UNIVERSITI PUTRA MALAYSIA

EFFECTS OF EDIBLE COATINGS ENRICHED WITH CALCIUM CHLORIDE ON PHYSIOLOGICAL, BIOCHEMICAL AND QUALITY RESPONSES OF MANGO (Mangifera Indica L. cv. Choke Anan) FRUIT DURING COLD STORAGE

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By

GHULAM KHALIQ

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2015

DEDICATION

This thesis is dedicated to all I love especially to
My beloved mother
The wind below my wings and
My father
The trust on me

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

EFFECTS OF EDIBLE COATINGS ENRICHED WITH CALCIUM CHLORIDE ON PHYSIOLOGICAL, BIOCHEMICAL AND QUALITY RESPONSES OF MANGO (*Mangifera Indica* L. cv. Choke Anan) FRUIT DURING COLD STORAGE

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December 2015

Chairperson: Professor Mahmud Tengku Muda Mohamed, PhD
Faculty : Agriculture

Mango is a climacteric fruit and various biochemical changes during ripening affect the fruit composition. It is very sensitive to chilling temperature during storage. In the first experiment, the effects of gum arabic (GA) 10%, chitosan (CH) 1%, calcium chloride (CA) 3 %, GA 10% + CH 1%, GA 10% + CA 3%, CH 1% + CA 3% and distilled water as a control on the physico-chemical properties of „Choke Anan" mango fruit were investigated. After dipping treatments, the fruits were stored at 2, 6 and 13 °C for 28 days and then transferred to 25 °C for 5 days shelf life. Mango stored at 2 or 6 °C, inhibited physico-chemical changes and delayed the ripening process than those stored at 13 °C. The results showed that GA 10% or CH 1% coatings significantly reduced weight loss, colour changes, soluble solid concentration, respiration rate, ethylene production and maintained higher firmness or titratable acidity than the control. GA 10% coatings formulation improved with CA 3% supplement. Another experiment was conducted to evaluate the effect of GA 10%, CA 3%, GA 10% + CA 3% or distilled water as a control on the accumulation of reactive oxygen species (ROS) and oxidative damage of mango. Mango stored at 2 °C, significantly accumulated higher ROS, malondialdehyde (MDA) content or ion leakage than those stored at 6 and 13 °C. GA 10% and CA 3% treatments decreased hydrogen peroxide (H_2O_2) content and superoxide anion ($O_2^{\bullet-}$) production in all the three temperatures. The combined application of CA 3% and GA 10% alleviated chilling injury (CI) and oxidative damage. The third experiment was carried out to find out the effect of GA 10%, CA 3% and their combinations on enzymatic and non-enzymatic antioxidant defense system of mango stored at 6, 10 and 13 °C. The enzymatic and non-enzymatic antioxidant properties of mango were induced at 6 °C than those stored at 10 or 13 °C. Mango treated with GA 10% or GA 10% + CA 3%, enhanced catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) enzyme activities. GA 10% or CA 3% treatments triggered 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and preserved total phenolic content or ascorbic acid. In the fourth experiment, the ultra-structural changes in the peel of mango were investigated

by transmission electron microscope (TEM). Rapid changes can be seen in the structures of cell membranes and mitochondria of mango stored at 13 °C than those stored at 6 °C. Treated mango maintained cell membranes and mitochondria structure integrity than the control. The results from all experiments suggest that mango fruit treated with GA 10% plus CA 3% can be stored at 6 °C without much deterioration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN PENYALUTAN YANG DIPERKAYA DENGAN KALSIUM KLORIDA KE ATAS TINDAK BALAS FISIOLOGI, BIOKIMIA DAN KUALITI BUAH MANGGA (*Mangifera Indica* L. cv. Choke Anan) SEMASA PENYIMPANAN SEJUK

Oleh

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Mangga merupakan buah klimakterik dan pelbagai perubahan biokimia berlaku semasa ranum yang dapat menjejaskan komposisi buah. Ia adalah sangat sensitif kepada suhu penyejukan semasa penyimpanan. Dalam kajian pertama, kesan gam arab (GA) 10%, kitosan (CH) 1%, kalsium klorida (CA) 3%, GA 10% + CH 1%, GA 10% + CA 3%, CH 1% + CA 3% dan air suling sebagai kawalan ke atas sifat fiziko-kimia buah mangga 'Choke Anan' telah disiasat. Setelah direndam dengan rawatan, buah disimpan pada suhu 2, 6 dan 13 °C selama 28 hari dan kemudian dipindahkan ke 25 °C selama 5 hari. Mangga yang disimpan pada suhu 2 atau 6 °C, perubahan fiziko-kimia dihalang dan proses ranum dilambatkan berbanding dengan buah yang disimpan pada suhu 13 °C. Hasil kajian menunjukkan bahawa buah yang dirawat dengan GA 10% atau CH 1% telah mengurangkan kehilangan berat, perubahan warna, kepekatan pepejal larut, kadar pernafasan dan pengeluaran etilena, dan meningkatkan kekerasan atau asid tertitrat berbanding dengan kawalan. Fomulasi penyalut GA 10% diperbaiki dengan tambahan CA 3%. Satu lagi eksperimen telah dijalankan untuk menilai kesan GA 10%, CA 3%, GA 10% + CA 3% atau air suling sebagai kawalan pada pengumpulan spesies reaktif oksigen (ROS) dan kerosakan oksidatif mangga. Mangga disimpan pada suhu 2 °C adalah jauh lebih tinggi dalam pengumpulan ROS, kandungan malondialdehid (MDA) atau kebocoran ion berbanding dengan buah yang disimpan pada suhu 6 dan 13 °C. GA 10% dan 3% CA rawatan menunjukkan penurunan kandungan hidrogen peroksida (H_2O_2) dan superoxide anion ($O_2^{\bullet-}$) pada ketiga-tiga suhu. Penggunaan kombinasi CA 3% dan GA 10% mengurangkan kecederaan dingin (CI) dan oksidatif kerosakan. Kajian ketiga telah dijalankan untuk mengetahui kesan GA 10%, CA 3% dan kombinasi mereka terhadap enzim dan antioksidan sistem pertahanan bukan enzim mangga yang disimpan pada suhu 6, 10 dan 13 °C. Enzim dan antioksidan bukan enzim mangga telah didorongkan pada suhu 6 °C berbanding dengan buah yang disimpan pada suhu 10 atau 13 °C. Mango yang dirawat dengan GA 10% atau GA 10% + CA 3% menyebabkan penambahan aktiviti enzim katalase (CAT), askorbat peroxidase (APX) dan glutation reduktase (GR).

Rawatan GA 10% atau CA 3% mencetuskan aktiviti 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radikal dan mengekalkan jumlah kandungan fenolik atau asid askorbik. Dalam kajian keempat, perubahan ultra-struktur pada kulit mangga telah disiasat dengan mikroskop elektron transmisi (TEM). Perubahan pesat dapat dilihat dalam struktur membran sel dan mitokondria mangga yang disimpan pada suhu 13 °C berbanding dengan buah yang disimpan pada suhu 6 °C. Mangga dirawat mengekalkan membran sel dan integriti struktur mitokondria daripada. Keputusan daripada semua ujikaji menunjukkan bahawa buah manga yang dirawat dengan GA 10% ditambah dengan CA 3% boleh disimpan pada suhu 6 °C tanpa banyak kerosakan.

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I certify that a Thesis Examination Committee has met on 16 December 2015 to conduct the final examination of Ghulam Khaliq on his thesis entitled "Effects of Edible Coatings Enriched with Calcium Chloride on Physiological, Biochemical and Quality Responses of Mango (*Mangifera indica* L. cv. Choke Anan) Fruit During Cold Storage" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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I hereby confirm that:

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1-MCP	1-Methylcyclopropene
ACO	Aminocyclopropanecarboxylate oxidase
ACS	Aminocyclopropanecarboxylate synthase
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
ASH-GSH	Ascorbate-glutathione
ATP	Adinosine triphosphate
C*	Chroma
CA	Calcium chloride
CA	Controlled atmosphere
CAT	Catalase
CH	Chitosan
CI	Chilling injury
CM	Cell membrane
CMC	Carboxymethyl cellulose
CW	Cell wall
DPPH	2, 2-diphenyl-1-picryl hydrazyl
FW	Fresh weight
GA	Gum arabic
GC	Gas chromatograph
GR	Glutathione reductase
GSH	Glutathione
h°	Hue angle
L*	Lightness
LOX	Lipoxygenase
LSD	Least significant differences
M	Mitochondria
MA	Modified atmosphere
MDA	Malondialdehyde
MM	Mitochondria membrane
NO	Nitric oxide
Pas	Polyamines
PG	Polygalacturonase
PLD	Phospholipase D
PME	Pectin methyl esterase
POD	Peroxidase
PPO	Polyphenoloxidase
PVPP	Polyvinylpolypyrrolidone
RCBD	Randomize complete block design
ROS	Reactive oxygen species
SAS	Statistical analysis system
SOD	Superoxide dismutase
SSC	Soluble solids concentrations
TA	Titratable acidity
TEM	Transmission electron microscope

CHAPTER 1

GENERAL INTRODUCTION

Mango (*Mangifera indica* L.) belongs to family Anacardicceae and the most popular fruit throughout the world. Mango enjoys a unique status among other fruits since it is called the king of fruits. It has a high commercial value in the international fruit market (Baldwin et al., 1999). Mango is considered as one of the choicest fruits for its attractive colour, delicious taste, good aroma and excellent nutritional properties. Mango fruit are rich source of carotenoids (precursor of vitamin A), vitamin C, organic acids, carbohydrates, phenolic compounds and minerals (Pott et al., 2003). Mango fruit composition depends on location, maturity stage, storage condition and cultivar. Mango fruit contains a fair amount of β carotene, gallic acid, sucrose, fructose and glucose (Singh et al., 2013). The most popular varieties of mango grown in Malaysia are „Harumanis‟, „Choke Anan‟, „MAHA‟ and „Masmuda‟ (Ding and Darduri, 2013). Choke Anan mango is one of the most common cultivar grown in Malaysia for local and export market (Teoh and Syaifudin, 2007). The increasing demand for this cultivar is due to its vibrant colors, exotic flavors, distinctive taste and nutritional properties (Arauz, 2000). Choke Anan is a sweet mango also known as honey mango. Ripe fruit peel and flesh are light yellow colour with sweet taste. Choke Anan is a miracle mango, because it often fruit twice a year fruiting in summer, and then gives way to another crop in winter time (Teoh and Syaifudin, 2007).

The current mango industry is facing several challenges and needs specific strategies to overcome these problems. Postharvest management of mango fruit is one of the major problems faced by the industry (Wongmetha and Ke, 2012). International trade and mango production are increasing rapidly, however qualitative and quantitative postharvest losses occur along the mango supply chain, especially during export (Singh et al., 2013). Air transportation of mangoes is expensive and shipping in sea freight takes two to three weeks to reach destination, which restricts its transportation in long distance due to limited storage life. Short storage life, postharvest diseases, and softening are the main issues affecting mango fruit quality in domestic as well as in international market. Mango is a climacteric fruit and sudden rise in respiration rate and ethylene production, result in changes in colour, softness and aroma volatiles (Mitra, 1997). The respiration rate is inversely associated with shelf life due to which mango fruit have limited postharvest life (Yahia, 2011). Additionally, desiccation of mango during transportation and storage causes it to shrivel and reduces the market value of the fruit (Wang et al., 2006). The soft texture of mango reduces the postharvest life and increase susceptibility to various pathogenic infections. Mango fruit softening is associated with the destruction of cell wall structure, middle lamella and changes in the pectin composition (Ali et al., 2004). Mango fruit shows rapid deterioration after harvest due to ripening and senescence processes. Mango is a tropical fruit and ripens rapidly, which limits the storage life, handling and transportation potential during marketing (Djioua et al., 2010). The optimum storage temperature for mango is 13 °C, however at this temperature the fruits cannot be stored for a very long time due to accelerated ripening (Weor, 2007). So, low temperature storage is the only possible option for quality maintenance and extension storage life of mango. Internationally, low temperature storage is the usual method for maintaining quality and extending storage life of fruits. Low temperature slows down respiration, ethylene production, ripening, senescence, decay and other undesirable metabolic changes of mango fruit (Nunes et al., 2007). However, mango is a tropical fruit and extremely sensitive to chilling injury (CI), when the fruits are exposed to temperature below 13 °C, which affect the postharvest qualities (Phakawatmongkol et al., 2004). CI symptoms in mango fruit appear as sunken lesions or surface pitting, browning, greyish scald like discoloration of the skin, poor aroma and flavor, uneven ripening and increased susceptibility to fungal decay (Nunes et al., 2007).

Several techniques have been used to reduce deterioration, CI and maintain quality of mango fruit during storage. For example, modified or controlled atmosphere are common techniques for maintaining quality and reducing CI in mango (Pesis et al., 2000). However, mango fruit stored in modified atmosphere can cause undesirable flavor, anaerobic respiration and CO_2 accumulation (Lalel et al., 2005). Heat treatments can be used for keeping quality and alleviating CI of mango fruit (Jacobi and Wong, 1992; Kim et al., 2007). Though, mango fruit show sensitivity to heat treatment (Bender et al., 1994). Hypobaric is low pressure storage technique, which inhibiting ethylene production, extending shelf life and reducing CI in mango (Singh et al., 2013). However, hypobaric storage is costly and cannot be used on the large scale commercial basis (Wills et al., 2007). Continuous use of fungicides has been used to reduce postharvest decay and extend the storage life of fruit. On the other hand, fungicide resistance by pathogens, along with consumer concerns about possible risks associated with the residue of fungicides on the fruit surface (Mari et al., 2014).

Alternatively, natural products are useful and taking place as biopreservative approaches for delaying ripening, retaining quality, and reducing postharvest disorders of fruits (Tripathi and Dubey, 2004). In developed and developing countries, edible coating has the potential to keep quality and extend shelf life of mango (Singh et al., 2013). Edible coatings are created a modified atmosphere by decreasing gas exchange CO_2 and O_2, thus reducing water loss, oxidation reaction and respiration rate (Martínez-Romero et al., 2013). Many studies have indicated that edible coatings can preserve fruit quality, delay ripening and reduce biochemical changes, such as alginate in plum (Valero et al., 2013), aloe vera gel in raspberry (Hassanpour, 2015), wheat gluten in strawberry (Tanada-Palmu and Grosso, 2005) and carnauba-shellac wax in apple (Jo et al., 2014). Coatings are used as postharvest managements to maintain fruit quality and minimize the size of non-biodegradable packaging materials (Campos et al., 2011). However, the commercial uses of edible coatings are still very limited. Thus, edible coatings in combination with low storage temperature are needed to further explore its effect on mango post harvest qualities.

Gum arabic (GA) is a polysaccharide natural secretion from *Acacia* sp and used in industries for film forming, emulsification, and encapsulation properties (Motlagh et al., 2006). It is cheap and natural polymer for preservation of fresh produce. GA coatings effectively reduced weight loss, colour changes and ascorbic acid loss in tomato fruit during storage (Ali et al., 2010). It has been observed that GA combined with chitosan extended the shelf life of banan during storage (Maqbool et al., 2011). GA enriched with natamycin potentially preserved the quality and enhanced the storage life of shiitake mushroom as reported by Jiang et al. (2013). Chitosan (CH) is a polysaccharide containing (1,4)-linked 2-amino-deoxy-β-d-glucan, derived by deacetylation of chitin (Aider, 2010). CH is a natural polymer, biodegradable, biocompatible, non-toxic and has strong antimicrobial and antifungal properties (Elsabee and Abdou, 2013). CH has been known to preserve perishable fruits from quick deterioration, reduce dehydration, respiration and maintain high antioxidant properties of fruits such as guava, longan and strawberries (Hong et al., 2012; Shi et al., 2013; Wang and Gao, 2013). CH enhances defense mechanisms in fruits against pathogens and fungal infections (Aider, 2010).

Calcium plays a significant role in signaling, reducing physiological disorders, and regulating biochemical function in fruits during postharvest life (Aghdam et al., 2012). It has been observed that calcium is associated with fruit firmness, stress tolerance, ripening and senescence (Martin-Diana et al., 2007). CA dip treatment reduced flesh browning of peach (Manganaris et al., 2007) and increased firmness of dragon fruit (Awang et al., 2011). CA combined with chitosan maintained a high level of vitamin C and reduced sensitivity to CI of peach fruit during refrigerated storage (Ruoyi et al., 2005). Various studies showed that CA combined with coating materials, improved quality, delayed ripening and reduced biochemical changes of fruits, such as in strawberry (Hernández-Muñoz, et al., 2008), fresh cut banana (Bico et al., 2009) and pears (Kou et al., 2015).

To our knowledge, there is no work done on the use of GA coatings enriched with CH or CA for maintaining postharvest quality and extending the storage life of mango. Therefore, the present study was conducted with the general objective of assessing the effect of CH, GA and CA application on mango fruit stored at three different temperatures to retain the overall mango fruit quality and to reduce postharvest losses by evaluating suitable postharvest novel technologies. The specific objectives of this study were:
(1). to determine the effects of CH, GA and CA on physico-chemical and postharvest quality attributes of mango fruit during low temperature storage.
(2). to investigate the accumulation of reactive oxygen species (ROS) and capability of GA and CA to reduce oxidative damage at low temperature stress.
(3). to study the mechanism of selected treatments to induce antioxidant enzymatic and non-enzymatic defense system and chilling tolerance.

(4). to find out the ultra-structural changes in cells of mango fruit after dipping treatments and storage temperatures.

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