

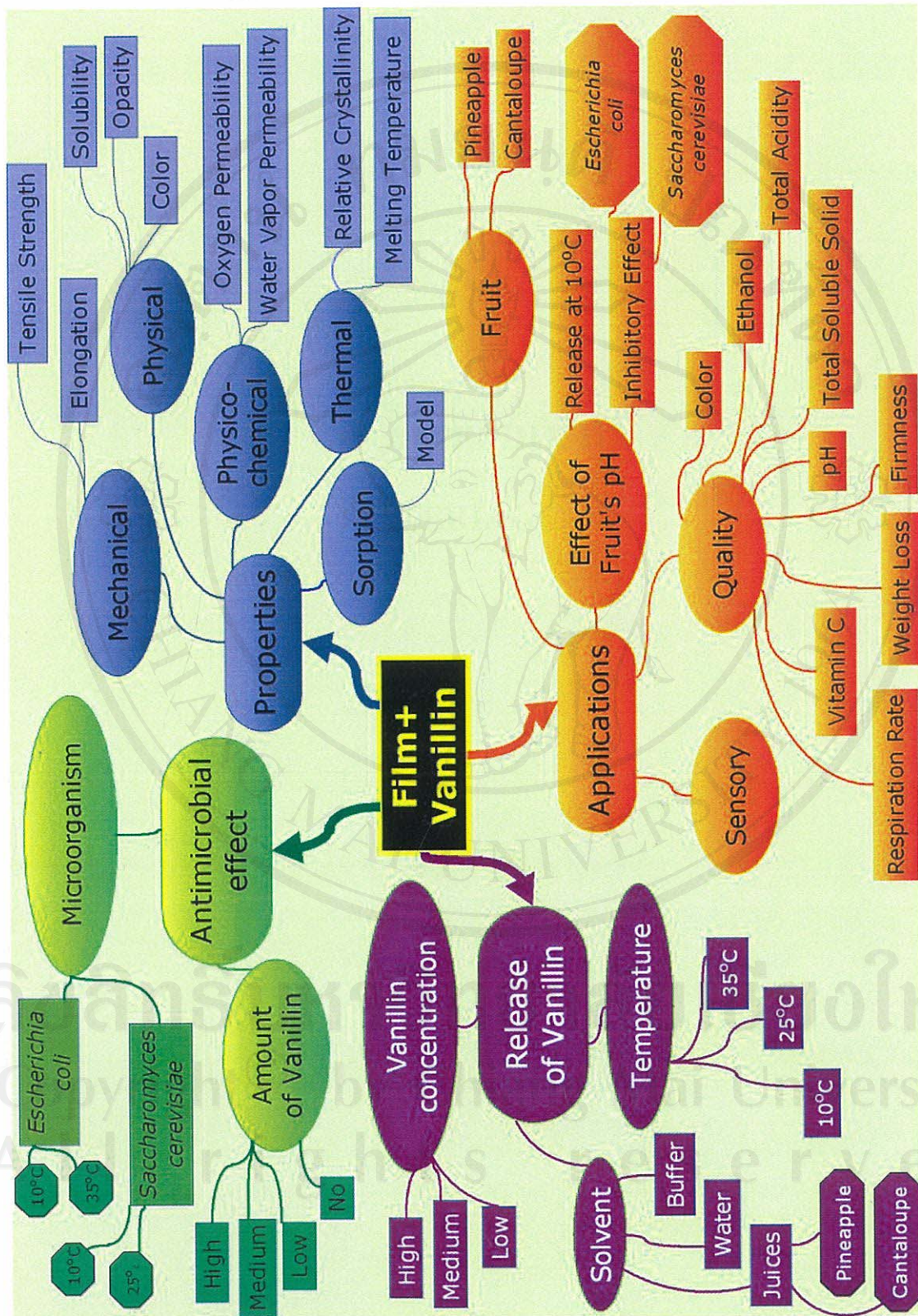
**APPENDIX 1**

**MIND MAP**

**ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่**

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ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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**Chitosan/methylcellulose film**

Formulations			Material Cost (Baht/g or ml)	Material Cost (Baht)
Chitosan	1.5	g	0.70	1.05
Methylcellulose	0.5	g	1.00	0.50
Ethanol	50	ml	0.12	6.11
PEG	0.99	g	0.22	0.22
Stearic acid	0.075	g	0.15	0.01
Acetic acid	1	ml	0.16	0.16
			Total cost/5 films	8.05
			<b>Total cost/film</b>	<b>1.61</b>

**Chitosan/methylcellulose film containing high natural vanillin**

Formulations			Material Cost (Baht/g or ml)	Material Cost (Baht)
Chitosan	1.5	g	0.70	1.05
Methylcellulose	0.5	g	1.00	0.50
Ethanol	50	ml	0.12	6.11
PEG	0.99	g	0.22	0.22
Stearic acid	0.075	g	0.15	0.01
Natural vanillin	0.9	g	9.98	8.99
Acetic acid	1	ml	0.16	0.16
			Total cost/5 films	17.04
			<b>Total cost/film</b>	<b>3.41</b>

**Chitosan/methylcellulose film containing high synthetic vanillin**

Formulations			Material Cost (Baht/g or ml)	Material Cost (Baht)
Chitosan	1.5	g	0.70	1.05
Methylcellulose	0.5	g	1.00	0.50
Ethanol	50	ml	0.12	6.11
PEG	0.99	g	0.22	0.22
Stearic acid	0.075	g	0.15	0.01
Synthetic vanillin	0.9	g	0.90	0.81
Acetic acid	1	ml	0.16	0.16
			Total cost/5 films	8.86
			<b>Total cost/film</b>	<b>1.77</b>

## PUBLICATIONS

**International oral presentations**

**Sangsuwan, J., Rattanapanone, N., Harte, B. R., Auras, R. A. and Rachtanapun, P.** (2008). Antimicrobial effect and migration of vanillin in chitosan-methyl cellulose films. *IAPRI World Conference on Packaging*. Bangkok. June 8-12, 2008.

**National poster presentations**

**Sangsuwan, J., Rattanapanone, N. and Rachtanapun, P.** (2008). Effects of vanillin in chitosan/methyl cellulose films on microbial control of fresh-cut cantaloupe and pineapple. *RGJ-Ph.D. Congress IX*. Chonburi. April 4-6, 2008.

**Sangsuwan, J., Rattanapanone, N. and Rachtanapun, P.** (2006). Effect of vanillin on properties of chitosan-methyl cellulose based film. *4<sup>th</sup> National Technical Seminar on Postharvest / Post Production Technology*. Chiangmai. June 8-9, 2006.

**Sangsuwan, J., Rattanapanone, N., Rachtanapun, P. and Boonprasom, P.** (2005). Development of biodegradable films from chitosan and cellulose derivatives. *3<sup>rd</sup> National Technical Seminar on Postharvest / Post Production Technology*. Petchaburi. October 10-11, 2005.

**Journal publications**

**Sangsuwan, J., Rattanapanone, N. and Rachtanapun, P.** (2008). Effects of vanillin and plasticizer on properties of chitosan-methyl cellulose based film. *Journal of Applied Polymer Science*. 109: 3540-3545.

**Sangsuwan, J., Rattanapanone, N. and Rachtanapun, P.** (2008). Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. *Postharvest Biology and Technology*. 49: 403-410.

**Sangsuwan, J., Rattanapanone, N., Auras, R., Harte, B. and Rachtanapun, P.** Factors affecting migration of vanillin from chitosan/methylcellulose films. (In preparation).

# Effects of Vanillin and Plasticizer on Properties of Chitosan-Methyl Cellulose Based Film

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**ABSTRACT:** Chitosan-methyl cellulose based films which incorporate vanillin as an antimicrobial agent and polyethylene glycol 400 (PEG) as a plasticizer were developed in this study. The effects of vanillin and plasticizer concentration on mechanical, barrier, optical, and thermal properties of chitosan-methyl cellulose film were evaluated. When the vanillin concentration was increased at a given PEG level, film flexibility decreased while tensile strength increased slightly. Vanillin increased the barrier to oxygen but not water vapor. Increasing vanillin content resulted in less transparency and a more yellowish tint. The bulky na-

ture of vanillin reduced film crystallization. When PEG concentration was increased at a given vanillin level, it resulted in greater film flexibility but reduced film strength. Water vapor permeability (WVP) and oxygen permeability (OP) increased with increase in PEG content. PEG contributed less to the opacity, yellowness, and crystallization of the film than did vanillin. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 109: 3540–3545, 2008

**Key words:** chitosan; vanillin; polyethylene glycol; biodegradable; mechanical and barrier properties

## INTRODUCTION

Recent food-borne microbial outbreaks have stimulated interest in searching for innovative ways to inhibit microbial growth in foods. At the same time, consumers are demanding foods that do not contain synthetic chemical preservatives, so natural preservatives are receiving more attention. However, antimicrobials, directly incorporated into food may be rapidly lost because of evaporation into the atmosphere or thorough reaction with food components.<sup>1,2</sup> One option is to use packaging materials that have antimicrobial properties to help assure food safety and quality. Antimicrobials can be designed so as to be gradually released from the packaging material so as to remain on the food surface as long as possible. Research and development of antimicrobial materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymer

materials and antimicrobials. The next generation of food packaging may include materials with antimicrobial properties.<sup>3</sup> These packaging technologies could help extend the shelf-life of foods while reducing the risk from pathogens. The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research.

Some researchers have incorporated essential plant oils in biodegradable packaging as antimicrobial active agents, including anise, basil, coriander, oregano, and garlic oil.<sup>4,5</sup> Antimicrobial chitosan edible films incorporating garlic oil up to levels at least 100 µL/g were found to have antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. Films with 1 and 2% oregano essential oil decreased the number of *L. monocytogenes* by 3.6 to 4 logs and *Escherichia coli* O157:H7 by 3 logs. Garlic oil components did not affect the physical and mechanical properties of chitosan films. Addition of oregano essential oil to chitosan films decreased water vapor permeability, puncture and tensile strength while increasing the elasticity of the film. However, they still faced a sensory problem when these films were used with food since essential oils have strong odors.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major flavor constituent of vanilla beans and a principle flavor compound used in numerous foods. Recent reports have shown that vanillin can be effective

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in inhibiting bacteria, yeasts, and molds.<sup>6-9</sup> Vanillin is hydrophobic and structurally similar to eugenol. Rupasinghe et al.<sup>10</sup> examined the antimicrobial effect of vanillin on four pathogenic or indicator organisms; *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Salmonella enterica* and four spoilage organisms: *Candida albicans*, *Lactobacillus casei*, *Penicillium expansum*, and *Saccharomyces cerevisiae*. They found that the minimum inhibitory concentration of vanillin was dependent on the microorganism (6–18 mM). Microbial growth was inversely proportional to vanillin concentration. Oral LD50 values for vanillin in animals were reported to be 3.0 g/kg for rabbits, 1.58–2.8 g/kg for rats, and 1.4 g/kg for guinea pigs, which indicates that these animals have low oral toxicity.<sup>11</sup> They also found that vanillin was found to be a moderate skin allergen in animals. Vanillin is not responsible for most cases of natural vanilla sensitivity.

Direct incorporation of vanillin as an antimicrobial agent into chitosan-methyl cellulose mixtures is an economical method for producing antimicrobial packaging. However, the interaction between vanillin and film may affect the film properties. Therefore, effects of vanillin and plasticizer content (PEG) in chitosan-methyl cellulose based film on mechanical (tensile strength and percent elongation), physicochemical (water vapor permeability and oxygen permeability), optical, and thermal properties were investigated.

## MATERIALS AND METHODS

### Film preparation

Chitosan with 90% deacetylation and purity of more than 99.75% (Bannawach Bio-line Co., Chonburi, Thailand) was prepared by dissolving 1.5 g of chitosan in 100 mL of a 1% acetic acid solution (MERCK, Darmstadt, Germany). One half gram of methyl cellulose (M-043, BENECEL®, Wilmington, DE) was dissolved in 50% ethanol (MERCK). Polyethylene glycol 400 (PEG) was used as a plasticizer according to Table I (low plasticized film) and (high plasticized

TABLE I  
Film Formulation

Film	No vanillin	Low vanillin	Medium vanillin	High vanillin
Low plasticized film with different vanillin concentrations				
%PEG <sup>a</sup>	0.17	0.17	0.17	0.17
%Vn <sup>a</sup>	0	0.15	0.30	0.45
High plasticized film with different vanillin concentrations				
%PEG <sup>a</sup>	0.50	0.50	0.50	0.50
%Vn <sup>a</sup>	0	0.15	0.30	0.45

<sup>a</sup> % in the table expressed % w/v of film forming solution.

film). Solutions of chitosan and methyl cellulose were mixed and heated to 72°C. Stearic acid was added (15% of cellulose derivatives) to improve the water barrier property. Vanillin (Vn), purchased from Sigma, St. Louis, was incorporated into the chitosan-methyl cellulose solution after the temperature of solution reached 83°C. The film-forming solution was then filtered to remove particles, degassed, poured onto glass plates, and dried at 40°C for 42 h. Dried films obtained were peeled off and conditioned at (25 ± 2)°C, (50 ± 5)% RH for at least 48 h before testing. Film thickness was measured using a gauge micrometer (GT-313-A, Gotech testing machines, Taichung City, Taiwan) with an accuracy of 0.01 mm. The reported thickness values are the average of at least 30 measurements.

### Determination of mechanical properties

Tensile strength (TS) and percent elongation (%E) were tested by using the Instron Universal Testing Instrument (Model 5565, Instron, Canton, MA) following ASTM D638M (ASTM, 1993). Film specimens were cut into rectangular strips, 1 × 10 cm<sup>2</sup>. Initial grip separation was 5 cm and cross-head speed was 25 mm/min. TS was calculated by dividing the peak load by the cross-sectional area (average thickness × 1 cm) of the initial specimen. %E was defined as the percentage of a change in the length ( $\Delta L$ ) of the specimen to the original length ( $L$ ) between the grips (5 cm). Data of TS and %E were obtained from 8 replications of samples.

### Determination of water vapor permeability and oxygen permeability

Water vapor permeability (WVP) was determined according to ASTM E96-00 (ASTM, 2000). Film specimens with approximately 8 cm diameter were mounted on the aluminum cups containing 10 mL of distilled water. Paraffin was used to fix a film specimen with the wide rim of aluminum cup. They were weighed and then placed in a desiccator containing saturated magnesium nitrate salt. The relative humidity of the chamber was kept at 53% and 23°C throughout the experiment. Weight loss of the aluminum cups covered with films was recorded daily for 5 days. The test was performed in triplicate. WVP was calculated by using the following equation.

$$WVP = \left( \frac{W}{t} \right) \left( \frac{x}{A\Delta P} \right)$$

where

$W/t$  = the slope of the plot between weight loss and time,

$x$  = the average thickness of the films,

$A$  = the permeation area, and

$\Delta P$  = the partial pressure difference of distilled water in the cup and atmosphere in desiccator.

Oxygen permeability (OP) was tested by using Gas Permeability Tester VAC-V1 (M and E Instruments, Jinan, China) according to ASTM D1434-82 (ASTM, 2003). Film specimen with 10 cm diameter was fixed between upper and lower chamber. Oxygen in both chambers was removed by vacuum for 8 h. After 8 h, oxygen was filled in the upper chamber. The amount of oxygen that permeated through the film in the lower chamber was determined. The test was done in duplicate at 23°C.

#### Determination of opacity and color

Opacity and color of film were measured by HunterLab color meter (ColorQuest® XE, HunterLab, Reston, VA). Absolute measurements were displayed as tristimulus color values which closely represents human sensitivity. All data were obtained from 8 replications of samples.

#### Thermal properties

The determination of the thermal properties of films was accomplished by differential scanning calorimetry (TA Instruments, New Castle, DE), using a DSCQ100 (TA Instrument). Approximately 10 mg film ( $\pm 0.001$  mg) was weighed in a precision balance model xs205 (METTLER TOLEDO, Menlo Park, CA). Aluminum pan containing film was heated up at 10°C/min from -50 to 350°C in inert atmosphere (50 mL/min of N<sub>2</sub>).

## RESULTS AND DISCUSSION

The inhibition effect of two experimental films (chitosan-methyl cellulose film and chitosan-methyl cellulose film with high vanillin concentration) on *Escherichia coli* and *Saccharomyces cerevisiae* was tested by wrapping the films around inoculated fresh-cut cantaloupe and pineapple. Both films provided an inhibitory effect against *Escherichia coli* on fresh-cut cantaloupe. The chitosan-methyl cellulose film rapidly reduced the number of *Saccharomyces cerevisiae* yeast inoculated on cantaloupe and pineapple. Chitosan-methyl cellulose film with vanillin was more efficient than chitosan-methyl cellulose alone in reducing the number of yeast, which decreased by 4 logs in fresh-cut pineapple on day 6.<sup>12</sup>

Chitosan-methyl cellulose film was apparently more hydrophilic than films with vanillin when applied on fresh-cut fruit. After absorbing some water from fruit wedges, chitosan-methyl cellulose film swelled and no longer had a smooth surface. Therefore, chitosan-methyl cellulose film should only

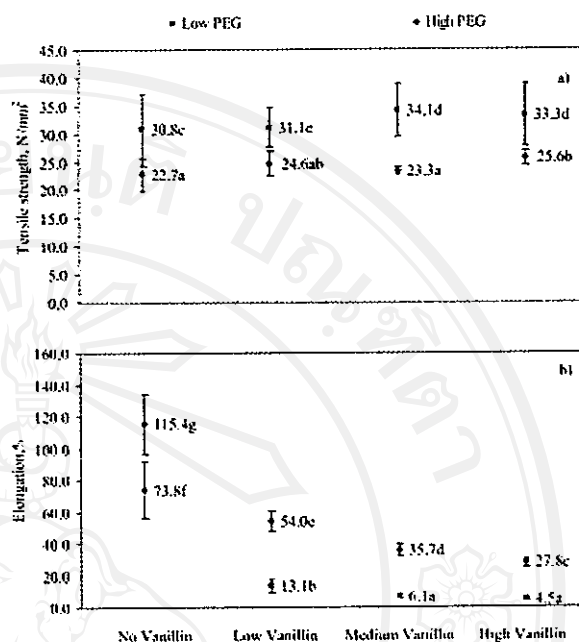


Figure 1 (a) Tensile strength and (b) percent elongation of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

be used with low moisture products because it has poor wet strength. Film containing vanillin maintained its strength when wet much better than film without vanillin. It can be used as a wrap with high moisture foods such as fresh-cut fruits.<sup>12</sup>

#### Mechanical properties

Thickness of films containing low PEG (0.17%) and high PEG (0.5%) varied from 30 to 42 and 37–47 micron, respectively. At a given vanillin level, films with higher amount of PEG were thicker than those containing low PEG. At a given plasticizer (PEG) level, film thickness increased with increasing amount of vanillin in formula. Therefore, both PEG and vanillin contributed to film thickness.

The tensile strength of the chitosan-methyl cellulose film was affected by the concentration of vanillin, the antimicrobial agent, and PEG, the plasticizer [Fig. 1(a)]. The tensile strength of film containing low PEG was greater than those containing high PEG regardless of the vanillin content. The tensile strength of film with low PEG was not affected by low vanillin content, but increased when the vanillin concentration was increased to medium or high level. With film containing high level of PEG, addition of vanillin had an effect on the tensile strength, but was not consistent with the level of vanillin.



The percent elongation, indicator of flexibility, was affected by the various combinations of vanillin and PEG [Fig. 1(b)]. The percent elongation of film containing low PEG was less than those containing high PEG at a given vanillin content. The percent elongation of low PEG film decreased substantially when low vanillin content was added and decreased more with additional amount of vanillin in the film. Addition of vanillin to high PEG film also decreased the percent elongation and the decrease continued as the vanillin level increased. High vanillin film which had the very low %E cracked instantly when it was folded, thus these film required more PEG to remain flexible.

Incorporation of the antimicrobial vanillin into the film changed the functional characteristics of the packaging materials. Molecular structure of vanillin is composed of an aromatic benzene ring like styrene monomer. The bulky structure of vanillin made the film more rigid and contributed to the loss of its segmental mobility.<sup>13</sup> TS of all films were comparable with those of commercial HDPE films (20–37.2 N/mm<sup>2</sup>).<sup>14</sup> The high TS values were attributed to the numerous hydrogen bonds between methyl cellulose chains. These bonds contribute to the cohesiveness and low flexibility of unplasticized films.<sup>15</sup> Our results were similar to that of Zivanovic et al.<sup>5</sup> who found that addition of oregano essential oil into the chitosan films decreased puncture and TS, but increased elasticity of the films. Pranoto et al.<sup>4</sup> found a greater reduction of TS and increment of %E of chitosan film when incorporating potassium sorbate and nisin but TS and %E of chitosan film with garlic oil did not alter because garlic oil components have no interaction with the functional groups of chitosan. However, Chen et al.<sup>16</sup> demonstrated that addition of 4% benzoate and sorbate into chitosan/methyl cellulose film resulted in higher TS and %E. The effect of plasticizer on mechanical properties of film agreed with Park et al.<sup>17</sup>

#### Water vapor permeability and oxygen permeability

The presence of vanillin was expected to increase the water vapor barrier property due to hydrophobicity of vanillin entities. However, vanillin did not significantly alter the water barrier property of films [Fig. 2(a)]. Plasticizer had dominant effect on water barrier property. Higher PEG content in film significantly allowed water to pass through. Butler et al.<sup>18</sup> reported that higher plasticizer concentration in chitosan film yielded higher water vapor permeability value. Pranoto et al.<sup>4</sup> incorporated garlic oil which was hydrophobic like vanillin into chitosan films. They found that garlic oil did not affect WVP of film as well. Even though the WVP of the films did not differ significantly, film with vanillin was superior

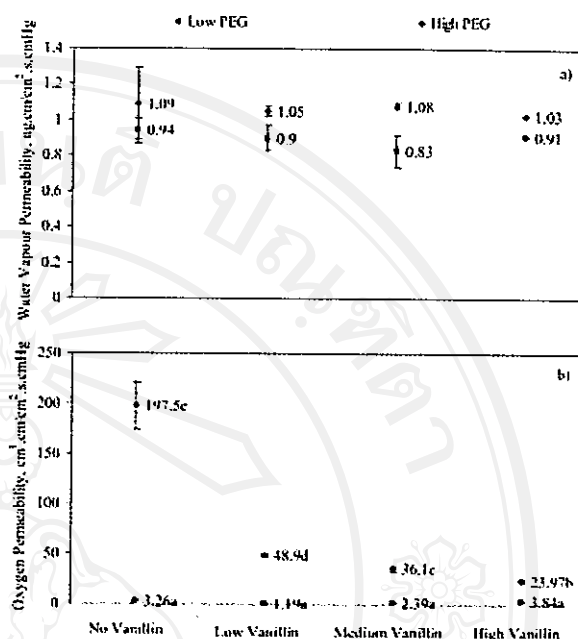
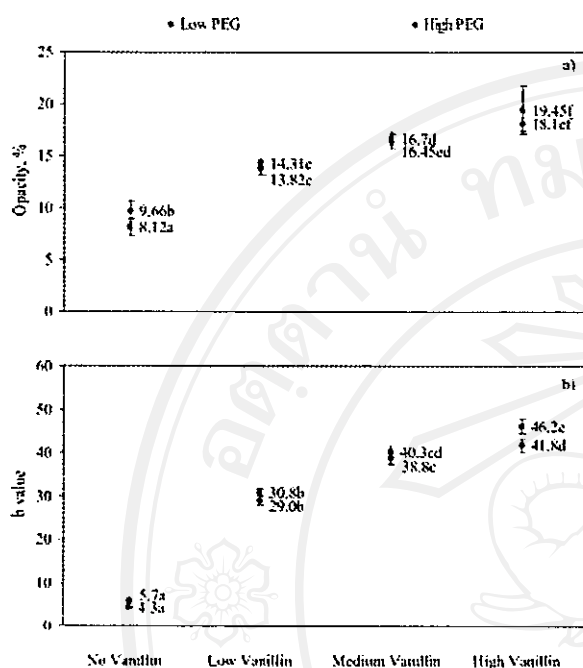


Figure 2 (a) Water vapor permeability and (b) oxygen permeability of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

in maintaining wet strength than film without vanillin. Chitosan-methyl cellulose film without vanillin was easy to tear apart when wet.

In films containing low PEG, OP did not change with increase in vanillin concentration [Fig. 1(b)]. In contrast, OP was reduced significantly with addition of vanillin to high PEG film and the decrease was significant with each incremental increase in vanillin. The molecular structure of vanillin contains a hydroxyl group which might increase polarity and hydrogen bond formation of film. Park et al.<sup>17</sup> reported that OP and WVP values of methyl cellulose containing PEG generally were not affected by plasticizer concentration.

At a given vanillin content, OP of chitosan-methyl cellulose film increased as concentration of PEG was increased. Park and Chinan<sup>19</sup> also reported that OP and WVP increased as the concentration of plasticizer increased. Permeability ( $P$ ) is equal to the product of the diffusion coefficient ( $D$ ), which represents the mobility of permeant molecules in the polymer, and the solubility coefficient ( $S$ ), which represents the permeant concentration in the film in balance with the external pressure:  $P = DS$ . Increased permeability could thus be related to an increase in the diffusion coefficient, due to structural changes in the polymer matrix, and also to an increase in the oxygen solubility in the film because of the increased



**Figure 3** (a) Opacity and (b) *b* value of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

water content in the high PEG film (oxygen solubility in water = 1.25 mM at 25°C and 1 atm). Water molecules in the polymer interact with hydrophilic groups and thus may disrupt hydrogen bonding, creating additional sites for the dissolution of oxygen and increase mobility of oxygen molecules within the polymer.<sup>20</sup>

Chitosan-methyl cellulose film provided an excellent oxygen barrier compared with commercial plastics. All films in this study had better oxygen barrier compared with PETP, PA, PCTFE, OPP, HDPE, PS, PC, and LDPE which is commonly used as food packaging.

#### Opacity and color

Opacity and *b* value of films are shown in Figure 3. Opacity is a reflection of the transparency of films while the *b* value represents the yellowish color of the films. The increase in the *b* value indicates that the color of the film became more yellow. Without vanillin in the formula, high PEG film was more opaque and yellow than low PEG film. Migration of PEG to film surface was observed in high PEG film with no vanillin which caused the greasy appearance. The opacity and yellowness of film increased significantly with increasing percent of vanillin in the film-forming solution. At a given level of vanil-

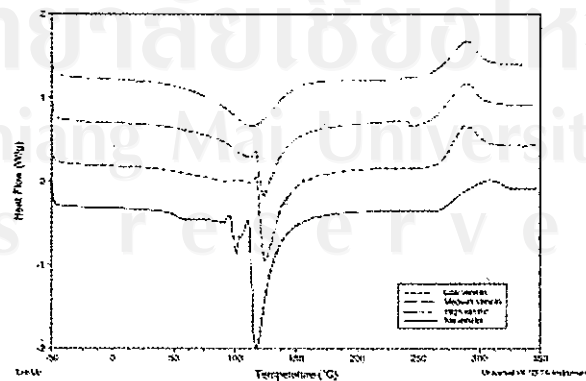
lin, both films containing high- and low-level of PEG had almost the same color and opacity. Therefore, PEG had less effect on film color and opacity than vanillin. Zivanovic et al.<sup>5</sup> found that addition of oregano essential oil into the chitosan films resulted in opaque film.

#### Thermal properties

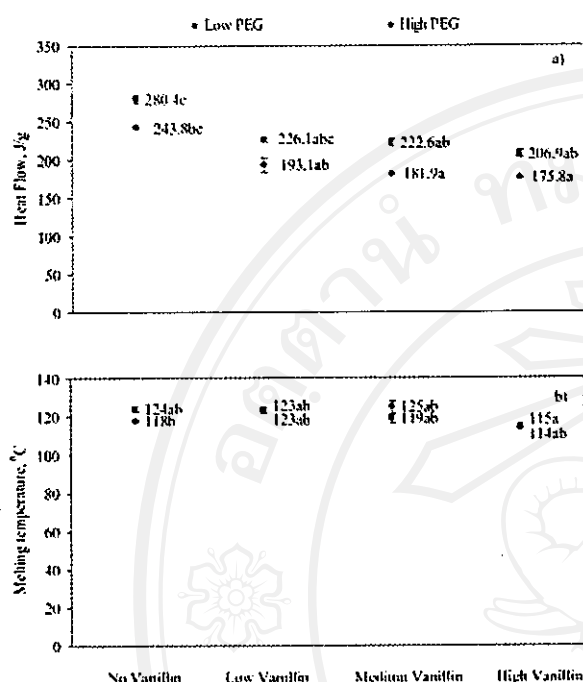
DSC thermograms of highly plasticized films with different vanillin concentrations are shown in Figure 4. A temperature scan from -50 to 350°C at 10°C/min with no preheat treatment was used to observe the thermal properties of the film, including the miscibility of vanillin in chitosan-methyl cellulose film and relative crystallinity of film. Chitosan-methyl cellulose film had an endothermic peak around 100°C which is associated with the loss of absorbed water from the film matrix. Thermograms of chitosan-methyl cellulose film containing high vanillin concentration observed in this study had only one peak, which indicated well miscibility of film component. Thermogram area expressed the heat of fusion of film. At a given PEG level, thermogram area decreased with increase in vanillin concentration [Fig. 5(a)]. The reduction of area should be the effect of lower crystallization due to vanillin has an aromatic benzene structure. As the result, the bulky benzene structure interrupted the rearrangement of polymer chain.

At a given level of vanillin, both high- and low-PEG film had insignificant different heat of fusion.

Effects of vanillin and plasticizer on melting temperature were not obvious [Fig. 5(b)]. Nothing was observed in the second scan at the cooling rate of 2°C/min. This cooling rate should be too fast to allow the rearrangement of polymer chains.



**Figure 4** DSC thermograms of high plasticized chitosan-methyl cellulose films containing different levels of vanillin. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



**Figure 5** (a) Heat flow and (b) melting temperature of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

### CONCLUSIONS

The concentration of vanillin and PEG affected mechanical, barrier, optical, and thermal properties of chitosan-methyl cellulose based film. Vanillin reduced and PEG increased film flexibility. Addition of vanillin content in film slightly improved film strength while PEG decreased film strength. Vanillin did not improve water barrier of film but improve oxygen barrier. Higher plasticized film provided the negative effect on both water and oxygen barrier. Vanillin affected film opacity and yellowness more

than PEG. Both vanillin and PEG reduced crystalline formation of film.

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## Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple

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Antimicrobial film

## ABSTRACT

Two experimental films were applied on fresh-cut cantaloupe and pineapple and their effects on microbial control and fruit quality were investigated during storage at 10 °C. Three types of films were used in this study: a commercial stretch film, an experimental chitosan/methyl cellulose film, and a chitosan/methyl cellulose film incorporating vanillin (vanillin film) as a natural antimicrobial agent. Fresh-cut fruit without any film wrapping served as controls. Chitosan/methyl cellulose film and vanillin film provided an inhibitory effect against *Escherichia coli* on fresh-cut cantaloupe. The chitosan/methyl cellulose film rapidly reduced the number of *Saccharomyces cerevisiae* yeast inoculated on cantaloupe and pineapple. Vanillin film was more efficient than chitosan/methyl cellulose in reducing the number of yeast, which decreased by 4 logs in fresh-cut pineapple on day 6. Vanillin film increased the intensity of yellow color of pineapple. Pineapple removed from stretch film had higher respiration rates and ethanol contents than other treatments. Unsurprisingly, the stretch film maintained the moisture content in fruit better than other treatments. However, vanillin film reduced the ascorbic acid content in pineapple. At the end of storage, ascorbic acid in pineapple wrapped with vanillin film was only 10% of its original concentration.

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## 1. Introduction

Chitosan is the second most abundant polysaccharide on earth and is inherently antimicrobial (Goldberg et al., 1990). Furthermore, it provides films with good mechanical and oxygen barrier properties (Caner et al., 1998; Chen et al., 1996). Chitosan, however, has poor tensile strength when wet. It is rigid and has poor elongation properties. Blending cellulose with chitosan can be expected to correct these weaknesses; film flexibility has been shown to increase with increasing methyl cellulose content (Garcia et al., 2004).

The growth of microorganisms on the cut surfaces is a main cause of food spoilage for fresh-cut produce. The application of antibacterial substances directly onto a food has some limitations because the active substances can be neutralized, evaporated or they may inadequately diffuse into the bulk of the food (Torres et al., 1985; Siragusa and Dickson, 1992). The incorporation of antimicrobial agents into packaging can create an environment inside the package that may delay or prevent the growth of microorganisms on the product's surface and, hence, lead to an extension of its shelf-life. Antimicrobial packaging has attracted much attention from

the food industry because of the increase in consumer demand for minimally processed and preservative-free products. Reflecting this demand, preservative agents (preferably natural preservatives) must be applied at the lowest effective level possible (Cha and Chinnan, 2004). According to Brody et al. (2001), the antimicrobial effect of chitosan occurs when organisms are in direct contact with the active sites of chitosan. When antimicrobial agents are incorporated into film, they diffuse out of the film, thus improving its antimicrobial efficacy. Zivanovic et al. (2005) applied chitosan-oregano essential oil (EO) in comparison with chitosan films on inoculated bologna meat samples stored for 5 d at 10 °C. Pure chitosan films reduced *Listeria monocytogenes* by 2 logs, whereas the films with 1 and 2% oregano EO decreased the numbers of *L. monocytogenes* by 3.6 to 4 logs and *Escherichia coli* by 3 logs. Pranoto et al. (2005) incorporated garlic oil, potassium sorbate and nisin in chitosan films. The activity of the antimicrobial films was tested against the food pathogenic bacteria, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *L. monocytogenes*, and *Bacillus cereus*. They found that the pure chitosan film had no inhibitory effect. Incorporation of 100 µL of garlic oil/g, 100 mg potassium sorbate/g or nisin at 51,000 IU/g of chitosan had antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*.

Many consumers have concerns over the addition of chemical additives to food, and this has driven the food industry

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Table 1  
Numbers of *Escherichia coli* on inoculated cantaloupe (log cfu/piece) during storage at 10 °C

	No film	Stretch film	Chitosan/methyl cellulose film	Vanillin film
0	5.18 ± 0.00 f	5.18 ± 0.00 f	5.18 ± 0.00 f	5.18 ± 0.00 f
1	4.50 ± 0.23 e	5.45 ± 0.19 fg	3.88 ± 0.52 de	4.43 ± 0.20 e
2	3.94 ± 0.01 de	5.95 ± 0.05 g	2.29 ± 0.83 c	3.54 ± 0.58 d
4	2.86 ± 0.15 c	7.19 ± 0.22 h	1.00 ± 0.00 b	1.00 ± 0.00 b
6	2.56 ± 0.15 c	8.98 ± 0.05 i	0.74 ± 0.00 b	0.74 ± 0.00 b
8	1.00 ± 0.00 b	9.27 ± 0.05 i	0 a	0 a

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

Food research towards the search for natural antimicrobial compounds (Devlieghere et al., 2004). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major constituent of vanilla beans and is a flavor compound used in many baked or processed foods. Prindle and Wright (1977) found that the effect of phenolic compounds was concentration dependent. At low concentrations, phenols affected enzyme activity, especially those enzymes associated with energy production, while at greater concentrations, they caused proteins to denature. The antimicrobial activity of vanillin depended on the time of exposure, concentration and the target organism. Recent reports have shown that vanillin can be effective in inhibiting bacteria, yeasts and molds (Jay and Rivers, 1994; Cerrutti and Alzamora, 1996; Matamoros-Leon et al., 1999; Fitzgerald et al., 2004). Vanillin has been used to inhibit *E. coli* O157:H7 and *L. monocytogenes* in 'Granny Smith' apple juice (Moon et al., 2006). Rupasinghe et al. (2006) reported that total aerobic counts of fresh-cut apple slices decreased from 4.3 log cfu/g fresh weight (untreated) to 1.6 log/cfu by using NatureSeal (an anti-aging agent) plus 12 mM vanillin after 19 d at 4 °C. Cerrutti et al. (1997) treated strawberry puree with a mild heat treatment combined with 3000 mg/L vanillin and 500 mg/L ascorbic acid. They found that the inhibition of native and inoculated flora was for at least 60 d storage at room temperature. Penney et al. (2004) found that vanillin at 2000 mg/L suppressed fungal and bacterial microbial growth in yoghurt significantly over the 3-week period.

Research on the application of antimicrobial biodegradable films on fresh-cut fruit is limited. The objectives of this work were to evaluate the inhibitory effect of chitosan/methyl cellulose film and chitosan/methyl cellulose film with vanillin against *E. coli* and *Saccharomyces cerevisiae*, and to determine the effect of these films on fresh-cut cantaloupe and pineapple quality.

## Material and methods

### Film preparation

Chitosan with a degree of deacetylation of 90% and purity 97.5% (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 methyl cellulose, 1.5 g. (M-043, BENECEL®) was dis-

solved in 50% ethanol. One gram of polyethylene glycol (PEG) 400 was used as a plasticizer. Solutions of chitosan and methyl cellulose were mixed in a beaker with a stir bar and heated to 72 °C. Stearic acid, 0.075 g was added to improve the water barrier properties of the film. Vanillin, 0.9 g (Sigma, St. Louis, USA) was incorporated after the temperature of the solution reached its melting point (83 °C). The film-forming solution was filtered through a cheese cloth to remove undissolved parts, homogenized with a homogenizer, degassed, cast onto glass plates, and dried at 40 °C for 42 h. Dried films were peeled off and conditioned at 25 ± 2 °C, 50 ± 5% RH for at least 48 h prior to use. Film thickness was measured with a gauge micrometer GT-313-A (Taiwan) with an accuracy of 0.01 mm.

### 2.2. Fruit preparation

Cantaloupe (*Cucumis melo*) and pineapple (*Ananas comosus*) fruit were purchased from a wholesale market in Chiang Mai province, Thailand. Total soluble solids were measured to indicate fruit maturity. Cantaloupe and pineapple used in this study had total soluble solids in the range of 7.0–8.2 and 17.0–19.4%, respectively. Whole fruit were washed with 500 mg/L chlorine solution. The blossom and stem ends were discarded. Cantaloupe and pineapple were sliced longitudinally into 12 wedges and 8 wedges, respectively using a sanitized sharp knife and cutting board. Then, the seeds or core, and peel were removed. All knives, cutting boards and other equipment which come into contact with the fruit were sanitized by immersion in 1000 mg/L chlorine solution for 30 min before cutting.

### 2.3. *E. coli* and *S. cerevisiae* inoculation and determination of *E. coli* and *S. cerevisiae* number through incubation

Cantaloupe and pineapple wedges were cut into 2.5 cm × 2.5 cm × 0.5 cm pieces. They were then inoculated with 20 µL of approximately 10<sup>5</sup> cfu/mL *E. coli* (TISTR 780) or *S. cerevisiae* (TISTR 5240) suspensions on the top surface of each piece (Zivanovic et al., 2005). Then, commercial stretch film, M wrap®, chitosan/methyl cellulose film and chitosan/methyl cellulose film with vanillin were wrapped around each piece. Wrapped fruit were placed on polystyrene trays and stored at 10 °C up to 20 d. Inoculated fruit without any wrapping served

Table 2  
Numbers of *Saccharomyces cerevisiae* on inoculated cantaloupe (log cfu/piece) during storage at 10 °C

	No film	Stretch film	Chitosan/methyl cellulose film	Vanillin film
0	5.36 ± 0.00 bc	5.36 ± 0.00 bc	5.36 ± 0.00 bc	5.36 ± 0.00 bc
1	5.07 ± 0.06 bc	5.26 ± 0.04 bc	2.83 ± 0.95 a	4.81 ± 0.19 bc
2	5.11 ± 0.03 bc	5.38 ± 0.04 bc	3.00 ± 0.00 a	4.85 ± 0.22 bc
4	5.39 ± 0.16 bc	6.81 ± 0.10 de	2.26 ± 1.16 a	4.31 ± 0.12 bc
8	7.07 ± 0.49 e	8.47 ± 0.23 fg	4.24 ± 1.29 b	4.85 ± 0.11 bc
12	6.72 ± 0.85 de	9.03 ± 0.11 gh	5.65 ± 0.58 bcd	4.45 ± 0.11 bc
16	7.45 ± 0.38 ef	8.87 ± 0.14 gh	5.68 ± 0.55 cd	4.75 ± 0.05 bc
20	7.27 ± 0.63 e	9.72 ± 0.16 h	5.20 ± 0.62 bc	4.71 ± 0.12 bc

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

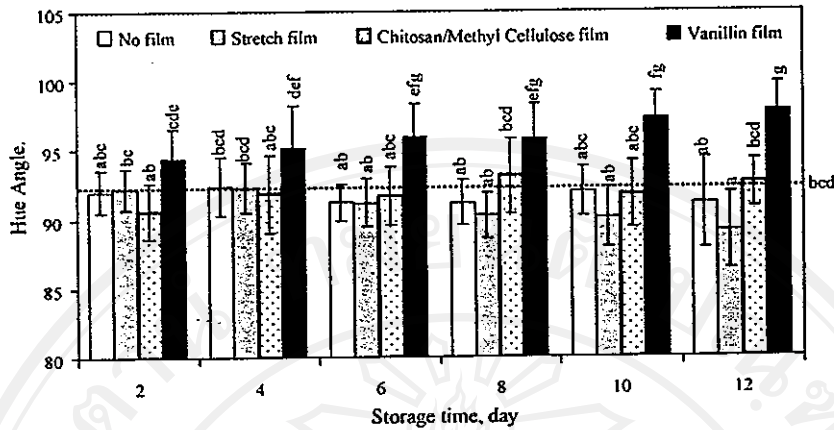


Fig. 1. Hue angle of fresh-cut pineapple during storage at 10°C. Dash line represents hue angle on day 0. Bars with the same letters are not significantly different according to the Tukey's-*b* test ( $p > 0.05$ ).

is controls. At specific time intervals, fruit pieces and films were washed with sterile 0.1% peptone. The plate counts of *E. coli* were performed on violet red bile agar with MUG (Criterion, JSA) after incubated at 37°C for 48 h. MUG generally permits the rapid detection of *E. coli* when the medium is observed for fluorescence under long wavelength UV light. The numbers of yeast cells were determined by surface plating of 0.1 mL washed peptone solution on Sabouraud agar (MERCK, Germany) with 1% yeast extract. They were incubated at 25°C for 48 h prior to counting.

#### 2.4. Quality evaluation of fruit wrapped with antimicrobial film

Fresh-cut cantaloupe and pineapple wedges were wrapped with the three types of films (Stretch film, chitosan/methyl cellulose film and vanillin film), and unwrapped fruit served as controls. All fruit pieces were placed on polystyrene trays and stored at 10°C for up to 20 d. Since Thailand is a tropical country and fresh-cut fruit are stored in open chiller displays or even placed on ice-cubes, 10°C was selected in this study. Measurements of all attributes were done every 2–4 d until the end of storage:

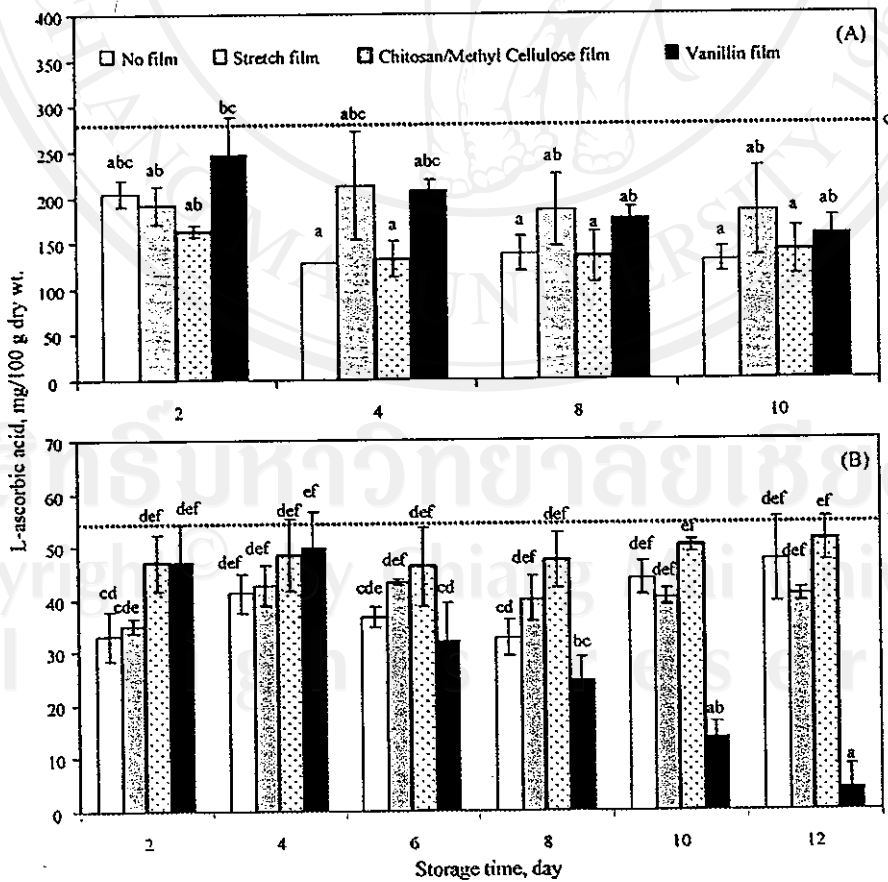


Fig. 2. L-Ascorbic acid content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C. Dash line represents L-ascorbic acid content on day 0. Bars with the same letters are not significantly different according to the Tukey's-*b* test ( $p > 0.05$ ).

flesh color ( $L^*$ ,  $a^*$ ,  $b^*$ ) of both fruit species was measured on longitudinally cut surfaces after removal from the wraps using Hunterlab color meter ColorQuest XE (The Color Management Company, Virginia, USA) calibrated with a white tile. Readings were taken from six wedges of each treatment.

Firmness was measured as the maximum force required to penetrate into fruit wedges using a Texture Analyser TA.XT2i (Texture Technologies Corp., Scarsdale, NY, USA) with a 50-kg load cell. A 5 mm diameter flat head stainless steel cylindrical probe was set to penetrate into the fruit at a speed of 0.5 mm/min.

L-Ascorbic acid (AA) was determined using an Agilent 1100 HPLC equipped with a quaternary pump system, an autosampler and a UV detector at 254 nm (Agilent Technologies, Palo Alto, CA). The analytical column used was a Restek Ultra Aqueous C18 (150 mm × 4.6 mm, 5 μm particle size). The sample preparation was done at cold temperature and under reduced lighting. Fifteen grams of each flesh was blended with cold 0.4% oxalic acid solution and adjusted to a final volume of 50 mL. The homogenate was filtered through a Whatman 1 filter paper and then a 0.45-μm syringe filter. The filtrate, 20 μL, was injected into the HPLC system. Isocratic separation was carried out using a mobile phase of Milli-Q water with 0.1% (v/v) oxalic acid. The eluent flow rate was 0.7 mL/min and the column temperature was 25 °C (Romeu-Nadal et al., 2006). HPLC grade L-ascorbic acid (MERCK, Germany) was used to make the calibration curve. Calibration curve was created by diluting L-ascorbic acid in a concentration range from 5 to 100 mg/L. Standard solutions were prepared fresh under cold and dark conditions to avoid AA degradation in samples. The relative retention time was 4.5 min. The determination of linearity ( $R^2$ ) of the standard curve was 0.993.

Fruit respiration rate was determined using a static method. Each unwrapped cantaloupe and pineapple wedges were put into a 10-mL airtight glass container.  $CO_2$  released from the products was absorbed by calibrated 0.01N NaOH solution for 1 h at 10 °C. The solution was then titrated with 0.005N oxalic acid. The respiration rate was expressed as mg  $CO_2$ /kg h (Zhang et al., 2005).

Ethanol content of the fruit was determined by gas chromatographic analysis of the headspace according to a method developed by Davis and Chace (1969) with some modification. Five grams of flesh were placed in a 10-mL amber glass bottle with rubber cap and incubated in a water bath at 60 °C for 45 min. Headspace gas was withdrawn using a 1-mL syringe and injected into a TRACE GC gas chromatograph (ThermoQuest Italia S.p.A., Italy) equipped with a flame ionization detector. The temperature of the oven, injector and detector were 150, 175 and 200 °C, respectively. The column used was a 30 m × 0.53 mm i.d. × 1 μm OV-1 (100% dimethylpolysiloxane) capillary column. Retention times and a standard curve of solute ethanol (31–2000 mg/L) in water solution were used for peak identification and quantification.

Total soluble solids (TSS) and pH were measured. TSS was determined using a digital refractometer (Pocket PAL-1, Japan). Ten

grams of flesh were blended with 40 mL distilled water in a blender. The pH was measured at 25 °C with a pH meter (Consort C831, Belgium).

Weight loss of fresh-cut cantaloupe and pineapple was determined by weighing the samples at specific time intervals and plotting weight losses against time.

### 2.5. Statistical analysis

All experiments were conducted by triplicate determinations and data were subjected to analysis of variance and Tukey's *b* multiple range test ( $p < 0.05$ ).

## 3. Results and discussion

Chitosan/methyl cellulose film was colorless and transparent while the film containing vanillin (vanillin film) was more opaque and yellow. Film thickness varied from 40 to 50 μm. Chitosan/methyl cellulose film was apparently more hydrophilic than vanillin film because it absorbed some water from the fruit wedges, causing it to swell and its surface to become rough. It also adhered to the surface of the fruit and was difficult to remove. Vanillin film adhered to fruit wedges like the other synthetic plastic films. Unlike the chitosan/methyl cellulose film, it was easy to remove from the fruit surface. Mold incidence was visually observed on day 12 in all cantaloupe treatments except with the vanillin film. At the same time, even though there was no mold on fresh-cut pineapple, an off-odor was detected.

### 3.1. Inhibitory effect of antimicrobial film against *E. coli* on fruit at 10 °C

The number of *E. coli* on each cantaloupe piece on day 0 was  $1.5 \times 10^5$  cfu/piece. To disregard the difference in weight loss of each treatment during storage, the microbiological counts were expressed per piece instead of per gram. As storage time increased, the number of *E. coli* on cantaloupe wrapped with stretch film increased, while the populations of *E. coli* on cantaloupe without film, wrapped over with chitosan/methyl cellulose film and vanillin film decreased (Table 1). The reduction of *E. coli* populations might be due to the loss of water content on fruit during storage. The decline rate was faster on fruit wrapped with chitosan/methyl cellulose film during the first 2 d. After that, the populations of *E. coli* on cantaloupe wrapped with chitosan/methyl cellulose film and vanillin film were not different. After 4 d storage, cantaloupe in chitosan/methyl cellulose and vanillin films had a significantly lower number of *E. coli* than fruit without wrapping.

The initial number of *E. coli* on pineapple pieces was equivalent to that on cantaloupe. However, *E. coli* populations of all treatments decreased over time. This seemed to be the effect of fruit pH which was too low for this microorganism. Presser et al. (1997) reported that *E. coli* grew at pH 4.0 but not at pH 3.7. Pineapple used in this

Table 3  
Numbers of *S. cerevisiae* on inoculated pineapple (log cfu/piece) during storage at 10 °C

	No film	Stretch film	Chitosan/methyl cellulose film	Vanillin film
0	5.08 ± 0.00 <sup>efgh</sup>	5.08 ± 0.00 <sup>efgh</sup>	5.08 ± 0.00 <sup>efgh</sup>	5.08 ± 0.00 <sup>efgh</sup>
1	4.73 ± 0.03 <sup>ef</sup>	4.93 ± 0.17 <sup>efg</sup>	2.93 ± 0.99 <sup>bc</sup>	4.89 ± 0.15 <sup>efg</sup>
2	5.30 ± 0.14 <sup>fgh</sup>	5.11 ± 0.18 <sup>efgh</sup>	3.00 ± 0.00 <sup>bc</sup>	4.27 ± 0.21 <sup>de</sup>
4	5.53 ± 0.17 <sup>fghi</sup>	5.98 ± 0.31 <sup>hi</sup>	3.15 ± 0.12 <sup>bc</sup>	3.20 ± 0.44 <sup>bc</sup>
6	6.36 ± 0.33 <sup>ij</sup>	7.06 ± 0.21 <sup>jk</sup>	3.56 ± 0.21 <sup>cd</sup>	1.00 ± 0.00 <sup>a</sup>
8	5.82 ± 0.49 <sup>ghi</sup>	7.36 ± 0.05 <sup>k</sup>	3.08 ± 0.16 <sup>bc</sup>	1.43 ± 0.75 <sup>a</sup>
10	5.39 ± 0.31 <sup>fghi</sup>	7.33 ± 0.36 <sup>k</sup>	3.10 ± 0.61 <sup>bc</sup>	1.00 ± 0.00 <sup>a</sup>
12	5.82 ± 0.35 <sup>fghi</sup>	7.96 ± 0.19 <sup>k</sup>	2.52 ± 0.50 <sup>b</sup>	1.23 ± 0.40 <sup>a</sup>

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

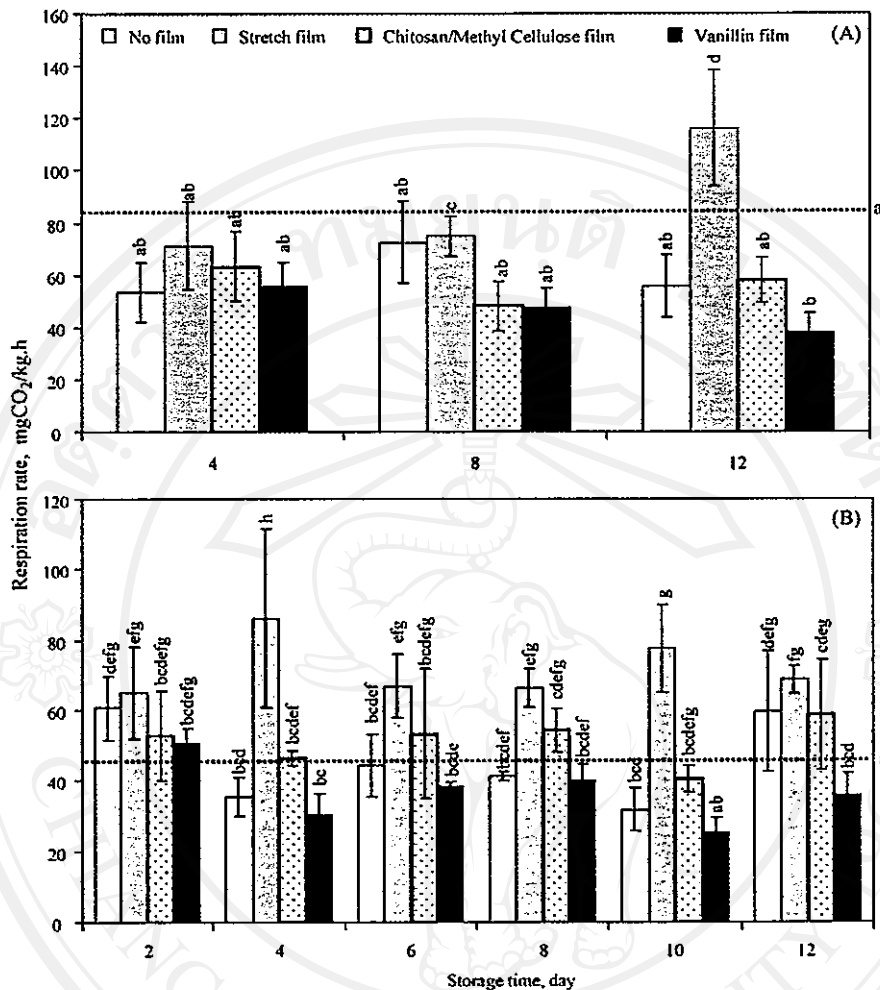


Fig. 3. Respiration rate of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C. Dash line represents respiration rate on day 0. Bars with the same letters are not significantly different according to the Tukey's-b test ( $p > 0.05$ ).

study had pH values of 3.3–3.8. Therefore, the inhibitory effect of the antimicrobial films studied was not relevant (data not shown).

### 3.2. Inhibitory effect of antimicrobial film against *S. cerevisiae* on fruit at 10°C

The initial numbers of yeast on cantaloupe and pineapple pieces after inoculation with *S. cerevisiae* were  $2.3 \times 10^5$  and  $1.2 \times 10^5$  cfu/piece, respectively. After 2 d, the numbers began to increase on the control cantaloupe and cantaloupe wrapped with commercial stretch film (Table 2). The increase in the latter film was faster because it maintained higher moisture contents and lower oxygen concentrations. On the other hand, the numbers of yeast on cantaloupe in the vanillin film remained constant over time, while those wrapped with chitosan/methyl cellulose film decreased over the first 4 d and then increased afterwards. After that, chitosan/methyl cellulose and vanillin films provided the same inhibition. Similar to cantaloupe, the numbers of yeast on pineapple in stretch film increased (Table 3). The yeast populations on pineapple wrapped with chitosan/methyl cellulose film decreased almost 2 logs cfu/piece on the first day and remained constant afterwards. Film containing vanillin resulted in a decrease of 4 logs more than the other films. Vanillin film was more effective but took a longer time to show the effect than chitosan/methyl cellulose film. Vanillin

film may be useful for food with longer storage life. Application of vanillin film on cantaloupe and pineapple showed a different behavior. Vanillin film maintained the amount of yeast or inhibited cell division of yeast on cantaloupe while it decreased the number of yeast on pineapple. These results agreed with Lopez-malo et al. (1998) and Matamoros-Leon et al. (1999) who reported that vanillin was more effective in inhibiting microorganisms in lower pH foods. Visual observation showed that the yellowness of vanillin film used to wrap pineapple decreased. Therefore, the greater inhibition might be the result of a higher release rate of vanillin out of the film.

### 3.3. Flesh color

Color of fresh-cut cantaloupe in all treatments remained unchanged over the storage period. Hue angle of fresh cantaloupe was 67.13. However, the hue angle of pineapple flesh wrapped with stretch film tended to decrease over time while that of tissue wrapped with vanillin film increased significantly from 92.3 to 97.9 (Fig. 1). This may be the result of the yellow vanillin completely migrating from the film. On the contrary, the color of vanillin film was still yellow after removed from the cantaloupe flesh. The sensory changes could possibly be expected as a result of vanillin migration from film to fruit surface. However, sensory analysis by taste panel was not performed in this study.



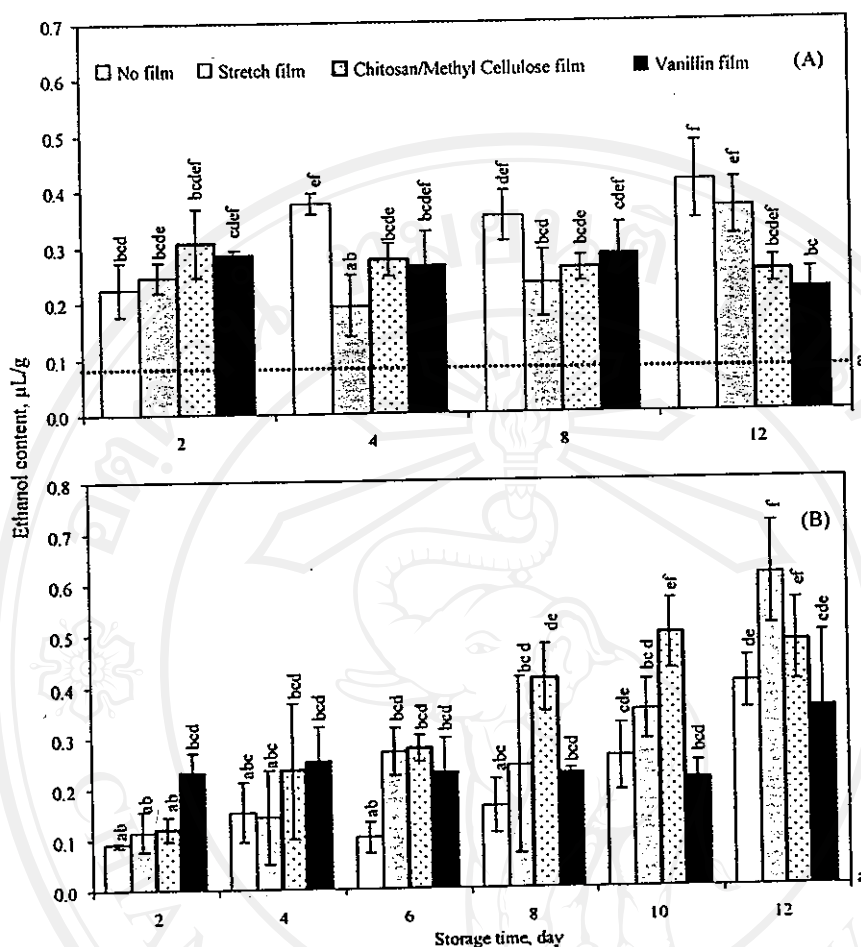


Fig. 4. Ethanol content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10 °C. Dash line represents ethanol content on day 0. Bars with the same letters are significantly different according to the Tukey's-*b* test ( $p > 0.05$ ).

### 3.1. Firmness

Firmness of cantaloupe flesh in all treatments remained unchanged over time. Even though the force measured did not show any difference, it is possible that the mouth-feel of the fruit is completely different due to water loss. None of the film had an effect on pineapple firmness, which decreased by approximately 20% over the first 2 d and was almost unchanged after that (data not shown).

### 3.2. L-Ascorbic acid (AA)

Initial AA content of fresh-cut cantaloupe was 17.6 mg/100 g fresh weight or 281.3 mg/100 g dry weight. The values were in the range of 14–19.8 mg/100 g fresh weight published by Saftner et al. (2006). AA content decreased over the first 2 d (Fig. 2A). The reduction in AA content at the end of storage was about half of the initial concentration.

Initial AA content of fresh-cut pineapple was only 8.6 mg/100 g fresh weight or 54.3 mg/100 g dry weight (Fig. 2B). This seemed to be low compared with that found by Vinci et al. (1995) who reported that the AA contents of fresh and artificially ripened pineapple were 30.6 and 18.1 mg/100 g fresh weight. The low AA content might be due to a higher storage temperature at the market before purchasing. In a preliminary study, it was found that AA rapidly degraded in pineapple samples. Therefore, the sample

must be carefully prepared and immediately injected into the HPLC system. From Fig. 2B, the AA content in pineapple wrapped with chitosan/methyl cellulose film, stretch film and no film slightly decreased during storage. Unexpectedly, the AA content in pineapple wrapped with vanillin film diminished drastically. Only 10% of the initial concentration was left in the fruit after 12 d storage. The reaction of vanillin and AA should be investigated. Any hydrogen exchange or bonding could be considered. However, Burri et al. (1989) reported that vanillin also acts as an antioxidant.

### 3.6. Respiration rate

The respiration rate of fresh-cut cantaloupe was unchanged during storage except in cantaloupe wrapped with stretch film on day 12, where a spike in respiration was observed (Fig. 3A). Similarly, the respiration rate of fresh-cut pineapple wrapped with stretch film was generally higher than that for other treatments, while that wrapped with vanillin film was the lowest (Fig. 3B). In general, chitosan/methyl cellulose and vanillin films were better gas barriers than the stretch film in dry conditions (Sangsuwan et al., unpublished data), but the barrier properties decline with moisture absorption. Thus, the gas protection of these films is limited. Too high a gas barrier in stretch film might result in depletion of oxygen, resulting in fermentation of the product. Therefore, films should have an appropriate oxygen permeability which is very important

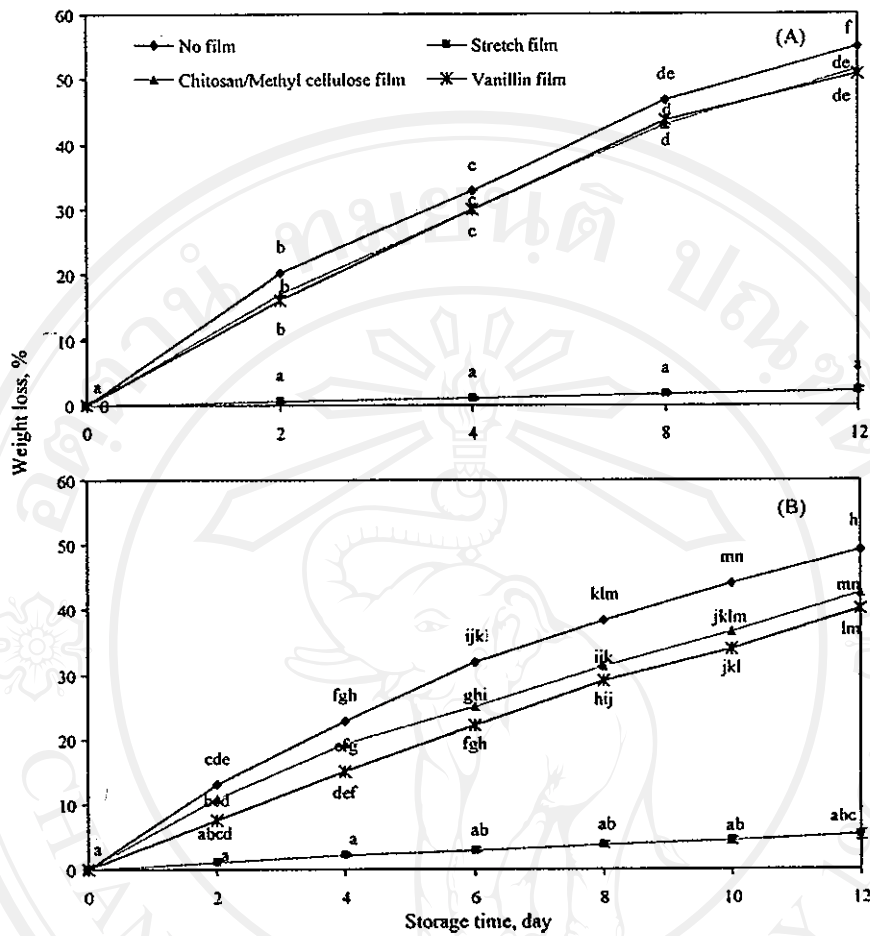


Fig. 5. Weight loss of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10 °C.

for respiring products. The burst of CO<sub>2</sub> production in fruit wrapped with stretch film could also be the result of microbial activity, since these fruit had higher microbial counts.

### 3.7. Ethanol content

The initial ethanol content of fresh-cut cantaloupe, immediately after processing, was 0.08 μL/g. Ethanol contents increased in all treatments and varied from 0.19 to 0.41 μL/g afterwards (Fig. 4A). In pineapple, ethanol increased over time, indicating fermentative metabolism associated with senescence (Fig. 4B). Ethanol content of pineapple wrapped with vanillin film increased on day 2 and remained unchanged until day 12. The final content was slightly lower than for other treatments.

### 3.8. Total soluble solids and pH

Initial TSS of cantaloupe and pineapple was 7.8 and 18.0%, respectively. TSS in all treatments, except in the stretch film, increased with time because the fruit lost approximately 50% water content. Therefore, fruit in the stretch film maintained the best TSS. The pHs of cantaloupe and pineapple flesh were in the range of 5.3–6.1 and 3.3–3.8, respectively (data not shown).

### 3.9. Weight loss

Weight loss of both cantaloupe and pineapple in the commercial stretch film was significantly lower than that in fruit

tissue wrapped with vanillin or chitosan/methyl cellulose film, or without film (Fig. 5). Pineapple without any wrapping lost the most moisture (Fig. 5B). Stretch film provided a better barrier to water than vanillin and chitosan/methyl cellulose film. Water vapor permeabilities at 23 °C, 53% RH of vanillin film and chitosan/methyl cellulose film were 1.03 and 1.09 ng cm/cm<sup>2</sup> s cmHg, respectively (Sangsuwan et al., unpublished data). The water barrier of a biopolymer was impaired in a higher relative humidity environment or with higher moisture content of the food (García et al., 2004). A poor water vapor barrier property allows the movement of water vapor across the film, thus, preventing water condensation that can be a potential source of microbial spoilage in horticultural commodities (Park et al., 1994). With respect to synthetic polymers, chitosan/methyl cellulose films had WVP values similar to those of cellophane, as expected due to the similar chemical structure of the components. However, chitosan/methyl cellulose films are better water vapor barriers than hydrophilic films based on starch, casein and wheat gluten (Greener and Fennema, 1989; Kester and Fennema, 1989; Aydt et al., 1991; Gontard and Guilbert, 1994).

## 4. Conclusion

Chitosan/methyl cellulose and vanillin films provided an inhibitory effect against *E. coli* bacteria and *S. cerevisiae* yeast. Vanillin film reduced microorganisms levels (higher log reduction) to a greater extent, but over a longer time. Use of vanillin film on cantaloupe and pineapple showed different responses.

a low pH fruit, vanillin was more effective at inhibiting microorganisms. Quality attributes of fresh-cut cantaloupe and apple were generally acceptable. An extreme reduction of L-ascorbic acid, which represents vitamin C, of pineapple wrapped in vanillin film, was observed, and would be worth further investigation.

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