

Synergistic Effect of Methyl Cellulose and Carvacrol Coating on Physicochemical and Microbial Attributes of Mango (*Mangifera indica*) Fruit in Postharvest Storage

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Abstract

Decay on mango (*Mangifera indica*) fruit mostly derived from a fungal disease which was caused by anthracnose invasion and infestation. The falling quality of mango fruit during postharvest preservation was commonly associated with weight loss, softening, vitamin C degradation and decay. This research evaluated the synergistic effect of methyl cellulose (MC) and carvacrol (Car) in the preparation of the edible coating on the physicochemical and microbial characteristics of mango fruit during 28 days of storage at 18°C. Five groups of coating treatments were prepared as follows: A (4% MC), B (4% MC + 0.5% Car), C (4% MC + 0.75% Car), D (4% MC + 1.0% Car), E (4% MC + 1.25% Car). These coating solutions were set 40°C for mango dipping. Mango fruits were individually dipped in the respected MC-Car solutions for 15 s and left out to air condition for 30 min to create the coating film. These mango fruits were then kept at 18°C for 28 days. In 7 day-interval, experimental fruits were sampled to estimate weight loss, firmness, ascorbic acid content, decay index. Mango fruit pre-coated by 4% MC + 1.0% Car showed the least weight loss (1.61±0.03 %) and decay index (2.19±0.03 mark) while the highest retention of firmness (47.13±0.23 N) and ascorbic acid (25.60±0.13 mg/100 g) at the end of 28 days of storage. Results showed that incorporation of 1.0% carvacrol into 4% methyl cellulose-based edible coating would extend the shelf-life of mango fruit for 28 days of preservation. The edible coating would be a promising and green alternative with minimal environmental pollution.

Keywords: Anthracnose, carvacrol, coating, mango, methyl cellulose, physicochemical

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INTRODUCTION

Mango (*Mangifera indica*) was a climacteric fruit widely cultivated in climate, tropical and subtropical areas. Mango fruit was greatly appreciated due to its nice looks, enrich sense, palatable sensory attributes, and the balanced proximate of nutritional and phytochemical components.¹ External appearance and internal composition contributed to consumer's acceptance of mango fruit.² Mango fruit was commonly harvested at the technical maturity and ripened quickly within few days at room condition.³ Inappropriate postharvest manipulation (from farm to pork), physiological disorder caused uncountable loss on both nutritional quality and commercial value of mango fruit.⁴

Filamentous fungi was one of the most prevalent agents causing mango fruit spoilage.⁵ Anthracnose caused by *Colletotrichum* spp. was a common fungal disorder on mango fruit in pre-harvest and post-harvest periods.⁶ Symptoms of anthracnose could be easily noticed by the appearance dark brown to black irregular lesions of various dimensions. These lesions accumulated to create massive spots initiating for the fruit decaying.⁷ Chemical fungicide was effective in controlling fungal infection⁸ however this implementation was strictly prohibited due to food safety concerns. Edible coating revealed an appropriate candidate to minimizing fungal pathogen invasion and manifestation while regulating physiological behaviors to obtain the best physicochemical and sensory attributes of fruit during storage. Edible coating also showed less vulnerability to environment because of its biodegradability.^{9,10}

Carvacrol (2-methyl5-(1-methylethyl)-phenol) was an essential oil based on monoterpenic phenolic naturally originated from aromatic herbs.¹¹ Carvacrol (Car) revealed a great antimicrobial potential against food such as gram [+], gram [-] bacteria as well as fungi.¹² The antimicrobial property of carvacrol was due to its interaction on the structural and functional attributes of the microbial cytoplasmic membranes.¹³ Methyl cellulose (MC) was a polysaccharide derivative of cellulose esters mostly applied in the edible coating. Methyl cellulose was proven to be effective to slow down senescence, minimize

moisture removal, improved overall acceptance and extended shelf-life of different kinds of fruits such as plum,¹⁴ banana,¹⁵ and pomegranate.^{16,17} Methyl cellulose was received from methylation of 30% of hydroxyl groups which was dissolved in cold water to form an apparent solution. Methyl cellulose was not soluble in hot water and became saturated solution. Due to its hydrophobic property, methyl cellulose had a great capability to prevent water and oxygen permeability as well as microbial and chemical resistance.¹⁸

Proper packaging could protect fruit against detrimentally-environmental factors such as moisture removal, oxygen exchange, invasion of pathogens and spoilage microbes while ensuring physicochemical quality properties and shelf-life extension during postharvest storage.^{19,23} Active coating consisting of bioactive ingredients originated from natural sources was proven to be effective in retarding harmful microorganisms.²⁴ A progressively emission of bioactive components into sample kept in the active coating permitted their availability in antimicrobial efficiency during storage.^{25,26}

Mango fruit was highly perishable during postharvest handling via quick senescence, more weight loss, less firmness, high total soluble solid accumulation, and serious rot.²⁷ It was very urgent and necessary to find out the appropriate strategy to extend the shelf-life of mango fruit while maintaining the most physicochemical quality attributes during storage. Purpose of our study examined the possibility of methyl cellulose (MC) and carvacrol (Car) in the preparation of the edible coating on the physicochemical and microbial characteristics of mango fruit during storage. Through edible coating, the shelf-life of mango fruit would be extended. Moreover, the commercial value of mango fruit would be improved.

MATERIAL AND METHOD

Material

Mango fruits were collected from orchard in Ke Sach district, Soc Trang province, Vietnam. Mango fruits must be in technical maturity, uniformity (weight 350±50 g) without defect. After harvesting, mango fruits were covered by sponge to avoid mechanical bruise and quickly moved to laboratory for experiments. Methyl cellulose

(96% purity), carvacrol (99% purity) was supplied from Merck (Germany). Chemical reagents such as oxalic acid, ethanol, glycerol, Tween 80, 2,6-dichlorophenol indophenol were all analytical grade supplied from De Phat Co. Ltd. Ho Chi Minh city, Vietnam.

Researching method

Coating solutions were prepared by dissolving 8 g methyl cellulose in 100 ml ethanol 90%, heating at 55°C for 2 min. Glycerol 0.5% was supplemented to the solution as a plasticizer followed by 0.05% Tween 80. Different amounts (0, 1.0, 1.5, 2.0, 2.5 ml) of carvacrol and 100 ml distilled water were added into the mixture. There were 5 groups of coating solutions (A: 4% MC; B: 4% MC + 0.5% Car; C: 4% MC + 0.75% Car; D: 4% MC + 1.0% Car; E: 4% MC + 1.25% Car). The coating solutions were cooled to 40°C ready for mango dipping. Mango fruits were individually dipped in the MC-Car solutions for 15 s and left out to air condition for 30 min to create the coating film on the mango fruits. These fruits were stored at 18°C for 28 days. In 7 day-interval, samples were taken to determine weight attrition, firmness, ascorbic acid content, decay index. Decay index was determined by visual appearance of fruit from completely fresh (1 mark), decay 5-10% (2 marks), decay 10-30% (3 marks), decay 30-50% (4 marks), decay surpass 50% (5 marks). Weight attrition (%) was defined as the difference of weight at the beginning and the time of interval sampling. Firmness (N) was measured by texture penetrator (Stable Micro Systems, model: TA.XTplusC). Ascorbic acid content (mg/100 g) was measured by volumetric method using a 2,6-dichlorophenol indophenol visual titration method described by AOAC.²⁸ Take 1 ml of the testing pure liquid into a 20 mL of flask. Supplement 2 mL of 5% oxalic acid

and titrate the reagent (V₁, ml). The final point was the occurrence of pink pigment. The quantity of chemical titrated was equal to the quantity of vitamin C. Pattern was weighed (M, g) and filled with 4% oxalic acid to volume (20 ml), separated by centrifugator. Take 1ml of this supernatant with 2 mL of 5% oxalic acid and then titrate one more time the reagent (V₂, ml).

$$\text{Vitamin C content (mg/100 g)} = \frac{0.5 \cdot V_2 \cdot 20 \cdot 100}{V_1 \cdot M}$$

Note: We totally understand the importance of measurement CO₂ and O₂ to better know the mode of action of edible coating. Due to laboratory insufficiency of high-tech equipments, we lack the O₂/CO₂ analyzer therefore we were not able to arrange this experiment.

Statistical analysis

The demonstrations were established in 3 replications with various sets of samples. The figure was illustrated as mean±standard deviation. Statistical summary was done by the Statgraphics Centurion version XVI. The mean value and standard deviation of a set of data obtained by analysis of random samples estimating the population statistics. 95% of results would be expected to lie within the range $x \pm 2s$ we described the lower and upper bounds of this range as the 95% confidence limits of the results.

RESULT AND DISCUSSION

Weight attrition

Weight loss in mango fruits increased at a higher rate in the control (group A) as compared to the coated samples (group B, C, D and E). There was not a significant difference in weight loss between group D (4% MC + 1.0% Car) and E (4% MC + 1.25% Car) (see Table 1). Mango fruit pre-

Table 1. Weight attrition (%) of mango fruit pre-coated by different MC-Car solutions during storage

Storage (days)	0	7	14	21	28
A	0	0.93±0.03 ^a	3.02±0.08 ^a	5.24±0.06 ^a	8.35±0.04 ^a
B	0	0.57±0.04 ^b	1.48±0.05 ^b	2.71±0.05 ^b	3.18±0.05 ^b
C	0	0.39±0.02 ^{bc}	0.95±0.04 ^{bc}	1.84±0.06 ^{bc}	2.39±0.03 ^{bc}
D	0	0.15±0.03 ^c	0.44±0.05 ^c	0.97±0.05 ^c	1.61±0.03 ^c
E	0	0.12±0.01 ^c	0.37±0.03 ^c	0.85±0.02 ^c	1.46±0.05 ^c

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

coated by 4% MC + 1.0% Car showed the least weight loss ($1.61 \pm 0.03\%$) at the end of 28 days of storage. Weight loss was not beneficial for mango fruit because it caused a remarkable tissue shrinkage leading to negative appearance as well as commercial value. Therefore, weight loss should be minimal. Weight loss in fruit was normally managed by epidermal overlay of the peel which coupled with stomata and protective tissue control the oxygen and carbon dioxide barter attributes of fruit.¹⁴ Permeability to moisture vapor was one of the most important properties of edible coating because it directly affected to respiration rate, weight loss of fruit during storage. Moreover, permeability to moisture vapor of film also related to microbial proliferation. Our results were in accordance with other literatures. Carvacrol incorporated to chitosan significantly decreased the moisture vapor permeability of coating film.²⁹ Carvacrol supplemented to sago starch-guar gum led to the lower moisture vapor permeability of coating film.³⁰ Methyl cellulose was a polysaccharide with hydrophobic property to limit water and oxygen permeability.¹⁵ Methyl cellulose controlled weight loss by establishing a mechanical hurdle against water moisture removal.³¹ Reducing weight loss via the application of methyl cellulose on avocado was mentioned.³² Methyl cellulose-based edible coating significantly minimized weight loss on strawberry fruit during 11 days of storage at 4°C.³³

Firmness

During storage, there was a declining trend of texture firmness on mango fruits. A significant retention of firmness was shown in mango fruits coated with edible film prepared

from methyl cellulose and carvacrol. There was not a significant difference in firmness retention between group D (4% MC + 1.0% Car) and E (4% MC + 1.25% Car). Meanwhile, a rapid softening occurred on the mango fruits coated by methyl cellulose only (group A) (see Table 2). Mango fruit pre-coated by 4% MC + 1.0% Car showed the highest retention of firmness (47.13 ± 0.23 N) at the end of 28 days of storage. This implied that carvacrol participated on the reinforcement of texture firmness on mango fruit effectively. Firmness degradation was strongly correlated to a deterioration of polymeric carbohydrates building cell walls; and moisture evaporation leading loose cell integrity.³⁴ Moisture and oxygen exchange through the edible coating film affected to texture degradation, biochemical and enzymatic reactions. Superior moisture vapor barrier of the edible coating film played a key role in fruit firmness retention. Carvacrol inclusion contributed to the hydrophobicity of the coating.³⁵ Oxygen permeability of the edible coating film also directly affect to oxidative and destructive reactions.³⁶ The less oxygen exchange via the coating film would slow down the respiration rate leading to the retention of the fruit texture. Numerous hydrogen bonds existing along the coating facilitated the surrounding sequences constraint closely to each other.³⁷ This phenomenon limited ethylene emission and oxygen permeability. Our results were in accordance with other literatures. Carvacrol supported for starch-based coating in maintaining firmness of mango and papaya.³⁸ Methyl cellulose-based edible coating greatly maintained firmness on strawberry fruit during 11 days of storage at 4°C.³³

Table 2. Firmness (N) of mango fruit pre-coated by different MC-Car solutions during storage

Storage (days)	0	7	14	21	28
A	72.89±0.34 ^a	50.97±0.35 ^c	37.61±0.37 ^c	30.07±0.19 ^c	21.05±0.24 ^c
B	72.89±0.34 ^a	59.46±0.48 ^{bc}	43.26±0.42 ^{bc}	36.12±0.27 ^{bc}	28.60±0.22 ^{bc}
C	72.89±0.34 ^a	62.18±0.39 ^b	51.15±0.50 ^b	43.81±0.36 ^b	36.84±0.30 ^b
D	72.89±0.34 ^a	65.03±0.51 ^a	58.87±0.38 ^a	52.04±0.31 ^a	47.13±0.23 ^a
E	72.89±0.34 ^a	65.41±0.47 ^a	59.28±0.41 ^a	52.33±0.28 ^a	47.62±0.25 ^a

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

Table 3. Vitamin C content (mg/100 g) of mango fruit pre-coated by different MC-Car solutions during storage

Storage (days)	0	7	14	21	28
A	37.15±0.19 ^a	26.59±0.18 ^c	22.87±0.20 ^c	19.35±0.13 ^c	12.57±0.09 ^c
B	37.15±0.19 ^a	29.74±0.19 ^{bc}	26.23±0.21 ^{bc}	22.98±0.19 ^{bc}	16.34±0.12 ^{bc}
C	37.15±0.19 ^a	32.08±0.21 ^b	29.86±0.17 ^b	26.40±0.21 ^b	20.29±0.16 ^b
D	37.15±0.19 ^a	35.27±0.27 ^a	32.94±0.20 ^a	30.09±0.16 ^a	25.60±0.13 ^a
E	37.15±0.19 ^a	35.42±0.24 ^a	33.08±0.19 ^a	30.27±0.20 ^a	25.89±0.18 ^a

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

Ascorbic acid content

Ascorbic acid content in mango fruits decreased at a higher rate in the control (group A) as compared to the coated samples (group B, C, D and E). There was not a significant difference in acidity between group D (4% MC + 1.0% Car) and E (4% MC + 1.25% Car) (see Table 3). Mango fruit pre-coated by 4% MC + 1.0% Car showed the highest retention of ascorbic acid (25.60±0.13 mg/100 g) at the end of 28 days of storage. The variation of ascorbic acid content could be explained due to the reaction between ascorbic acid and reactive oxygen species. Our result was similar to other findings on peach,³⁹ litchi,⁴⁰ cherry.⁴¹ The peach fruit coated by methyl cellulose had the highest retention of titrable acidity while the lowest one was noticed at the control sample.¹⁴ Ascorbic acid content was greatly decomposed by light transmission. Carvacrol had potential to prevent the penetration of visible and ultraviolet beam from the coating enter the sample; hence light transmission greatly minimized.⁴² By retardation of light invasion into fruit sample, the more ascorbic acid content would be highly preserved. Moreover, degradation of ascorbic acid content during

storage also derived from respiration activity. Ascorbic acid might involve in enzyme-catalyzed reactions during aerobic respiration in mango tissue, leading to a reduction in ascorbic content during senescence. Edible coating could limit oxygen and moisture permeability consequently slower the respiration rate that facilitated for higher ascorbic acid retention.

Decay index

During 28 days of preservation, there was a ascending trend of decay index on mango fruits. A significant retardation of decay index was observed on mango fruits coated with edible film prepared from methyl cellulose and carvacrol. There was not a significant difference in decay index between group D (4% MC + 1.0% Car) and E (4% MC + 1.25% Car). At the 28th day of storage, decay index of group D were 2.19±0.03 marks that implied the deterioration rate was around 10%. Meanwhile, a high deterioration was noticed on the mango fruits coated by methyl cellulose only (group A) with decay index 4.83±0.02 marks that implied over 50% of fruit was rotten (see Table 4). The microbial proliferation curve included 4 sections: lag phase, log phase, stable phase, and lethal phase. In the

Table 4. Decay index (mark) of mango fruit pre-coated by different MC-Car solutions during storage

Storage (days)	0	7	14	21	28
A	1.00±0.00 ^a	2.25±0.01 ^a	3.04±0.04 ^a	3.96±0.01 ^a	4.83±0.02 ^a
B	1.00±0.00 ^a	1.84±0.03 ^b	2.36±0.01 ^b	2.79±0.03 ^b	3.08±0.00 ^b
C	1.00±0.00 ^a	1.57±0.00 ^{bc}	1.91±0.03 ^{bc}	2.43±0.02 ^{bc}	2.72±0.01 ^{bc}
D	1.00±0.00 ^a	1.21±0.01 ^c	1.52±0.02 ^c	1.97±0.00 ^c	2.19±0.03 ^c
E	1.00±0.00 ^a	1.18±0.02 ^c	1.45±0.00 ^c	1.89±0.01 ^c	2.11±0.02 ^c

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

lag phase, the microbe regulated to the fresh condition with non-count increment. In the log phase, microbe utilized substrate to increase their count exponentially and logarithmically. In the stable phase, the proportion of live formation was comparable to the death percentage, and in the final phase, the microbial count declined. Carvacrol had antimicrobial potential to retard the lag phase and decrease the colony forming unit in the log phase. Carvacrol reacted with the fat profile of microbe, mitochondrial cell membranes, and intracellular substances; leading cell matrix damage, ion barter; limiting respiration; accelerating permeability, loosing intracellular substances, and ultimately microbial cell lethal.⁴³ The antimicrobial principle of carvacrol was based on the reaction of hydrophobic groups with microbial membranes. This intervention modified the pump of H⁺ and K⁺ leading to the disruption of fundamental properties and cell lethal.⁴⁴ Carvacrol inhibited the proliferation of *E. coli* more effective than *Salmonella*.⁴⁵ Carvacrol greatly improved the microbial inactivation of the chitosan film in respect to *Staphylococcus*.⁴⁶ Carvacrol was proven to improve antioxidant and antibacterial activities of chitosan nanoparticles.⁴⁷ Our results were in accordance with other literatures. Lesion of anthracnose symptoms on mango and papaya fruits caused by *Colletotrichum gloeosporioides* would be thoroughly minimized by carvacrol-starch based coating.³⁸ Polyethylene coating included with high concentrations of carvacrol led to better antimicrobial efficacy towards *Saccharomyces*, *E. coli*, *Listeria* and *Staphylococcus*.³⁵

CONCLUSION

The utilization of carvacrol in preparation methyl cellulose coating greatly improved the physicochemical and antimicrobial characteristics on the mango fruit during 28 days of storage. We found that carvacrol incorporated with methyl cellulose enhanced durability, resistance to moisture vapor permeability and oxygen permeability hence slowing down the weight attrition (1.61±0.03%), retaining better firmness (47.13±0.23 N) of mango fruit. MC-Car complex also created an excellent barrier against visible and ultraviolet light transmission that implicated ascorbic acid content would be preserved effectively (25.60±0.13 mg/100 g). Mango

coated by MC-Car film with 4% MC and 1.0% Car demonstrated good antifungal activity against anthracnose with low decay index (2.19±0.03 mark) in this research. Carvacrol incorporated with methyl cellulose created a synergistic effect superior to individual usage. Shelf-life of the coated mango fruit was stable for 28 days at 18°C. MC-Car coating would create a favorable condition for long time shipping of this item in a shelf-life extension to the international market with a competitive price instead of airfreight.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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