INTRODUCTION

Chilli pepper (Capsicum annuum L. cv. Superhot) is a major domestic vegetable and exported fruit in Thailand. Organic production induced the increase of market demands due to people awareness and preferences to healthy and free-toxic chemicals food products. Chilli peppers are usually harvested and marketed as fresh fruit at the red ripening stage and these resulted in short postharvest life (7-10 days) even under proper temperature management (Rodoni, Vicente, Azevedo, Concellón, & Cunha, 2015). At tropical temperatures, shelf life is around 2 days due to rapid weight loss and decay. Since organic produces are not treated with chemical pesticides, they are expected to be more perishable than conventional produces (Arnoldio, Colelli, Hasey, & Kader, 2007; Krongyut & Duangsi, 2020).

Several safe techniques have been developed to enhance quality and shelf life of fruit and vegetables including organic produces, and among these techniques are heat treatment and modified atmosphere packaging (MAP). In organic green and red peppers, Rodoni et al. (2016) combined the use of hot water dip (HWD) and cold storage and they found that HWD at 45°C for 3 minutes reduced soft rot, shriveling, weight loss, softening, colour changes and respiration rate during storage at 4°C. A different rate of HWD treatment of 50°C water for 4 minutes was found to be effective in maintaining quality of red ripe organic chilli pepper cv. Superhot (Krongyut & Duangsi, 2015). On the other hand, MAP uses semi-permeable polymeric films to create an atmosphere of high carbon dioxide and low oxygen levels which slow metabolic rates and prolong shelf life of fresh produce (Kader & Watkins, 2000). Recent research focused on the application of active MAP on a broad array of food systems (Atarés & Chiralt, 2016). In a study in organic chilli pepper cv. Superhot, active EMA film using a propriety product (active PAK™) developed by the

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ABSTRACT

Organic chilli peppers (Capsicum annuum cv. Superhot) at full-red stage were submerged in 50°C water for 4 minutes followed by surface coating with carboxymethyl cellulose (CMC) or chitosan at 0.25-1.0% for 2 minutes. The fruits were then packed in 32-34 µm thick polyethylene bag (Active PAK™) before cold storage at 10°C for 12 days followed by 5 days holding at 25°C. The decrease of fruit quality was due to weight loss and decay and these could be reduced by edible coating. Coated fruit had higher titratable acidity, vitamin C content, total phenols, and activities of phenylalanine ammonia lyase, superoxide dismutase and catalase as compared to uncoated fruit. Chitosan was more effective than CMC in bringing these effects, and 0.5% was the most effective concentration. The results indicated that fruit coating by 0.5% chitosan and CMC maintained quality of chilli peppers by slowing physicochemical quality changes and improving the antioxidant system.

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National Metal and Materials Technology Center (MTEC), Thailand, combined with HWD at 50°C for 4 minutes effectively reduced weight loss and decay during 16-day cold storage (10°C, 95% RH) as compared to passive MAP (62 µm-thick PE) and 45°C or 55°C HWD (Krongyut & Duangsi, 2015). It was contended that prolonged storage at 10°C may have induced non-visible chilling damage as there was no chill-induced fruit discoloration observed. It was recommended to integrate other safe techniques, such as edible coatings, to increase the resistance of the fruit to chilling storage and extend post-cold storage distribution and marketing at ambient.

Edible coatings have gained more interest in food preservation because of their nontoxic, biodegradable and biocompatible properties. They serve as barriers against water vapor, oxygen and carbon dioxide, thereby reducing water loss, respiration and other oxidative processes and extending shelf life of fruit and vegetables (Dhall, 2013; Petriccione et al., 2015). Among naturally derived edible coatings, carboxymethyl cellulose (CMC) and chitosan have dominated the food industry.

CMC is an anionic polysaccharide produced by alkalization of cellulose followed by carboxymethylation using chloroacetic acid (Lakshmi, Trivedi, & Reddy, 2017). Due to its diverse physicochemical and biological properties, CMC has a wide range of applications in the food industry, including maintaining quality and extending shelf life of many fruits and vegetables (Mondal, Yeasmin, & Rahman, 2015; Tesfay & Magwaza, 2017). However, CMC has no antimicrobial property and has high water vapor permeability (Raeisi, Tajik, Aliakbarlu, Mirhosseini, & Hosseini, 2015). At high concentration (e.g. 2% or 2 g/100 g), CMC substantially increased the risk of fermentation (Banks, Cutting, & Nicholson, 1997).

Chitosan is a cationic polysaccharide produced by alkaline deacetylation of chitin (Muzzarelli et al., 2012). Chitosan is the second most abundant biopolymer found in nature after cellulose (de Oliveira Pedro et al., 2013) and resembles cellulose in chemical structure, but they differ in the ratio of β(1,4)-linked monosaccharides and N-acetyl-D-glucosamine (Mellegård, Strand, Christensen, Granum, & Hardy, 2011). Chitosan has an average molecular weight of 3.8 to 20 kDa, and degree of deacetylation of 66% to 95% (Mohammadpourdounighi, Behfar, Ezabadi, Zolfagharian, & Heydari, 2010). In contrast to CMC, chitosan has antimicrobial activity against a wide range of fungi, yeasts and bacteria and could replace the use of synthetic chemicals (Mohammadi, Hashemi, & Hosseini, 2016; Xin, Chen, Lai, & Yang, 2017). In addition, chitosan can elicit defence responses (Terry & Joyce, 2004) and has antioxidant activity as it can bind metal ions and prevent initiation of lipid oxidation (Schreiber, Bozell, Hayes, & Zivanovic, 2013). Chitosan coating has great promise for their application in food preservation including shelf life extension of fruit and vegetables (Verlee, Mincke, & Stevens, 2017).

To our knowledge, there is no available report on the use of CMC or chitosan coating in combination with HWD and active EMA packaging on chilli peppers. Most studies also dealt with conventionally produced peppers. The present study explored the potential of applying combined HWD, active EMA packaging and CMC or chitosan coating in improving the shelf life of organic chilli pepper cv. Superhot during cold storage and subsequent holding at ambient.

MATERIALS AND METHODS

Fruit Sampling

Chilli pepper cv. Superhot at the full-red stage were freshly harvested from a local organic farm and transported to the Postharvest Laboratory of Ubon Ratchathani Rajabhat University, Thailand in February 2018. Uniform sized and defect-free selected fruits were washed with running tap water; dipped in 100 ppm chlorinated solution for 2 minutes to reduce the microbial load; rinsed with clean water; and air dried at ambient.

Preparation of CMC and Chitosan Solution

Three concentrations of CMC (Sigma-Aldrich, Thailand) and chitosan (>90% deacetylation, > 900 kDa molecular weight, Bio Safer Co., Ltd., Thailand) at 0.25, 0.5 and 1.0% (w/v), were tested and prepared by dissolving 0.25, 0.5 or 1.0 g CMC powder in 100 ml distilled water or 0.25, 0.5 and 1.0 g chitosan in 100 ml distilled water containing 0.5 ml (v/v) glacial acetic acid with heating and rigorous stirring for one hour) pH of the solution was adjusted at 5.6 with 1N NaOH (Ali, Muhammad, Sijam, & Siddiqui, 2011).
Application of Treatments

Fruits were dipped in 50°C water for 4 minutes and then air-dried at room temperature for 30 minutes (Krongyut & Duangsi, 2015). They were then coated with CMC or chitosan by dipping for 2 minutes. Uncoated fruit served as control. After coating, triplicate samples of 110 g fruit per fruit tray were packed in active EMA film using Active PAK™ (MTEC, Thailand). The active EMA film created an equilibrium modified atmosphere (5-15% O₂ and 5-15% CO₂), resulting in a reduction of respiration rate and was essentially made of PE of 32-34 μm thickness and 8 x 12 inches size, with O₂ transmission rate of 10,000-12,000 cm³/m²/day. atm, CO₂ transmission rate of 29,000-32,000 cm³/m²/day.atm, and water vapor transmission rate of 10 g/m².day. The packed fruits were then stored at 10°C with 95% relative humidity (RH) for 12 days. Thereafter, the fruits were held at 25°C with 75% RH for 5 days. Ten fruits from each bag were randomly sampled every 4 days to determine quality and biochemical changes.

Measurement of Quality Changes

**Overall quality and shelf life.** Overall quality was assessed based on visual quality and decay by eight trained panellists using a 5-point hedonic scales, i.e. 5 = excellent, no defect; 4 = good, defects slight; 3 = fair, defects moderate; 2 = limit of marketability and 1 = non-marketable (González-Aguilar, Ayala-Zavala, Ruiz-Cruz, Acedo-Félix, & Díaz-Cinco, 2004). Shelf life was estimated as the number of days to reach the limit of marketability.

**Peel colour.** CR-300 Chromameter (Minolta, Hong Kong) was used to measure peel colour expressed as the average of five readings of a*, b* and L* values at the mid portion of each fruit.

**Weight loss.** Fruit weight was taken before storage (initial weight) and at each observation period during storage. Weight loss was determined as percentage of of weight decrease from the initial weight.

**Decay incidence.** Fruit with decay was counted and expressed as percentage of the number of decayed fruits from number of stored samples.

**Titratable acidity (TA).** Pulp tissues (10 g) were homogenized using a blender with 40 ml of distilled water. The mixture was filtered through cotton wool; 5 ml filtrate was added with 1-2 drops of 0.1% phenolphthalein indicator and titrated with 0.1 N NaOH to an endpoint of faint pink colour (pH 8.1). TA was expressed as percentage citric acid.

**Vitamin C content.** The measurement of vitamin C content was carried out based on Roe, Mills, Oesterling, & Damron (1948). A 100 mg fruit sample was extracted in 5% metaphosphoric-glacial acetic acid solution. The extracts were then centrifuged and the clear supernatant was collected. Aliquots of the supernatant were placed in a test tube containing 5% metaphosphoric-glacial acetic acid solution, 2% dinitrophenyl hydrazine and 10% thiourea. The test tubes were incubated for 3 hours at 37°C in a water bath and then 85% sulfuric acid was added to terminate the reaction. The optical density of the reaction mixture was measured against a blank at 540 nm using a spectrophotometer (model DU 640B; Beckman Coulter Canada, Ltd, Mississauga, ON, Canada). Ascorbic acid content was calculated from a standard curve of pure ascorbic acid.

Measurement of Biochemical Changes

**Total phenols.** The phenolic content was measured bases on Folin-Ciocalteu method (Toivonen & Stan, 2004). In 20 ml of 70% methanol, 4 g fruit sample was refluxed for 1 hour and then the volume was brought up to 26 ml with methanol. This was followed by 1 minute homogenisation (Brinkmann Instruments Homogenizer) at a speed setting of 2 and centrifugation at 12,000 g for 15 minutes using Model RC-5 centrifuge (Sorvall Products, Wilmington, DE, USA). The supernatant was taken and filtered through Whatman #4 filter paper and 0.5 ml of filtrate was diluted to 7 ml with distilled water. The diluted extract was added with 0.5 ml of Folin-Ciocalteau reagent (Sigma) and then shaken with a vortex mixer. After allowing to stand for 3 minutes, the extract was added with 1 ml of aqueous saturated sodium carbonate solution. The distilled water was added until the volume reached 10 ml. After 1 hour, spectrophotometric reading at 725 nm was taken. To estimate phenolic content, a response curve from chlorogenic acid standards (Sigma) was used.

**Phenylalanine ammonia lyase (PAL) activity.** The procedure of Cheng & Breen (1991) was followed with some modifications. A 5 g frozen pulp sample was grounded with mortar and pestle, placed on dry ice, homogenised with chilled 80% acetone (1:10 w/v) and stored in a freezer for 15 minutes. This was followed by filtering the
homogenate and drying the pellet under vacuum to produce the acetone powder. Protein extract was obtained by homogenising at 4°C 0.5 g acetone powder in 5 ml 0.1 M sodium borate buffer, pH 8.8, containing 5 mM mercaptoethanol, 2 mM EDTA and 4% (w/v) PVP. After 1 hour, the homogenate was centrifuged at 27,000 g for 30 minutes at 4°C. The reaction mixture contained 0.5 ml of 30 mM L-phenylalanine, 30 mM sodium borate buffer, pH 8.8, and 1 ml crude extract in a total volume of 3 ml. The substrate was added after 10 minutes of pre-incubation and the reaction was stopped with 0.1 ml 6 N HCl. PAL activity was determined by the production of cinnamate for 90 minutes at 30°C with continuous shaking, measured by the absorbance change at 290 nm (Zucker, 1965). Enzyme activity was expressed as ΔOD290/min·mg protein.

Total protein content of the enzyme extract was determined by the method of Bradford (1976) using bovine serum albumin standard (BSA, Fluka, Buchs, Switzerland).

Superoxide dismutase (SOD) activity. SOD activity was assayed by the procedure of Rao, Paliyath, & Ormrod (1996). Fruit sample (1 g) was ground in 5 ml of 50 mM sodium phosphate buffer (pH 7.8). The reaction medium contained 50 mM sodium phosphate buffer, pH 7.8, 14 mM methionine, 3 mM EDTA, 1 mM nitro blue tetrazolium (NBT), 60 mM riboflavin and 0.1 ml crude enzyme extract. The formation of blue formazan was monitored by taking the absorbance at 560 nm. One unit of SOD activity was the amount of enzyme that caused a 50% inhibition of NBT.

Catalase (CAT) activity. Enzyme extraction was conducted following the method of Wang et al. (2016). Fruit tissue (2 g) was grounded in 6 ml of 0.1 M ice-cold sodium phosphate buffer (pH 7.8). The extract was homogenised and centrifuged at 13,000 g for 30 minutes at 4°C. The supernatant was subjected to enzyme assay by the method of Ali, Mohd Noh, & Mustafa (2015). CAT reaction solution (3 ml) contained 1.9 ml of 0.1 M sodium phosphate buffer (pH 7.8), 1 ml of 0.3% H2O2, and 0.1 ml enzyme extract. The reaction solution was observed based on the change of absorbance at 240 nm every 5 seconds. A decrease of 0.01 absorbance unit in one minute represented one unit of enzyme activity.

The experiments were conducted in completely randomized design (CRD) with three replicates. Results were subjected to analysis of variance (ANOVA) and treatment mean comparison by the least significant difference (LSD) test (P ≤ 0.05) using Statistica 7 software (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Fruit Quality and Shelf Life

Overall quality of chilli pepper decreased with increasing period of cold storage which was most rapid in uncoated fruit and fruit coated with 1% CMC or 1% chitosan (Table 1). At the end of cold storage, fruit from these three treatments reached the limit of marketability (score of 2), consequently no fruit was subjected to post-cold storage ambient holding. Fruit coated with 0.25-0.5% CMC or chitosan were still fair (score of 3) to good (score of 4) in overall quality at the end of cold storage. Fruit coated with 0.5% CMC or chitosan had significantly higher overall quality ratings than fruit coated with 0.25% CMC or chitosan. This trend was maintained during subsequent holding at ambient so that fruit coated with 0.25% concentration reached the limit of marketability after only one day at ambient whereas fruit coated with 0.5% concentration were still fair to good in overall quality at the end of the 5-day ambient holding period. Chitosan appeared to be more effective than CMC in maintaining quality and extending shelf life of fruit.

The decrease in quality and shelf life was due to water loss (weight loss) and decay which were highest in uncoated fruit (Table 1). CMC and chitosan coating significantly decreased weight loss but at the highest concentration of 1%, they increased weight loss relative to that at lower concentrations. The same trend was obtained for decay incidence which was lowest in fruit coated with 0.5% CMC or chitosan. Chitosan was more effective than CMC in reducing weight loss and decay with the 0.5% concentration as the most effective.

Changes in peel colour did not contribute to fruit quality loss as no wide variation in a*, b* and L* values was obtained (Table 2). During cold storage, a*, b* and L* values in all treatments ranged from 21.6-25.8, 9.0-9.6 and 40.3-41.8, respectively, while during subsequent ambient holding, the remaining treatments had a*, b* and L* values of 17.9-21.5, 9.0-9.3 and 40.2-40.9, respectively.

Titratable acidity was not markedly affected by the coating treatments except on the last day of cold storage when significant treatment differences
were obtained. Only fruit coated with 1% CMC had significantly higher titratable acidity (0.23%) than the uncoated fruit (0.19%) (Table 3). In general, titratable acidity in all treatments was maintained or slightly increased with cold storage to 0.18-0.25% from an initial level of 0.15-0.20% at the beginning of storage. During subsequent ambient holding, titratable acidity ranged from 0.15-0.19%.

**Table 1.** Overall quality, weight loss and decay incidence during and after cold storage of organic chilli pepper in response to CMC and chitosan treatment.

| Treatments   | Day at 10°C | Days at ambient after 10°C storage |
|--------------|-------------|-----------------------------------|
|              | 0 | 4 | 8 | 12 | 1 | 3 | 5 |                |
| Overall quality (score) |   |   |   |    |   |   |    |                |
| Control      | 5.0 | 4.0b | 3.0d | 2.0d | ND | ND | ND |                |
| 0.25% CMC    | 5.0 | 4.0b | 3.3cd | 3.0c | 2.0c | ND | ND |                |
| 0.5% CMC     | 5.0 | 3.7b | 4.0b | 3.7b | 3.3b | 2.7b | 2.7b |                |
| 1.0% CMC     | 5.0 | 4.0bc | 3.0d | 1.3e | ND | ND | ND |                |
| 0.25% Chitosan | 5.0 | 4.0b | 3.7bc | 3.0c | 2.3c | ND | ND |                |
| 0.5% Chitosan | 5.0 | 5.0a | 4.7a | 4.3a | 4.0a | 3.7a | 3.7a |                |
| 1.0% Chitosan | 5.0 | 3.3c | 3.0d | 2.0d | ND | ND | ND |                |
| F-test       | ** | ** | ** |    | ** | ** | ** |                |
| CV (%)       | 7.4 | 10.7 | 13.7 | 18.5 | 25.9 | 25.9 |    |                |
| Weight loss (%) |   |   |   |    |   |   |    |                |
| Control      | 1.3 | 3.1d | 8.8a | ND | ND | ND |    |                |
| 0.25% CMC    | 1.9 | 3.4bc | 7.5b | 8.2a | 10.8a | ND |    |                |
| 0.5% CMC     | 1.3 | 2.5e | 3.2d | 4.3b | 4.4b | 5.8a |    |                |
| 1.0% CMC     | 1.5 | 3.6a | 7.8b | ND | ND | ND |    |                |
| 0.25% Chitosan | 1.7 | 3.2cd | 3.4d | 4.5b | ND | ND |    |                |
| 0.5% Chitosan | 1.1 | 2.0f | 1.9e | 2.2c | 3.3c | 3.3b |    |                |
| 1.0% Chitosan | 1.4 | 3.5ab | 6.6c | ND | ND | ND |    |                |
| F-test       | ns | ** | ** |    | ** | ** | ** |                |
| CV (%)       | 22.3 | 4.2 | 6.2 | 7.6 | 8.8 | 16.3 |    |                |
| Decay (%)    |   |   |   |    |   |   |    |                |
| Control      | 6.6ab | 10.7b | 14.5a | ND | ND | ND |    |                |
| 0.25% CMC    | 7.2a | 10.7b | 13.5b | 16.9a | ND | ND |    |                |
| 0.5% CMC     | 5.6b | 7.2c | 10.6c | 12.8c | 13.8a | 14.4a |    |                |
| 1.0% CMC     | 7.2a | 13.2a | 13.0b | ND | ND | ND |    |                |
| 0.25% Chitosan | 6.4ab | 10.2b | 13.4b | 14.4b | ND | ND |    |                |
| 0.5% Chitosan | 4.3c | 6.6c | 6.5d | 8.2d | 10.9b | 12.3b |    |                |
| 1.0% Chitosan | 6.7ab | 13.8a | 13.7ab | ND | ND | ND |    |                |
| F-test       | ** | ** | ** |    | ** | ** | ** |                |
| CV (%)       | 11.2 | 4.3 | 4.6 | 8.7 | 9.4 | 5.9 |    |                |

Remarks: ND = not determined as fruit reached the end of shelf life in preceding period of observation. Values in the same column followed by different letters, differ significantly based on LSD (5%); ns = not significant; ** = highly significant; Overall quality rating: 5 = excellent, no defect; 4 = good, defects slight; 3 = fair, defects moderate; 2 = limit of marketability; 1 = unmarketable.
Table 2. Peel colour changes during and after cold storage of organic chilli pepper in response to CMC and chitosan treatment.

| Treatments          | Day at 10°C | Days at ambient after 10°C storage |
|---------------------|-------------|-----------------------------------|
|                     | 0  | 4  | 8  | 12 | 1  | 3  | 5  |
| **a** values        |    |    |    |    |    |    |    |
| Control             | 24.4 | 24.2 | 23.2 | 21.6 | ND | ND | ND |
| 0.25% CMC           | 24.2 | 23.6 | 23.2 | 21.9 | 19.6 | ND | ND |
| 0.5% CMC            | 24.8 | 24.5 | 23.2 | 22.6 | 21.0 | 20.2 | 17.9b |
| 1.0% CMC            | 24.4 | 23.5 | 23.1 | 23.1 | ND | ND | ND |
| 0.25% Chitosan      | 24.0 | 23.3 | 22.8 | 22.1 | 21.5 | ND | ND |
| 0.5% Chitosan       | 25.8 | 24.1 | 23.3 | 22.8 | 21.4 | 20.4 | 20.0a |
| 1.0% Chitosan       | 25.5 | 24.7 | 23.5 | 23.5 | ND | ND | ND |
| **F-test**          | ns | ns | ns | ns | ns | ns | ** |
| **CV (%)**          | 5.6 | 3.6 | 3.7 | 6.6 | 15.8 | 21.6 | 14.6 |
| **b** values        |    |    |    |    |    |    |    |
| Control             | 9.5 | 9.4 | 9.4 | 9.3 | ND | ND | ND |
| 0.25% CMC           | 9.6 | 9.5 | 9.4 | 9.4 | 9.3a | ND | ND |
| 0.5% CMC            | 9.4 | 9.2 | 9.2 | 9.1 | 9.0b | 9.0 | 9.0 |
| 1.0% CMC            | 9.5 | 9.4 | 9.3 | 9.0 | ND | ND | ND |
| 0.25% Chitosan      | 9.6 | 9.4 | 9.2 | 9.1 | 9.0b | ND | ND |
| 0.5% Chitosan       | 9.4 | 9.4 | 9.4 | 9.2 | 9.2ab | 9.0 | 9.0a |
| 1.0% Chitosan       | 9.4 | 9.2 | 9.1 | 9.1 | ND | ND | ND |
| **F-test**          | ns | ns | ns | ns | ** | ns | ns |
| **CV (%)**          | 2.2 | 1.6 | 2.5 | 2.4 | 2.5 | 4.5 | 5.0 |
| **L** values        |    |    |    |    |    |    |    |
| Control             | 41.4 | 41.6 | 40.7 | 40.3 | ND | ND | ND |
| 0.25% CMC           | 41.4 | 41.4 | 41.5 | 40.7 | 40.6 | ND | ND |
| 0.5% CMC            | 41.3 | 41.0 | 41.2 | 41.1 | 40.6 | 40.3 | 40.2 |
| 1.0% CMC            | 41.3 | 41.1 | 41.5 | 41.4 | ND | ND | ND |
| 0.25% Chitosan      | 41.1 | 41.0 | 40.8 | 40.6 | 40.9 | ND | ND |
| 0.5% Chitosan       | 41.2 | 40.9 | 41.6 | 41.0 | 40.9 | 40.9 | 40.8 |
| 1.0% Chitosan       | 41.8 | 41.6 | 41.6 | 41.7 | ND | ND | ND |
| **F-test**          | ns | ns | ns | ns | ns | ns | ns |
| **CV (%)**          | 1.0 | 1.1 | 1.6 | 1.6 | 2.7 | 4.1 | 4.2 |

Remarks: ND = not determined as fruit reached the end of shelf life in preceding period of observation. Values in the same column followed by different letters, differ significantly based on LSD (5%); ns = not significant; ** = highly significant.
Vitamin C content significantly differed with treatment after 8-12 days of cold storage and after one day of ambient holding (Table 3). After 8 days of cold storage, fruit coated with 1% CMC or 0.5-1% chitosan had significantly lower vitamin C content than uncoated fruit, but 4 days later, fruit coated with 0.25-0.5% CMC or chitosan had significantly higher vitamin C content than uncoated fruit and those coated with 1% CMC or chitosan. Following ambient holding, 0.5% chitosan was more effective than 0.25% chitosan in maintaining higher vitamin C content while the effect of 0.25% and 0.5% CMC was negligible.

The results are in agreement with previous findings in other fruit and vegetables, though treatment conditions were different (Ali, Mohd Noh, & Mustafa, 2015; Dong & Wang, 2017; Hong, Xie, Zhang, Sun, & Gong, 2012). In a study comparing three edible coatings in strawberries stored at 4°C for 16 days, chitosan (2%) was found to have the most positive effects on fruit quality and shelf life (Li et al., 2017). In a study of broccoli, CMC (0.75%) and chitosan (2%) were applied after HWD (50°C, 1.5 minutes) and before MAP (25 μm-thick multilayered polyolefin bags) and cold storage (5°C for 18 days) (Ansorena, Marcovich, & Roura, 2011). CMC and chitosan improved quality and shelf life, viewed from weight loss, green colour retention, rate of yellowing and vitamin C content. Chitosan, however, maintained quality attributes and extended shelf life more effectively than CMC which is similar to the results of the present study. The higher effectiveness of chitosan could be attributed to its antimicrobial activity (Mohammadi, Hashemi, & Hosseini, 2016; Xin, Chen, Lai, & Yang, 2017) which CMC does not possess (Raeisi, Tajik, Aliakbarlu, Mirhosseini, & Hosseini, 2015). Furthermore, from previous findings the effective concentration of CMC and chitosan...
ranged from 0.75-3% which is higher than that found in the present study which is 0.5% for both CMC and chitosan. This indicates that the effective coating concentration varies with the type of produce and the treatment conditions. At higher concentration of 1%, CMC and chitosan seemed to have detrimental effects on pepper quality, with some similar responses to that of the uncoated fruit. It has been pointed out that excessive rate of application of edible coatings could restrict gas permeation resulting in the accumulation of CO₂ and stimulation of fermentation (Aron, Zaitsev, Porat, & Poverenov, 2014). Banks, Cutting, & Nicholson (1997) earlier reported that CMC at high concentration (e.g. 2%) substantially increased the risk of fermentation. Thus, CMC or chitosan at 1% may be excessive already for chilli pepper and may have caused anaerobic metabolism resulting in the production of toxic metabolites (e.g. ethanol and acetaldehyde) and induction of abnormal metabolism that may have contributed to fruit quality loss.

**Biochemical Changes**

**Total Phenols and PAL Activity**

Total phenolics content generally increased with storage from an initial level of 75.6-76.8 mg/100g fresh weight in all treatments, except in fruit coated with 0.25% CMC (Table 4). Significant treatment differences were obtained after 12 days of cold storage and during subsequent 5-day holding at ambient. Fruit coated with 0.5% CMC or chitosan had the highest phenolics content, while the uncoated fruit and fruit coated with 1% CMC or chitosan had the lowest at the end of cold storage. Following storage at ambient, 0.5% coating induced higher phenolics content than 0.25% coating. Chitosan was more effective than CMC in maintaining high phenolics content.

PAL activity, a key enzyme in phenolics biosynthesis, followed similar trend as that of phenolics content as it increased with storage (Table 4). Significant treatment differences were also obtained at the end of cold storage and during subsequent ambient holding. PAL activity was highest in fruit coated with 0.5% CMC or chitosan, while uncoated fruit and fruit coated with 1% CMC or chitosan had the lowest PAL activity at the end of cold storage. During ambient holding, 0.5% chitosan maintained higher PAL activity than CMC or lower chitosan concentration.

Phenolic compounds contribute to fruit and vegetable quality in terms of colour and taste as well as to the antioxidant system in plant tissues as some phenolics (e.g. benzoic and cinnamic acid derivatives) are potent antioxidants (Natella, Nardini, Di Felice, & Scaccini, 1999). PAL catalyses the first step in the transformation of phenylalanine into the caffeoyl moiety of chlorogenic acid (Zucker, 1965). Increased PAL activity has been implicated in the increased chilling tolerance of some fruit and vegetables (Lafuente, Sala, & Zacarias, 2004). In response to edible coatings particularly chitosan, total phenols and PAL activity increased (Li et al., 2017). In the present study, 0.5% chitosan caused the greatest increase in phenolics content and PAL activity followed by 0.5% CMC which coincided to their effects on fruit quality and shelf life. These results suggest that chilling storage at 10°C induced invisible chilling injury since the usual chill injury symptom (i.e. fruit discoloration) was not detected. Invisible chilling injury may involve the initial stage of injury development, particularly damage to cell membranes as a result of free radical action and loss of defence system (e.g. phenolics as antioxidants). Consequently, quality loss proceeded more rapidly as in the case of uncoated fruit which reached the end of their shelf life on the 12th and last day of cold storage (Table 1). CMC and chitosan coating apparently inhibited this chill-induced damage most effectively at 0.5% concentration. Chitosan was more effective than CMC in maintaining higher phenolic content and PAL activity and consequently inhibited chilling damage and quality loss. Chitosan has been reported to have antioxidant activity and can elicit defence responses in plant tissues (Schreiber, Bozell, Hayes, & Zivanovic, 2013; Terry & Joyce, 2004). A different mechanism of quality deterioration at higher coating concentration of 1% is speculated earlier to be due to anaerobic metabolism rather than chill-induced cell membrane damage.

**SOD and Catalase Activity**

SOD and catalase activity increased with storage and SOD showed more dramatic increase than catalase activity (Table 5). SOD activity increased from an initial level of 58.5-60.3 units/mg protein at the beginning of cold storage to 69.8-80.4 units/mg protein at the end of cold storage. Treatment effects differed significantly only at the end of cold storage, and fruit coated with 0.5% CMC or chitosan had the highest SOD activity while fruit coated with 1% CMC or chitosan showed the lowest and even lower than that of uncoated fruit.
During post-cold storage ambient holding, 0.5% coating resulted in higher SOD activity than 0.25% coating and chitosan induced higher SOD activity than CMC. On the other hand, catalase activity did not differ with treatment during cold storage and increased from an initial level of 30.2-31.0 units/mg protein to 32.4-35.8 units/mg protein after 12 days. Following storage at ambient, catalase activity differed in chitosan treatments, with 0.5% concentration causing higher catalase activity than 0.25%. After 3 days at ambient, 0.5% chitosan resulted in higher catalase activity than that of 0.5% CMC.

SOD and catalase are the most efficient antioxidant enzymes which counter the action of free radicals (Masia, 1998). Free radicals such as reactive oxygen species (ROS, e.g. O^2-') cause oxidative injury and accelerate senescence and senescence-associated disorders such as chilling injury which arises from membrane damage and loss of membrane functions. Antioxidant enzymes are critical in inhibiting oxidative stress by scavenging ROS, retarding peroxidation of membrane lipids, and maintaining membrane function (Li et al., 2017; Sun et al., 2011). SOD catalyses the dismutation of superoxide radical (O^2-) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) which is then detoxified by catalase by reducing H_2O_2 into water (H_2O) and O_2 (Axelrod, Cheesbrough, & Laakso, 1981). Chitosan coating has been found earlier to induce a significant increase in the activities of SOD and catalase, and inhibited superoxide free radical production (Hong, Xie, Zhang, Sun, & Gong, 2012). Li et al. (2017) also showed that polysaccharide-based coatings increased the activity of SOD and catalase during cold storage of strawberries, with chitosan as the most effective in eliciting this response. These findings are supported by the results of the present study.

**Table 4.** Total phenolics content and phenylalanine ammonia lyase (PAL) activity during and after cold storage of organic chilli pepper in response to CMC and chitosan treatment

| Treatments                  | Day at 10°C | Days at ambient after 10°C storage |
|-----------------------------|-------------|-----------------------------------|
|                             | 0 | 4 | 8 | 12 | 1 | 3 | 5 |
| **Total phenolics (mg/100 g fresh weight)** | | | | | | | |
| Control                     | 76.2 | 83.9 | 86.8 | 83.5d | ND | ND | ND |
| 0.25% CMC                   | 75.9 | 83.5 | 85.5 | 89.5bc | 63.5b | ND | ND |
| 0.5% CMC                    | 75.7 | 84.1 | 84.7 | 96.7a | 96.3ab | 97.5b | 96.4b |
| 1.0% CMC                    | 76.8 | 83.2 | 85.2 | 83.3d | ND | ND | ND |
| 0.25% Chitosan              | 75.6 | 83.5 | 85.6 | 91.7b | 90.1ab | ND | ND |
| 0.5% Chitosan               | 76.7 | 84.6 | 86.5 | 100.5a | 121.4a | 120.3a | 99.2a |
| 1.0% Chitosan               | 76.2 | 84.3 | 85.8 | 85.3cd | ND | ND | ND |
| F-test                      | ns | ns | ns | * | ** | ** | ** |
| CV (%)                      | 0.70 | 0.58 | 0.95 | 1.92 | 8.64 | 13.47 | 2.69 |
| **PAL (µOD290/min.mg protein)** | | | | | | | |
| Control                     | 80.2 | 83.6 | 86.5 | 86.4d | ND | ND | ND |
| 0.25% CMC                   | 80.9 | 83.5 | 85.5 | 89.5bc | 95.0b | ND | ND |
| 0.5% CMC                    | 80.3 | 83.4 | 84.7 | 95.0a | 97.3b | 102.8b | 98.1a |
| 1.0% CMC                    | 79.8 | 83.3 | 85.2 | 86.8cd | ND | ND | ND |
| 0.25% Chitosan              | 80.3 | 83.5 | 85.7 | 91.7b | 83.8c | ND | ND |
| 0.5% Chitosan               | 80.4 | 83.3 | 86.5 | 97.8a | 108.1a | 108.6a | 99.2a |
| 1.0% Chitosan               | 80.9 | 84.0 | 85.8 | 85.1d | ND | ND | ND |
| F-test                      | ns | ns | ns | * | ** | ** | ** |
| CV (%)                      | 0.7 | 0.4 | 0.9 | 2.0 | 2.4 | 12.0 | 3.3 |

Remarks: ND = not determined as fruit reached the end of shelf life in preceding period of observation. Values in the same column followed by different letters, differ significantly based on LSD (5%); ns = not significant; * = significant; ** = highly significant.
Table 5. Superoxide (SOD) and catalase activities during and after cold storage of organic chilli pepper in response to CMC and chitosan treatment.

| Treatments          | Day at 10°C | Days at ambient after 10°C storage |
|---------------------|-------------|-----------------------------------|
|                     | 0 | 4 | 8 | 12 | 1 | 3 | 5 |
| **SOD (units/mg protein)** |     |     |     |     |     |     |
| Control             | 59.6 | 63.7 | 74.8 | 74.7bc | ND | ND | ND |
| 0.25% CMC           | 59.0 | 64.1 | 75.2 | 77.4ab | 81.0b | ND | ND |
| 0.5% CMC            | 58.5 | 63.8 | 75.6 | 80.4a | 84.7a | 82.3b | 80.6b |
| 1.0% CMC            | 59.7 | 64.0 | 73.2 | 69.8d | ND | ND | ND |
| 0.25% Chitosan      | 60.3 | 63.7 | 75.8 | 75.5bc | 79.8b | ND | ND |
| 0.5% Chitosan       | 58.8 | 63.1 | 74.3 | 80.4a | 80.3b | 82.9a | 82.0a |
| 1.0% Chitosan       | 60.2 | 64.2 | 75.1 | 73.6c | ND | ND | ND |
| **F-test**          | ns | ns | ns | ** | ** | ** | * |
| **CV (%)**          | 1.3 | 0.9 | 2.4 | 2.8 | 1.4 | 1.2 | 1.0 |

| **Catalase (units/mg protein)** |     |     |     |     |     |     |
| Control             | 30.2 | 31.5 | 33.2 | 35.2 | ND | ND | ND |
| 0.25% CMC           | 30.5 | 31.3 | 33.6 | 35.8 | 35.3ab | ND | ND |
| 0.5% CMC            | 30.2 | 31.5 | 33.5 | 33.9 | 35.0ab | 38.4b | 39.6a |
| 1.0% CMC            | 31.0 | 30.5 | 33.7 | 32.4 | ND | ND | ND |
| 0.25% Chitosan      | 30.3 | 30.6 | 33.4 | 34.8 | 33.9b | ND | ND |
| 0.5% Chitosan       | 30.4 | 31.5 | 33.0 | 34.7 | 36.4a | 40.1a | 40.0a |
| 1.0% Chitosan       | 30.5 | 31.6 | 33.9 | 35.5 | ND | ND | ND |
| **F-test**          | ns | ns | ns | ** | * | * |
| **CV (%)**          | 2.8 | 1.3 | 1.1 | 5.0 | 4.0 | 6.5 | 6.0 |

Remarks: ND = not determined as fruit reached the end of shelf life in preceding period of observation. Values in the same column followed by different letters, differ significantly based on LSD (5%); ns = not significant; * = significant; ** = highly significant.

At the most effective concentration of 0.5%, chitosan was more effective than CMC in increasing SOD and catalase activity. This may have increased the tolerance to chilling condition during storage thereby contributing to better quality of chilli pepper during cold storage and subsequent ambient holding. Chitosan was more effective than CMC in enhancing fruit quality and storability as a result of improved antioxidant system. Similar to antioxidant substances, chitosan has more hydroxyl groups and amino acids that bind reactive oxygen species (ROS) thereby making this free radical stable and non-toxic to cells (Xie, Xu, & Liu, 2001). On the other hand, CMC does not exhibit antioxidant activity as it has less hydroxyl group (Olivas & Barbosa-Cánovas, 2005).

**CONCLUSION AND SUGGESTION**

CMC or chitosan coating in combination with HWD and active EMA film improved the shelf life of organic chilli pepper during cold storage and subsequent ambient holding. Most effective concentration for both edible coatings was 0.5%. Chitosan was more effective than CMC in enhancing fruit quality and storability as a result of improved antioxidant system.

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