CHAPTER 4

RESULTS AND DISCUSSION

4.1 Formulations and properties of chitosan/cellulose derivative based films

4.1.1 Film formulations

In preliminary study, a concentration of methylcellulose higher than 1.0% was difficult to dissolve in 50% aqueous ethanol and resulted in a non-homogeneous film. To obtain a homogeneous film, methylcellulose concentrations of 0.5 and 1.0% were selected for this study. Chitosan film, in general, usually contains 1-2% chitosan (Park and Zhao, 2004; Pranoto *et al.,* 2005; Zivanovic *et al.,* 2005). Blends of cellulose derivatives and chitosan were expected to result in a more flexible and controlled release film. Films prepared from mixtures of chitosan and the cellulose derivatives; methylcellulose and hydroxypropyl cellulose (in different ratios) are shown in Figure 4.1. Chitosan film is transparent and easy to wrinkle. Films prepared from chitosan and methylcellulose or hydroxypropyl cellulose mixtures are less transparency. The 1.5:0.5 chitosan : methylcellulose ratio which resulted in the thinnest and clearest film. All film formulas produced homogeneous films except for formula H (chitosan : hydroxypropyl cellulose = 2.0:3.0).

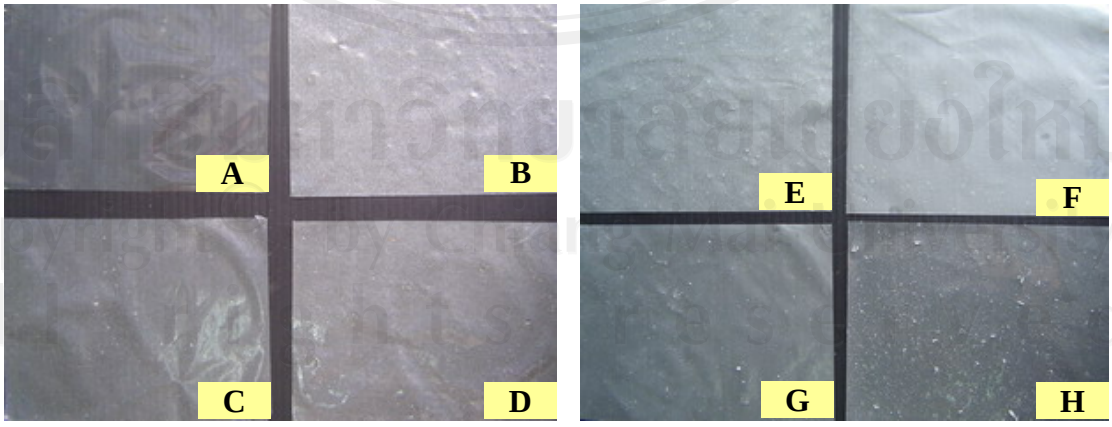


Figure 4.1 Films prepared from chitosan (CH) and methylcellulose (MC) or hydroxypropyl cellulose (HPC).
(A) CH:MC =1. 5:0.5 (B) CH:MC =1.5:1.0 (C) CH:MC =2.0:0.5 (D) CH:MC =2.0:1.0
(E) CH:HPC=1.5:2.0 (F) CH:HPC=1.5:3.0 (G) CH:HPC=2.0:2.0 (H) CH:HPC=2.0:3.0

4.1.2 Thickness and mechanical properties of the films

The thickness of the chitosan film was 38±6 µm. The thickness of the blended films depended mostly on the amount of cellulose derivative added (Table 4.1). Film containing the same amount of chitosan (film A and B or film C and D or film E and F or film G and H) had thicker films with increasing amounts of methylcellulose or hydroxypropyl cellulose. The high standard deviation in film H was possibly due to a non-homogeneous film matrix.

Table 4.1 Thickness of composite films from different ratios of chitosan and cellulose derivatives.

Film CH:MC	1.5:0.5 (A)	1.5:1.0 (B)	2.0:0.5 (C)	2.0:1.0 (D)
Thickness, µm	39 ± 4^{a}	73 ± 6^{c}	57 ± 4^{b}	71 ± 5^{c}
Film CH:HPC	1.5:2.0 (E)	1.5:3.0 (F)	2.0:2.0 (G)	2.0:3.0 (H)
Thickness, µm	77 ± 5^{ab}	87 ± 6^{c}	71 ± 4^{a}	83 ± 9^{bc}

Means ± SD with different letters in each row are significantly different at p=0.05.

The tensile strength and percent elongation are shown in Figures 4.2 and 4.3. The tensile strength and percent elongation of chitosan film were 25.41±3.32 N/mm^2 and 47.2±3.15%, respectively. Blending 0.5% methylcellulose into chitosan did not change the film strength while significantly improving film flexibility. Increasing the methylcellulose content to 1.0% reduced film strength and flexibility. Film made from chitosan : methylcellulose = 1.5:0.5 had the best mechanical properties with percent elongation more than 100. Composite films from chitosan and methylcellulose (all formulations) had greater elongation than pure chitosan film. However, the tensile strengths of chitosan/hydroxypropyl cellulose films were the worse while percent elongation was not apparently improved when compared to pure chitosan film. The chitosan : methylcellulose ratio which was selected to use in the next experiment was 1.5:0.5.

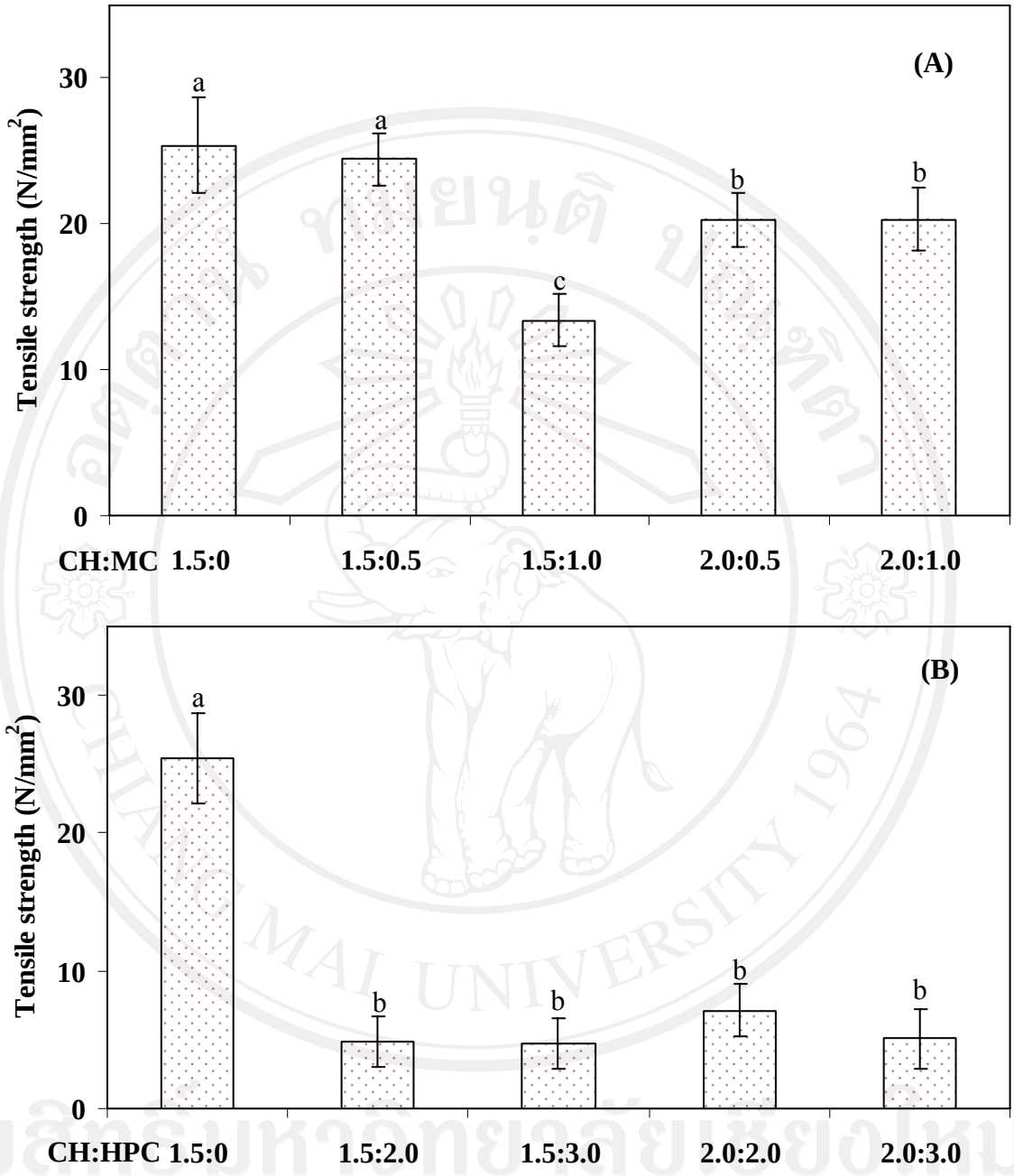


Figure 4.2 **Tensile strength of (A) chitosan/methylcellulose mixture (CH:MC) and (B) chitosan/hydroxypropyl cellulose mixture (CH:HPC).**

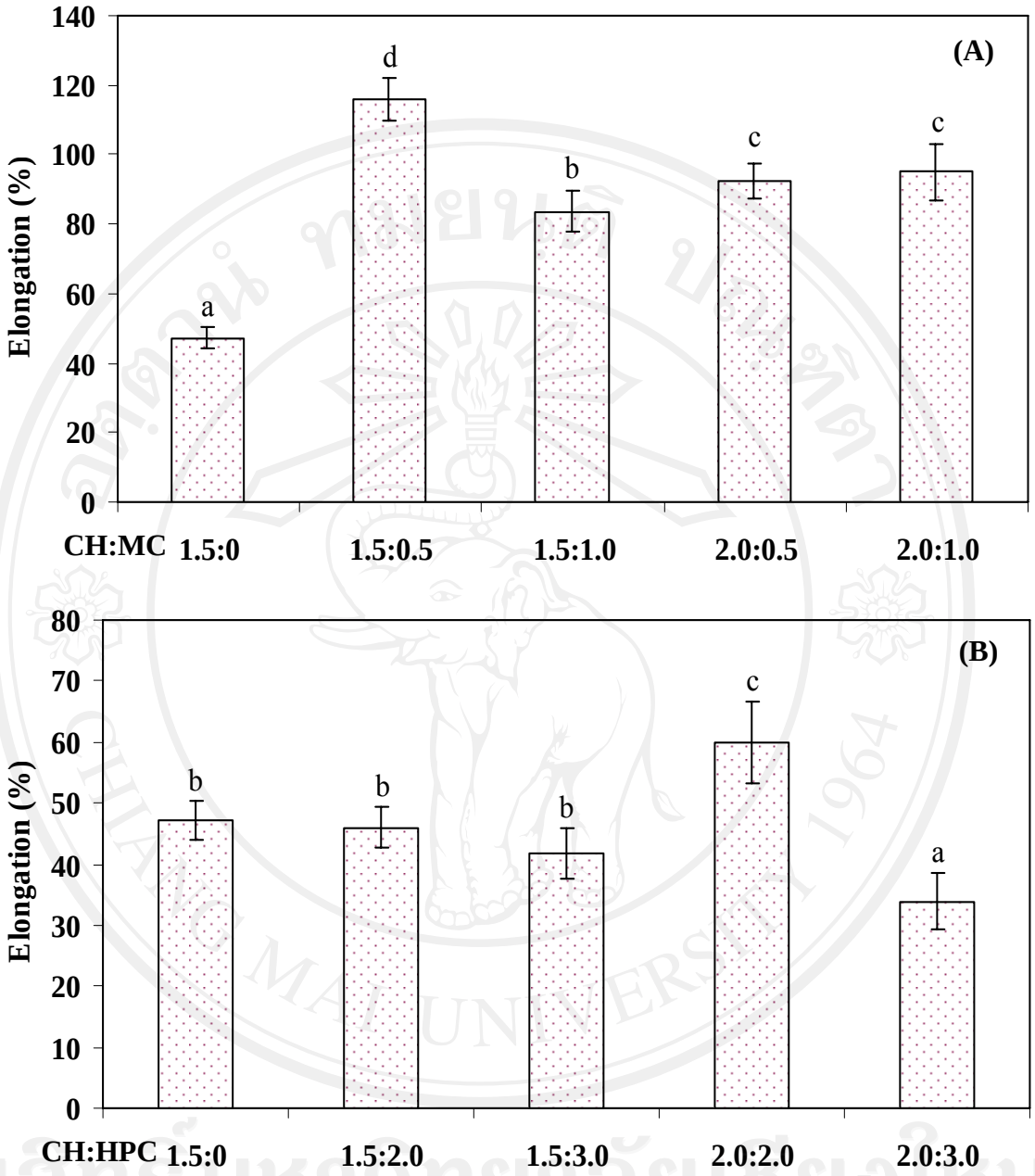


Figure 4.3 **Percent elongation of (A) chitosan/methylcellulose mixture (CH:MC) and (B) chitosan/hydroxypropyl cellulose mixture (CH:HPC).**

4.1.3 Effect of plasticizer on properties of chitosan/methylcellulose films

Three levels of plasticizer, polyethylene glycol 400 (PEG): low (0.17% w/v), medium (0.33% w/v) and high (0.50% w/v), were used with chitosan/methylcellulose film with no PEG. The amount of PEG was reported as the percent by weight of PEG per volume of film forming solution. The tensile strength and percent elongation of chitosan/methylcellulose film with no PEG were 35.63±2.95 N/mm^2 and 25.67±2.97%, respectively. Addition of PEG in film sample tended to lower the tensile strength, however, a low level of PEG did not significantly reduced film strength compared to film with no PEG (Figure 4.4A). Incorporation of PEG significantly improved film flexibility (Figure 4.4B). Films containing medium and high PEG had the highest flexibility. Addition of PEG (higher than 0.33%) further did not impact either film flexibility or strength. After drying, a greasy surface was observed for high plasticized film which reflected the excess amount of plasticizer. The migration of PEG to the film surface was also observed in medium plasticized film after storage at 53% RH, 23^{o}C for 2 weeks.

4.2 Effects of vanillin and plasticizer on chitosan/methylcellulose films

4.2.1 Thickness

The thickness of films containing different combination levels of vanillin, the antimicrobial agent, and PEG are shown in Table 4.2. At a given vanillin level, films with higher amounts of PEG were thicker than those containing low PEG. At a given PEG level, film thickness tended to increase with increasing vanillin in the formula. Therefore, it appears that both PEG and vanillin contributed to film thickness.

Table 4.2 Thickness of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).

Plasticizer content	Vanillin content			
	No vanillin	Low vanillin	Medium vanillin	High vanillin
Low plasticizer	35±6a	34±4a	34±2a	42±3b
High plasticizer	36±3a	44±7bc	48±3c	47±2bc

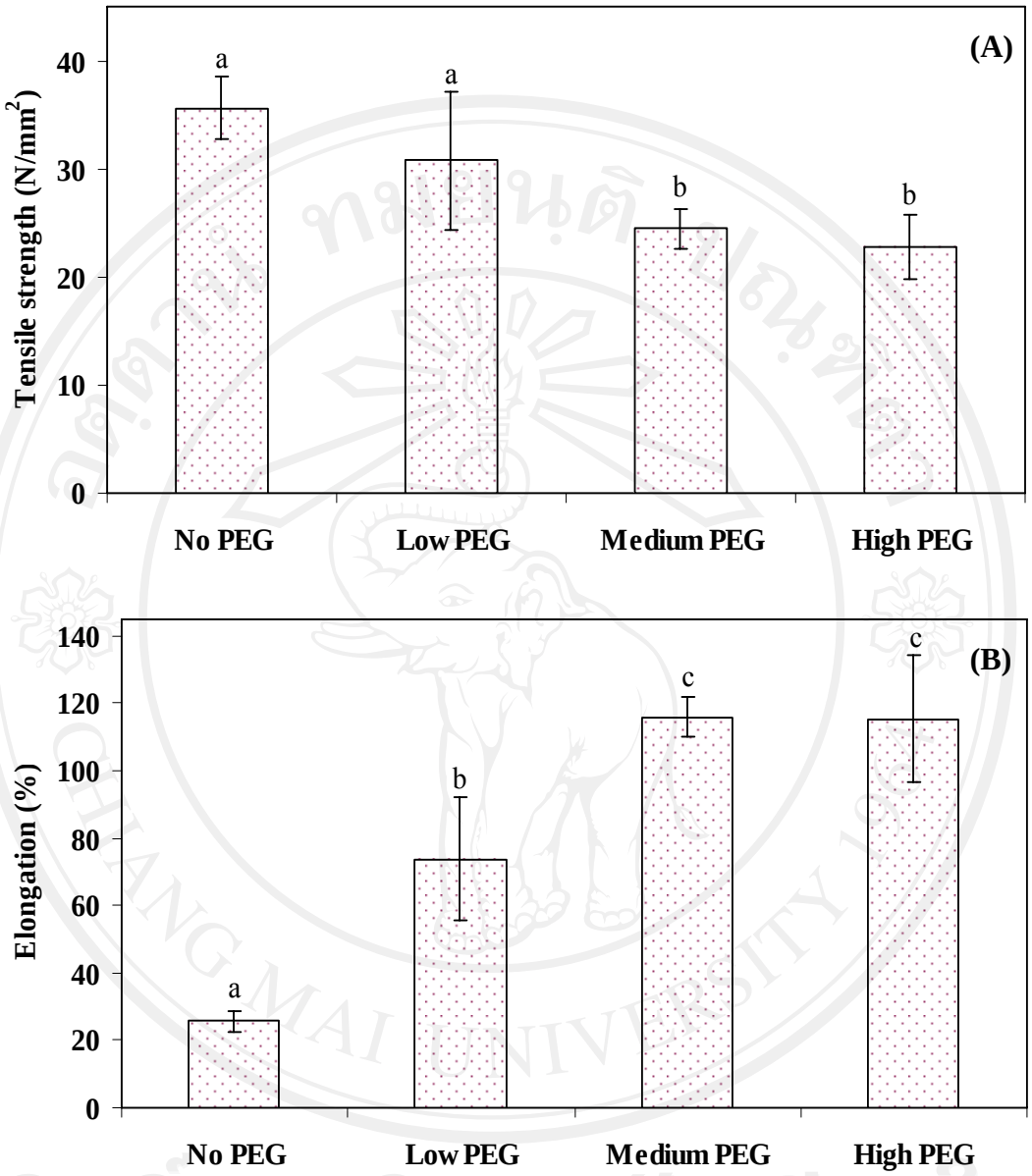


Figure 4.4 Tensile strength (A) and percent elongation (B) of chitosan/methylcellulose films with varying plasticizer (PEG) concentrations.

4.2.2 Mechanical properties

The tensile strength of the chitosan/methylcellulose film was affected by the concentration of vanillin and PEG (Figure 4.5A). The tensile strength of films containing low PEG were greater than those containing high PEG regardless of the vanillin content. The tensile strength of films with low PEG was not affected by low vanillin content, but increased when the vanillin concentration was increased to medium or high levels. With film containing a 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 was affected by the various combinations of vanillin and PEG (Figure 4.5B). The percent elongation of films containing low PEG was less than those containing high PEG at a given vanillin content. The percent elongation of low PEG film decreased substantially from 73.79 to 13.12% when a low vanillin content was added and decreased more with additional vanillin in the film. Addition of vanillin to high PEG films also decreased the percent elongation from 115.42 to 54.01% and the decrease continued as the vanillin level increased. Low plasticized and high vanillin containing films which had very low percent elongation (4.53%) cracked instantly when folded, thus these films required more PEG to maintain flexibility.

Incorporation of the antimicrobial vanillin into the film changed the functional characteristics of the packaging materials. The 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 (Birley *et al*., 1992). The tensile strength values of all the films were comparable to those of commercial HDPE films (20-37.2 N/mm^2) (Ahvenainen, 2003). The high tensile strength values were attributed to the numerous hydrogen bonds between methylcellulose chains. These bonds contribute to the cohesiveness and low flexibility of unplasticized films (Turhan *et al.,* 2001). Results in this study were opposed to those of Zivanovic *et al.* (2005) who found that addition of oregano essential oil to chitosan films decreased puncture and tensile strength. Pranoto *et al.* (2005) found greater reduction in tensile strength and increased elongation of chitosan film with incorporation of potassium sorbate and nisin. The tensile strength and percent elongation of chitosan film containing garlic oil did not change because garlic

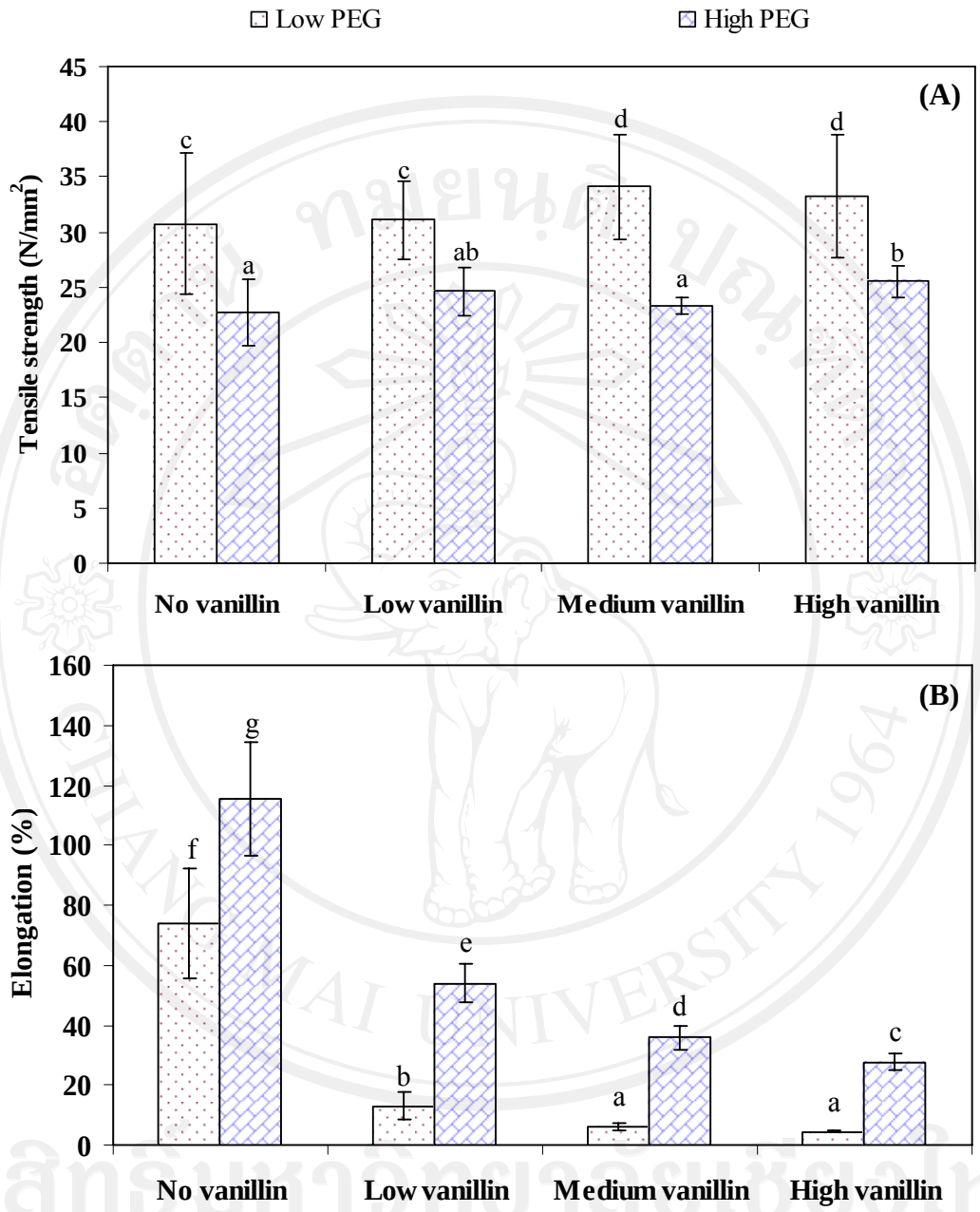


Figure 4.5 Tensile strength (A) and percent elongation (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).

oil components do not interact with the functional groups of chitosan. However, Chen *et al.* (1996) demonstrated that addition of 4% benzoate and sorbate into a chitosan/methylcellulose film resulted in higher tensile strength and percent elongation. The effect of plasticizer on mechanical properties of the film agreed with Park *et al.* (1993).

4.2.3 Water vapor permeability

Plasticizer had the dominant effect on water barrier. A higher PEG film content allowed water to pass through quicker. Butler *et al*. (1996) reported that higher plasticizer concentration in chitosan film yielded a higher water vapor permeability value. The incorporation of vanillin was expected to increase the water vapor barrier property due to the hydrophobicity of vanillin. However, vanillin did not significantly alter the water barrier property of the films (Figure 4.6A). Hydroxyl group in vanillin could produce hydrogen bond and increase water solubility. Pranoto *et al*. (2005) incorporated garlic oil, which was hydrophobic like vanillin, into chitosan films. They found that garlic oil did not affect the water vapor permeability of the film. Even though the water vapor permeability of the films did not differ significantly, films with vanillin were superior in maintaining wet strength. Chitosan/methylcellulose films without vanillin were swollen, rough and easy to tear apart when they wet.

4.2.4 Oxygen permeability

In films containing low PEG, oxygen permeability did not change with increase in vanillin concentration (Figure 4.6B). In contrast, oxygen permeability was reduced significantly from 197.50E-12 to 48.90E-12 $cm^3.cm/cm^2.s.cmHg$ with addition of vanillin to high PEG films 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 thus, the hydrogen bond formation occurs in the film.

At a given vanillin content, oxygen permeability of the chitosan/ methylcellulose film increased as concentration of PEG increased. Park and Chinnan (1995) also reported that oxygen permeability and water vapor permeability 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 increase in the oxygen solubility in the film because of the increased water content in the high PEG film (oxygen solubility in water = 1.25 mmol/L at 25°C and 1 atm). Water molecules in polymers can interact with hydrophilic groups and thus may disrupt hydrogen bonding, creating additional sites for the dissolution of oxygen and increase the mobility of oxygen molecules within the polymer (Gontard *et al.*, 1996). However, Park *et al*. (1993) reported that oxygen permeability and water vapor permeability values of methylcellulose containing PEG were generally not affected by plasticizer concentration.

Chitosan/methylcellulose films had excellent oxygen barrier compared with commercial plastics. All of the films made in this study had better oxygen barrier compared to HDPE, LDPE, OPP, PS, PC, PA, PETP and PCTFE (Birley *et al.*, 1992).

4.2.5 Opacity and color

Table 4.3 represented L, a*, b* chroma, hue and opacity of films with varying plasticizer and vanillin concentrations. Opacity and the Hunter b* value of the films are shown in Figure 4.7. Opacity is the opposite of transparency. A transparent material is defined as having a transmittance value above 90% or opacity less than 10%. The b* value represents the yellowish color of films. An increase in the b* value indicates that the color of the film is becoming more yellow.

Chitosan/methylcellulose films without vanillin had percent opacity less than 10 which makes a transparent film. Without vanillin in the formula, high PEG film was more opaque and yellow than low PEG film. Migration of PEG to the film surface was observed in high PEG films without vanillin, thus caused the greasy surface appearance. The opacity and yellowness of the films increased significantly with increasing percent of vanillin in the film-forming solution. At a given level of

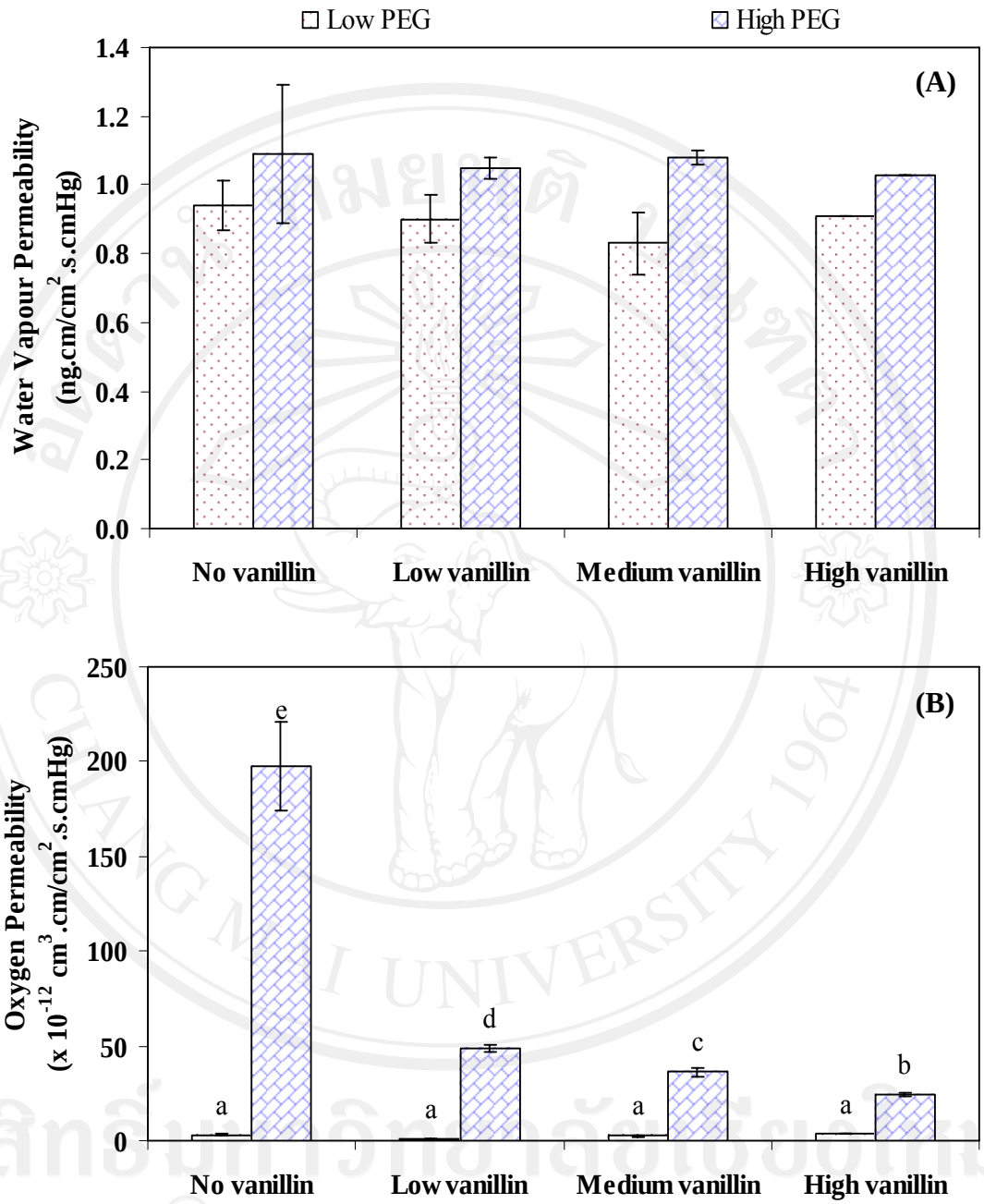


Figure 4.6 Water vapor permeability (A) and oxygen permeability (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).

Table 4.3 L, a*, b*, chroma, hue angle and opacity of films containing different combination levels of vanillin and plasticizer (PEG).

	L	a*	b*	chroma	hue angle	opacity
Low plasticized film containing						
No vanillin	88.17 ± 0.73^{c}	-1.47 ± 0.08^{h}	4.30 ± 0.22^{a}	4.54 ± 0.23^{a}	108.94 ± 0.37^{d}	8.12 ± 0.78^{a}
Low vanillin	88.05 ± 0.11^{c}	-10.34 ± 0.25^{e}	30.81 ± 1.03^{b}	32.50 ± 1.03^{d}	108.56 ± 0.44^{d}	14.31 ± 0.45^{c}
Medium vanillin	87.52 ± 0.10^{ab}	-12.67 ± 0.23^{b}	40.34 ± 1.35^{cd}	42.28 ± 1.35^{f}	107.45 ± 0.34^{c}	16.70 ± 0.61^{d}
High vanillin	87.88 ± 0.31^{bc}	-13.42 ± 0.29^{a}	46.16 ± 1.62^{e}	48.07 ± 1.52^{g}	106.24 ± 0.75^{ab}	19.45 ± 2.34^{f}
High plasticized film containing						
No vanillin	89.00 ± 0.47^{d}	-2.34 ± 0.28^{g}	5.73 ± 0.63^{a}	6.19 ± 0.69^{b}	112.18 ± 0.33^{e}	9.66 ± 1.08^{b}
Low vanillin	88.63 ± 0.27^{d}	-9.89 ± 0.21^{f}	29.03 ± 1.00^{b}	30.67 ± 0.97^{c}	108.83 ± 0.56^{d}	13.82 ± 0.55^{c}
Medium vanillin	87.46 ± 0.21^{ab}	-11.61 ± 0.13^{d}	38.75 ± 1.28^{c}	40.45 ± 1.25^{e}	106.69 ± 0.44^{b}	16.45 ± 0.70^{cd}
High vanillin	87.25 ± 0.19^{a}	-12.01 ± 0.22^{c}	41.81 ± 1.49^{d}	43.50 ± 1.47^{f}	106.04 ± 0.47^{a}	18.10 ± 0.63^{ef}

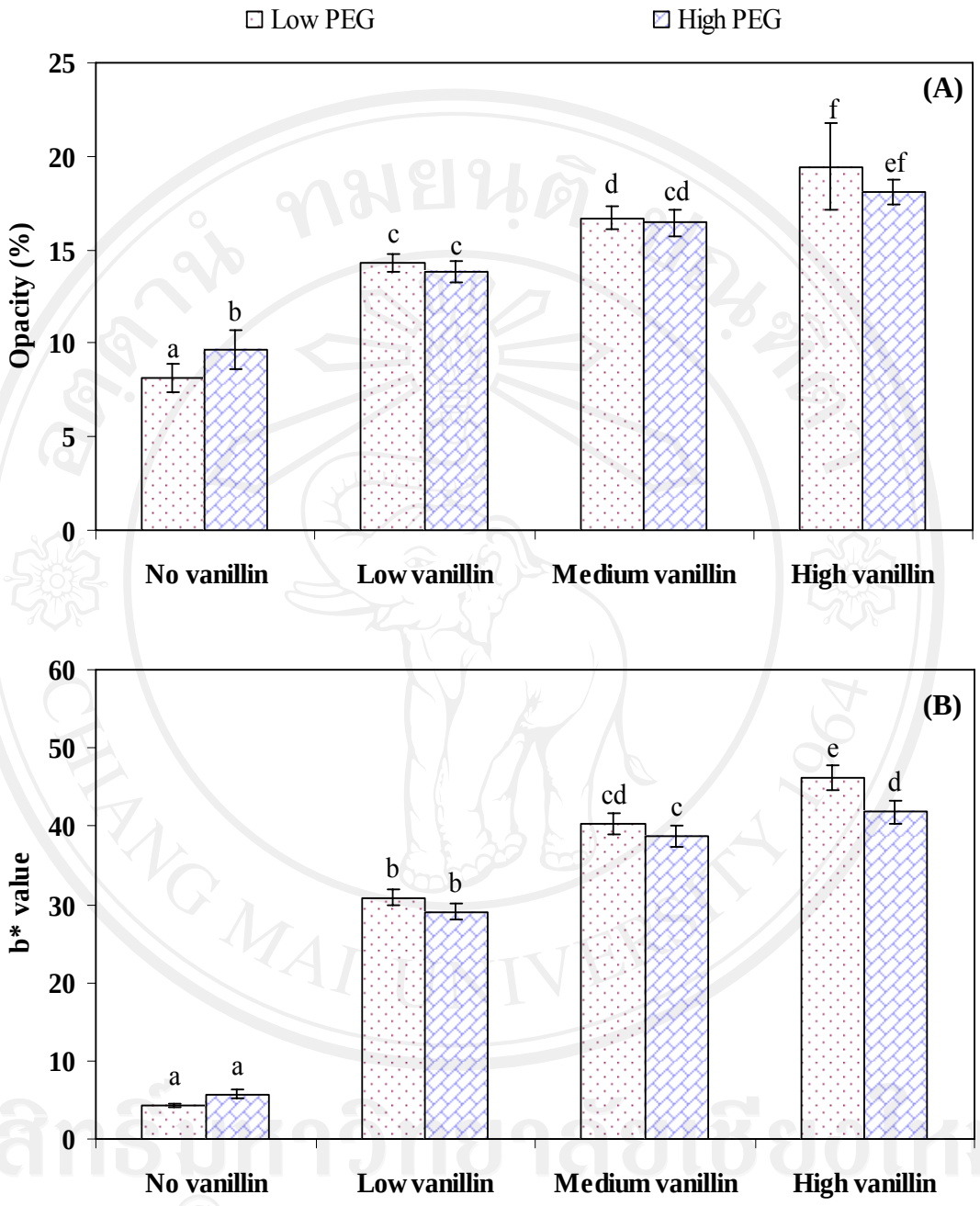


Figure 4.7 **Opacity (A) and b* value (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).**

vanillin, both films containing high and low levels of PEG had almost the same color and opacity. Therefore, PEG had less effect on film color and opacity than vanillin. Zivanovic *et al.* (2005) found that addition of the oregano essential oil to the chitosan resulted in an opaque film.

4.2.6 Film thermal properties

DSC thermograms of highly plasticized films with different vanillin concentrations are shown in Figure 4.8. A temperature scan from -50 to 350°C at 10°C/minute with no preheat treatment was used to determine the thermal properties of the film, including the miscibility of vanillin in chitosan/methylcellulose film and relative crystallinity of the film. Chitosan/methylcellulose film had an endothermic peak around 100°C which is associated with the loss of absorbed water from the film matrix. Endothermic peak area can be used to express the heat of fusion of the films. Endothermic peak of chitosan/methylcellulose film containing high vanillin concentration had only one peak, which indicates good miscibility of the film components. At a given PEG level, heat of fusion decreased with increase in vanillin concentration (Figure 4.9). The reduction in heat of fusion should be due to the effect of lower crystallization because vanillin has an aromatic benzene structure. The bulky benzene structure interrupts the rearrangement of the polymer chains.

At a given level of vanillin, the heat of fusion for both high and low PEG films were not significantly different. Addition of vanillin and plasticizer did not have any obvious effect on melt temperature. No peak was observed in the second scan at a cooling rate of 2°C/minute. This cooling rate may have been too fast to allow rearrangement of the polymer chains.

4.2.7 Film solubility

Film solubility in water is an important property for edible films. However, some potential applications may require water insolubility to enhance product integrity and water resistance. The solubility of films containing different combinations of vanillin and plasticizer are shown in Table 4.4. At a given level of vanillin, the solubility of high plasticized films was higher than that of low plasticized films.

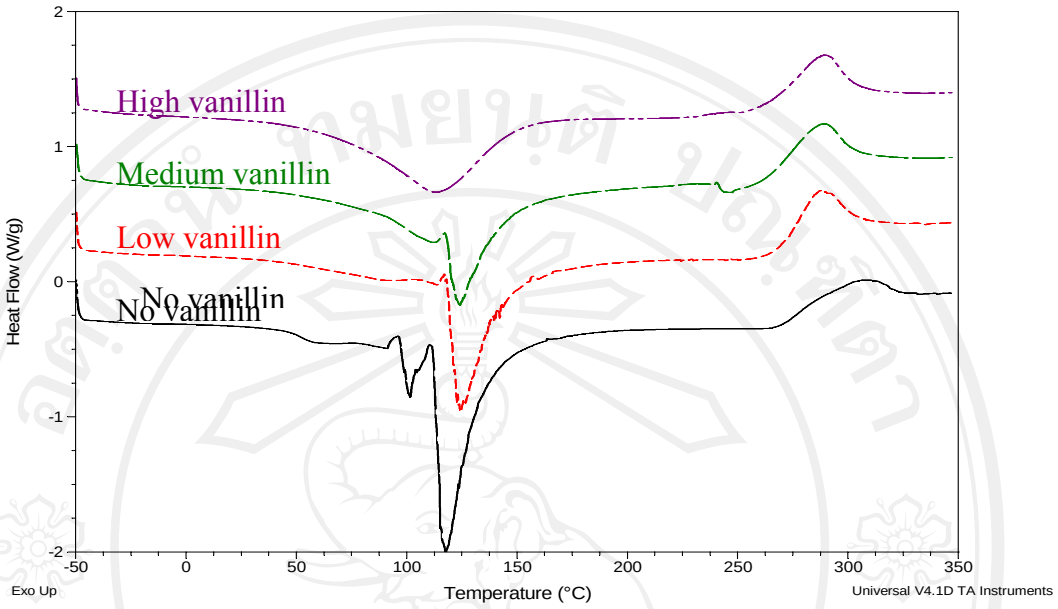


Figure 4.8 DSC thermograms of high plasticized chitosan/methylcellulose films containing different levels of vanillin.

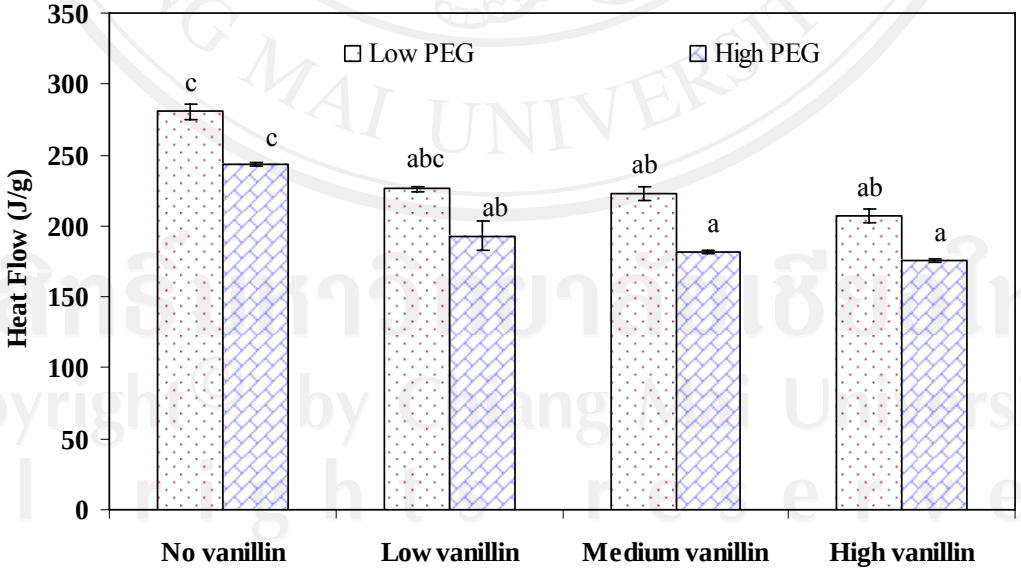


Figure 4.9 Heat flow of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).

This agreed with Turhan and Sahbaz (2004) who reported that the concentration of PEG significantly increased the solution rate of methylcellulose film. The increase in dissolution rate by the addition of PEG was due to the disruption of the native three-dimensional structure of methylcellulose through hydrogen bond formation with PEG. Garcia *et al.* (2004) reported that methylcellulose film was completely soluble in water at 25°C while chitosan film had low solubility below 4.5% at 25°C. They also reported that chitosan/methylcellulose film had solubility values between the values of one component film. Film of chitosan and methylcellulose in the ratio of 1:3 gave the highest solubility (39.9%). However, they did not use any plasticizer to produce the film. Turhan and Sahbaz (2004) reported that film without plasticizer had low dissolution due to high cohesion within the methylcellulose matrix via the numerous hydrogen bonds between the methylcellulose chains.

The total solubility and the solubility of vanillin in water were previously known in this study. Thus, the solubility of other components such as chitosan, methylcellulose and PEG without vanillin was determined. The solubility of vanillin in high plasticized film containing no, low, medium and high vanillin was 0, 3.3, 5.9 and 9.5%, respectively. The solubility of other components (no vanillin) was 46.2, 39.0, 39.1 and 35.8%, respectively. Thus, it can be concluded that vanillin reduced the solubility of other components in the film matrix.

Table 4.4 Solubility of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG).

Plasticizer content	Vanillin content			
	No vanillin	Low vanillin	Medium vanillin	High vanillin
Low plasticizer	26.2±2.3a	24.8±1.8a	30.2±2.1b	35.7±1.8c
High plasticizer	46.2±1.3e	42.3±2.6d	45.0±1.3e	45.3±1.3e

4.2.8 Sorption behavior

Sorption isotherms of high vanillin films containing low and high plasticizer levels were shown in Figure 4.10A. Initially both isotherms (0-50% RH) increased linearly as relative humidity increased. During the later stage, the films

absorbed water exponentially. At a given level of vanillin, high plasticized film absorbed more water than low plasticized film. At 90% RH, high plasticized film absorbed almost twice as much water as did low plasticized film. Therefore, the ability of films to absorb water depended on PEG content in films. Figure 4.10B depicts the sorption isotherms of high plasticized chitosan/methylcellulose films containing different levels of vanillin. The shape of the isotherms is similar to those shown in Figure 4.10A. Incorporation of vanillin in chitosan/methylcellulose film resulted in significantly lower moisture absorption because vanillin is hydrophobic. At 90% RH, high, medium, low and no vanillin films gained 39.18, 47.88, 56.46 and 63.00% by weight, respectively. The effect of vanillin on the absorption behavior of the films was obvious at relative humidity above 50%. Modeling of the moisture sorption isotherms was done using the BET (Brunauer-Emmett-Teller) and GAB (Guggenheim-Anderson-deBoer) equations. All isotherms fit the GAB equation (equation 4.1) but not the BET equation. BET equation is similar to GAB equation without *K* constant. All variables are shown in Table 4.5 and 4.6. Higher PEG level resulted in higher monolayer moisture content (V_m) and lower *C* constant. Constant *C* related to the water-substrate interaction energy. Monolayer moisture content decreased when increasing vanillin content in film. Constant *C* increased as vanillin concentration increased. PEG and vanillin provided the opposite effect on monolayer moisture content and constant *C*.

GAB equation

$$M = \frac{C \cdot K \cdot V_m \cdot a_w}{(1 - K \cdot a_w)(1 - K \cdot a_w + C \cdot K \cdot a_w)} \quad (4.1)$$

Where M = moisture content

a_w = water activity

C = constant related to excess enthalpy of sorption

K = GAB constant related to the properties of multilayer water molecules with respect to bulk liquid

V_m = monolayer moisture content

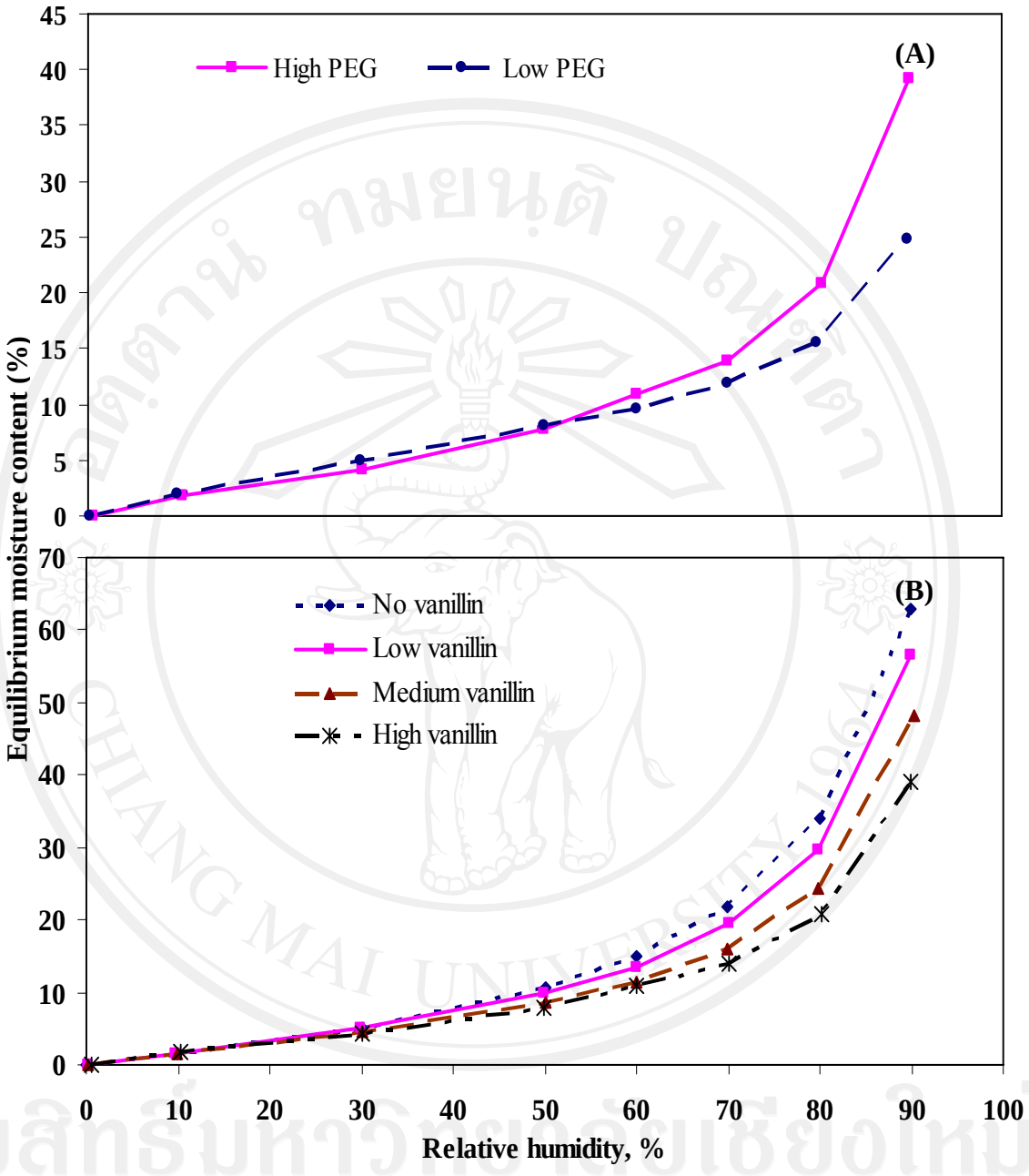


Figure 4.10 Sorption isotherms of chitosan/methylcellulose films containing different levels of plasticizer (A) and vanillin (B).

Table 4.5 **K, C, Vm and SSE of chitosan/methylcellulose films containing different plasticizer concentrations.**

	Plasticizer content	
	Low PEG	High PEG
K	0.907±0.001 [a]	0.977±0.003 [b]
C	8.439±0.040 [a]	3.148±0.100 [b]
V_m	4.696±0.015 [a]	4.988±0.123 [b]
SSE	1.067±0.075 [a]	0.000±0.000 [b]

SSE = sum square of error

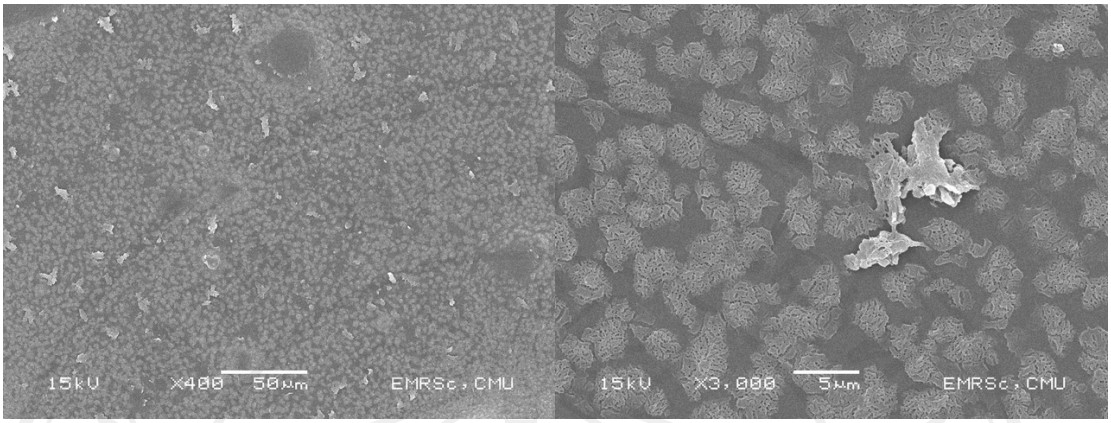
Table 4.6 **K, C, Vm and SSE of chitosan/methylcellulose films containing different vanillin concentrations.**

	Vanillin content			
	No vanillin	Low vanillin	Medium vanillin	High vanillin
K	0.963±0.002 [a]	0.973±0.006 [ab]	0.982±0.002 [b]	0.977±0.003 [b]
C	1.605±0.081 [a]	2.204±0.151 [b]	3.037±0.060 [c]	3.148±0.100 [c]
V_m	9.269±0.090 [a]	7.499±0.258 [b]	5.749±0.030 [c]	4.988±0.123 [d]
SSE	0.000±0.000 [a]	0.030±0.033 [a]	0.011±0.016 [a]	0.000±0.000 [a]

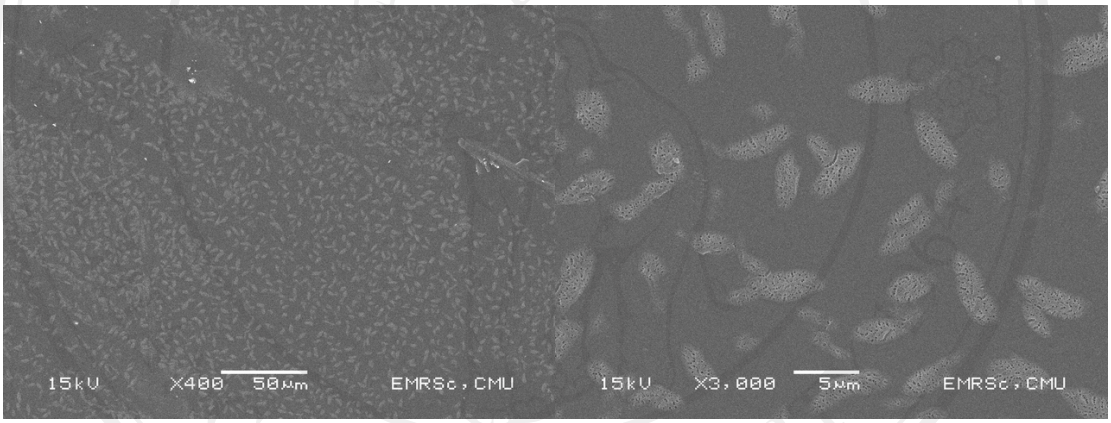
SSE = sum square of error

4.2.9 Morphology observation

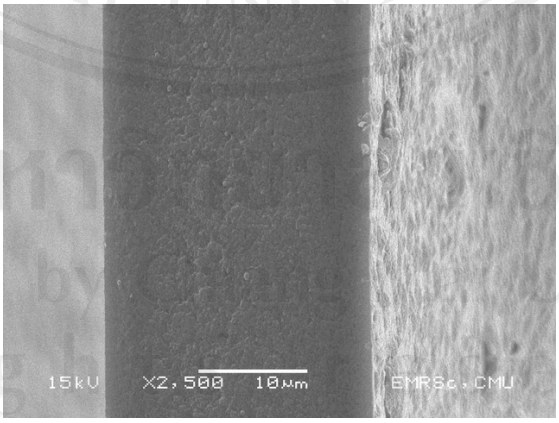
The surface morphologies of low plasticized and high plasticized chitosan/methylcellulose and vanillin films are shown in Figure 4.11, 4.12 and 4.13, respectively. The top surfaces (A and B) were relatively rough while the bottom surfaces of these films (C and D) were very smooth. Film surface plasticizer aggregates were found more in high plasticized chitosan/methylcellulose film. In the vanillin film, the dispersion of micro-domains which was approximately 1 micron throughout the film matrix could be vanillin. The cross-section of these films was very integral and dense. Cross-section of film containing vanillin (Figure 4.13E) indicated that film was fragile which related to mechanical properties. Incorporation of vanillin into chitosan/methylcellulose film did not cause a discontinuity in the film



A : Top surface (x400) **B** : Top surface (x3,000)

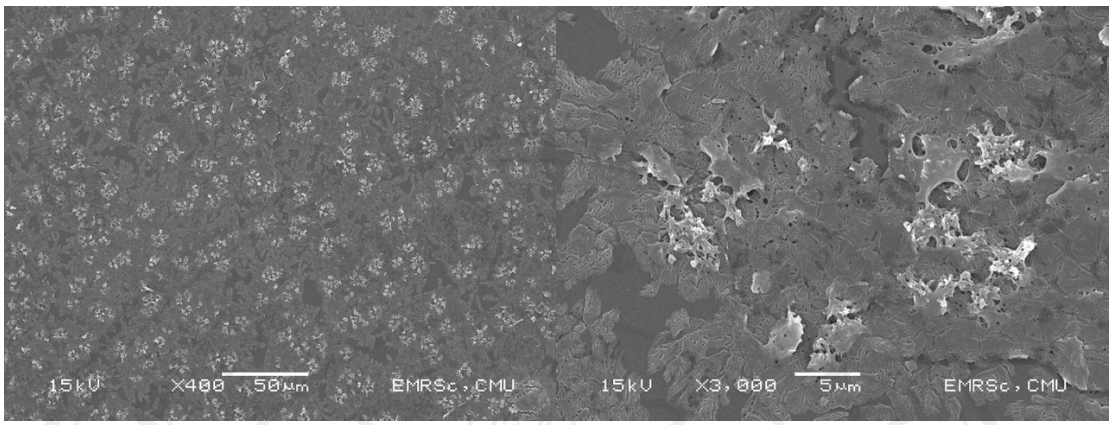


C : Bottom surface (x400) **D** : Bottom surface (x3,000)

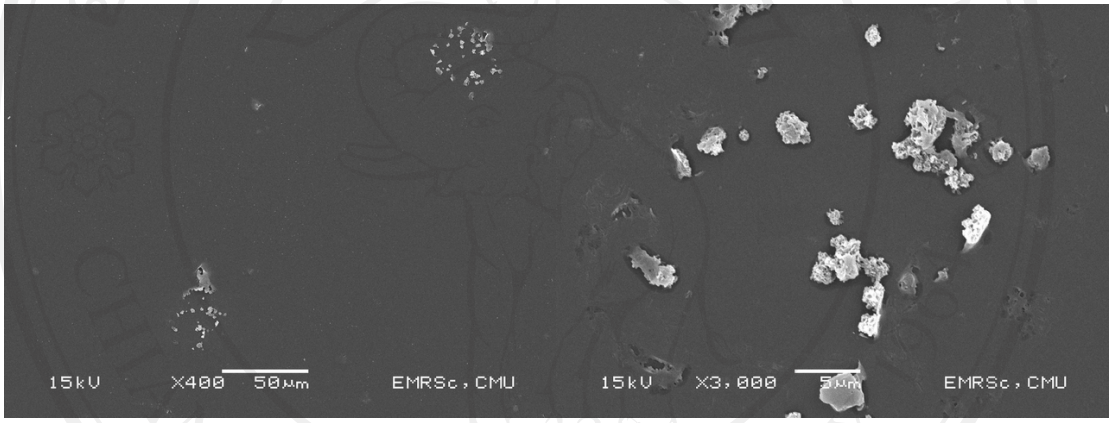


E : Cross-section (x2,500)

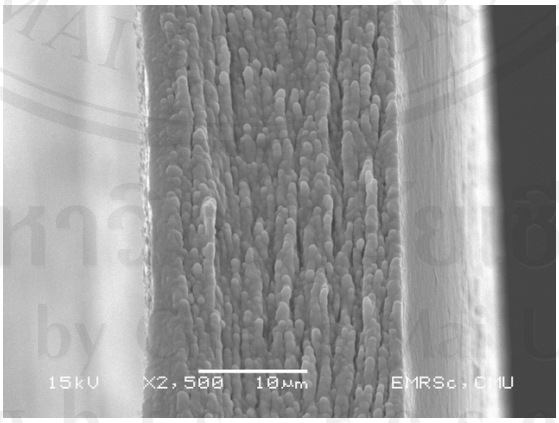
Figure 4.11 Morphologies of low plasticized chitosan/methylcellulose film.



A : Top surface (x400) **B** : Top surface (x3,000)

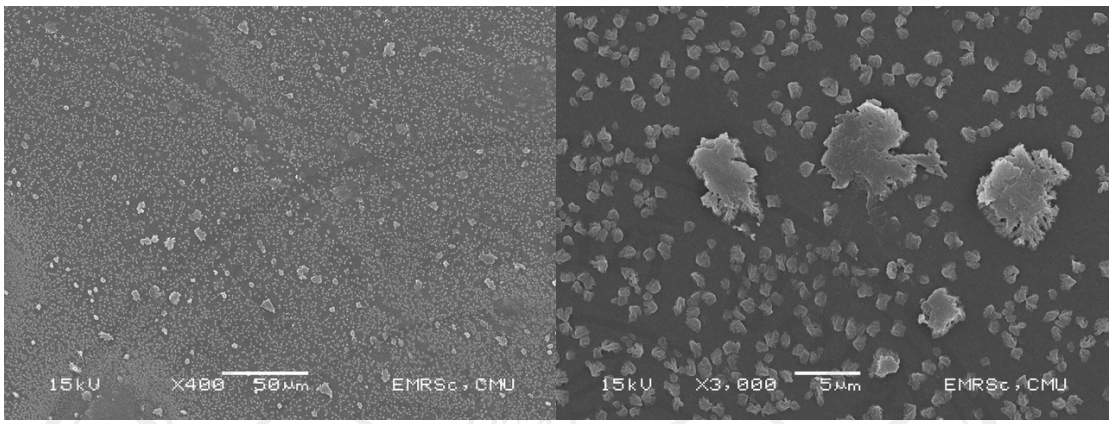


C : Bottom surface (x400) **D** : Bottom surface (x3,000)

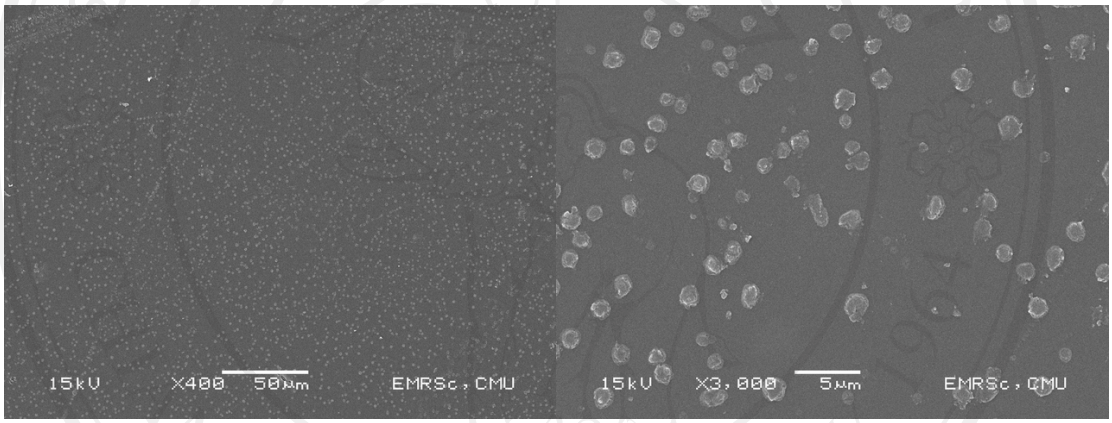


E : Cross-section (x2,500)

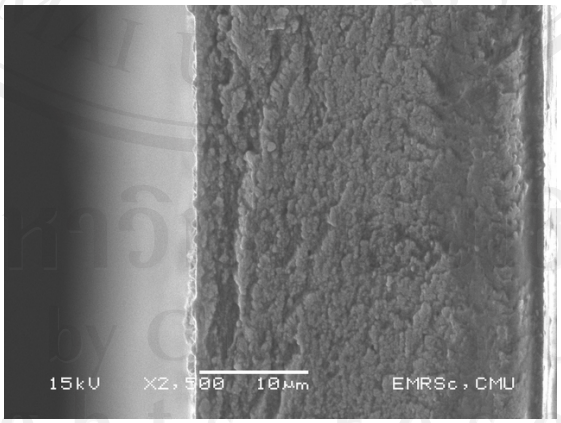
Figure 4.12 Morphologies of high plasticized chitosan/methylcellulose film.



A : Top surface (x400) **B** : Top surface (x3,000)



C : Bottom surface (x400) **D** : Bottom surface (x3,000)



E : Cross-section (x2,500)

Figure 4.13 Morphologies of high plasticized chitosan/methylcellulose film incorporating vanillin as an antimicrobial agent.

matrix. Yin *et al.* (2006) studied the morphology of blended chitosan/methylcellulose film compared with chitosan/hydroxypropyl methylcellulose. They concluded that the morphology of the chitosan and cellulose ether blends was homogeneous. Shu *et al.* (2001) reported that the surface and cross-section morphologies changed significantly due to the incorporation of model drugs into the citrate/chitosan film. They observed large pores in both the top and bottom surfaces and a very rough cross-section.

4.3 Release of vanillin and migration test

4.3.1 Factors affecting release of vanillin from the films

4.3.1.1 Kinetics of vanillin released from the chitosan/methylcellulose film

In general, the mechanism involved in the diffusion process of the migrant from the packaging film into liquid can be determined by fitting the early part of the release curve (M_t/M_∞ <0.6) to equation 4.2.

$$\frac{M_t}{M_\infty} = kt^n \qquad (4.2)$$

where M_t = amount of vanillin released at time t

M_∞ = amount of vanillin released at equilibrium

k = constant which characterizes the polymer network (s^{-1})

t = time (s)

n = diffusion exponent

Diffusion exponent can be classified into 3 cases. A value of $n \leq 0.5$ is representation of Case I or Fickian diffusion in which the rate of diffusion is much less than that of the film relaxation. A diffusion exponent in the range of 0.5 to 1 is classified as non-Fickian (anomalous) diffusion which occurs when diffusion and relaxation are comparable. The last case in which n>1 shown that diffusion is very rapid compared with relaxation processes. Values of n and k can be obtained from the slope and intercept of the ln M_t/M_∞ and ln t plot. The diffusion exponent (n) and k in different films and solvent systems are shown in Table 4.7. Also shown in the table is the goodness of the fit R^2 which shows the deviation from the linearity plot of ln M_t/M_∞ and ln t. Films in water, cantaloupe juice and citrate buffer, pH 5.0 and

pH 6.5, had lower diffusion exponents (0.33-0.67). R^2 for these determinations were higher than 0.95. No swelling effect was found in these film/solvent systems (see section 4.3.1.6 regarding swelling). Films in pineapple juice (at all temperatures) and citrate buffer pH 3.5 had high diffusion exponents (0.76-1.17). Swelling was found in films immersed in pineapple juice and citrate buffer pH 3.5. As temperature increased, more swelling was observed. At 10, 25 and 35°C, films immersed in pineapple juice gained 41.5, 63.7 and 69.2% of its original weight.

Table 4.7 Diffusion exponent (*n*) and constant (*k*) of chitosan/methylcellulose films containing vanillin in different systems and temperatures.

Film	Solvent	Temperature, °C	*n*	*k*, s^{-1}	R^2
H	water	10	0.44	0.0072	0.98
H	water	25	0.57	0.0026	0.97
H	water	35	0.60	0.0032	0.97
H	cantaloupe juice	10	0.49	0.0028	0.96
H	cantaloupe juice	25	0.42	0.0090	0.98
H	cantaloupe juice	35	0.36	0.0163	0.97
H	pineapple juice	10	0.76	0.0015	0.95
H	pineapple juice	25	0.91	00010	0.98
H	pineapple juice	35	1.01	0.0011	0.95
L	water	10	0.35	0.0242	0.99
M	water	10	0.33	0.0219	1.00
H	water	10	0.42	0.0089	0.99
H	buffer pH 3.5	10	1.17	0.0001	0.99
H	buffer pH 5.0	10	0.67	0.0014	0.95
H	buffer pH 6.5	10	0.46	0.0049	0.97

L, M and H = high plasticized film containing low, medium and high vanillin level, respectively.

4.3.1.2 Comparison of experimental data with theoretically calculated values

Figure 4.14, 4.15 and 4.16 illustrate the release profiles of vanillin from chitosan/methylcellulose films as a function of time. The lines show the theoretical profiles calculated from equation (4.3) using the optimum diffusion coefficient value obtained from equation (4.4) (Crank 1975).

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty}\left(\frac{8}{(2n+1)^2\pi^2}\exp\left[-(2n+1)^2\pi^2\frac{D\cdot t}{l^2}\right]\right) \quad (4.3)$$

$$D = \frac{0.049\cdot l^2}{t_{0.5}} \quad (4.4)$$

where M_t = amount of vanillin released at time t

M_∞ = amount of vanillin released at equilibrium

D = diffusion coefficient (cm^2/s)

t = time (s)

l = film thickness (cm)

$t_{0.5}$ = the time at which $M_t/M_\infty = 0.5$ (s).

To calculate the optimum diffusion coefficient from equation (4.4), a procedure based on the sum of squares technique was applied (Barr *et al*., 2000; Auras *et al.*, 2006). This technique estimates the diffusion coefficient value, which provides the least difference between the experimental and the theoretical data. Time values for the experimental M_t/M_∞ above, equal and below 0.5 were used in equation (4.4). Each diffusion coefficient obtained was used to calculate M_t/M_∞ using equation (4.3), and then the sum square error between the experimental and theoretical data was calculated. The diffusion coefficient value which minimized the sum square error was labeled as optimum. Goodness of fit (R^2) for the theoretical and experimental mass fraction released are shown in Figures 4.14, 4.15 and 4.16.

4.3.1.3 Effect of temperature

Figure 4.14 illustrated the release profiles of vanillin from chitosan/methylcellulose films in different solvent types and storage temperatures as a function of time. At equilibrium, 100% of the vanillin leached into the pineapple

juice at all temperatures studied (pH 3.7±0.1). However, only 73.0, 74.5 and 80% of the vanillin leached into cantaloupe juice (pH 6.2±0.2) at 10, 25 and 35°C and 40.7, 48.4 and 57.3% vanillin leached into water at 10, 25 and 35°C, respectively. Vanillin release was complete in about 17.3E+4 seconds in water, 34.6E+4 seconds in cantaloupe juice and 2.9E+4 seconds in pineapple juice. The release rate of vanillin from chitosan/methylcellulose film was slow compared to the release of acetic acid and propionic acid from chitosan film as reported by Ouattara *et al.* (2000). Slow release of the antimicrobial might not be as effective as direct addition of the antimicrobial to the food when the initial concentration of the microorganisms is high. However, a major advantage of slow release over direct addition of antimicrobial is the continuous microbial inhibition expected during an extended storage period (Chung *et al.*, 2001). At higher temperature, equilibrium was reached faster than at lower temperature. In addition, a higher level of vanillin was released at equilibrium at higher storage temperature. Temperature significantly impacted the release rate of vanillin from the chitosan/methylcellulose film in all solvents studied (water, cantaloupe juice and pineapple juice). The faster release rate (steeper slope Figure 4.14 A, B, C) was observed as the temperature increased. The diffusion coefficient for the film in each solvent type increased as temperature increased (Table 4.8). At a given temperature, pineapple juice has the highest release rate.

An Arrhenius plot is shown in Figure 4.17. Slope and intercept of the Arrhenius plot (ln D and 1/T) was used to calculate activation energy (E_a) and D_0, respectively. From the Arrhenius plot, the activation energies of the films in water, cantaloupe juice and pineapple juice were 33.46, 23.75 and 40.67 kJ/mol, respectively. Greater E_a indicated more sensitivity of the diffusivity to temperature change. The E_a values in this study were close to the E_a of potassium sorbate in a K-carrageenan film in the pH range 3.8 to 7 (E_a = 23.6-36.9 kJ/mol) reported by Choi *et al.* (2005). D_0 indicated maximum diffusion coefficient. D_0 of the film in these solvents were 1.09E-4, 9.03E-7 and 1.91E-2 cm^2/s, respectively. A lack of breaking points in the Arrhenius plots indicates that no morphological changes occurred within this film over the temperature range studied (10-35°C).

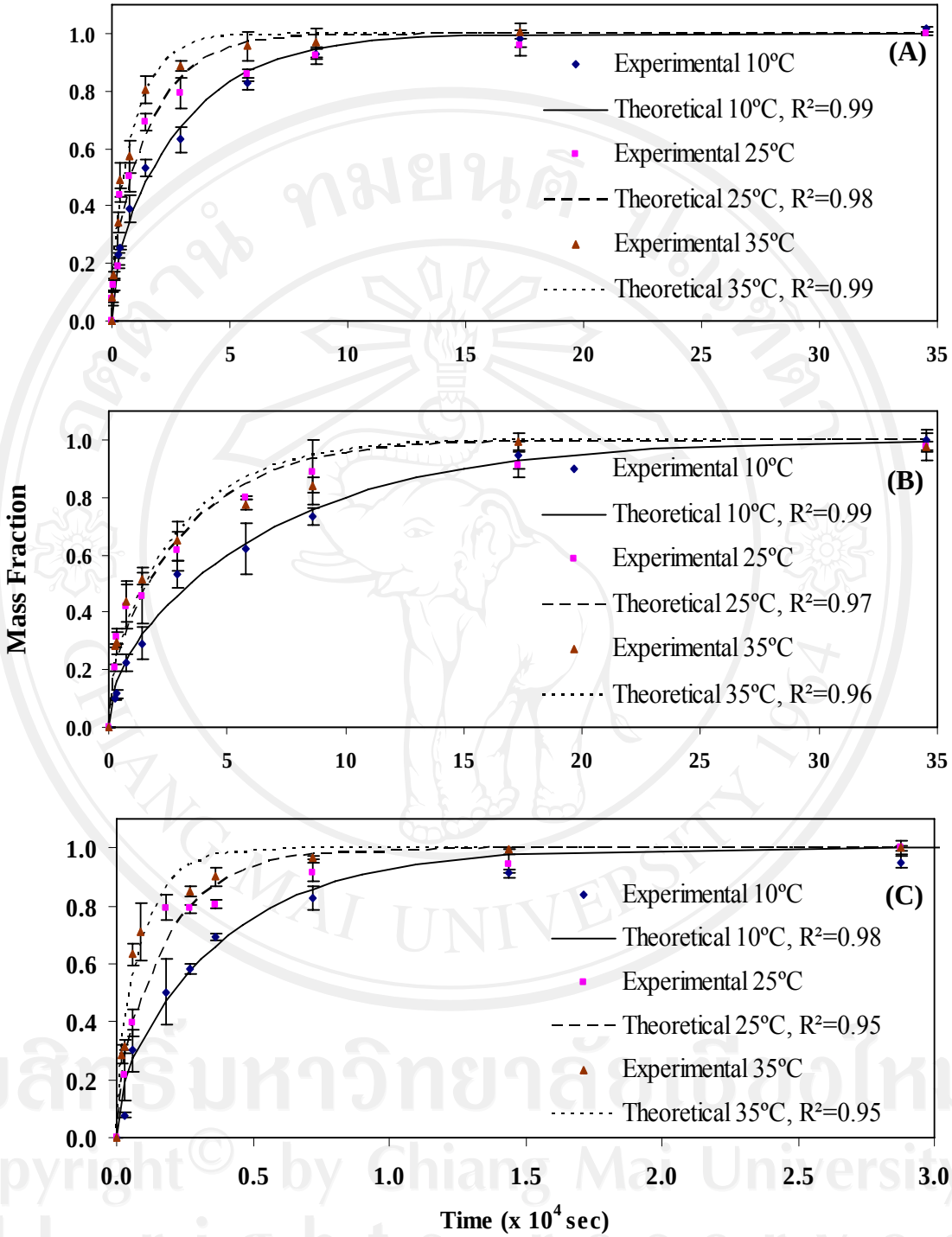


Figure 4.14 **Release profiles for vanillin in chitosan/methylcellulose films immersed in water (A), cantaloupe juice (B) and pineapple juice (C) at 10, 25 and 35°C.**

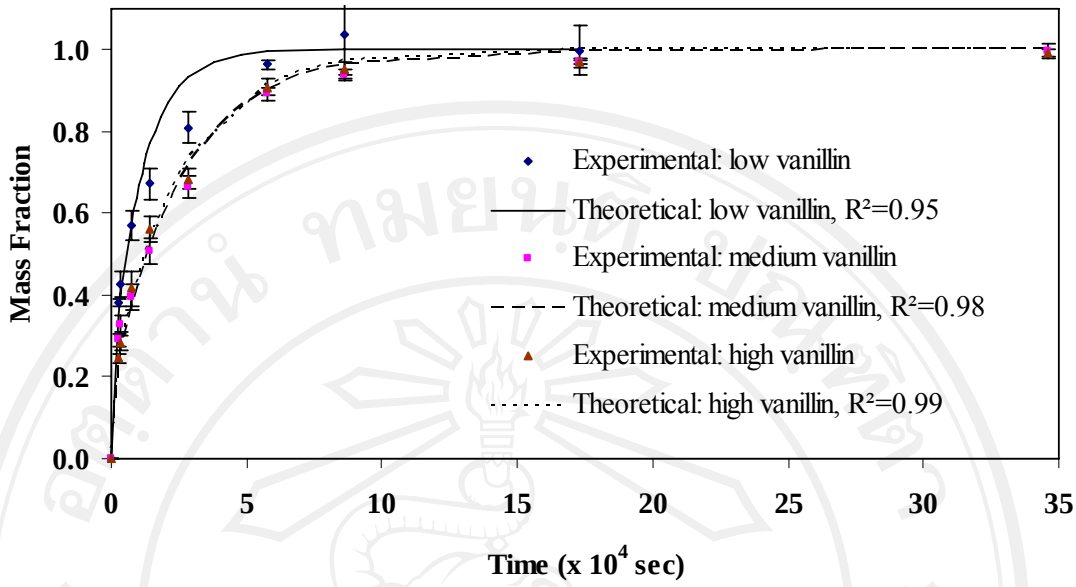


Figure 4.15 Release profiles for vanillin in chitosan/methylcellulose films containing 3 vanillin concentrations at 10°C.

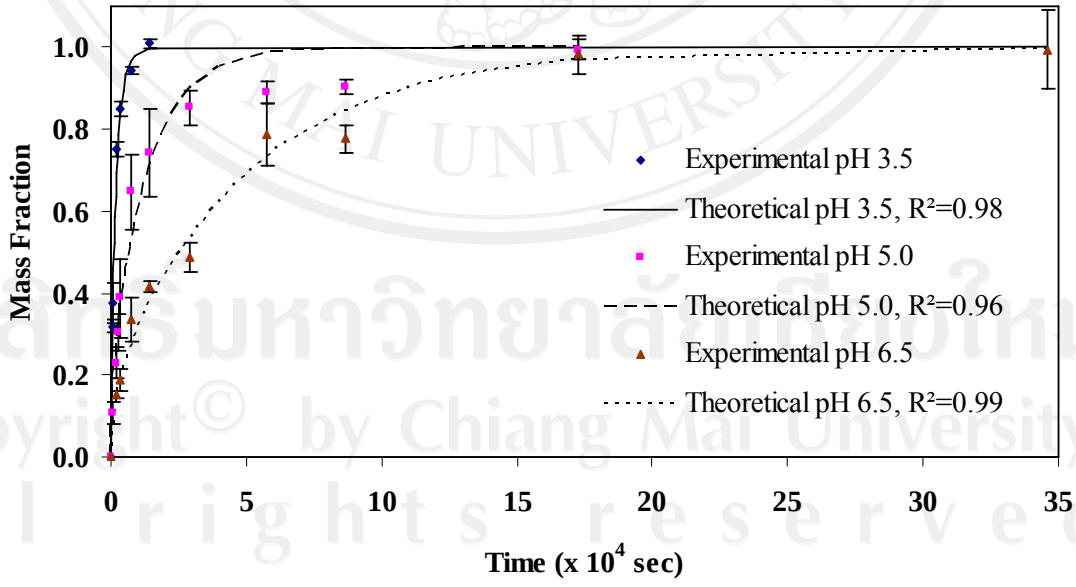


Figure 4.16 Release profiles for vanillin in chitosan/methylcellulose films immersed in citrate buffer pH 3.5, 5.0 and 6.5 at 10°C.

4.3.1.4 Effect of initial vanillin concentration in film

In this study, the release profiles of vanillin from films containing low, medium and high vanillin concentrations as a function of time are shown in Figure 4.15. The amount of vanillin in films containing low, medium and high levels of vanillin were 8.9%, 17.0% and 23.6% w/w dried film. Film containing low vanillin level had a faster release rate. Diffusion coefficients of film containing low, medium and high levels of vanillin were 12.72E-11, 8.35E-11, and 8.82E-11 cm^2/s, respectively. Diffusion coefficient of low vanillin film was 1.5 times higher than that of films containing medium and high vanillin concentrations. Since vanillin is rather hydrophobic, film containing low vanillin concentration absorbed more water. Since water acted as a plasticizer in the polymer matrix, it facilitates the release of vanillin from low level vanillin film. This behavior was similar to the release of acetic and propionic acids from chitosan films as reported by Ouattara *et al.* (2000). They explained that water first enters the chitosan matrix and dissolves the acids, thus allowing their subsequent release from the polymer. Therefore, the diffusion rate should increase with increasing penetration of water into the chitosan film as shown in this study. However, vanillin content higher than 17% w/w did not lower further the diffusion coefficient values.

Table 4.8 Diffusion coefficient of film containing high vanillin concentration in water, cantaloupe juice and pineapple juice at 10, 25 and 35°C.

Solvent	Diffusion coefficient, $x10^{-10}$ cm^2/s		
	10°C	25°C	35°C
Water	$0.80\pm0.12^{a,A}$	$1.43\pm0.28^{a,A}$	$2.39\pm0.50^{b,A}$
Cantaloupe juice	$0.35\pm0.07^{a,A}$	$0.73\pm0.23^{ab,A}$	$0.80\pm0.18^{b,A}$
Pineapple juice	$6.11\pm1.14^{a,B}$	$12.85\pm1.86^{a,B}$	$24.86\pm6.48^{b,B}$

Mean ± SD with different lower case letters in each row or different upper case letters in each column are significantly different at p=0.05.

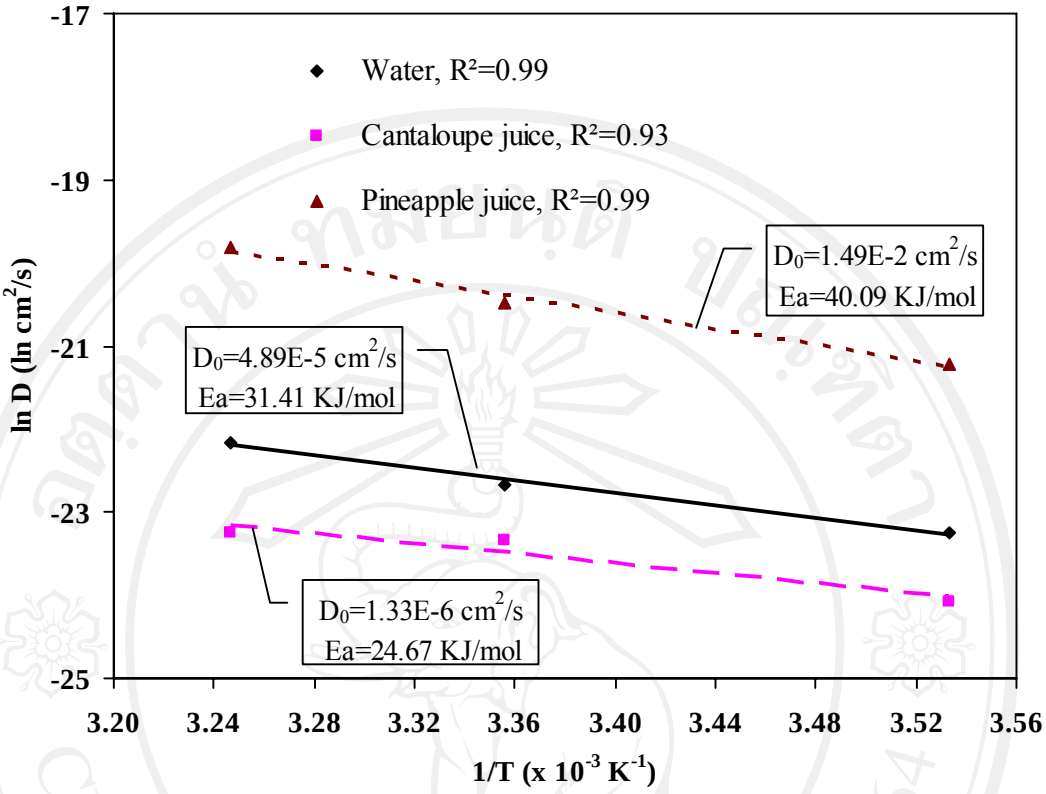


Figure 4.17 Arrhenius plot of chitosan/methylcellulose films containing vanillin in water, cantaloupe juice and pineapple juice at 10, 25 and 35 °C.

4.3.1.5 Effect of pH

Figure 4.16 presents the profiles of films in citrate buffer pH 3.5, 5.0 and 6.5 as a function of time. The pH of the buffer influenced the diffusion process. The diffusion coefficient of the vanillin in the films containing high vanillin in citrate buffer pH 3.5, 5.0 and 6.5 were 11.55E-10, 1.69E-10 and 0.494E-10 cm^2/s, respectively. The diffusion coefficient of the film in the pH 3.5 buffer was 6 times and 23 times higher than that of pH 5.0 and pH 6.5, respectively. This can partially attributed to the swelling of the films. Films immersed in buffer pH 3.5 had a higher swelling ratio (see section 4.3.1.6), indicating a more open structure of the films. Shu *et al.* (2001) found that the swelling ratio of citrate cross-linked chitosan film was lowest at pH 5.5 due to significant electrostatic attraction between citrate and chitosan. The decrease of the pH weakened salt-bonds and facilitated the film

swelling. They also found dissociation of chitosan below pH 3.5 which caused higher leaching of the migrant in the chitosan network while no leaching occurred at pH 5.5 and above. They also reported that the model drugs (brilliant blue and riboflavin) which had been incorporated into the film released quickly at low pH.

Figure 4.18 depicts the percent release of vanillin into citrate buffer with varying acid concentrations and pH. At a given period of time, citrate buffer pH 3.5 leached vanillin greater than citrate buffer pH 6.5 at any acid concentration. Film swelling was observed when immersed in buffer pH 3.5. However, increasing the acid concentration from 0.01 M to 0.09 M resulted in little change in percent release. Acid concentration had less influence on vanillin migration than pH. Varshosaz and Alinagari (2005) modified the chitosan structure using citric acid as a cross-linking agent while Muzzarelli *et al.* (2003) reported that citrate could cross-link chitosan and impart insolubility to the microsphere.

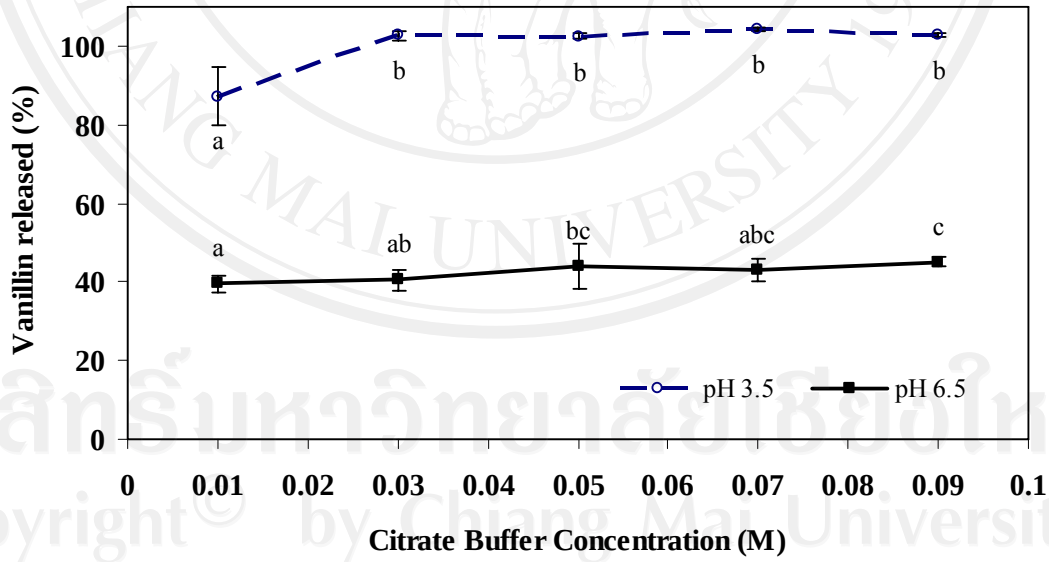


Figure 4.18 Percentage of vanillin released out of vanillin films in different concentrations of citrate buffer at pH 3.5 and 6.5.

4.3.1.6 Swelling behavior

Swelling behavior of chitosan/methylcellulose films containing vanillin were found to depend on solvent pH and temperature. No swelling occurred in films immersed in water and cantaloupe juice at 10, 25 and 35°C and citrate buffer pH 5.0 and 6.5 at 10°C. However, significant swelling was observed in film immersed in pineapple juice and citrate buffer pH 3.5. Figure 4.19 shows the swelling of chitosan/methylcellulose films containing high vanillin concentration immersed in pineapple juice at 10, 25 and 35°C. At 35°C, the film gained up to 70.2% of its initial weight within 25 minutes immersion. At 25°C, it took double the time to gain the same weight. In pineapple juice, increasing in film size from 1x1 cm to 1.1x1.1 cm were observed after 60, 50 and 30 minutes immersion at 10, 25 and 35°C, respectively. Figure 4.20 demonstrates the swelling of chitosan/methylcellulose films containing high vanillin concentration immersed in citrate buffer pH 3.5, 5.0 and 6.5. The swelling occurred in citrate buffer pH 3.5 while no swelling was observed after immersion in buffer pH 5.0 and 6.5. After 60 minutes immersion in citrate buffer pH 3.5, the dimension change of film was observed.

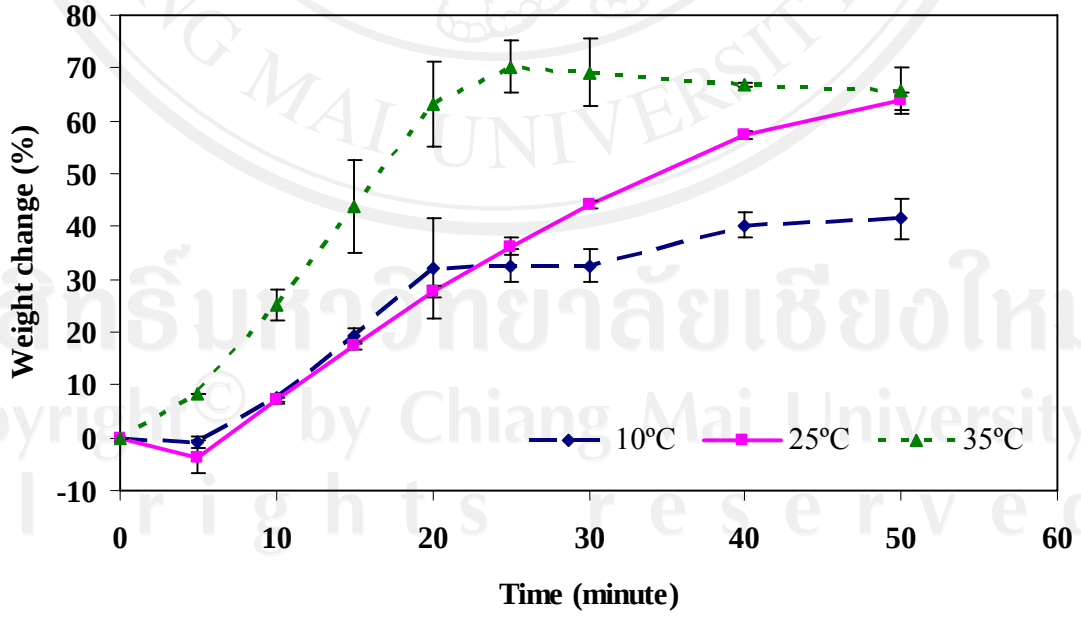


Figure 4.19 Swelling behavior of chitosan/methylcellulose film containing high vanillin immersed in pineapple juice at 10, 25 and 35 °C.

Shu *et al.* (2001) reported that the swelling ratio of citrate cross-linked chitosan film was pH sensitive. Under acidic conditions, the film swelled and dissociated in pH less than 3.5. At neutral conditions the swelling ratio of the film was less significant. At pH 5.5 and 6.5, the swelling ratio of citrate cross-linked chitosan film was lowest due to significant electrostatic attraction between citrate and chitosan. The decrease in pH weakened salt-bonds and therefore facilitated the film swelling. Moreover, films lose weight after immersion in buffer pH 5.0 and 6.5 (Figure 4.20). There are 2 possible reasons that could cause the weight losses; migration of vanillin out of the film and the leaching of film components such as chitosan, methylcellulose or PEG from the film matrix. Weight losses also occurred in films immersed in water at 10, 25 and 35°C (data not shown). Shu *et al.* (2001) found that chitosan leached from films. At pH 1.0 and 3.5, the film dissociated quickly (within 5 hours) while at pH 4.5, the leaching percent of chitosan was less than 20% in 24 hours due to relatively weak electrostatic attraction forces between citrate and chitosan. However, at pH 5.5, 6.5 and 7.4 no leaching of chitosan occurred. In this study, chitosan/methylcellulose films containing vanillin did not dissociate after immersion in citrate buffer pH 3.5 for 3 days.

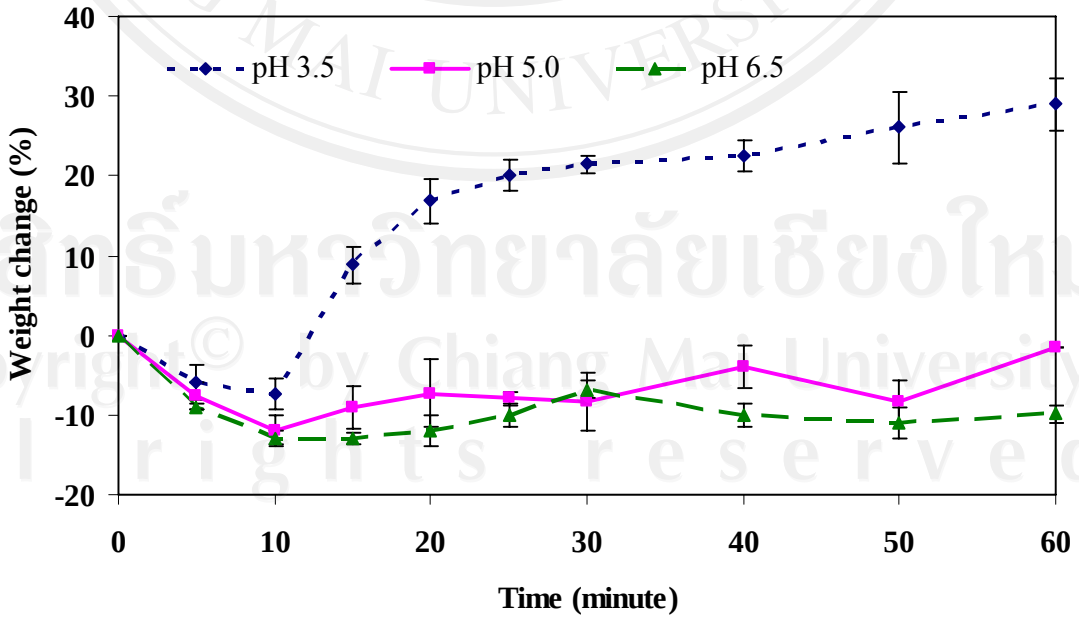


Figure 4.20 Swelling behavior of chitosan/methylcellulose film containing high vanillin immersed in citrate buffer pH 3.5, 5.0 and 6.5 at 10 °C.

4.3.2 Migration of vanillin from film

Figure 4.21 shows the release of vanillin from chitosan/methylcellulose films as a function of time into food simulants at 20 and 40°C. Three types of food simulants were used in this study; 10% ethanol represented acid food, 50% ethanol represented high alcoholic food and corn oil represented oily food (ASTM D4754). In this experiment, the food simulants were not replaced after sampling and the total stimulant volumes were corrected in calculation after each sampling. Gandek (1986) found that a smaller error was created when the stimulant was not replaced compared with replacing the food stimulant during the diffusion experiment. In Figure 4.21, it is shown that only 17.6 and 20.8% vanillin leached into corn oil at 20 and 40°C while 42.5 and 55.1% vanillin leached into 10% ethanol at 20 and 40°C and 64.5 and 81.2% vanillin leached into 50% ethanol at 20 and 40°C, respectively. The highest level of vanillin leached into 50% ethanol which represents food with high ethanol content followed by 10% ethanol which represents acid food. Temperature also affected release of vanillin out of the films. In all solvents studied, greater release was observed at higher temperature.

4.3.3 Release of vanillin on fruit pieces

Figure 4.22 shows the percent vanillin released from chitosan/methylcellulose film as a function of time on fresh-cut cantaloupe and pineapple surfaces stored at 10°C. On day 12, 52.2% of the vanillin was released from the film into the cantaloupe flesh. The release rate was found to be faster into pineapple flesh, and vanillin was completely released from the film on day 6. The release rates on fruit pieces shown in this study were relatively slower compared to the release rates into fruit juices (Figure 4.23). With cantaloupe juice, it took only 8 hours to release the same amount as it did for pieces in 12 days. Vanillin completely released into pineapple juice within 24 hours. There were 2 main factors that could possibly cause the difference. Release on fruit pieces occurred from only one side of the film while release into fruit juices occurred from both sides. Further more, the juices being liquids were free to move while the fruit pieces being solid did not move.

Ouattara *et al.* (2000) found that slower acid release was observed when chitosan films were applied on to the surfaces of processed meats compared with release into an aqueous medium. Significantly more acetic acid remained in the chitosan film during storage when the film was applied on bologna than on ham or pastrami due to the lower moisture content in bologna. Diffusion was not as complete as that for propionic acid when the chitosan films were applied used with processed meats.

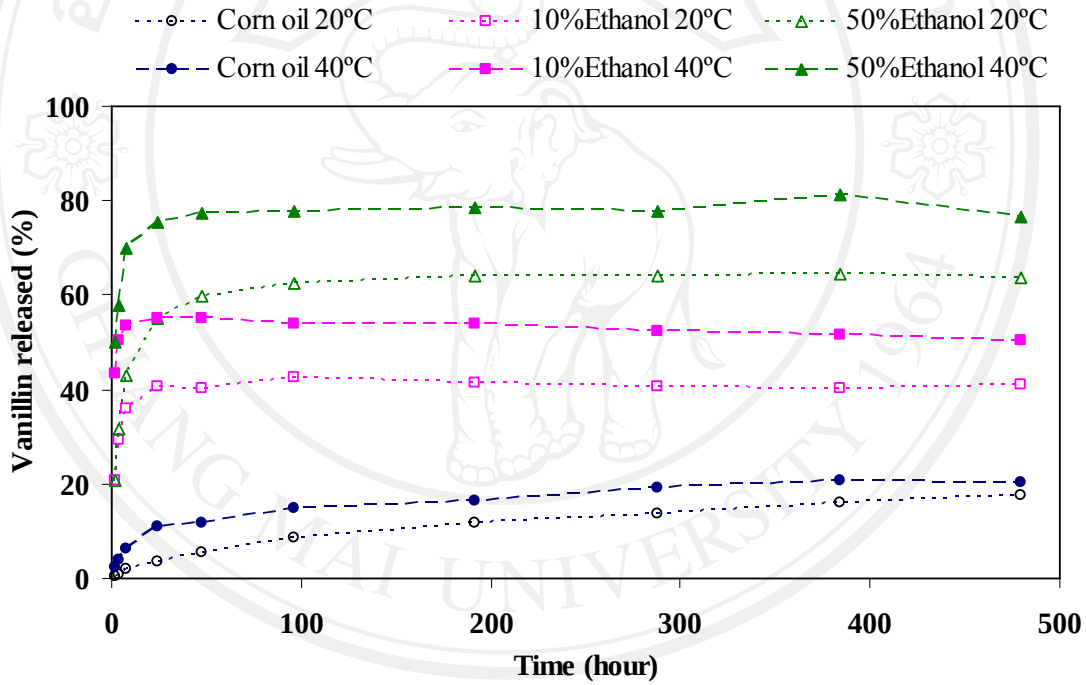


Figure 4.21 Migration of vanillin from chitosan/methylcellulose films into food simulants.

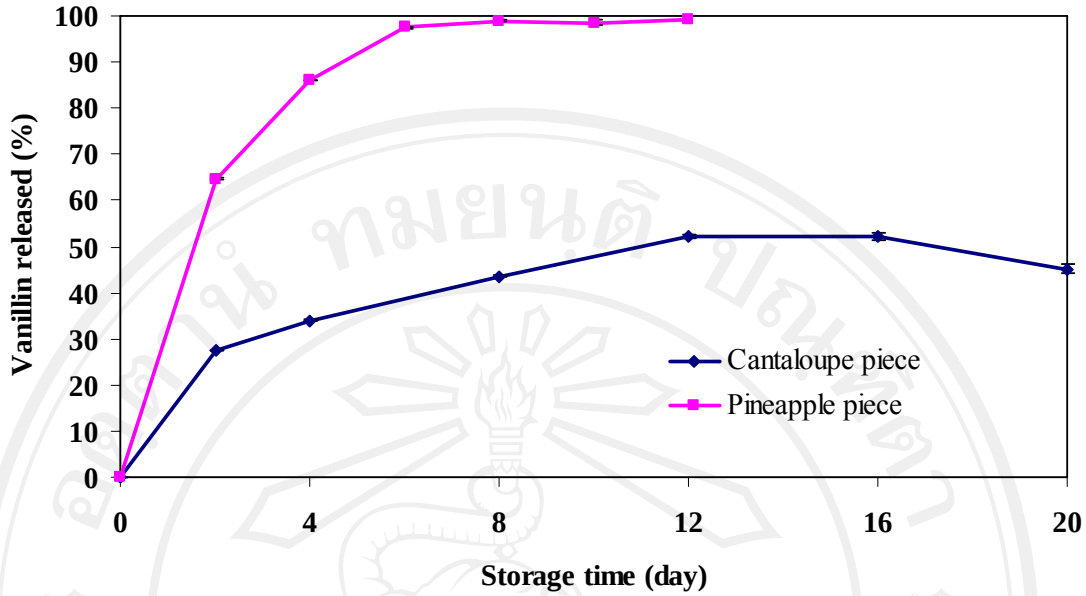


Figure 4.22 **Percentage of vanillin released out of chitosan/methylcellulose films containing high vanillin to fresh-cut cantaloupe and pineapple pieces stored at 10°C.**

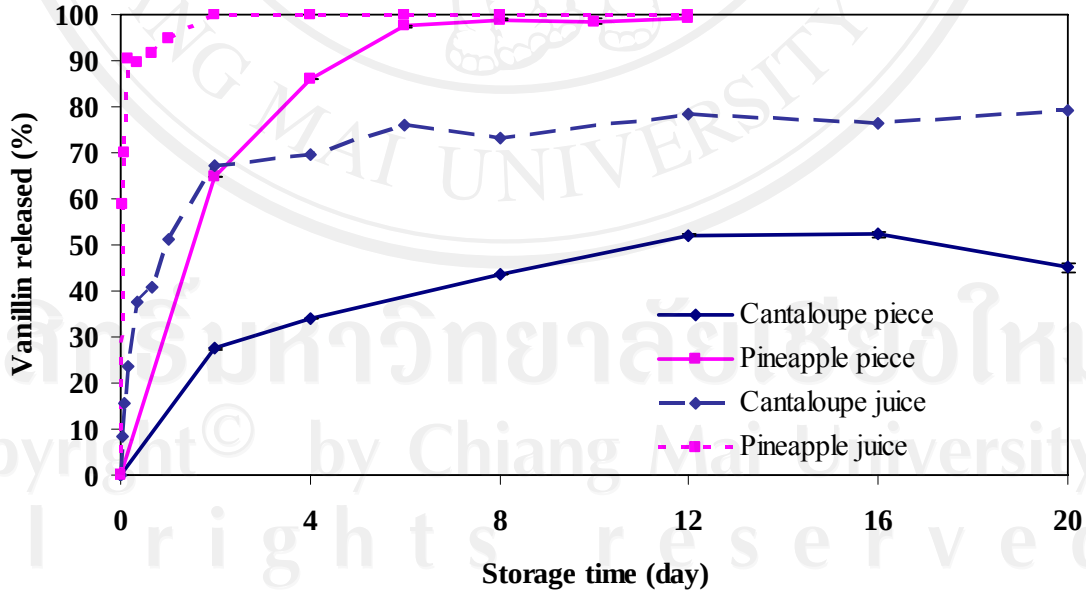


Figure 4.23 **Percentage of vanillin released out of chitosan/methylcellulose films containing high vanillin to fruit pieces (solid line) compared with fruit juices (dash line) stored at 10°C.**

4.4. Inhibition effect of chitosan/methylcellulose film and chitosan/ methylcellulose film incorporating vanillin as an antimicrobial agent

4.4.1 Standard disk diffusion technique

To study the inhibition effect of films containing varying amounts of plasticizer and vanillin, films (1 x 1 cm) were placed on the top of inoculum and incubated at 10 and 37°C. Two concentrations of *Escherichia coli*, $2.2x10^6$ and $2.2x10^5$ CFU/ml, were evenly spread on nutrient agar. The growth of *Escherichia coli* on agar was observed on day 6 and day 1 at 10°C and 37°C, respectively. The inhibition effect of films was recorded on the following day to make sure that the colonies were big enough. The experiment was done in duplicate and the results with the most organisms are shown in Table 4.9 and 4.10. PP film and chitosan/methylcellulose film with no vanillin did not inhibit the growth of *Escherichia coli*. Film containing medium and high vanillin levels inhibited the growth of *Escherichia coli* (Figure 4.24). Inhibition effect of films containing different amount of plasticizer was not obvious. A clear zone was observed around the film containing medium and high vanillin levels at 10°C but not at 37°C. The growth of *Escherichia coli* surrounding the film was different from other parts which was less condense and had smaller colonies (Figure 4.24D and E). No growth was observed for the film containing low vanillin on the first day. However, the growth could be seen on the next day. Films containing medium and high vanillin levels inhibited the growth of *Escherichia coli* for a longer period of time. Films containing medium and high vanillin provided the same inhibition against *Escherichia coli*. The effect of initial concentration of inoculum was the same for plates that were incubated at 10°C.

At 37°C, the inhibition effect of films on different inoculum concentrations was observed. Films containing medium vanillin (Figure 4.25), for example, inhibited *Escherichia coli* at the lower inoculum concentration ($2.2x10^5$ CFU/ml) but not at the higher inoculum concentration ($2.2x10^6$ CFU/ml). There were no difference inhibitions by films containing different concentrations of PEG.

Table 4.9 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Escherichia coli* at 10°C.**

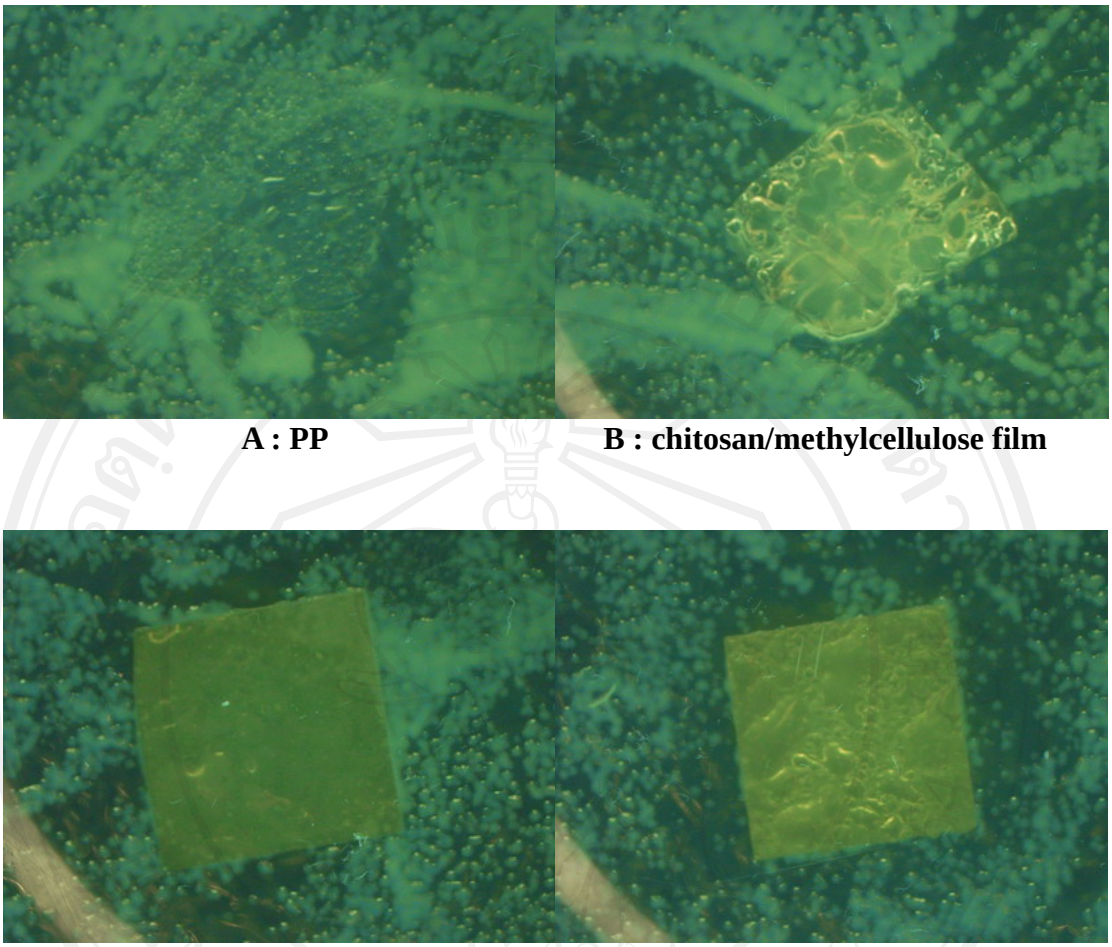
Initial count	Plasticizer content	Chitosan/methylcellulose film containing			
		No Vn	Low Vn	Medium Vn	High Vn
2.2E+6	Low	-	#	+*	+*
	High	-	#	+*	+*
2.2E+5	Low	-	#	+*	+*
	High	-	#	+*	+*

Vn = vanillin, (-) no inhibition, (#) partial inhibition, (+*) complete inhibition with clear zone

Table 4.10 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Escherichia coli* at 37°C.**

Initial count	Plasticizer content	Chitosan/methylcellulose film containing			
		No Vn	Low Vn	Medium Vn	High Vn
2.2E+6	Low	-	-	#	#
	High	-	#	#	+
2.2E+5	Low	-	#	+	+
	High	-	#	+	+

Vn = vanillin, (-) no inhibition, (#) partial inhibition, (+) complete inhibition

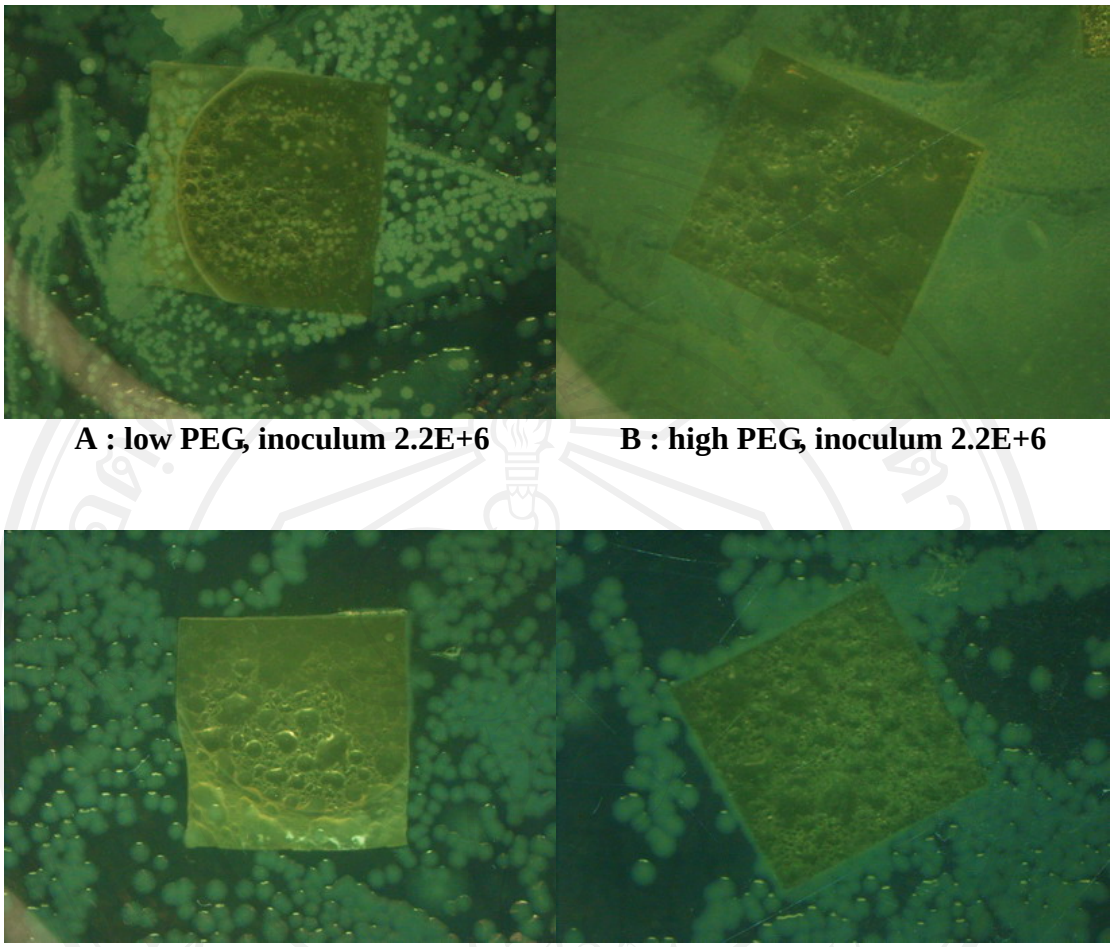


A : PP **B : chitosan/methylcellulose film**

C : chitosan/methylcellulose film containing low vanillin **D : chitosan/methylcellulose film containing medium vanillin**

E : chitosan/methylcellulose film containing high vanillin

Figure 4.24 ***Escherichia coli*** **growth underneath different films during storage at 10^{o}C for 7 days.**



A : low PEG, inoculum 2.2E+6 **B : high PEG, inoculum 2.2E+6**

C : low PEG, inoculum 2.2E+5 **D : high PEG, inoculum 2.2E+5**

Figure 4.25 ***Escherichia coli*** **growth underneath chitosan/methylcellulose films containing medium vanillin with different plasticizer concentrations during storage at 37^{o}C for 2 days.**

Similar to the technique used with *Escherichia coli*, two concentrations of *Saccharomyces cerevisiae*, $3.1x10^6$ and $3.1x10^5$ CFU/ml, were evenly spread on Sabouraud agar. The experiment was done in duplicate and the results showing the most growth of microorganisms are presented in Table 4.11 and 4.12. The growth of *Saccharomyces cerevisiae* on agar was observed on day 5 and day 1 at 10 and 25°C, respectively. The data in the Table 4.9-4.12 were recorded the following day after the growths were observed. PP film and chitosan/methylcellulose film with no vanillin did not inhibit the growth of *Saccharomyces cerevisiae* at both temperatures studied. At 25°C, only film with high vanillin inhibited the growth of yeast (Figure 4.26). The inhibitory effect of films containing different amounts of plasticizer was unclear. The inhibition effect of films with different inoculum concentrations was obvious. Films containing medium vanillin, for example, inhibited *Saccharomyces cerevisiae* at the lower inoculum concentration (3.1E+5) but not at the higher inoculum concentration at 10°C (Figure 4.27). At 25°C, films containing low vanillin did not inhibit yeast while films containing medium vanillin partially inhibit the growth of yeast.

Further more, film containing medium vanillin completely inhibited *Escherichia coli* at 10°C but not yeast. Only film containing high vanillin concentration inhibited yeast at both temperatures and initial inoculum concentrations (Table 4.11 and 4.12). Thus, it can be concluded that *Escherichia coli* was more sensitive to vanillin than *Saccharomyces cerevisiae,* and therefore, film containing high vanillin concentration was selected for further study.

Table 4.11 Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Saccharomyces cerevisiae* at 10°C.

Initial count	Plasticizer content	Chitosan/methylcellulose film containing			
		No Vn	Low Vn	Medium Vn	High Vn
3.1E+6	Low	-	#	#	+
	High	-	#	#	+
3.1E+5	Low	-	#	+	+
	High	-	#	+	+

Vn = vanillin, (-) no inhibition, (#) parial inhibition, (+) complete inhibition

A higher temperature resulted in faster growth of the microorganism studied. At the same time, it caused a higher release of vanillin from the film. Even though the release rate of vanillin from the film was faster at higher temperature, it might not be fast enough to inhibit the microorganism at its optimum growth temperature. To obtain enough vanillin to inhibit the growth, a film containing a higher vanillin concentration must be used with the products at high storage temperature.

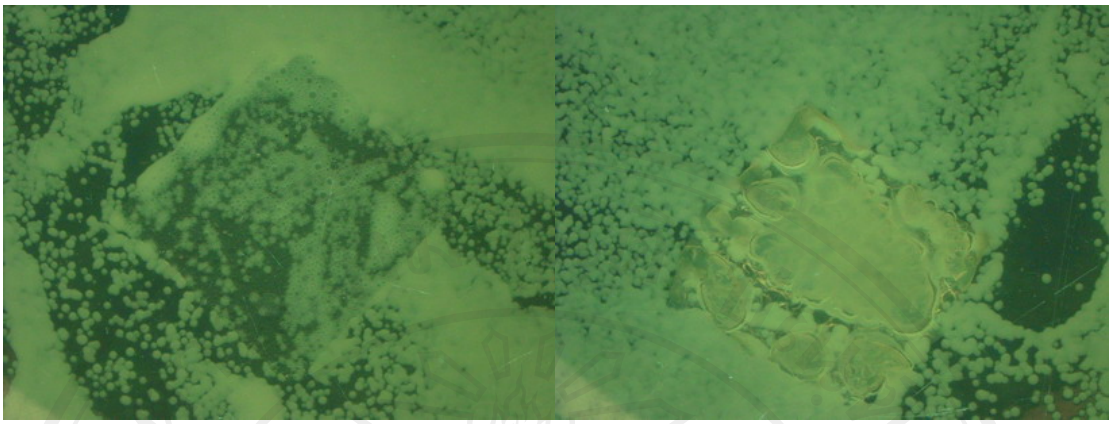
Table 4.12 Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Saccharomyces cerevisiae* at 25°C.

Initial count	Plasticizer content	Chitosan/methylcellulose film containing			
		No Vn	Low Vn	Medium Vn	High Vn
3.1E+6	Low	-	-	#	+
	High	-	-	-	+
3.1E+5	Low	-	-	#	+
	High	-	-	#	+

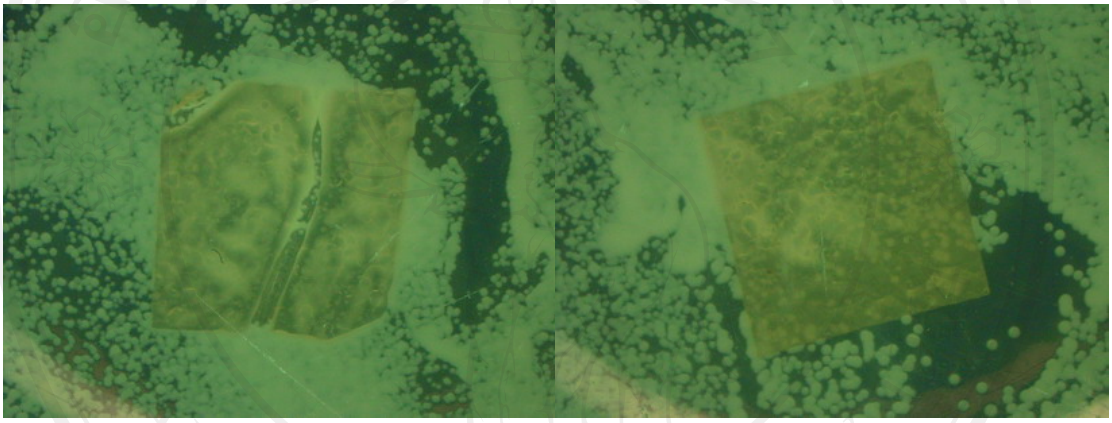
Vn = vanillin, (-) no inhibition, (#) partial inhibition, (+) complete inhibition

4.4.2 *Escherichia coli* inoculation and determination of number of *Escherichia coli*

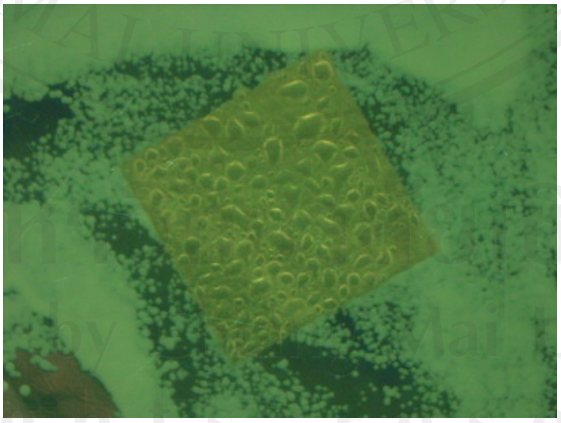
On day 0, the number of *Escherichia coli* on each cantaloupe piece was $1.5x10^5$ CFU/piece. To disregard the difference in weight loss of each treatment during storage, the microbiological counts are expressed per piece instead of per gram as some weight loss occurred during storage. With increased storage time, the number of *Escherichia coli* on cantaloupe over wrapped with stretch film increased, while the populations of *Escherichia coli* on cantaloupe, over wrapped with chitosan/methylcellulose film and vanillin film gradually decreased (Figure 4.28A). The reduction in the *Escherichia coli* population might be due to the loss of water from the fruit during storage. The loss rate was faster on fruit wrapped with chitosan/methylcellulose film during the first 2 days. After that, the populations of *Escherichia coli* on cantaloupe wrapped with chitosan/methylcellulose film and vanillin film were not different. After 4 days storage, cantaloupe in the



A : PP **B : chitosan/methylcellulose film**

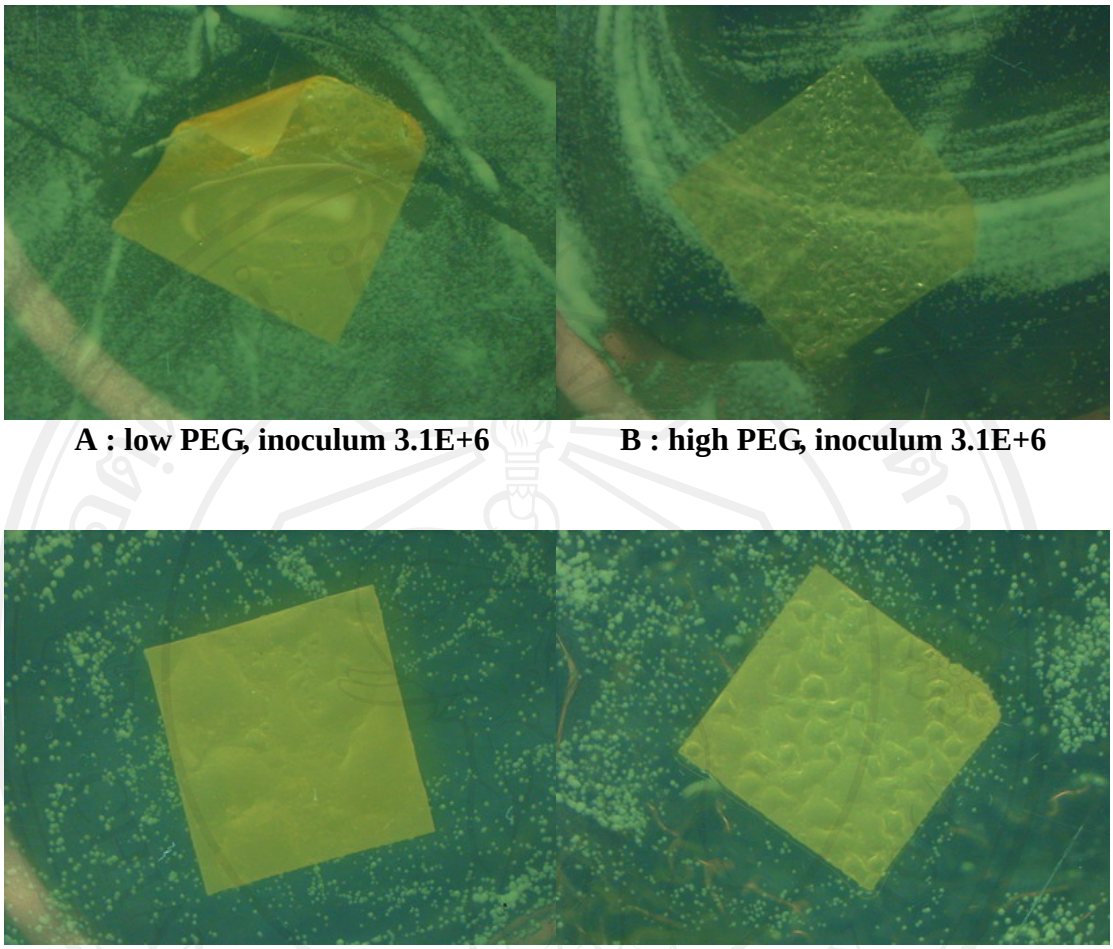


C : chitosan/methylcellulose film containing low vanillin **D : chitosan/methylcellulose film containing medium vanillin**



E : chitosan/methylcellulose film containing high vanillin

Figure 4.26 ***Saccharomyces cerevisiae*** **growth underneath different films during storage at 25°C for 2 days.**



A : low PEG, inoculum 3.1E+6 **B : high PEG, inoculum 3.1E+6**

C : low PEG, inoculum 3.1E+5 **D : high PEG, inoculum 3.1E+5**

Figure 4.27 ***Saccharomyces cerevisiae*** **growth underneath chitosan/ methylcellulose films containing medium vanillin with different plasticizer concentrations during storage at 10^{o}C for 6 days.**

chitosan/methylcellulose and vanillin films had a significantly lower number of *Escherichia coli* than fruit without wrapping.

The initial number of *Escherichia coli* on pineapple pieces was equivalent to that on cantaloupe. However, *E coli* populations of all treatments decreased over the storage period (Figure 4.28B). The fruit pH might be too low for this microorganism to grow. Presser *et al* (1997) reported that *Escherichia coli* grew at pH 4.0 but not at pH 3.7. The pineapple used in this study had a pH of 3.3-3.8. Therefore, any inhibitory effect of the antimicrobial films may not have been relevant.

4.4.3 *Saccharomyces cerevisiae* inoculation and determination of number of *Saccharomyces cerevisiae*

The initial number of yeast on cantaloupe and pineapple pieces after inoculation with *Saccharomyces cerevisiae* were $2.3x10^5$ and $1.2x10^5$ CFU/piece, respectively. After 2 days, the numbers began to increase on the control cantaloupe and cantaloupe wrapped with commercial stretch film (Figure 4.29A). The increase in the latter was faster because it maintained higher moisture content and lower oxygen concentration. In comparison, the numbers of yeast on cantaloupe wrapped with the vanillin film remained constant overtime, while those wrapped with the chitosan/methylcellulose film decreased over the first 4 days and then increased afterward. After day 4, the chitosan/methylcellulose film and vanillin film provided the same inhibition.

Similar to cantaloupe, the numbers of yeast on pineapple wrapped with stretch film increased (Figure 4.29B). The yeast populations on pineapple wrapped with chitosan/methylcellulose film decreased almost 2 logs CFU/piece on the first day and remained constant thereafter. Film containing vanillin resulted in a decrease of 4 logs more than the other films. The vanillin film was more effective but took a longer time to show that effect than chitosan/methylcellulose film. The vanillin film may be useful for food with longer storage life. Ngarmsak *et al.* (2006) found that fresh-cut mango dipped in 80 mM vanillin for 1 minute significantly delayed the development of total aerobic bacteria, yeast and mold populations.

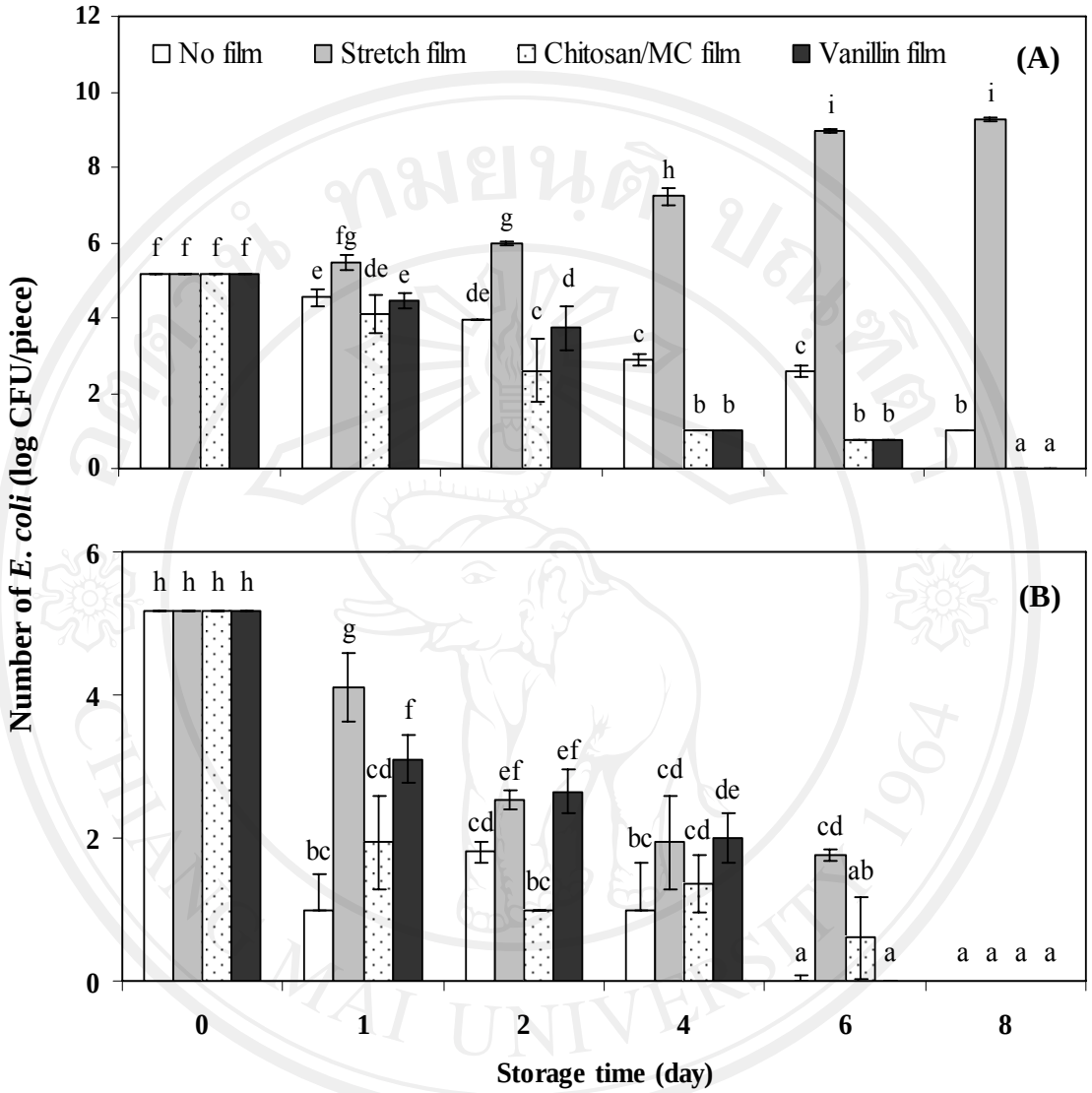


Figure 4.28 Numbers of *Escherichia coli* on inoculated cantaloupe (A) and pineapple (B) during storage at 10°C for 8 days.

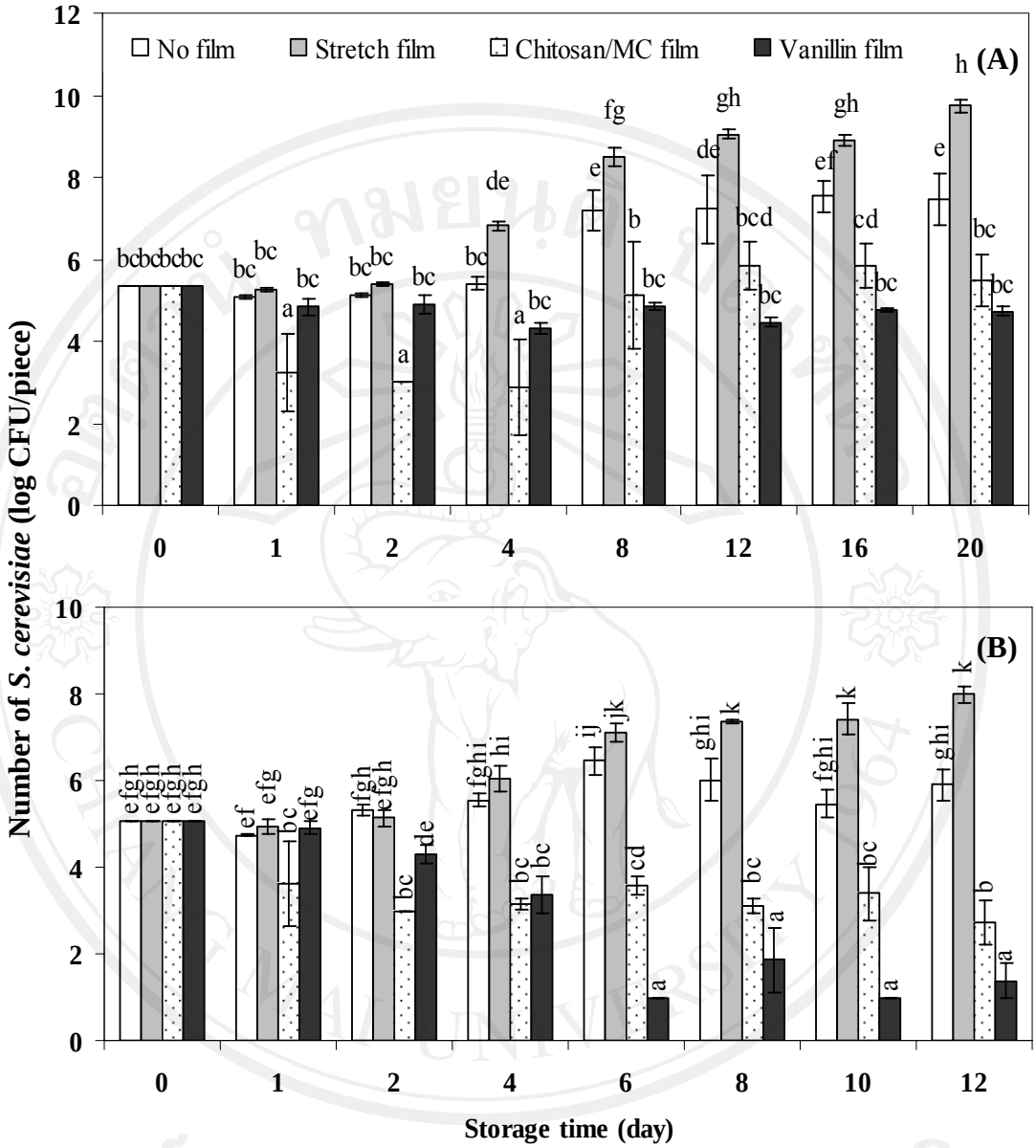


Figure 4.29 Numbers of *Saccharomyces cerevisiae* on inoculated cantaloupe (A) and pineapple (B) during storage at 10°C for 20 and 12 days, respectively.

Use of vanillin film with fresh-cut cantaloupe and pineapple resulted in a different behavior. Vanillin film did not reduce the number 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 microorganism in lower pH foods. Fitzgerald *et al.* (2004) found that 20 mM vanillin was required to achieve complete inhibition of *Saccharomyces cerevisiae* inoculated at a level of 10^4 CFU/ml in apple juice and a peach-flavored soft drink over an 8 week storage period at 25°C. They also reported that the increased activity of vanillin in the peach-flavored soft drink (pH 3.1), compared to the apple juice (pH 3.5) is probably a result of the lower intrinsic pH of the former. However, variation in vitamin and mineral levels or the presence of other phenolic compounds might also have contributed to the observed difference. Visual observation showed that the yellowness of the vanillin film used to wrap pineapple decreased (Figure 4.30). Therefore, the greater inhibition might be due to the higher release rate of vanillin out of the film.

Figure 4.30 Color of vanillin films after removal from fresh-cut pineapple.

4.5. Quality evaluation of fruit wrapped with antimicrobial films

Chitosan/methylcellulose film is 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 (Figure 4.31). It also adhered to the surface of the fruit and was difficult to remove. Vanillin film adhered to fruit wedges like other synthetic

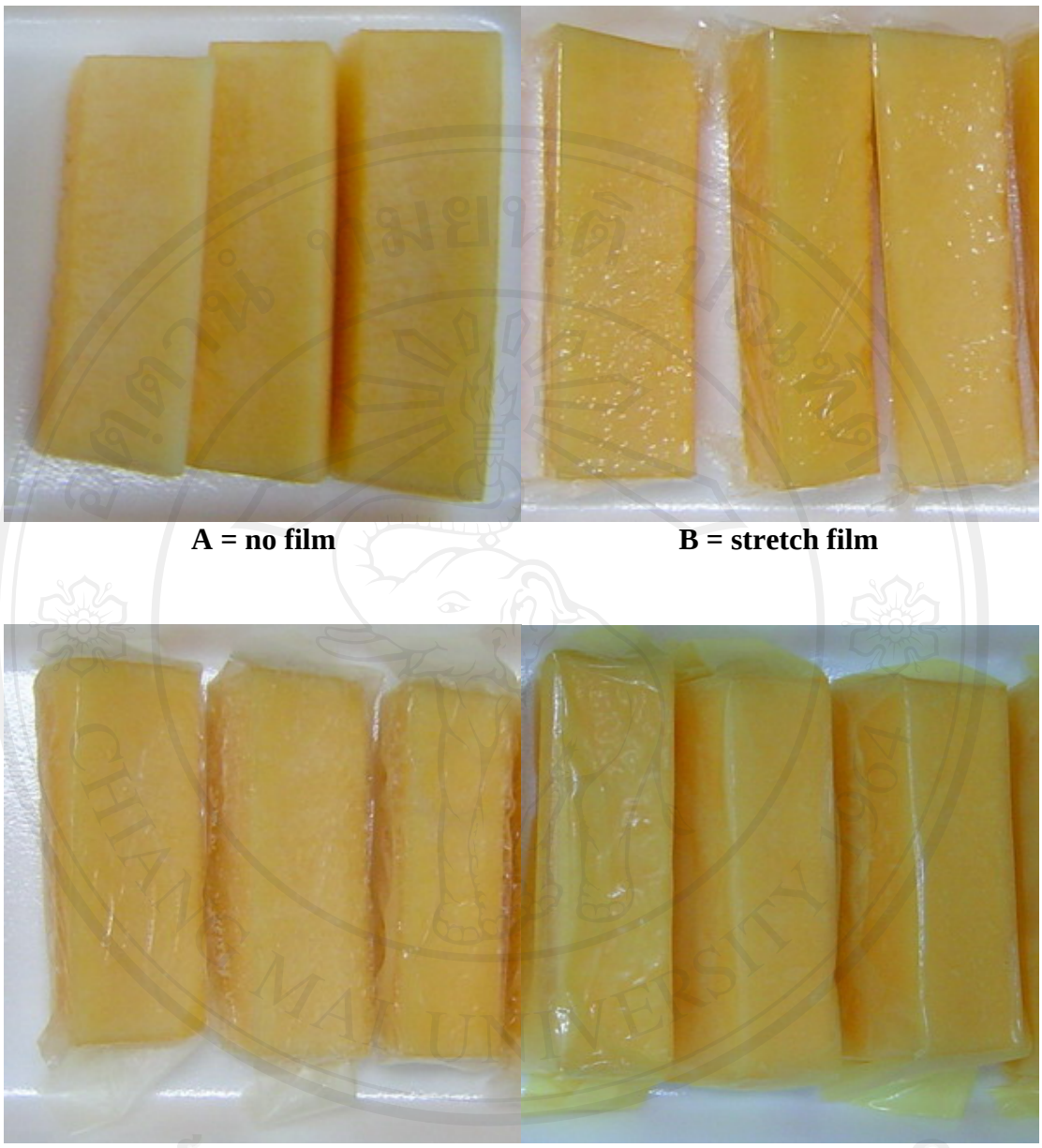
plastic films. Unlike the chitosan/methylcellulose film, it was easy to remove from the surface of the fruit. Mold was visually observed on day 12 for all cantaloupe treatments except the vanillin film treatment (Figure 4.32). Even though there was no mold on fresh-cut pineapple, an off-odor was detected.

4.5.1 Flesh color

The initial hue angle of cantaloupe flesh was 67.13. The color of fresh-cut cantaloupe for all treatments remained unchanged over the storage period. However, the hue angle of pineapple flesh wrapped with stretch film decreased overtime while that wrapped with vanillin film increased significantly from 92.3 to 97.9 (Figure 4.33). This may be the result of the yellow vanillin migrating from the vanillin film (Figure 4.30). On the contrary, the color of vanillin film was still yellow after removal from the cantaloupe flesh. Migration of the vanillin to the fruit might affected the sensory changes of the fruit.

4.5.2 Firmness

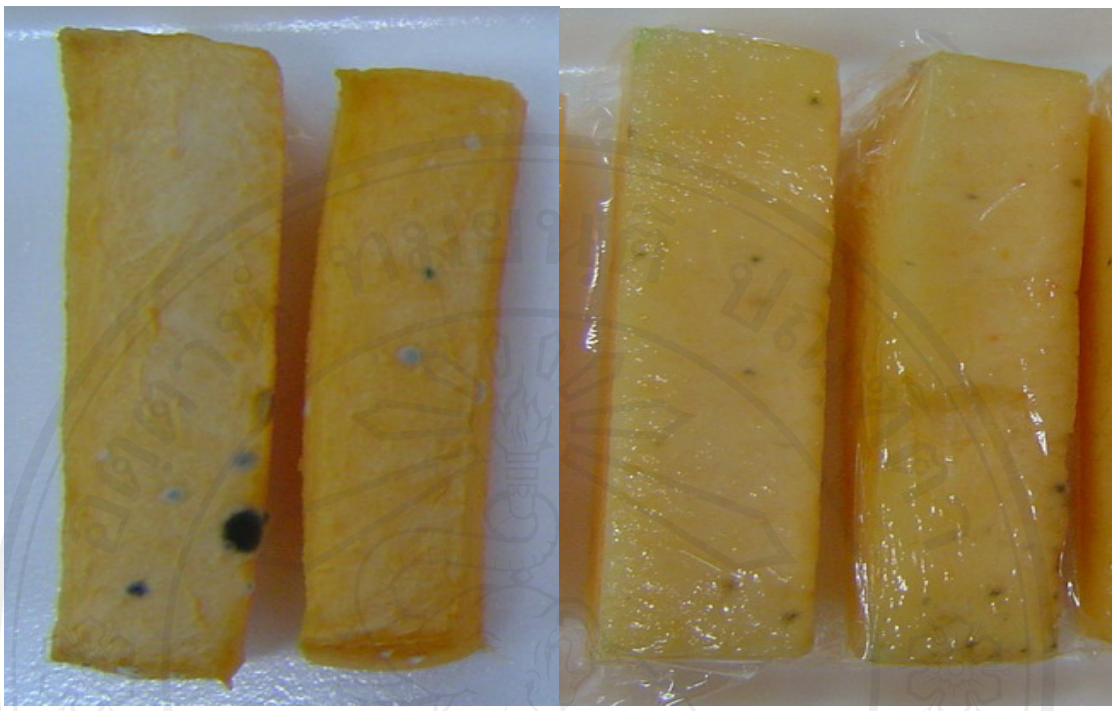
The firmness of the cantaloupe flesh, for all treatments, remained unchanged over time (Figure 4.34). Although the measured force did not show any difference, it is possible that the mouth-feel of the fruit was completely different due to water loss. None of the films had any effect on the firmness of the pineapple, which decreased by approximately 20% over the first 2 days and was almost unchanged after that. Maftoonazad and Ramaswamy (2005) treated avocados with an ethyl cellulose based coating. They found that coated avocados had higher firmness during 10 days storage.



A = no film **B = stretch film**

C = chitosan/methylcellulose film **D = vanillin film**

Figure 4.31 Fresh-cut cantaloupe wrapped with different film types on day 0.



A = no film **B = stretch film**

C = chitosan/methylcellulose film **D = vanillin film**

Figure 4.32 Mold incidence on fresh-cut cantaloupe after storage at 10°C for 12 days.

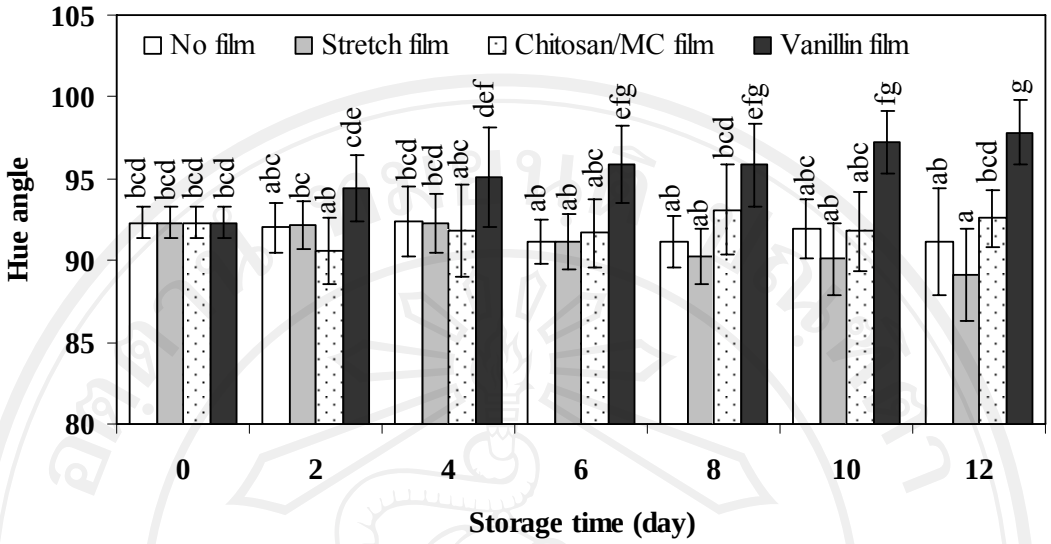


Figure 4.33 Hue angle of fresh-cut pineapple during storage at 10°C for 12 days.

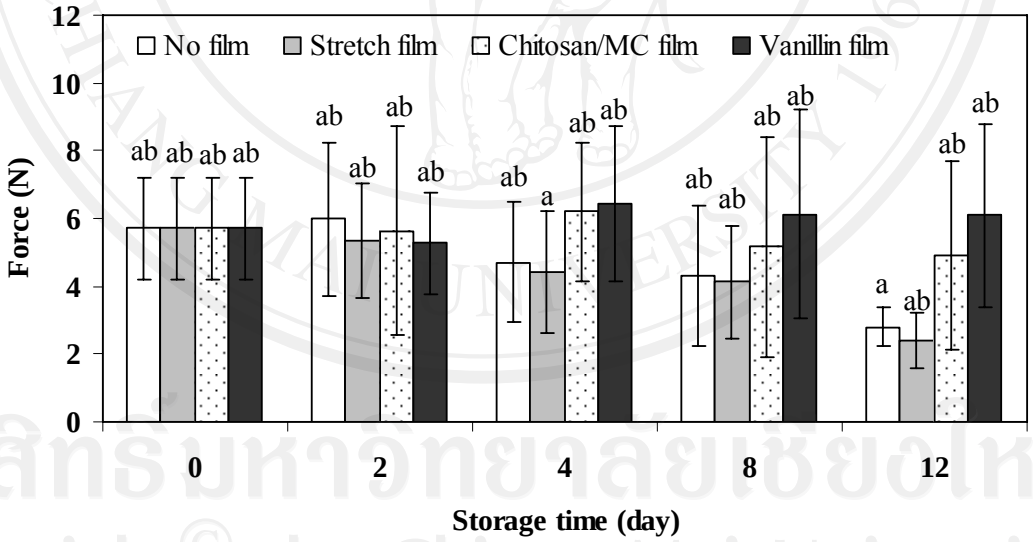


Figure 4.34 Firmness of fresh-cut cantaloupe during storage at 10°C for 12 days.

4.5.3 L-ascorbic acid

Standard solutions of L-ascorbic acid (AA) were prepared fresh under cold and dark conditions. The relative retention time was 4.5 minutes. The determination of linearity (R^2) of the standard curve was 0.999 (Figure 4.35).

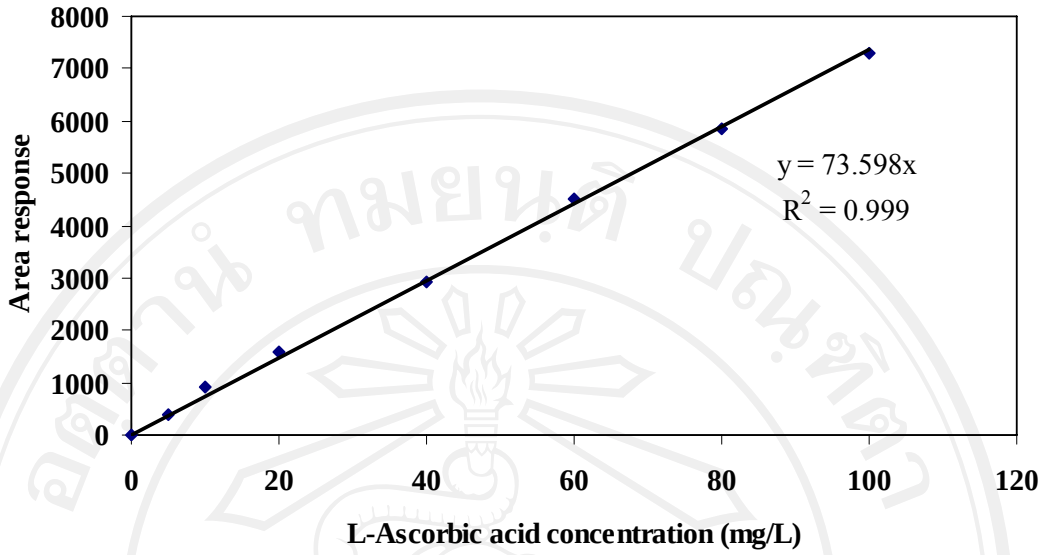


Figure 4.35 Standard curve of L-ascorbic acid.

The 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 are in the range of 14-19.8 mg/100 g fresh weight as found by Saftner *et al*. (2006). AA content decreased over the first two days (Figure 4.36A). The reduction in AA content at the end of storage was about 50% of its 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 (Figure 4.36B). This is low compared with that found by Vinci *et al.* (1995) who reported that the AA content of fresh and artificially ripened pineapple was 30.6 and 18.1 mg/100 g fresh weight. The low AA content might be due to a higher storage temperature in the supply chain. In a preliminary study, it was found that AA rapidly degraded in pineapple samples. The sample must be carefully prepared and immediately injected into the HPLC system. The AA content in pineapple wrapped with chitosan/methylcellulose film, stretch film and no film slightly decreased during storage (Figure 4.36B). Unexpectedly, the AA content in pineapple wrapped with vanillin film diminished drastically after day 6. Only 10% of the initial concentration was left in the fruit after 12 days of storage. This apparent interaction between vanillin and AA should be investigated, including hydrogen exchange or bonding. However, Burri *et al.* (1989) reported that vanillin also acts as an antioxidant.

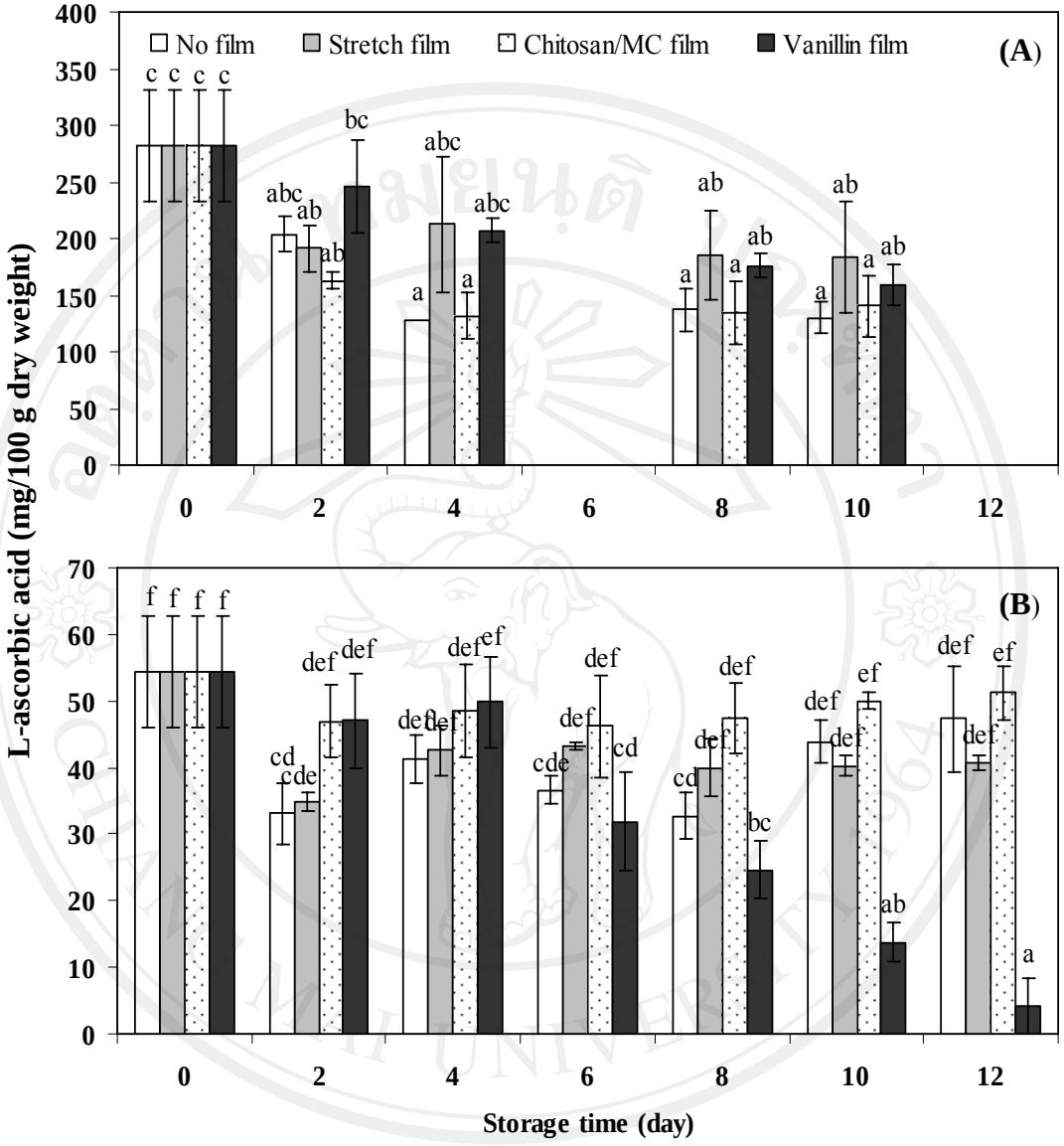


Figure 4.36 L-ascorbic acid content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 10 and 12 days, respectively.

A separate experiment was done by directly adding 1,000 and 2,000 mg/L vanillin into pineapple juice. It was found that AA content in both vanillin containing juices rapidly decreased, compared to the control (Figure 4.37). On day 12, only 10% of the AA was left in both treatments containing vanillin, while 60% of AA still remained in the control.

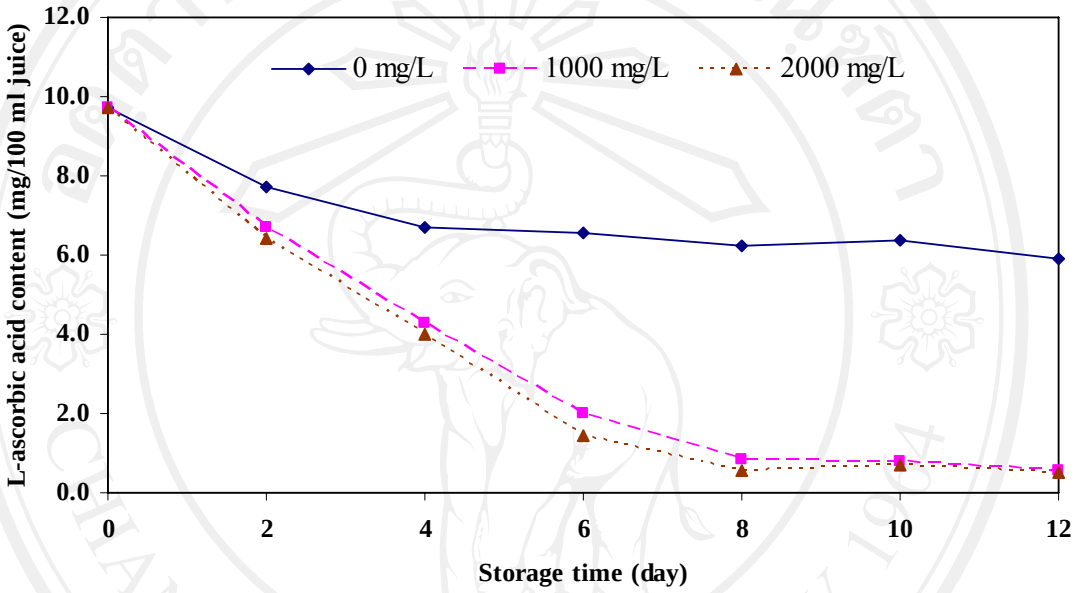


Figure 4.37 L-ascorbic acid content of pineapple juice supplemented with 1,000 and 2,000 mg/L vanillin during storage at 10°C for 12 days.

4.5.4 Respiration rate

The respiration rate of cantaloupe flesh 1 hour after cutting and wrapping was 0.101±0.007 g CO_2/kg.h. The respiration rate for all treatments significantly decreased on day 2 until day 8. On day 12, the respiration rate increased drastically for unwrapped and cantaloupe with stretch film (Figure 4.38A). The burst of CO_2 production in fruit wrapped with stretch film could also partially be the result of microbial activity, since these fruit had higher microbial counts.

The respiration rate of pineapple flesh 1 hour after cutting and wrapping was 0.088±0.012 g CO_2/kg.h. Similarly, the respiration rate for all treatments gradually decreased from day 2 to day 6. (Figure 4.38B). After day 6, the respiration rate increased again. The CO_2 production was slower in pineapple wrapped with vanillin

film compared with that wrapped with stretch film. This might be the result of better microbial inhibition. In general, chitosan/methylcellulose and vanillin films are better gas barriers than the stretch film in dry conditions, though the barrier properties worsen as they absorb moisture. Thus, the gas protection provided by these films is limited. Too high a gas barrier might result in depletion of oxygen, resulting in fermentation of the product. Therefore, films should have an appropriate oxygen permeability which is very important for respiring products.

4.5.5 Ethanol content

Initial ethanol content of fresh-cut cantaloupe was 0.08 µl/g flesh. Ethanol levels increased at all treatments and varied from 0.19 to 0.41 µl/g flesh (Figure 4.39A). On day 12, the ethanol content of cantaloupe wrapped in vanillin film was significantly lower than the control and that wrapped with stretch film. High ethanol content in control and stretch film treatments might be due to higher microbial count.

In pineapple, no initial ethanol was detectable. Ethanol increased with time which is indicative of a fermentative metabolism associated with senescence (Figure 4.39B). The 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 the other treatments. Pineapple wrapped with stretch film had significantly higher ethanol contents which reflect the level of fermentation from higher microbial growth and might result in an unacceptable flavor.

4.5.6 Total soluble solid (TSS), titratable acidity (TA) and pH

Initial TSS of cantaloupe and pineapple was 7.7±0.3% and 18.0±1.0%, respectively (Figure 4.40). The TSS of cantaloupe without film significantly increased after day 4 because the fruit lost approximately 50% of its water content. However, the TSS of cantaloupe wrapped in any of the films did not change during storage at 10^{o}C for 12 days. For pineapple, TSS for all treatments did not change during storage except for pineapple wrapped with vanillin film after day 8. Complete migration of vanillin (Figure 4.22) from film resulted in significantly increased TSS.

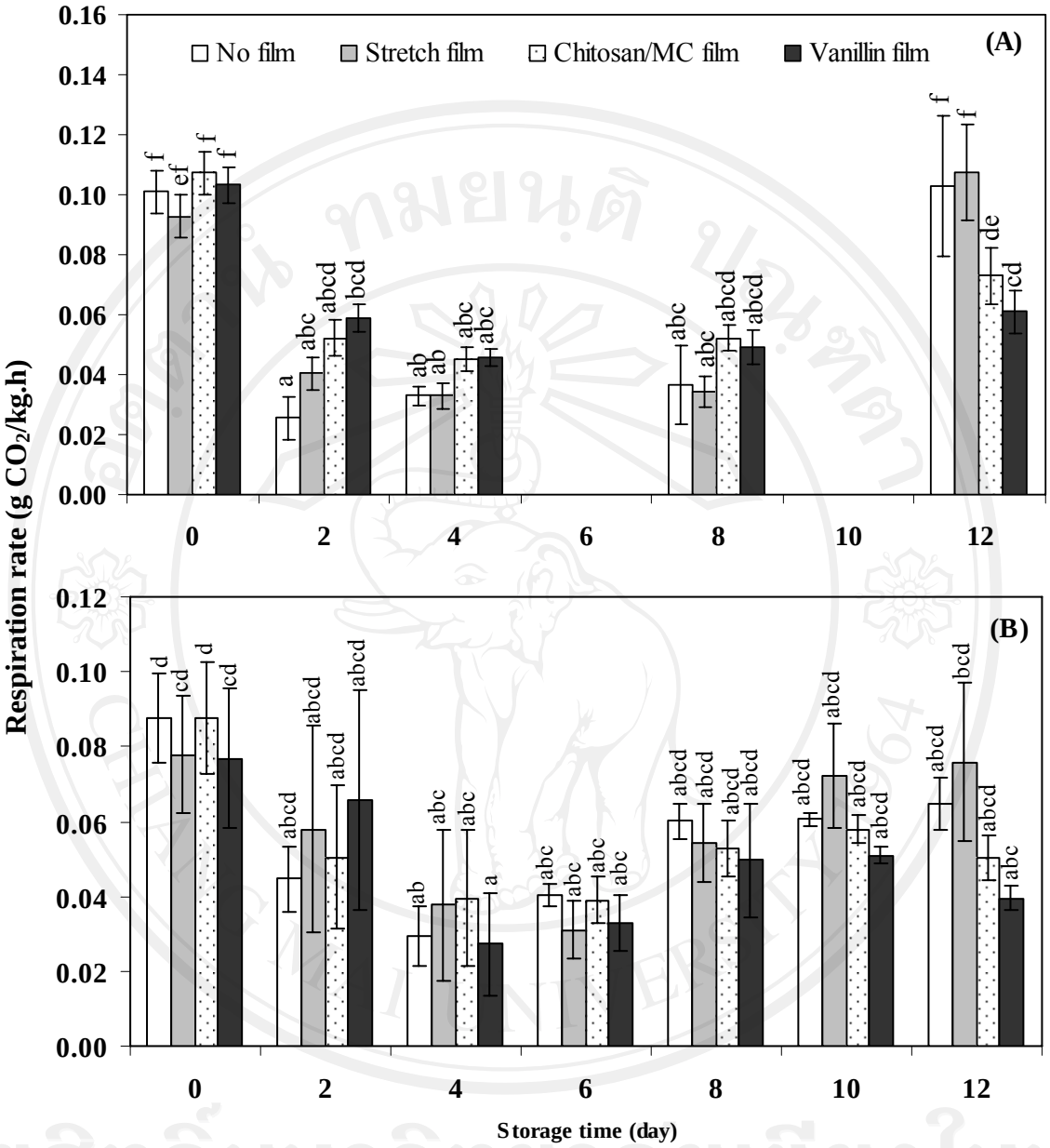


Figure 4.38 Respiration rate of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days.

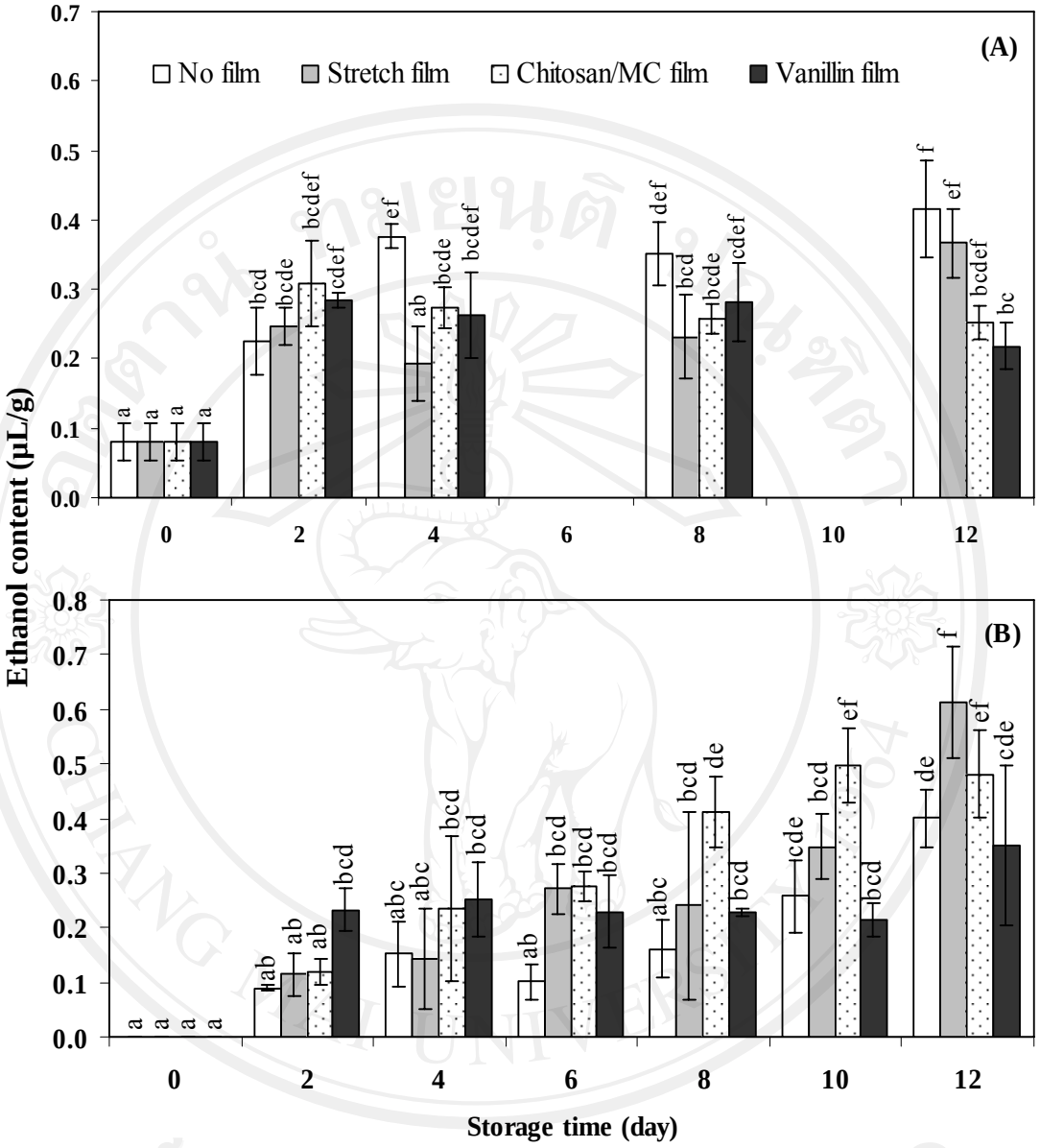


Figure 4.39 Ethanol content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days.

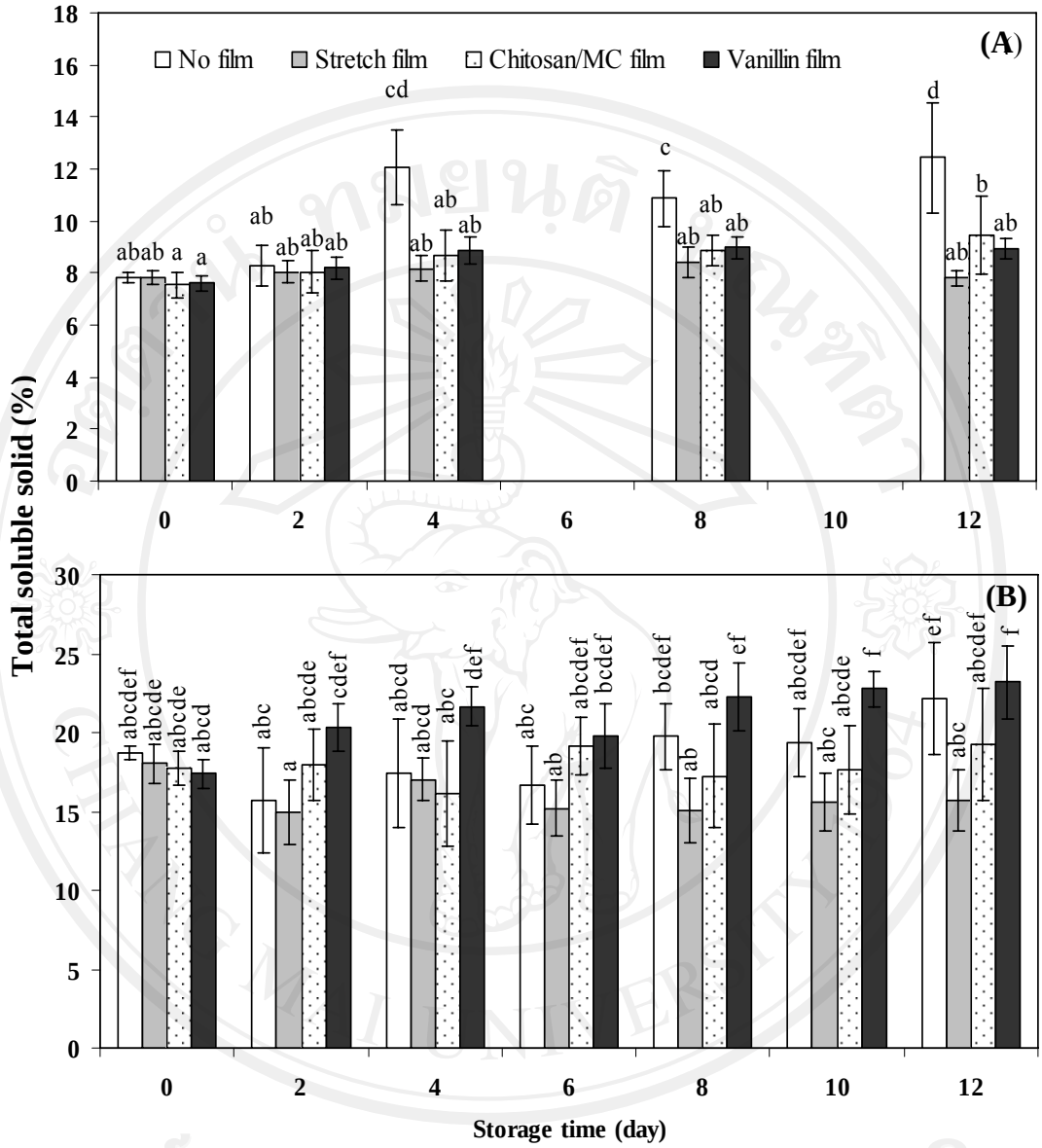


Figure 4.40 Total soluble solids of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days.

Initial TA of cantaloupe flesh was 1.83±0.31 g succinic acid/100 g dry weight or 0.11±0.02 g succinic acid/100 g wet weight. TA tended to decrease in cantaloupe without wrapping and for cantaloupe wrapped in the chitosan/methylcellulose film (Figure 4.41A). However, the TA of cantaloupe wrapped in stretch film and the vanillin film was unchanged. From Figure 4.41B, the initial TA of the pineapple flesh was 5.91 g citric acid/100 g dry weight or 0.94 g citric acid/100 g wet weight which is similar to values reported by Marrero and Kader (2006). The TA of pineapple for all treatments was not significantly different during 12 days storage.

The pH of cantaloupe and pineapple flesh are in the ranges of 5.92±0.27 and 3.57±0.24, respectively. Marrero and Kader (2006) reported that the pH values of pineapple cultivar SC2630 and premium select were 3.35 and 3.41, respectively. Fruits pH did not change during storage at 10^{o}C for 12 days.

4.5.7 Weight loss

The weight loss of both fresh-cut cantaloupe and pineapple in commercial stretch film was significantly lower than that wrapped with vanillin film or chitosan/methylcellulose film, and without film (Figure 4.42). On day 12, cantaloupe with no wrapping, wrapped with stretch film, chitosan/methylcellulose film and vanillin film lose 54.9, 2.3, 51.4 and 50.6% of their weight, respectively. The vanillin film which is more hydrophobic than chitosan/methylcellulose film did not show any significant difference in water barrier protection. However, they provided better protection than the control.

On day 12, pineapple with no wrapping, wrapping with stretch film, chitosan/methylcellulose film and vanillin film lose 49.2, 5.2, 42.6 and 40.0% of their weight, respectively. The highest weight loss was found in pineapple without any wrapping. Vanillin film did not provide better protection against moisture loss than chitosan/methylcellulose film. Stretch film had better barrier to water than vanillin and chitosan/methylcellulose films. Water vapor permeability values at 23^{o}C, 53% RH for vanillin film and chitosan/methylcellulose film were 1.03 and 1.09 ng.cm/cm^2.s.cmHg, respectively. The water barrier of a biopolymer is impaired in a higher relative humidity environment or with higher moisture content foods (Garcia *et al*., 2004). The poor water vapor barrier allows the movement of water vapor

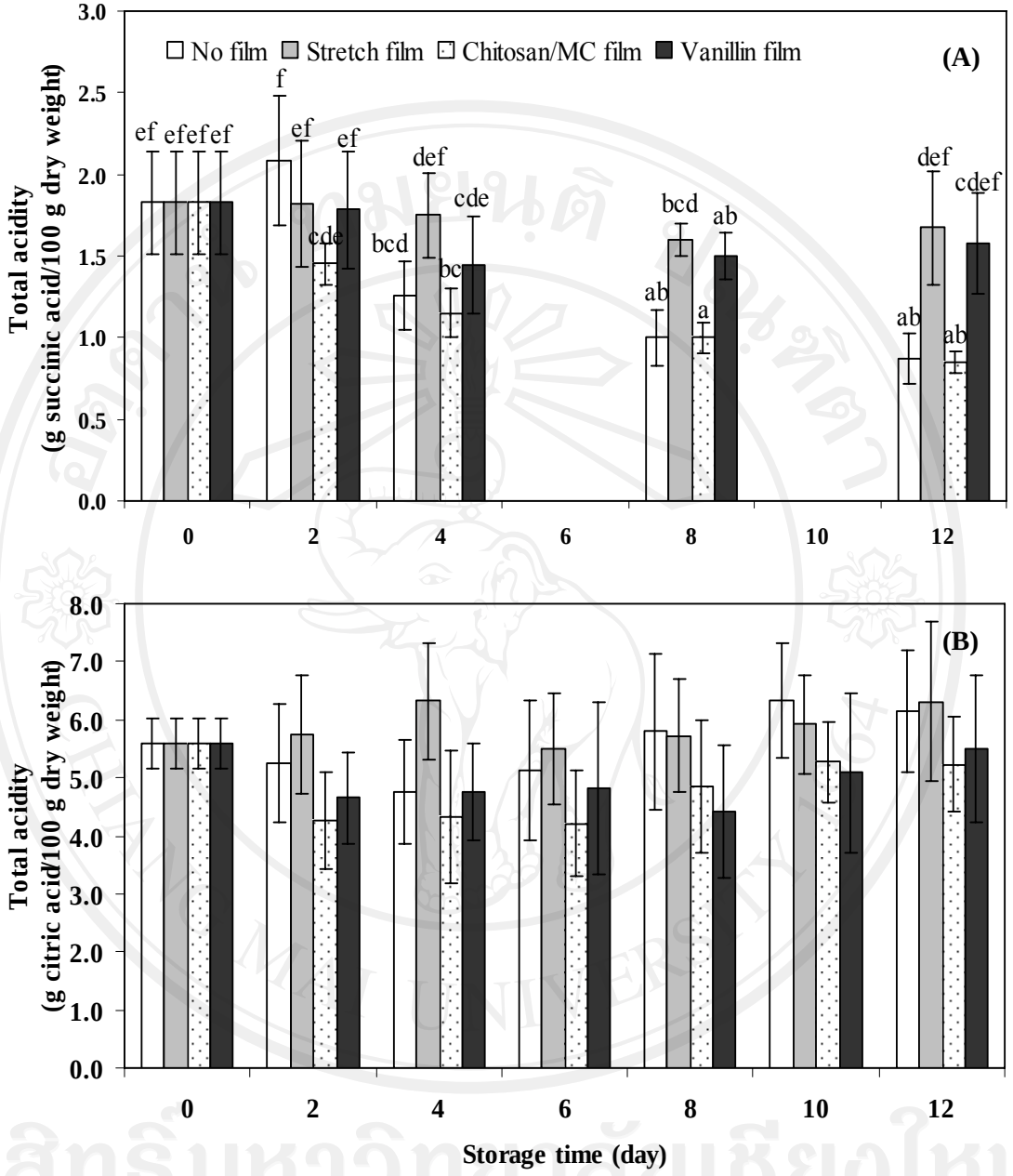


Figure 4.41 Total acidity of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days.

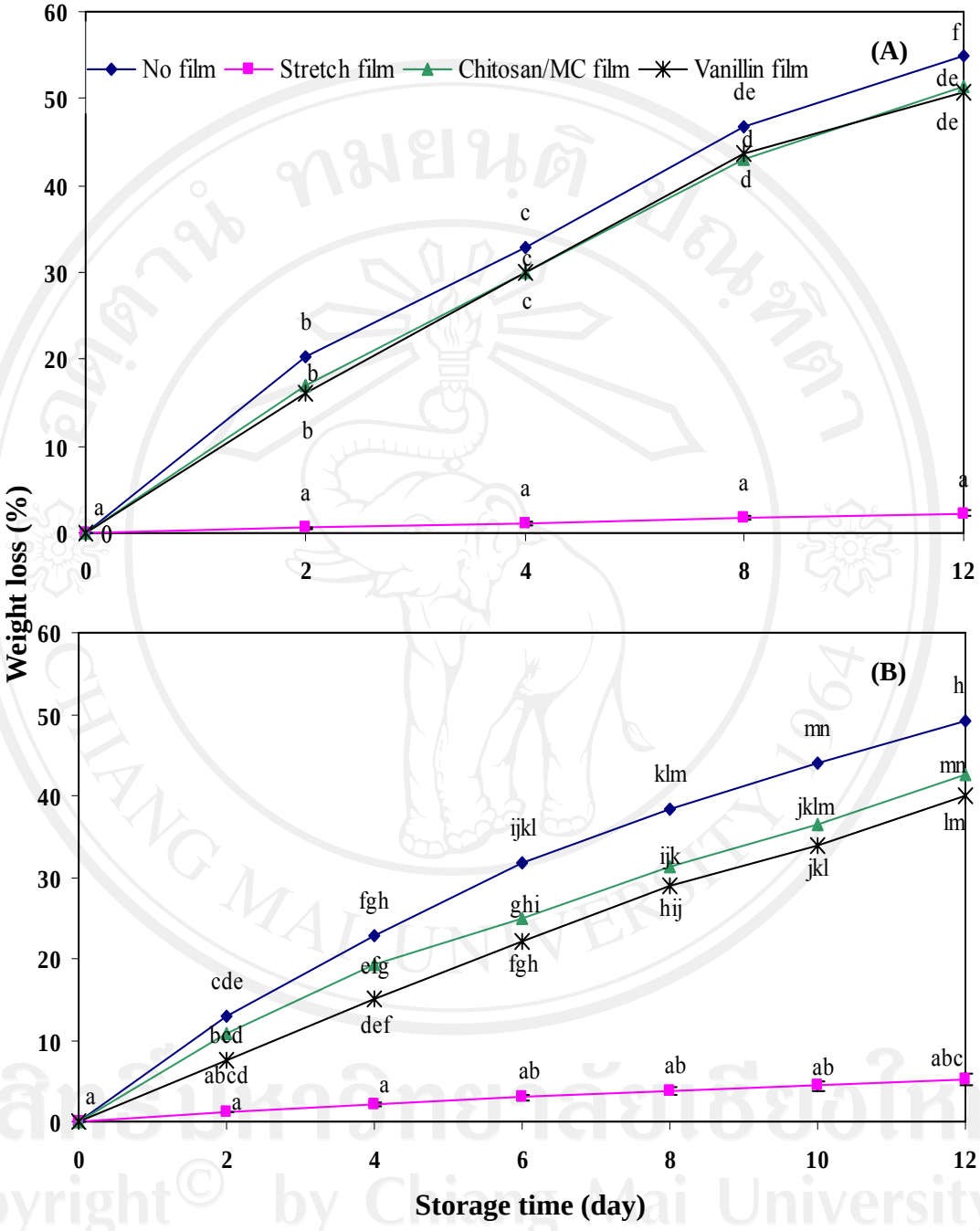


Figure 4.42 **Weight loss of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days.**

across the film. With respect to synthetic polymers, chitosan/methylcellulose films had WVP values similar to those of cellophane, as expected due to the similar chemical structure of the components. However, chitosan/methylcellulose 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).

4.5.8 Sensory evaluation

Cantaloupe and pineapple were unwrapped and were allowed to warm to room temperature (25^{o}C) before serving to enhance perception of aroma and taste characteristics. Cantaloupe that was wrapped with stretch film and chitosan/ methylcellulose film was not significantly different from the control in all attributes after storage at 10^{o}C for 2 and 8 days (Table 4.13). However, cantaloupe wrapped with vanillin film got significantly lower scores in overall, flavor and taste attributes after storage at the same temperature and time. Some panelists commented that cantaloupe wrapped with vanillin film had a bitter taste.

Pineapple wrapped with stretch film was not significantly different from the control in all attributes after storage at 10^{o}C for 2 and 6 days except color on day 6 (Table 4.14). Odor and firmness of pineapple wrapped with chitosan/methylcellulose film were significantly worsened on day 6. Overall attributes, odor and flavor had low acceptability for pineapple wrapped with vanillin film. In addition, its taste and texture changed after 6 days storage. Compared with the control, the decline in overall, flavor, odor and taste scores were greater for pineapple than cantaloupe due to the higher amount of vanillin migrating from the film into the pineapple. Vanillin had a negative effect especially on the taste and flavor of fresh-cut fruit. Ferrante *et al.* (2007) found that orange juice with 2,000 ppm vanillin inactivated *L. monocytogenes* by 4 log CFU/ml in less than 15 minutes but the flavor was not acceptable. They reported that 1,500 ppm vanillin was pleasant for the consumers. However, the pleasantness score was significantly lower than for orange juice with no preservative. Moon *et al.* (2006) found that supplementation with 10 mM vanillic acid in apple juice led to a 5-log reduction in populations of *Escherichia coli* O157:H7 within 7 days after storage at 4 and 15^{o}C. They reported that panelists detected the difference

between controls and all treated juices supplemented with 10 mM vanillic acid. Panelist's comments frequently included statements such as vanilla-like flavor or odor, tanginess or acidity and bitterness.

Fresh-cut fruits wrapped with vanillin film had lower sensory scores and were unacceptable to the panelists. To lower the amount of vanillin in the film and yet maintain the inhibition effect against microorganisms. It might be necessary to use it in combination with other compounds which have no taste and flavor. The use of vanillin film with bakery products might be beneficial because it helps to improve the flavor of bakery products in addition to its biocide effect.

Table 4.13 Sensory scores (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, and 1=dislike extremely) of cantaloupe during storage at 10°C for 2 and 8 days.

	Day	No film	Stretch film	Chitosan/ methyl-cellulose film	Vanillin film
Overall	2	5.8^{d}	5.7^{d}	5.4^{cd}	4.7^{bc}
	8	5.1^{bcd}	5.5^{cd}	4.4^{ab}	3.9^{a}
Color	2	6.1^{cd}	6.0^{bcd}	6.2^{d}	5.8^{bcd}
	8	5.1^{ab}	5.4^{abcd}	4.7^{a}	5.2^{abc}
Odor	2	5.9^{b}	5.7^{b}	5.6^{b}	5.0^{ab}
	8	5.0^{ab}	5.1^{ab}	5.0^{ab}	4.3^{a}
flavor	2	5.6^{f}	5.4^{ef}	5.0^{cde}	4.3^{bc}
	8	4.6^{bcd}	5.1^{cde}	4.1^{ab}	3.4^{a}
Taste	2	5.3^{c}	5.4^{c}	4.9^{bc}	4.0^{b}
	8	4.6^{bc}	5.1^{c}	4.1^{b}	3.0^{a}
Texture	2	6.2^{c}	6.1^{bc}	6.0^{ab}	5.3^{ab}
	8	5.9^{abc}	6.0^{abc}	5.4^{abc}	5.2^{a}

Means with different letters in each attribute are significantly different at p=0.05

Table 4.14 **Sensory scores (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, and 1=dislike extremely) of pineapple during storage at 10°C for 2 and 6 days.**

	Day	No film	Stretch film	Chitosan/ methyl-cellulose film	Vanillin film
Overall	2	6.6^{d}	6.6^{d}	6.2^{cd}	4.8^{b}
	6	6.2^{cd}	6.5^{d}	5.5^{bc}	3.3^{a}
Color	2	6.9^{c}	6.9^{c}	6.5^{ab}	6.7^{c}
	6	6.0^{ab}	6.7^{c}	6.1^{ab}	5.8^{a}
Odor	2	6.0^{c}	6.4^{c}	5.8^{c}	4.8^{b}
	6	5.9^{c}	6.0^{c}	5.1^{b}	3.7^{a}
flavor	2	6.4^{d}	6.3^{cd}	5.8^{cd}	4.2^{b}
	6	6.3^{cd}	6.4^{d}	5.4^{c}	2.7^{a}
Taste	2	6.4^{b}	6.2^{b}	6.0^{b}	4.4^{b}
	6	6.2^{b}	6.4^{b}	5.2^{b}	2.5^{a}
Texture	2	6.5^{d}	6.5^{d}	6.4^{cd}	6.1^{d}
	6	6.3^{d}	6.5^{d}	6.1^{bc}	4.6^{a}

Means with different letters in each attribute are significantly different at p=0.05