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Original Research Article

Application of active edible coating from chitosan incorporated with Cashew (*Anacardium occidentale*) leaf extracts for extending shelf life of lime fruits

Mooksupang Liangpanth*[1] *and Wirongrong Tongdeesoontorn* [1,2]

[1]*Program in Technology Management of Agriculture Produces, School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*Research Unit of Innovative Food Packaging and Biomaterials School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand*

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ABSTRACT

Chitosan coating is one of excellent technique to prolong shelf life of fruits. Many studies reported that chitosan incorporated with plant extracts such as guava leaves, papaya seeds, basil leaves, clove buds and cashew leaves can be used for coating fruits. The previous research found that cashew leaf extracts expressed the biological properties such as antioxidant, antimicrobial, analgesic and anti-inflammatory. Currently, lime fruit is one of the economic crops in Thailand and its marketing is limited due to climate change and diseases, which lower the quality of fruits. This research aimed to study and compare the antimicrobial properties of cashew leaf extracted with water and 70% ethanol. The effect of chitosan coating incorporated with cashew leaf extracts on lime (*Citrus latifolia* Tanaka) fruits qualities (firmness, color, TA, TSS, weight loss, vitamin C, and antioxidant property) during storage was also investigated. The result showed that 10% ethanolic and aqueous extracts could be inhibited the growth of *Aspergillus niger*. An ethanolic extract had lower minimum inhibitory concentration (MIC = 6.25 µg/100µl) than the aqueous extract (12.5 µg/100µl), but the had similar minimum fungicidal concentration (MFC = 25 µg/100µl). For application part, lime fruits were separated into four groups: uncoated (control), coated with 2% chitosan, 2% chitosan combined with 5% ethanolic extracts (CLE), and 2% chitosan combined with 5% aqueous extracts (CLW). All lime fruits were stored at 15°C, 90% RH for 42 days. The coated fruits combined with 5% CLE showed the higher firmness, color (a*), TA, vitamin C content, antioxidants activities, and the lower weight loss and TSS than those of other treatments. Furthermore, coating fruits with 5% CLE presented the inhibitory efficiency against the growth of *A. niger* in inoculated lime fruit as it showed the lowest percent disease incidence and disease severity index. Cashew leaf extract can be one choice of a natural antifungal agent which can be used in agricultural produce.

* *Corresponding author*: Tel.: +6-6-5391-6754
E-mail address: wirongrong.ton@mfu.ac.th

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INTRODUCTION

Fresh fruits and vegetables are perishable causing many problems during storage and transport for example loss of quality loss (water, nutrition, flavor), decay and disease. New postharvest technology is developed to fix the problems such as modified atmosphere, 1-Methylcyclopropene and chitosan coating (Xing et al., 2016). Chitosan substance is grouped in edible coating because it is a natural extract from exoskeletons of crustaceans (Davis, 2015). Moreover, the chitosan substance is a biodegradable, biocompatible, and non-toxic. The structure of chitosan is linear polysaccharide entailed of β-(1→4)-2-amino-2-deoxy-D-glucose (Elsabee & Abdou, 2013). Chitosan coating can prolong shelf life by reducing physiological disorders, gas exchange and respiration rate of fruit. The edible coating is a thin sheet acting as a barrier. This barrier used to control a gas exchange, moisture loss, and microbial growth (Raghav *et al*., 2016). Chitosan coating can be combined with many postharvest treatments such as modified atmosphere packaging (MAP), heat treatment, and gas fumigation (Xing *et al*., 2016). Santos *et al*., (2012) studied the qualities of chitosan-coated jujube, fresh cut carrot root, berry, and grape. The results showed that chitosan could preserve quality of fruits during storage. Furthermore, many studies reported the application of the chitosan coating combined with plants extract as papaya seed, clove buds, guava leaf and cashew leaf for maintaining quality of fruits during storage.

Cashew (*Anacardium occidentale L.*) is a native plant from South America before it extended in several countries such as Nigeria, Brazil, Vietnam, India and Thailand (Chaithra *et al*., 2013). Many research reported reported about bioactive compounds which contained in the leaf extracts such as tannins, phenolic, cinnamic acid, hydroxybenzoic acid, flavonoids, and etc. (Silva et al., 2016). Those compounds show the antiflammatory, antimicrobial, anti-disease, antioxidant properties, including protected memory problem (Mokhtar *et al*., 2008). According to Sika *et al*. (2014); Anand *et al*. (2015), antimicrobial properties of cashew leaf extracts against growth of *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albican*.

Lime Fruit (*Citrus latifolia* Tanaka) is an economics and tropical fruit in Thailand (Santos et al., 2015). The characteristic is elliptical shape, seedless, green color and glossy skin (Khan *et al*., 2017). The complication of climate change, diseases and quality losses affected by decreased productivity that it makes to increase a price due to shortage condition in market. From the previous study, chitosan (5 ppm) was applied to coated lime fruits to reduce weight loss and disease (Jongsri, 2011). The objectives of this research were to study and compare the antimicrobial properties of cashew leaf extracted with water and 70% ethanol. The effect of chitosan coating incorporated with cashew leaf extracts on lime fruits qualities (firmness, color, TA, TSS, weight loss, vitamin C, antioxidant property) during storage was also investigated.

MATERIALS AND METHODS

Extraction of cashew leaves

Cashew leaves (*Anacardium occidentale* L.) were picked after budding around 14 days from 'Ban Bang Klang' plantation, Ranong, Thailand. The leaves were separated from branch, then washed with tap water and dried at 50°C for 1 day. These cashew leaves were powdered and kept in a polyethelene zip-lock at 25°C. The leaves powder was soaked by water and 70% of ethanol (1:15 w/v). The mixture was shaken at 150 rpm for 48 hours. The solution was filtered, and only ethanol solvent was evaporated by rotary evaporator at 45°C for 20 min. The extracts were dried by used lyophilization process. The lyophilized powder was dissolved by using 50% dimethyl sulfoxide (DMSO) then stored at 4°C (Doss *et al*., 2011).

Disk diffusion assay

Aspergillus niger TISTR3281 was used to check the antimicrobial activity of cashew leaf extracts. The microbial was gained from Microbiological Laboratory, Mae Fah Luang University. Disk diffusion assays used the method from Tayel *et al*. (2015) with some modifications. The *A.niger* has suspended spores in sterile distilled water. The 10^6 spores mixture (200 μl) of fungi was mixed with PDA (150 ml), then poured into Petri dish (25 ml). The leaf extracts (50 μl) were added in the sterilized paper disk. The disks were placed on a petri dish and incubated at 25℃ for 48 hours.

Determination of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The leaf extracts were combined with potato dextrose broth (1:1 v/v) that loading in 96-well plate. Then two-fold dilution was done from 50 ug to 0.05 ug. The 10^6 spores/mL of *A. niger* was added into each concentration (1:10 v/v). The well plates was incubated 25 ºC for 48 hours. The minimum concentration which can protected visible growth of *A. niger* was explained as MIC. MFC was evaluated by using the similar concentration of leaf extracts from MIC determination in 96-well plate, which showed invisible growth of fungi. The carefully chosen concentration from MIC was sub-cultured on a potato dextrose agar (PDA) then incubated at 25°C for 48 hours. The lowest concentration cab inhibited the growth of *A. niger* was described as MFC. The MIC and MFC determination were examined by using the method of Tayel *et al*. (2015) with some modifications.

Preparation of lime fruits

Lime fruit (40 kg) were purchased from Chokcharoen Market, which collected not later than 1 day from a citrus orchard in Chiang Rai, Thailand. The lime was graded only green color, absent physical damage, and disease. The fruits were washed with tap water and then dipped in 50 ml/L of sodium hypochlorite solution for 5 min. The fruits were washed with distilled water and dried at 25°C for 1 hour (Supavanich *et al*., 2012).

Preparation of chitosan coating solution with cashew leaf extract

The chitosan powder (80,000 MW, ALDRICH) around 20 g was dissolved in 1 liter of lactic acid (1%), then the mixture was constantly homogenized (10000 rpm) for 1 hour and kept overnight. The leaf extracted solution was dissolved by 50% of DMSO. Then coating solution was done by 950 ml of chitosan mixture was combined with 50 ml of cashew leaf solution, then agitated until homogeneous. The preparation of coating solution was prepared followed Kaya *et al*. (2018) with some modifications.

Preparation of fruits coating

Limes fruits were dipped in coating solution for 2 min and then dried at room temperature (25°C). The coated and uncoated

fruits were divided into two parts. First, the coated and control (uncoated) fruits were stored in plastic bag for 42 days at a 15±2°C, 90% relative humidity (RH). The other part, coated and uncoated fruits were wounded by a stainless steel cutter (1 mm diameter and 2 mm depth) in the center area. The fruits were inoculated with 20 µl of 10^6 spores of *A. niger*, and then stored in the plastic bag at 25±2°C (95%RH) for 14 days. This method was modified from method of Taghinezhad & Ebadollahi (2017); Motlagh & Quantick (1988).

Weight loss

The percent weight loss during 42 days storage of coated and uncoated (control) fruits was calculated by the changes of initial weight before storage (Wi) and a final weight after storage (Wf) compared to the initial weight as the following equation (Taghinezhad & Ebadollahi, 2017):

Percent weight loss = ((Wf-Wi)/Wi) ×100

Color and firmness assessment

The color of coated and uncoated (control) fruits were taken by Colorimeter Hunter Lab (Miniscan EZ 4500L, Virgian, USA) and the data was explained in values of L*(lightness), a*(redness (+ve)/ greenness (-ve), and b*(yellowness (+ve)/blueness (-ve)) of the hunter scale. The firmness of fruits was measured three replications every treatment by using a compression test with a 50 mm-diameter probe. The average values were presented in Newton (N). For pre-test conditions used for the measurement as a test speed of 5 mm/s. Post-test speed as 10 mm/s, which used 16 mm of distance and 5 g of trigger force (Petriccione *et al*., 2015). The firmness of fruits were test by TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, England).

Titratable acidity (TA) assay

The titratable acidity was determined by titration of 5 ml of the lime juice with 0.1 N of NaOH. Phenolphthalein was used as an indicator. Percent acidity was calculated by the equation below (Akusu *et al*., 2016):

Percent acid = ((X x Y x B) / W) x 100
Where: X = mls NaOH used
Y = 0.1 N NaOH
B = Milliequivalent factor
W = Grams of sample

Total soluble solids (TSS) analysis

The fruits were cut to medium pieces, then squeezed. The total soluble solids content (ºBrix) ofjuice was determined by using a digital refractometer (Atago, Tokyo, Japan 1940). The percentage of soluble solid was calculated by using following equation (AOAC, 1980).

Percent of soluble solids = % °Brix × ((100-b))/100
Where b = % H2O-insoluble solid

Vitamim C determination

This method was obtained by Fatin & Azrina (2017) with some modification. The lime fruits (150 g) were cut and homogenized with metaphosphoric acid (6%) until getting 250 ml of mixture volume. A 30-ml of mixture was diluted to 100 ml with 3% HPO_3, then filtered. The homogenous solution (10 ml) was poured to Erlenmeyer flask, and titrated with a dye solution 2, 6-dichlorophenolindophenol, (SIGMA-Aldrich, Canada) until getting the end point (faint pink color). Vitamin C content was explained by followed the equation:

Total vitamin C content (mg/ 100 g sample) = ((X×Y×100))/W
Where: X = volume of dye used for titration (ml)
Y = vitamin C equivalent of dye solution (mg per ml dye solution)
W = weight of sample (g)

Total phenolic content (TPC)

Total phenolic content of the lime juice was determined according to Folin- Ciocalteu assay method described by the ISO 14502-1 (2005), the gallic acid was used as a standard. The juice (500 µl) was mixed with 2.5 ml of 10% (w/v) Folin-Ciocalteu reagent and 2 ml of 7.5% (w/v) sodium carbonate. The mixture was stirred and incubated in darkness for 1 hour at room temperature (25°C). The absorbance at 765 nm was measured using a microplate spectrophotometer (Thermo Fisher scienfic, Multiskan GO, USA).

2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH)

The Free Radical Scavenging was analyzed followed the method of Molyneux (2014). DPPH solution (60 mM) was prepared by dissolving 0.00236 g in 95% ethanol (v/v). The DPPH solution was mixed with lime juice (50 µl). Trolox (10,000 µM) was used as a standard solution, and methanol was used as a blank. The mixtures were left at room temperature for 30 min. The absorbance at 517 nm was measured using a microplate spectrophotometer (Thermo Fisher scienfic, Multiskan GO, USA).

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was evaluated by the method of Benzie & Strain (1999). The lime juice (400 µl) was mixed with 400 µl of the freshly prepared FRAP solution. FRAP solution contained as 300 mM acetate buffer (pH 3.6), 10 mM TPZ in 40mM HCl, and 20 mM FeCl3 in ratio of 10:1:1 (v/v/v) respectively. Distilled water was used as a blank. The mixture was incubated at 37°C for 30 min and then measured the absorbance at 595 nm by a microplate spectrophotometer (Thermo Fisher scienfic, Multiskan GO, USA).

Disease incidence and severity index

The percent of disease incidence and score of severity using the method of Mamza *et al*., (2008); Jongsri *et al*., (2011) with some modifications. Percent disease incidence and disease severity index was rated scores at 0, 3, 6, 9, 12 and 15 days after inoculation. The disease incidence was analyzed by estimating the defected fruit, the percent disease was calculated by used equation 1. Disease severity index was calculated (equation 2) and explained by scoring diseased

frutis using a 1-5 scales below:
1 = All fruit without symptoms.
2 = 1- 25% total areas with symptoms.
3 = 26-50% total areas with symptoms.
4 = 51-75% total areas with symptoms.
5 = 75% or more – total areas with symptoms.

Equation 1: Percent disease incident = $\frac{\text{Infected fruits}}{\text{Total fruits}} \times 100$

Equation 2: Percent severity = $\frac{\text{Sum of all disease scores}}{\text{Infected fruits} \times \text{Maximum scores}} \times 100$

Where: Disease scores = (Scores × Infected fruits)

Sensory Evaluation

Sensory evaluation for coated and uncoated lime frits were used by 9 point hedonic scale. The fruits were estimated by non-trained 30 panelists. All of lime fruits were investigated attributed color, texture, odor and overall acceptance.

Statistical analysis

The data were presented as mean ± SD at least triplicate determinations. Analysis of variance (ANOVA) and significant tests of the mean was used Duncan's Multiple Range Tests. The results were measured at $p<0.05$ statistical significance. The analysis was conducted by using SPSS 17 program.

RESULTS AND DISCUSSION

Antimicrobial analysis

This table 1 showed antifungal activities of the aqueous extract (CLW) and ethanolic extract (CLE) which can against the growth of *A. niger*. The result showed that the lowest concentration at 10% of CLW and CLE could be inhibited the growth of *A. niger*. The minimum MIC values were obtained in CLE (6.25 µg/100µl). However, CLE and CLW had the similar result of MFC (25 µg/100µl), the result showed Table 2. The result was the same as the previous study which described the capability of cashew leaf extract to against growth *A. niger* (Oladele & Ishola, 2017). According to Xing et al. (2016), an antimicrobial compound in cashew leaf extracts can destroy microbial by penetrating into the cytoplasm, then destroyed DNA and RNA process. Sanders & Cluff (1986) studied the mechanism of antimicrobial agents. The actions of antimicrobial agents contain with three major factors on the extinguished cell microbial. First steps, the antimicrobial agents can disrupt the replication process of genetic evidence. Second, the agents transfer abnormal genetic to stop protein synthesis. Moreover, the antimicrobial agent can changed activity and cell wall structure of microbial. Actually, Cashew leaf extracts consist of many antimicrobial agents as a phenolic compound, flavonoids, and tannins which can prevent the growth of microbial (Silva & Fernandes, 2010).

Weight loss

A cashew leaf extracts mixed with chitosan coating could delay the weight loss of lime fruit (Figure 1). After 42 day storage, the lowest percentage of weight loss was obtained in chitosan coating combined with 5% CLE (4.40%), followed by the coating with 5% CLW (5.41%). This result was similar to the previous studies about chitosan combined with plants extract which could retard weight loss of fruits (Taghinezhad and Ebadollahi, 2017). Khalifa et al (2017) reported that plant extracts combined with chitosan coating gave the lowest weight loss of fruit which can indicate the efficiency of chitosan coating on the weight loss reduction. After water loss, the quality of fruit was also changed, and the ability of microbial resistance also decreased. Chitosan coating was a thin layer on the surface of fruit which cloud be preserved water in the tissue of fruits (Youwei & Yinzhel, 2013). Normally, enzymes such as pectinase are produced in ripening stage of fruits that affected water loss and fruits softening (Paniagua et al., 2014). The bioactive compound in plant extracts can retard those enzymes activities (Banos et al., 2003). Tejirian & Xu (2011); Emilio et al. (2017) reported that phenolic compounds can inhibit many enzymes activities such as cellulases, α-amylase lipase, pepsin and pactinase.Moreover, CLE can maintain weight loss batter than CLW due to the higher amount of extracted bioactive compound (Ayepola & Ishola 2009). Ethanol had more ability to extract phenolic and triterpenoids compounds than water. Meanwhile, water had OH-ions that can dissolve tannin (JO et al., 2015).

Table 1. Antimicrobial activity of aqueous and ethanolic extract against *Aspergillus niger.*

No.	Concentration of Extract	Zone of inhibition (mm)
1	Control	0
2	1% Aqueous	0
3	10% Aqueous	8.33 ± 0.3^{d}
4	25% Aqueous	9.33 ± 0.6^{c}
5	50% Aqueous	10.83 ± 0.8^{b}
6	60% Aqueous	11.50 ± 0.3^{b}
7	1% Ethanolic	0
8	10% Ethanolilc `	10.00 ± 0.5^{c}
9	25% Ethanolic	11.17 ± 0.4^{b}
10	50% Ethanolic	11.50 ± 0.5^{b}
11	60% Ethanolic	12.17 ± 0.6^{a}

Data present mean values ± standard deviation (n=3).

Table 2. MIC and MFC values of cashew leaf extract against fungus.

Extracts	*Aspergillus niger*	
	MIC (µg/100 µl)	MFC (µg/100 µl)
Water	12.5±0.5	25±1.3
Ethanol	6.25±1.2	25±1.2

Data present mean values ± standard deviation (n=3).

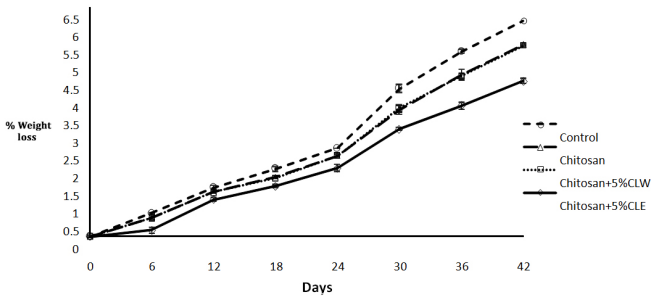


Figure 1 Weight loss of coated and uncoated lime fruits during 42 day storage at 15°C, 90% RH.

Fruit firmness

The firmness change of lime fruits after 42 days storage was showed in Figure 2. Lime fruits with chitosan coating mixed with 5% CLEshowed higher firmness than control (uncoated). This result was agreed with the study of Tesfay & Lembe (2017) which reported that chitosan coating with plant extract could maintain the firmness of fruits. Banos *et al*. (2003) presented that papaya fruit coated with chitosan alone had higher weight loss than the coating mixed with plants extract. Actually, primary cell wall of plant tissues had an important component which is pectin (Voragen et al., 1983). Many types of enzymes were produced in fruit ripening stages as α-amylase, pectinase, and cellulose. The enzymes can destroy cell wall structure of plant affected to fruit softening. The one of good characteristics of chitosan was reduced free radicals as reactive oxygen species (ROS), cause from respiration rate effect on retard softening enzyme activity. (Lycaon & Buret, 1990; Xie, 2011). Moreover, Banos *et al*. (2003); Mosch *et al*. (1993) described some properties of plant extracts. That it can motivate fruit resistance mechanism of enzyme activities.

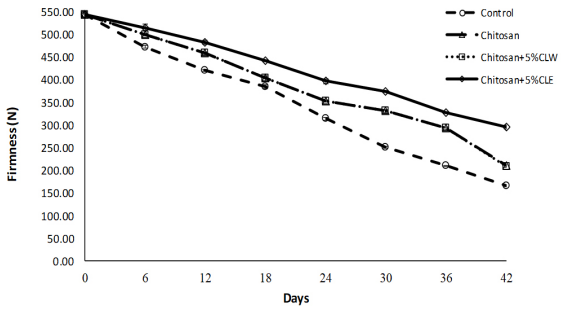
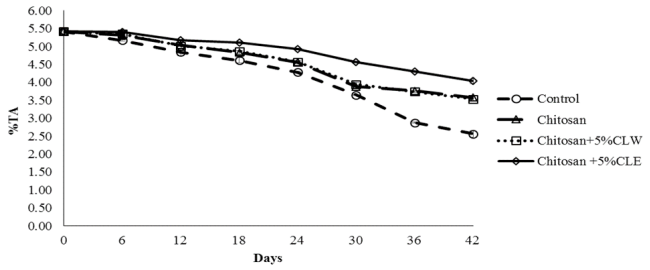


Figure 2 Firmness of coated and uncoated lime fruits during 42 days storage at 15°C, 90% RH.

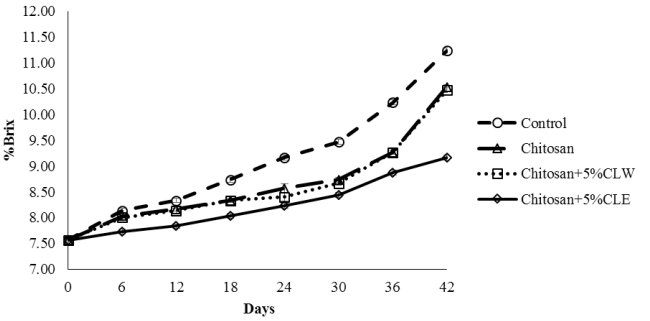
Titratable acidity, Total soluble solids and vitamin C content

Titratable acidity (TA) and vitamin C of treated and untreated fruits were showed in Figure 2 (a), (c). A percent of titratable acidity and vitamin C had a trend to reduce during storage. The chitosan coating with % 5 CLE had a significantly higher TA values and quantity of vitamin C after stored than the others treatment. An organic acid such as citric, malic and quinic acid was reduced by the ripening process (Wang *et al*., 1993). Normally, citrus fruits had a higher vitamin C loss during long storage (Ting & Attaway, 1971). This result was similar to the previous studies of using chitosan coating incorporated with plant extracts to preserve values of titratable acid and vitamin C on strawberry, bell paper, mango, and citrus fruits (Pasquariello, 2015; Manoj *et al*., 2016; Nongtaodum & Jangchud, 2009; Taghinezhad & Ebadollahi, 2017).

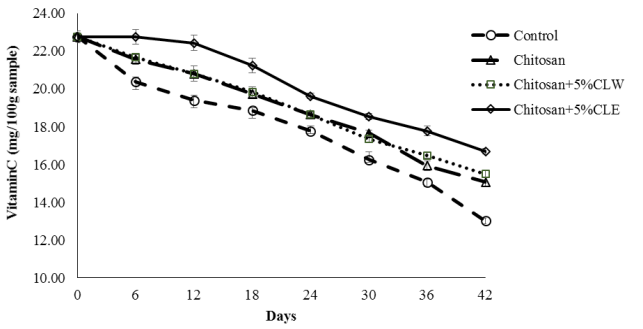
Total soluble solids (TSS) was shown in Figure 2 (b). The lowest values of TSS was obtained by chitosan coating with % 5 CLE also. Hulme (1978) described about the ripening stage of fruit. The starch contained in ripening fruit was hydrolyzed to sugar by enzymes. The enzymes were produced for degrades starch such as α-amylase, β-amylase, and phospolyase (Garcia & Lajolo, 1988). The result was followed with Molina & Soberanes (2004) who studied about chitosan coating on shelf life of lime fruits. Moreover, previous studies about logan treated with chitosan had lower TSS than untreated fruit (Young *et al*., 1982). Many plants extract can cooperate with chitosan coating as retardant of enzymatic process, affected to slows ripening stage (Ghasemnezhad *et al*., 2011).



(a)



(b)



(c)

Figure 3. Titratable acidity (a), Total soluble solid (b), and vitamin C content (C) on coated and uncoated lime fruits during 42 day storage at15°C.

Table 3. Color (L, a*, b*) values of peels coated and uncoated fruits during 42 day storage.

Days	Treatments	L	a*	b*
6	Control	41.57±1.11^{klm}	-12.17±0.12^{i}	33.53±0.37^{k}
	Chitosan	41.73±1.55^{kl}	-12.37±0.12^{kl}	30.97±0.37^{m}
	Chitosan+5%CLW	41.47±1.08^{klm}	-13.97±11.93^{kl}	30.60±0.64^{m}
	Chitosan+5%CLE	40.17±0.05^{m}	-13.87±0.29^{n}	27.30±0.86^{n}
12	Control	42.80±1.00^{hij}	-11.13±0.05^{i}	35.83±0.19^{j}
	Chitosan	41.37±0.83^{klm}	-12.17±0.02^{k}	33.63±0.37^{k}
	Chitosan+5%CLW	41.07±0.54^{lm}	-12.20±0.22^{k}	33.73±0.12^{k}
	Chitosan+5%CLE	40.27±0.54^{lm}	-13.43±0.40^{m}	32.50±0.29^{l}
18	Control	45.67±0.33^{f}	-10.37±0.12^{gh}	38.33±0.17^{i}
	Chitosan	43.27±0.25^{jkl}	-11.30±0.16^{i}	35.73±0.25^{j}
	Chitosan+5%CLW	43.63±0.33^{hi}	-11.67±0.12^{j}	35.43±0.48^{k}
	Chitosan+5%CLE	42.03±0.61^{jkl}	-12.67±0.21^{l}	37.37±0.12^{j}
24	Control	49.40±0.22^{cd}	-9.33 ±0.12^{de}	40.30±0.08^{fgh}
	Chitosan	45.50±0.22^{fg}	-10.40±0.08^{ij}	39.47±0.05^{h}
	Chitosan+5%CLW	45.70±0.08^{f}	-10.67±0.19^{i}	39.37±0.12^{h}
	Chitosan+5%CLE	42.73±0.39^{ijk}	-11.40±0.08^{h}	38.23±0.12^{ij}
30	Control	50.20±0.29^{c}	-8.43 ±0.17^{bc}	42.70±0.08^{c}
	Chitosan	46.47±0.31^{ef}	-9.60±0.29^{ef}	40.87±0.25^{ef}
	Chitosan+5%CLW	46.53±0.40^{ef}	-9.83±0.05^{f}	40.47±0.21^{fg}
	Chitosan+5%CLE	43.70±0.78^{hi}	-10.60±0.16^{h}	39.60±0.16^{gh}
36	Control	52.90±0.64^{b}	-8.10 ±0.08^{c}	43.20±0.22^{bc}
	Chitosan	47.50±0.22^{e}	-9.13±0.12^{d}	42.33±0.26^{cd}
	Chitosan+5%CLW	47.10±0.16^{e}	-9.50±0.16^{def}	42.60±0.45^{c}
	Chitosan+5%CLE	44.27±0.17^{gh}	-10.20±0.08^{g}	40.63±0.33^{ef}
42	Control	55.23±0.82^{a}	-7.13 ±0.12^{a}	45.50±1.48^{a}
	Chitosan	49.13±0.45^{cd}	-8.37±0.17^{bc}	43.97±0.63^{b}
	Chitosan+5%CLW	48.83±0.41^{d}	-8.60±0.22^{w}	43.73±0.39^{b}
	Chitosan+5%CLE	46.43±0.69^{ef}	-9.50±0.08^{def}	41.47±0.12^{de}

Data present mean values ± standard deviation (n=3).
For the same column, the different superscripts indicated significant differences ($p<0.05$).

Color of fruits

Table 3 showed the color change of lime fruit during 42 days storage, expressed as L, a*, b* values. The result showed that chitosan coating with 5% CLE could maintain green color (-ve a*), lightness (L*), and delayed yellowish (+ve b*) which represented the slower ripening of coated fruits. Meanwhile, uncoated fruits had the lowest –a*, and highest of L and b*. However, the values of L, a*, b* were not significantly different ($p>0.05$) between fruits coated by alone chitosan and chitosan with 5% CLW. Chien *et al*. (2007) suggested decrease greenness cause by inducing respiration rate and enzyme activities during fruit ripening. From previous study, chitosan combined with plant extracts delayed respiration rate and retard enzymatic process that the chitosan treatment can conserve color quality of lime fruits (Abebe & Mohammed, 2017).

Sensory evaluation quality

The sensory attributes described by color, texture, odor and overall acceptance of coated and uncoated lime fruits were showed figure 5. The results on day 0 was significantly different in each judge. After 18, 30 and 42 day storage, the highest intensity of the judges obtains in chitosan treatment with 5% CLE. The minimum sensory scores were showed in uncoated fruit (control). Consumer considered to like or unlike due to knowledge culture and experience to samples (Bárcenas *et al*., 2001). Chitosan coating on citrus fruits (orange, mandarin, and graph fruit) can prolong shelf life and main quality of fruits, affected to higher sensory evaluated than uncoated fruit (Hadar *et al*., 2014). Tayel *et al*. (2015) found that chitosan coting combined with plants extracts gave a higher consumer acceptance than untreated lemon fruits, because of its more glossiness and absence of disease.

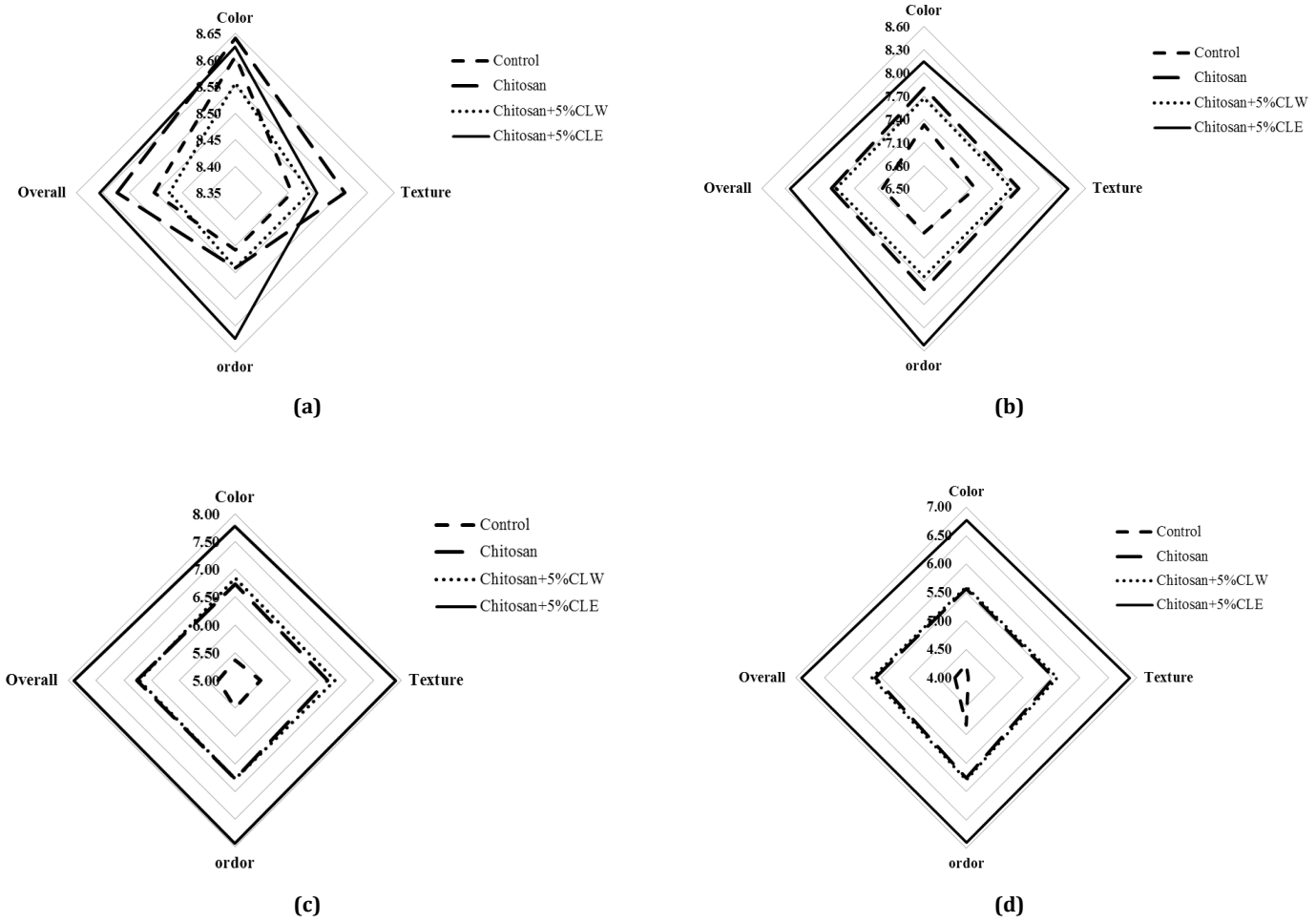


Figure 4. Sensory profile of coated and uncoated lime fruits during storage at 15°C (90% RH) for 0 day (a); 18 days (b); 30 days (C) and 42 days.

Antioxidant properties

The antioxidants properties were evaluated by using TPC, DPPH, and FRAP determination (Figure 5). The result showed that coated fruit had more quantity of antioxidants than uncoated fruits after 42 days stored. Generally, antioxidants in lime decrease during the ripening stage (Banos et al., 2003). The chitosan coating combined with 5% CLE showed the highest TPC (9.76 mg GAE/100ml), DPPH (698 µmol/100ml) and FRAP (148.79 mmol/100ml). Chitosan combined with ethanolic extracts had high efficiency to slow ripening process. The bioactive compound from CLE can inactivated enzymes, which destroyed antioxidant (Tesfay et al., 2017). Sogvar et al. (2015) studied Aloe vera mixed with bioactive compound as ascorbic acid (AA) coating on strawberries fruits. This study found that ascorbic acid could maintain antioxidant in fruit because AA can activate to increasing antioxidant system and ability of free radical scavenging in strawberries fruits. Also, Sun et al., (2010) found ascorbic acid combined with chitosan can decreasing delay of antioxidant enzyme of Superoxide Dismutase (SOD) and Catalase (CAT) that affected to inhibited oxidative stress in the fruit.

Actually, antioxidants values of fruits presented a tendency to decline except the amount of DPPH from day 6. The previous research explained this phenomenon associated to environmental stress such as cold stress, water stress and nutrition stress that affected to enhance antioxidant compounds (López *et al*., 2016; Rahal *et al*., 2014). The result was in agreement with Šašić *et al*. (2012) who studied TPC and antioxidants in lime fruits. Wang et al. (2013) found that the antioxidant values decrease in uncoated strawberry fruit at the end of storage because fruits had decay and senescence. In opposite side, chitosan coating can maintain values of antioxidant and prolong shelf life in strawberries fruits. For the chitosan coating with 5% CLE may be referred to synergistic effect to maintain antioxidants compounds during storage.

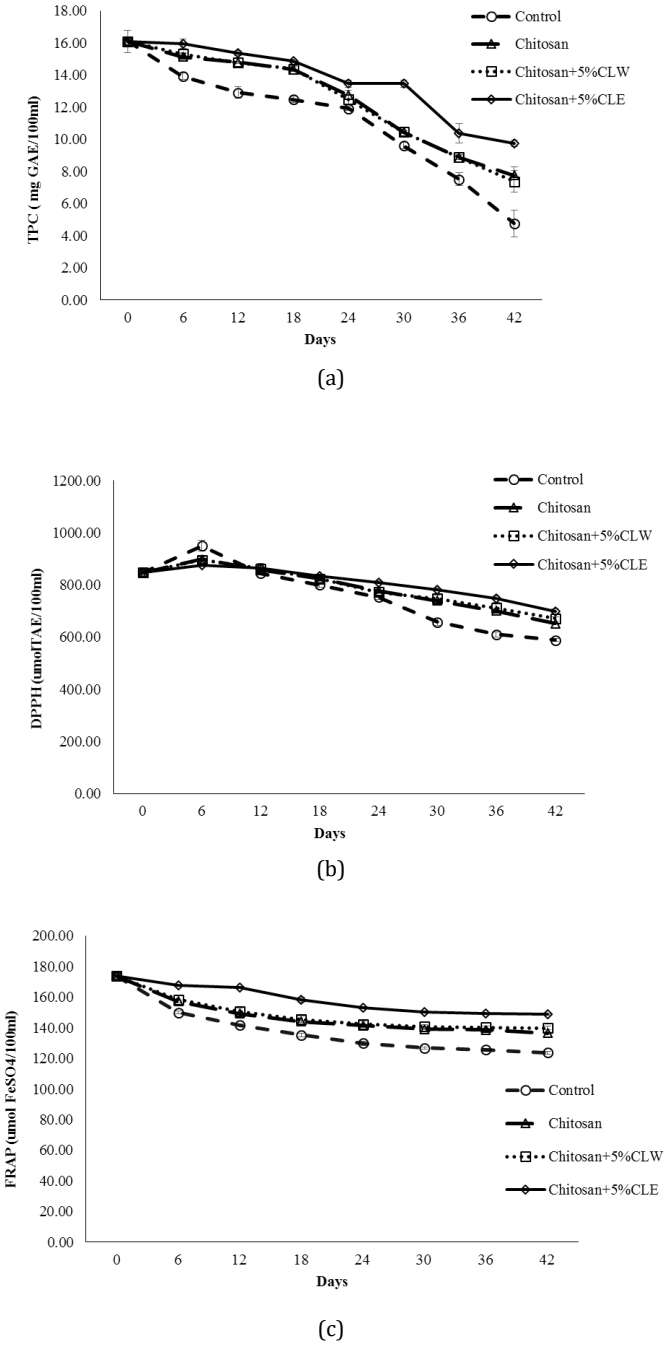


Figure 5. Antioxidant activities described by TPC (a), DPPH (b) and FRAP (C) on coated and uncoated lime fruits during 42 day stored at 15°C for 90% RH.

Disease incidence and severity index

Figure 6 (a) and (b) showed percent disease incidence and severity index of coated and uncoated lime fruits. The result showed that chitosan coating with 5% CLE had the lowest percent disease incidence and severity index (13.33%, 16.67%), followed by the coating with 5% CLW (60%, 61.11%) for 15-day storage. This results similar to Barrera *et al*. (2015) who studied chitosan treatment in papaya fruit. The result found that papaya fruit had a lower percent disease incidence than untreated fruits. Chitosan coating had antimicrobial activity. The mechanism to antimicrobial chitosan due to a cationic amino acid group of chitosan can neutralize anionic phospholipids on cell wall of the bacteria. The cell wall was fractured then the cytoplasm flow out affected to cell death (Chen, 2010). (Chen, 2010). Moreover, chitosan can transfer antimicrobial agents of cashew leaf extracted in to cell microbial. The antimicrobial agents destroyed DNA and RNA synthesis (Xing *et al*., 2016). In this research found that 5% of CLE had a higher improve ability of chitosan than 5% CLW. Normally, ethanol and water can extract antimicrobial compound in cashew leaves, but theirs provide in different quantity and types of compounds. Ayepola & Ishola (2009) reported methanolic and ethanolic extracts showed a higher ability to inhibited bacterial growth than aqueous extracts.

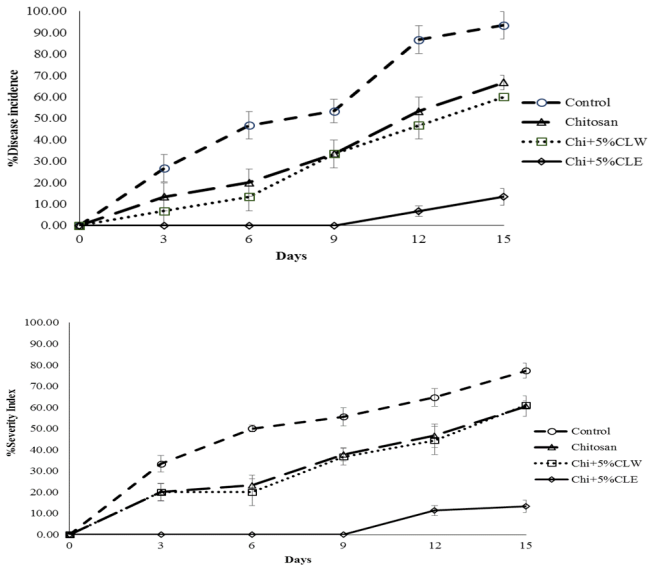


Figure 6. Percent disease incidence (a) and severity index (b) of coated and uncoated lime fruits during at 25°C (95% RH) for 15 day storage.

CONCLUSIONS

Cashew leaf extracts contained a greater antibacterial and antifungal properties. Both of ethanolic and aqueous extracts at 10% concentration could inhibitd growth of A. *niger.* The coated fruit with chitosan + 5% CLE showed minimum changes in weight loss, color, firmness, TSS, vitamin C, and antioxidants activities, but induced a decrease of TA values during storage. Moreover, the treated fruits had a percent of disease incidence and severity index lower than untreated fruits. The chitosan coating incorporated with 5% CLE showed a synergistic effect on extending shelf life of fruits, and preventing diseasea infection of A. *niger.*

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