UNIVERSITI PUTRA MALAYSIA

EFFECTS OF POLYSACCHARIDE-BASED COATING CONJUGATED WITH SILVER NANOPARTICLES ON SHELF-LIFE EXTENSION OF PAPAYA (Carica papaya L. cv. Eksotika)

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By

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September 2014

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Papaya is a tropical fruit that is well accepted worldwide for its good taste and nutritional content. However, like many other tropical fruits, papaya also has postharvest problems due to several reasons including short shelf life caused by disease mainly fungal infection. This problem also affects *Carica papaya* L. cv. Eksotika, which is one of the popular papaya cultivars in Malaysia. In the present study, polysaccharide-based coatings incorporated with silver nanoparticles were used to prolong the shelf life of Eksotika papaya.

In this study, a two-factor central composite design (CCD) was used to optimize two different edible coating formulations for coating of Eksotika papaya. The effect of two different coating formulations, namely, chitosan-based [chitosan (0.5 – 2.5%) and glycerol (0 – 2%)] and carrageenan-based [carrageenan (0.2 – 0.8%) and glycerol (0 – 1%)] on texture and colour components (L, a and b values) of coated papaya were evaluated. From the multiple response optimization analysis, combination of 2.32% chitosan with 0.53% glycerol, and 0.78% carrageenan with 0.85% glycerol for chitosan-based and carrageenan-based coating respectively were predicted to give desirable effect to the response variables tested, with no significant ($p > 0.05$) difference between the experimental and predicted values. However, during shelf life monitoring of the coated samples, papaya coated with the optimized chitosan-based formulation failed to ripen. In order to overcome this, visual observation of papaya coated with different concentrations of chitosan (0.5, 1.0, 1.5 and 2.32%) were conducted. 1% chitosan was found to successfully coat papaya without retarding the ripening process.

Silver nanoparticles at the range of 0-80 ppm were incorporated into the chitosan- and carrageenan-based coatings to evaluate the antimicrobial properties of the formed polysaccharide-silver nanocomposites. Chitosan alone without synergistic interaction with silver nanoparticles was sufficient in giving bactericidal and antifungal effects. Carrageenan on the other hand requires addition of 2.5 and 30 ppm of silver nanoparticles to give antifungal and bactericidal effects respectively.
Application of chitosan, carrageenan-silver nanocomposite and carrageenan-based coating on Eksotika papaya were able to prolong the shelf life up to 13, 11 and 9

days at ambient condition (26±2°C;70±10% RH), respectively. During low temperature (15±1°C; 60±10% RH) storage, the shelf life was extended up to 21 days for chitosan and carrageenan-silver nanocomposite based coating and also 17 days for carrageenan-based coating. The maximum shelf life of uncoated papaya was 7 and 13 days at ambient and low temperature, respectively. Shelf life extension of coated papaya was judged based on no visible fungal decay.

Physiological and physico-chemical evaluations during storage showed that there was no significant ($p > 0.05$) difference between coated samples and control except for firmness at ambient condition and also total soluble solids. The microbial counts between coated and uncoated papaya were also found to be insignificant ($p > 0.05$).

Determination of silver concentration in papaya fruit coated with carrageenan-silver nanocomposite revealed that, silver migrated through the peel and into the innermost pulp layer of papaya during storage. Consumer acceptance test showed that scores of all tested attributes for coated samples were above the minimum acceptable value (>5). This indicated that the coating did not give negative visual or physical perception towards the consumer. Hence, coating gave positive contribution on shelf life extension of papaya especially for marketing purposes, but incorporation of silver nanoparticles needs to be reconsidered since silver migrated into the pulp due to the thin peel layer of papaya fruit.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

KESAN SALUTAN BERASASKAN POLISAKARIDA BERKONJUGASI DENGAN ZARAH PERAK NANO TERHADAP PERLANJUTAN JANGKA HAYAT BUAH BETIK (*Carica papaya* L. cv. Eksotika)

Oleh

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Betik ialah sejenis buah tropika yang diterima baik di seluruh dunia disebabkan oleh kandungan nutrisi dan rasanya yang sedap. Walau bagaimanapun, seperti buah-buahan tropika yang lain, betik juga mempunyai masalah lepas tuai yang berpunca daripada beberapa sebab termasuk jangka hayat penyimpanan yang pendek disebabkan oleh penyakit yang berpunca daripada jangkitan kulat. Masalah ini juga memberi kesan kepada *Carica papaya* L. cv. Eksotika yang merupakan salah satu daripada kultivar betik yang popular di Malaysia. Dalam kajian ini, salutan berasaskan polisakarida yang dikonjugasikan dengan zarah nano perak telah digunakan untuk memanjangkan jangka hayat simpanan betik Eksotika.

Dalam kajian ini, reka bentuk komposit pusat dua faktorial telah digunakan untuk mendapatkan formulasi optimum bagi dua salutan boleh makan yang berbeza untuk menyalut betik Eksotika. Kesan dua formulasi berbeza, iaitu yang berasaskan kitosan [kitosan (0.5-2.5%) dan glicerol (0-2%)] dan juga yang berasaskan karagenan [karagenan (0.2-0.8%) dan glicerol (0-1%)] terhadap tekstur dan komponen warna (nilai L, a dan b) buah betik yang disalut telah diuji. Daripada analisis respon optimum pelbagai, kombinasi 2.32% kitosan dengan 0.53% glicerol, dan kombinasi 0.78% karagenan dengan 0.85% glicerol, masing-masing bagi salutan berasaskan kitosan dan karagenan diramalkan member kesan yang diingini terhadap pembolehubah bergerak balas yang dikaji, dengan tiada perbezaan yang signifikan ($p>0.05$) antara nilai eksperimen dengan nilai yang diramal. Walau bagaimanapun, semasa tempoh pemerhatian jangka hayat simpanan betik yang telah disalut, didapati bahawa betik yang disalut dengan formulasi kitosan optimum gagal meranum. Bagi mengatasi masalah ini, pemerhatian secara visual bagi betik yang disalut dengan kepekatan kitosan yang berbeza (0.5, 1.0, 1.5 dan 2.32%) telah dijalankan. Didapati kitosan pada kepekatan 1% telah berjaya menyalut betik tanpa membantutkan proses peranuman.

Zarah perak nano pada julat 0-80 ppm telah dimasukkan ke dalam salutan berasaskan kitosan dan karagenan bagi menguji sifat antimikrobial nanokomposit polisakarida dan perak yang terbentuk. Kitosan semata-mata tanpa interaksi sinergi dengan zarah perak nano adalah mencukupi untuk member kesan anti bakteria dan

anti kulat. Walau bagaimanapun, karagenan memerlukan penambahan 2.5 dan 30 ppm zarah perak nano masing-masing bagi member kesan anti kulat dan bakteriasid.

Aplikasi salutan berasaskan kitosan, nanokomposit karagenan dengan perak dan karagenan pada betik Eksotika masing-masing mampu memanjangkan jangka hayat penyimpanan buah sehingga 13, 11 dan 9 hari pada keadaan ambien (26±2°C; 70±10% RH). Semasa penyimpanan pada suhu rendah (15±1°C; 60±10% RH), jangka hayat simpanan dapat dipanjangkan sehingga 21 hari bagi salutan berasaskan kitosan dan nanokomposit karagenan dengan perak. Manakala bagi betik disalut karagenan, jangka hayat dapat dipanjangkan sehingga 17 hari. Jangka hayat simpanan maksimum bagi betik yang tidak disalut ialah 7 dan 13 hari, masing-masing bagi penyimpanan pada keadaan ambien dan suhu rendah. Peningkatan jangka hayat simpanan betik yang disalut adalah berdasarkan tiadanya kesan kerosakan akibat kulat.

Penilaian fisiologi dan fiziko-kimia semasa tempoh penyimpanan menunjukkan tiada perbezaan siknifikan ($p>0.05$) antara sampel bersalut dan tidak bersalut kecuali untuk tekstur dan kandungan kesuluruhan pepejal larut. Pertumbuhan bakteria, yis dan kulat antara betik bersalut dan tidak bersalut juga didapati tidak siknifikan ($p>0.05$).

Penentuan kepekatan zarah perak di dalam betik bersalut nanokomposit karagenan dan perak semasa tempoh penyimpanann menunjukkan bahawa zarah perak berjaya menembusi kulit betik dan terus ke lapisan isi betik yang paling dalam. Ujian penerimaan pengguna menunjukkan bahawa skor bagi setiap ciri betik bersalut yang dinilai adalah lebih tinggi daripada nilai minimum yang diterima (>5). Ini menunjukkan bahawa dari segi visual dan fizikal salutan yang digunakan tidak memberi persepsi negatif kepada pengguna. Justeru, penggunaan salutan dapat memberi kesan positif kepada peningkatan jangka hayat simpanan betik terutamanya bagi tujuan pemasaran. Walau bagaimanapun, penambahan zarah perak nano perlu dipertimbangkan, memandangkan terdapat penghijrahan zarah perak ke dalam isi buah betik disebabkan oleh kulitnya yang nipis.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment for the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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1-MCP	1- Methylcyclopropene
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CCD	Central composite design
CFU	Colony forming units
DNA	Deoxyribonucleic acid
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
HNO_3	Nitric acid
ICP-OES	Inductively coupled plasma- optical emission spectrometry
LDPE	Low density polyethylene
MAP	Modified atmosphere packaging
MARDI	Malaysian Agricultural Research and Development Institute
MBC	Minimum bactericidal concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
NaOH	Natrium hydroxide/ sodium hydroxide
PCA	Plate count agar
PDA	Potato dextrose agar
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PVP	Polyvinylpyrrolidone
RSM	Response surface methodology

SEM	Scanning electron microscopy
TA	Titratable acidity
TEM	Transmission electron microscopy
TPC	Total plate count
TSS	Total soluble solids
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV-Vis	Ultraviolet-visible
w/v	weight/volume
YM	Yeast and mould

CHAPTER 1

INTRODUCTION

Fruits and vegetables can be classified as climacteric or non-climacteric. Climacteric fruits continue to ripen after harvest and show more dramatic changes in their physiological activity (Maftoonazad & Ramaswamy, 2005). Papaya is one of the climacteric fruit and has attained great popularity among growers due to the fact it can be intensively cultivated, its rapid returns and the increase demand for the fresh fruit and its processed products (Singh & Rao, 2011). The ripe fruit are usually eaten as a dessert but they can also be processed into jams, soft drinks, ice cream flavouring, crytallised and candied fruits, fruit rolls and as filler for sauces (Md. Yon, 1994). Papaya is an important fruit in ASEAN countries and can be divided into two groups. The first group consists of varieties which bear large, elongated fruits. The second group on the other hand bears small, pyriform or round shaped fruit with very good quality. The varieties in the first group include Subang, Sitiawan, Batu Arang, Kaegdum, Kaegnuan and Sainampeung. The common varieties in the second group are Solo and Eksotika (Chan *et al.*, 1994). Due to the small, uniform size and also its ease of handling and serving, Eksotika papaya has become very popular among local consumers (Chan, 2011).

Brazil, Mexico and Malaysia are the main papaya exporting countries mainly to the European market (Cia *et al.*, 2007) with Malaysia being the second most important exporter of Eksotika papaya. Although the export revenue from papaya is quite significant, the prospects for market expansion are rather restricted due to expensive and limited air-cargo space (Chan, 2008). Short storage life limits the export of papaya to distant markets by refrigerated sea containers (Mahmud *et al.*, 2008). The short postharvest life of papaya fruit is contributed by many factors including diseases. Postharvest losses due to diseases can reach about 97% depending upon the postharvest handling and packaging procedures (Alvarez & Nishijima, 1987). Anthracnose is an important postharvest fungal disease in most tropical and sub-tropical regions (Teixeira da Silva *et al.*, 2007). Anthracnose was found to be the main postharvest disease associated with papaya in Brazil and other countries (Cia *et al.*, 2007). In addition, inspection of papaya shipments that were delivered to the New York market revealed that anthracnose rot caused by *Colletotrichum gloeosporioides* was the major disorder (Cappellini *et al.*, 1988). It was reported that 40 to 100 % of papaya fruits were lost either due to its short postharvest life or due to lack of proper postharvest technology (Vyas *et al.*, 2014).

Low temperature storage, heat treatments and controlled/modified atmosphere packaging are some of the common techniques that have been used to prolong the shelf life of papaya and consequently reduces postharvest losses (Alvarez & Nishijima, 1987; Abd. Rahman *et al.*, 1994; Arpaia & Kader, 1997; Paull *et al.*, 1997; Gonzalez-Aguilar *et al.*, 2003; Singh & Rao, 2005). Even though these techniques were found to be successful in achieving their objectives, several limitations have made the needs for other technique necessary. Papaya fruits are sensitive to prolonged storage at too low temperature. The fruits exhibit signs of chilling injury when exposed to temperature of about 10°C or lower (Biglete *et al.*, 1994). On the other hand, exposure to heat during vapour heat treatment, hot water treatment or high ambient temperature causes hyperthermal injury in papaya (Biglete *et al.*, 1994).

These limitations have urged the needs for an alternative technique to maintain the quality of papaya. Edible coating is an alternative packaging methods for fresh products including whole fruits. It have attracted increasing attention because of environmental considerations and the trends toward the use of convenience food (Özden & Bayindirli, 2002). In addition, application of edible coatings on fresh fruit can provide an alternative to modified atmosphere storage by reducing quality changes and quantity losses through modification and control of the internal atmosphere of the individual fruits (Park, 1999). Studies have shown that semi permeable coatings are capable to improve the storage life of perishable fruits (Worrel *et al.*, 2002). Shelf life of mango was extended by coating treatments due to delayed ripening and softening, reduced decay and weight loss (Baldwin *et al.*, 1999). Edible coatings may contribute to extend the shelf life of fresh-cut fruits by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates (Baldwin *et al.*, 1996). The application of edible coating have been expanded due to its high potential to carry active ingredients such as antibrowning agents, colourants, flavours, nutrients, spices and antimicrobial compounds that can extend product shelf life and reduce the risk of pathogenic growth on food surfaces (Pranoto *et al.*, 2005).

Range of materials including essential oils and plant extracts has been incorporated into coating formulations to improve it antimicrobial properties (Bautista-Baños *et al.*, 2003; Rojas-Graü *et al.*, 2007 & Ponce *et al.*, 2008). However, due to the high resistance of some microbial strains towards these antimicrobial agents, new ones are needed (Sondi & Salopek-Sondi, 2004). Nowadays, nanotechnologies are being used to enhance the nutritional aspects of food by means of nanoscale additives and nutrients and also nanosized delivery systems for bioactive compounds (Bouwmeester *et al.*, 2007). Metal nanoparticles with silver nanoparticles in particular are well known for their antimicrobial properties. Numerous studies have been conducted to determine the antibacterial and antifungal activity of silver nanoparticles (Sondi & Salopek-Sondi, 2004; Cho *et al.*, 2005; Kim *et al.*, 2008, 2009 & Lamsal *et al.*, 2011). Their application as antimicrobial agent in various industries including food packaging is growing rapidly. Silver nanoparticles incorporated polymer packaging materials have been used to reduce microbial load and prolong shelf life of asparagus, carrot, pear and orange juice (An *et al.*, 2008; Mohammed Fayaz *et al.*, 2009 & Emamifar *et al.*, 2010, 2011). However, studies on the application of nanoparticles incorporated into edible coating on specific fruits are rather limited. In addition, very few studies were done on the migration of these nanoparticles into the coated or packed food.

Eksotika papaya has the potential to be applied with the silver nanoparticles incorporated edible coating. This is because the fruit are being largely exported and an efficient packaging that can help in extending their shelf life and maintaining the freshness by the time they reach consumer is required. Successful shelf life extension of papaya fruit will offer a chance to exploit the export of the fruits by less expensive sea freight (Chan, 2008). Thus, the study was carried out based on the hypothesis that the application of polysaccharides-based surface coating with the addition of silver nanoparticles will help to extend the shelf life of Eksotika papaya and reduces postharvest losses due to pathological disorder, mainly anthracnose.

Therefore, the objectives of this study are:

Overall:

To extend the shelf life of Eksotika papaya using polysaccharides-silver nanocomposite-based coating,

Specific:

1. To optimize edible coating formulations (chitosan- and carrageenan-based)for coating of Eksotika papaya,

2. To determine optimum silver nanoparticles concentration that are able to give effective antimicrobial effect,

3. To evaluate the effect of edible coating incorporated with silver nanoparticles on the quality aspects of Eksotika papaya and

4. To determine silver concentration in peel and pulp of papaya fruit samples and consumer acceptance towards the coated and uncoated papaya fruits.

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