MC, C/MC-vanillin and C/MC-carbendazim) for 5 minutes. Fruits were dipped in distilled water as a control. Following treatment, pineapples were dried for 2h at 25°C and then stored at 12°C and 85 ± 2% relative humidity for 30 days. For each treatment, three replications were used. Weight loss, total soluble solids (TSS), titratable acidity (TA), firmness, color, ethanol content and microbial growth were determined every 5 days during 30 days storage. Additionally, carbendazim residue of fruits coated by C/MC-carbendazim was analyzed after 30 days storage.

C. Analysis of Physical and Chemical Properties of Pineapples

The flesh color (L* and h) of the pineapples was measured on the cut-surface using a colorimeter, model CR-200 (Minolta, Osaka, Japan). Multiple readings were taken on the cut-surface of the fruits over six tests (in the basal, middle and upper positions). The firmness of the pineapples' flesh was determined using a stable micro-systems TA-TXT2i texture analyzer (Texture Technologies Crop, UK). Slivers of 3 cm thickness were taken from the basal, middle and upper parts of each pineapple, and then measured for their flesh texture. The maximum force (Newton) of each sliver was then recorded at 3 positions - inner, middle and outer positions.

Fresh cut pineapple pieces (50g) were minced and filtered, and the juice was used to determine the pH, titratable acidity (TA) and total soluble solids (TSS) content at 25°C. The pH was measured directly using a pH meter, while TA was measured using 5 ml of juice titrated with a standardized 0.1 N NaOH up to pH 8.2 by an auto-titrator (Titroline easy, Schott, Japan), expressed as a percentage of citric acid (g citric acid/100g fresh weight). The TSS content (%) was determined using a digital refractometer (Pocket PR-101, ATAGO Company, Japan). The weight of the pineapples was determined using a scale balance (± 0.01 g), model PB1502-S (Mettler-Toledo, Menlo Park, CA, USA).

Ethanol content of the fruit was determined by gas chromatographic analysis of the headspace [21]. Five grams of pineapple flesh were placed in a 10 mL amber glass bottle with a rubber cap, and then incubated in a water bath at 60°C for 45 minutes. Headspace gas was drawn using a 1 mL syringe and injected into a TRACE GC gas chromatograph (Agilent 6890N, USA) equipped with a flame ionization detector. The temperatures of the oven, injector and detector were set at 150, 175 and 200°C respectively, and the column used was a 30 m x 0.25 mm i.d. x 0.25 µm capillary column. A retention time and a standard curve of absolute ethanol in water solutions (0-2400 mg L^{-1}) were both used for peak identification and quantification.

D. Sensory Evaluation

The pineapple fruits coated with different coating solutions and stored at 12 ± 1°C, 85 ± 2% RH were sensory evaluated at the 10th, 20th and 30th day storage. Fruit samples were removed from refrigerator before serving to allow them to equilibrate to room temperature. The fruits were hand peeled and transversely cut into three slices from each fruit and each slice was cut into 2 - 4 segments. Samples were labeled with three-digit random codes and placed in small white plastic cups. A 15-trained-members panel of both genders and of ages from 20 – 60 will be selected to evaluate the pineapple fruit quality. The acceptability of color, odor, flavor, taste, texture and overall quality were evaluated using a nine-point hedonic scale where 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely. The variability of acceptance was analyzed by ANOVA and Tukey'sb test ($p<0.05$).

E. Statistical analysis

Data were initially evaluated using analysis of variance (ANOVA) and Duncan's Multiple Range Test (SPSS 16.0 software program).

III. Results and Discussion

The pineapples used in this study had fruit weight, total soluble solids, titratable acidity, and pH in the range of 920-990 g, 17.6-18.8% Brix, 0.79-0.82% and 4.0-4.2, respectively. The flesh fruit was crispy, with high firmness and light yellow in color. Regarding coating solutions, chitosan/methylcellulose (C/MC) solution was colorless and transparent while C/MC solution incorporated with carbendazim and vanillin were more opaque, light white and bright yellow color, respectively (data not show).

Flesh color

Flesh color is one of the major properties of pineapple during storage at low temperature. The change of flesh color (L* and hue angle) of Queen pineapple with and without coating maintained at 12°C, 85% RH during 30 days is illustrated in Fig 1. The L* parameter was used to describe the darkness to whiteness level with a range from 0 to 100. Hue angle parameter showed the actual color altered from red-yellow to yellow to yellowish-green color when hue angle increased from 45° to 90° and 135°, respectively. The presence of carbendazim in C/MC coating solution significantly affected on the L* value profile of pineapple flesh while there was no significant difference in L* value of fruit uncoated and coated with C/MC and C/MC-vanillin (Fig. 1a). The L* value increased slightly on the first 5 days and then decreased at the later stage for fruit uncoated and coated with C/MC

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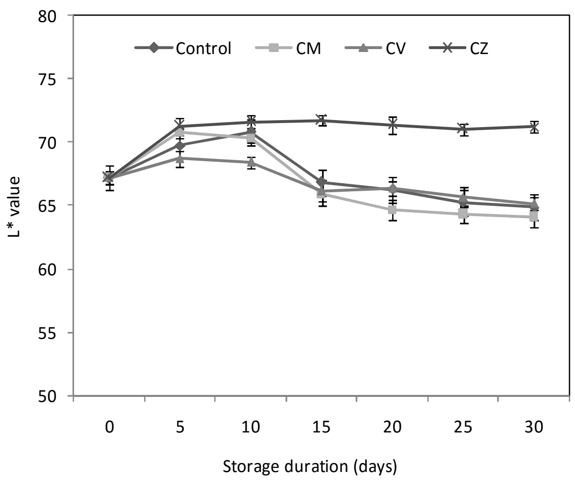
and C/MC-vanillin. At the 30th day storage, the fruit coated with C/MC-carbendazim still kept light-yellow flesh color, 71.18 in L* value, while fruits uncoated and coated with C/MC and C/MC-vanillin became less light, with L* values 64.8, 64.1 and 65.1, respectively. In addition, the flesh color of pineapple was also evaluated by hue angle value.

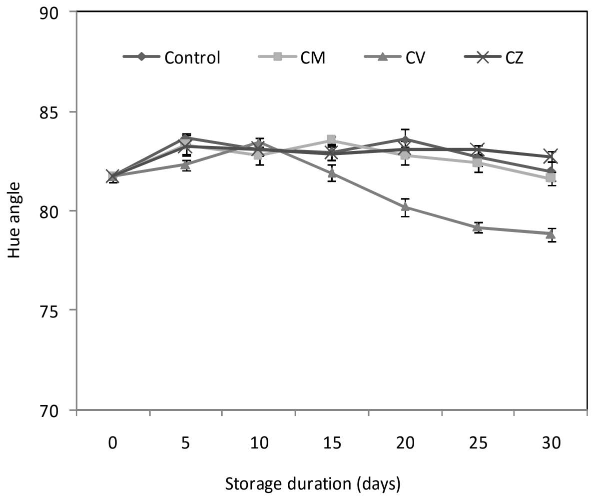
Figure 1b describes the change in hue angle value or actual color of the pineapple with and without coating. With all treatments, flesh color of the fruit was yellow (hue angle value = 45-90°), but there was a difference in the level of yellow color. At the end of the storage period, the flesh color of pineapples uncoated and coated with C/MC and C/MC-carbendazim still kept a slight yellow color, in the range of 81.65-82.70° hue angle, while flesh color of pineapples coated with C/MC-vanillin tended to orange-yellow color, at 78.84°. This result agrees with Sangsuwan et al. (2008a) who reported that vanillin film was more effective in flesh color of fresh-cut pineapple during storage at 10°C. The dramatic decrease in hue angle value of pineapple coated C/MC-vanillin solution may be related to vanillin migration from the solution to fruit surface during storage [21].

Fruit Firmness

Loss of firmness is one of the main factors limiting quality and the postharvest shelf-life of fruits and vegetables. Figure 2 illustrates the changes in flesh firmness of Queen pineapple being stored at 12 ± 1°C, 85 ± 2% RH and coated with different antimicrobial coating solutions of C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) The firmness decresed with increased storage time in all treatments. The initial firmness value of 15.95 N on the first day dropped to the range of 6.95-8.78 N on the 30th day of storage. The decrease in firmness of pineapple flesh might be related to the direct result of cell wall modification during fruit ripening that in turn decreased cells rigidity and eventually generated softness [3]. As shown in Figure 2, there was no significant difference in firmness value of fruit uncoated and coated with C/MC and C/MC-vanillin. However, expectedly, the presence of carbendazim in C/MC solution delayed the



(a)



(b)

Fig.1. Change of flesh color of Queen pineapple fruits uncoated and coated with different antimicrobial coating solutions: C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) at 12 ± 1°C and 85 ± 2% RH: L* value (a) and hue angle (b).

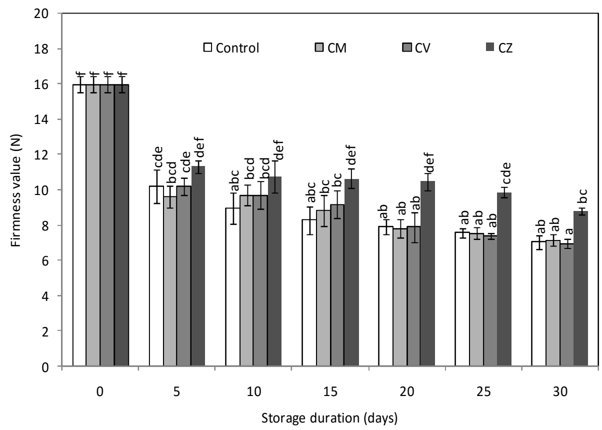


Fig. 2. Change of flesh firmness of Queen pineapple fruit uncoated and coated with different antimicrobial coating solutions: C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) at 12 ± 1°C and 85 ± 2% RH.

decline of firmness. After 30 days storage, the pineapples coated with C/MC-carbendazim achieved the highest value of firmness, (8.78 N) as compared with non-coated fruit and fruit coated by other antimicrobial coating solutions. Our results also are similar with the reported data and the trend on firmness of Smooth Cayenne (Comte de Paris) fruit treated by wax (Sta-Fresh 2952 and Sta-Fresh 7055) and stored at 7°C, 90% RH [13]. These authors found that pineapples coated by wax solutions delayed the change in firmness when compared with controls.

Weight loss, total soluble solids (TSS), pH and titratable acidity (TA)

The change of weight loss percentage and TSS value of pineapples coated with different antimicrobial films are showed in Table 1, 2. Coating processing was expected to decrease the weight loss of pineapples during storage.

However, at low temperature (12°C) and high relative humidity (85% RH) condition, C/MC, C/MC-carbendazim, C/MC-vanillin coating materials did not significantly alter the weight loss of the fruits. Results showed that the weight loss of pineapple increased with storage time in all treatments and the smallest loss of weight was obtained with uncoated fruit and C/MC-carbendazim coating (Table 1). There was no significant difference on weight loss between uncoated pineapple and pineapple coated with C/MC-carbendazim solution, which was 18.80 and 18.76%, respectively on the 30th day of storage. However, presence of vanillin in the C/MC solution increased the weight loss.

The highest value of weight loss was occurred in fruit coated with C/MC-vanillin solution, which was 21.91% on the 30th day storage, followed by C/MC and C/MC-carbendazim as compared with non-coated fruit. Our results also are in accordance with the reported data and the trend on weight loss of apricot fruit stored at 0°C, 80%RH and longan fruit stored at 5°C, 85%RH [2, 12]. However, according to Nimitkeatkai et al. (2006) and Hu et al. (2011), pineapples treated with Sta-fresh 7055 wax and then stored at 7°C and 10°C, 90-95% RH showed significantly delayed weight loss compared to the control fruit [13, 17].

Total soluble solids (TSS) in all treatments increased slightly during the first 15 days and then decreased in the last 10 days (Table 2). Higher TSS values were recorded for coated fruits, and the highest TSS value (16.73%) occurred in fruits coated with C/MC-carbendazim at the end of storage. In comparison, the published TSS values for control and waxed pineapples were 15.3 and 13.9 percent, respectively [13]. Thus, coating fruit by C/MC incorporated with carbendazim might be a useful technique to maintain the sweet taste during cold storage. Increasing the TSS values at the first stage might be concerned with metabolism processes, the ripening stage, and water loss of fruit during storage. By contrast, fruit fermentation by microorganisms is a main cause related to the decrease the TSS at the last stage. Coating pineapples with antimicrobial solutions did not significantly affect on TA and pH values. After 30 days storage, the TA and pH values were in the range of 0.69-0.74 and 3.4-3.7, respectively (data not show).

Ethanol content

In general, fermentation from the fruit sugar and formation of ethanol is a common process of pineapple ripening. The statistical analysis showed that there were significant differences among the mean of ethanol contents in pineapples in all treatments. During the storage time, the ethanol content of uncoated and coated fruits increased but the rate of increasing was different with various treatments (Fig. 3).

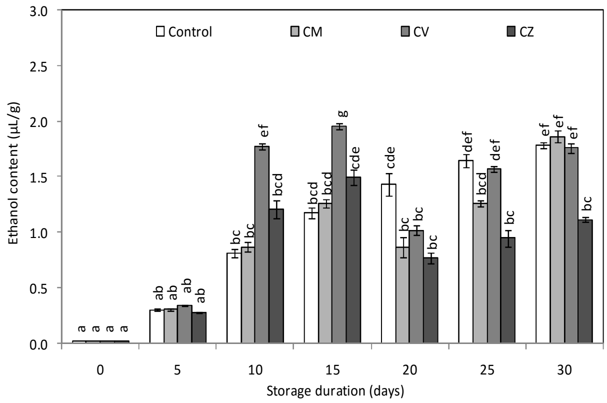


Fig. 3. Change of the ethanol content of Queen pineapple fruit uncoated and coated with different antimicrobial coating solutions: C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) at 12 ± 1°C and 85 ± 2% RH.

During the first 15 days of storage, the ethanol level in pineapples steadily increased and the lowest value of the ethanol was recorded for uncoated pineapple. The fruit coated with C/MC-vanillin contained the highest value of the ethanol (1.96 μL/g) at the 15th day, following by fruit coated with C/MC-carbendazim and C/MC solution. The increase in the ethanol content in fruit during storage might relate to the decrease of total soluble solid content in fruit and fermentation metabolism from microbial activities.

Table 1. Change in weight loss of Queen pineapple fruit uncoated and coated with different antimicrobial coating solutions: C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) at 12 ± 1°C and 85 ± 2% RH.

Day	No coating	Coating		
		C/MC	C/MC-vanillin	C/MC-carbendazim
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5	3.02 ± 0.05^{a}	3.15 ± 0.03^{a}	4.04 ± 0.04^{b}	3.24 ± 0.02^{a}
10	6.56 ± 0.14^{a}	8.35 ± 0.24^{b}	8.26 ± 0.30^{b}	6.46 ± 0.22^{a}
15	9.97 ± 0.27^{a}	11.12 ± 0.39^{b}	13.98 ± 0.31^{b}	9.82 ± 0.52^{a}
20	13.41 ± 0.64^{a}	14.51 ± 0.62^{b}	17.49 ± 0.57^{c}	13.36 ± 0.41^{a}
25	16.62 ± 0.85^{a}	17.51 ± 0.73^{b}	19.86 ± 0.53^{c}	16.53 ± 0.84^{a}
30	18.80 ± 0.86^{a}	19.77 ± 0.64^{b}	21.91 ± 0.75^{c}	18.76 ± 0.72^{a}

Means with different letters are significantly different at p = 0.05.

Table 2. Change in total soluble solids of Queen pineapple fruit uncoated and coated with different antimicrobial coating solutions: C/MC, C/MC-vanillin (CV) and C/MC-carbendazim (CZ) at 12 ± 1°C and 85 ± 2% RH.

Day	No coating	Coating		
		C/MC	C/MC-vanillin	C/MC-carbendazim
0	15.68 ± 0.00^{a}	15.68 ± 0.00^{a}	15.68 ± 0.00^{a}	15.68 ± 0.00^{a}
5	16.25 ± 0.05^{a}	16.55 ± 0.18^{b}	16.75 ± 0.20^{b}	16.80 ± 0.09^{b}
10	17.48 ± 0.10^{c}	16.65 ± 0.05^{a}	17.13 ± 0.04^{b}	17.45 ± 0.14^{c}
15	17.25 ± 0.05^{a}	17.55 ± 0.12^{a}	17.20 ± 0.07^{a}	17.75 ± 0.15^{a}
20	16.83 ± 0.18^{a}	17.38 ± 0.29^{bc}	16.93 ± 0.32^{a}	17.58 ± 0.32^{c}
25	16.48 ± 0.15^{a}	17.03 ± 0.17^{b}	16.50 ± 0.19^{a}	16.98 ± 0.12^{b}
30	15.88 ± 0.14^{a}	16.48 ± 0.12^{c}	16.2 ± 0.15^{b}	16.73 ± 0.10^{d}

Means with different letters are significantly different at p = 0.05.

Table 3. Sensory scores of pineapple uncoated and coated with different coating solutions, and stored at 12 ± 1°C and 85 ± 2% RH for 10, 20 and 30 days.

	Day	Uncoated	C/MC	C/MC-carbendazim	C/MC-vanillin
Acceptance	10	6.60^{d}	6.33^{cd}	6.60^{d}	5.53^{bc}
	20	5.87^{c}	6.07^{cd}	6.27^{cd}	4.80^{b}
	30	3.53^{ab}	3.67^{ab}	3.93^{ab}	3.07^{a}
Color	10	6.53^{bc}	6.67^{bc}	6.73^{bc}	6.07^{bc}
	20	6.87^{c}	6.87^{c}	6.93^{c}	5.53^{bc}
	30	5.13^{ab}	5.07^{ab}	5.40^{ab}	4.33^{a}
Flavor	10	6.07^{bc}	5.87^{bc}	5.80^{bc}	4.93^{b}
	20	6.33^{c}	5.93^{bc}	5.87^{bc}	4.93^{b}
	30	4.13^{a}	4.27^{a}	4.20^{a}	3.93^{a}
Taste	10	6.47^{d}	6.40^{d}	6.40^{d}	6.13^{c}
	20	5.87^{bc}	5.93^{bc}	6.20^{c}	4.87^{b}
	30	3.67^{ab}	3.53^{ab}	3.80^{ab}	2.93^{a}
Firmness	10	6.80^{cd}	6.87^{cd}	7.27^{d}	6.67^{c}
	20	5.93^{b}	6.20^{b}	6.67^{c}	6.07^{b}
	30	3.67^{a}	3.73^{a}	3.93^{a}	3.67^{a}

Total soluble solid amount in initial stage of Queen pineapple was very high (16.25-17.48% Brix) and it becomes an available nutrient sources for microbial growth and then increases the fermentation and ethanol production. However, in the last 15 days, the coating slowed the increase of the ethanol content in fruit. It was found that the ethanol content in coated pineapple was lower than that in uncoated fruit. Especially, the fruit coated with C/MC-carbendazim (CZ) remained the lowest amount of ethanol until the end of the storage (1.12 μL/g) as compared with non-coated fruit and other treatments. Higher ethanol content in control fruit and fruit coated by C/MC and vanillin solutions might be due to higher microbial count. Similar results on ethanol content of fresh-cut pineapple cv. Smooth Cayenne were reported by Sangsuwan et al.,(2008a), which were in the range of 0.35-0.6 μL/g on the 12th day storage at 10°C [21].

Sensory values

Pineapple coated with chitosan/methylcellulose incorporated with carbendazim was not significantly different from the control (uncoated) in most of attributes after storage at 12°C, 85% RH for 10, 20 and 30 days storage (Table 3).

Comparing with the control and other treatments, pineapple coated with C/MC-carbendazim showed the higher acceptability for color and firmness. The scores of acceptability of fruits coated with C/MC-carbendazim at the 10th, 20th and 30th day storage were 6.60, 6.27 and 3.93 while fruits fruits coated with C/MC-vanillin were 5.53, 4.80 and 3.07, respectively. At the 30th day storage, there was a drop in acceptance, color, taste, flavor and firmness scores for all treatments, which was lower than 5. Overall attributes, color, flavor and taste had low acceptability for pineapple coated with C/MC incorporated with vanillin. Our results agreed with the change of sensory evaluate for fruit wrapped or treated with vanillin. The fresh-cut pineapple declined its taste and flavor and had low acceptability after 6 days storage at 10°C [22, 10].

IV. Conclusions

The influence of coating and low storage temperature on quality of Queen pineapple planted in Ninh Binh province, Vietnam was studied. Addition of vanillin or carbendazim into chitosan/methylcellulose solution reduced the changes in total soluble solids, flesh firmness, flesh color and microorganisms levels, thus delaying the ethanol production. C/MC incorporated with carbendazim (1.6 g/100 g solid of C/MC) was more effective at inhibiting microorganisms. Lower ethanol content, higher total soluble solids, acceptable flesh firmness, and lighter flesh color were found in pineapples coated with C/MC incorporated with carbendazim. Quality attributes of fresh pineapple were generally acceptable. Therefore, these results suggested that combining the coating for Queen pineapple with C/MC-carbendazim and maintaining the fruit at 12°C, 85% RH might be a useful technique to extend the shelf life for fruit while maintaining good quality.

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